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## GETTING ABOUT IN TOWNS

THE Greek gods would appreciate some of the irony which now attends the muddle in Britain on transport policy, but people who have to move about on the surface of the Earth are much less likely to be amused. Absurdity has reached a kind of climax in the past week or so, when Mrs Barbara Castle, the Minister of Transport in the British Government, has been launching a fistful of public documents on road transport at a time at which the nationalized railway industry has been unable to win the consent of the National Union of Railwaymen to a scheme for the efficient working of new rail freight terminals in big cities. To be fair, Mrs Castle has entirely the right ideas about the need to see that the balance which is struck between different forms of transport, particularly road and rail, is determined by rational considerations, most of them economic. So much she announced in her White Paper on transport policy less than a year ago and, even if she is not given to the vocabulary of systems analysis, much of what she has to say would be considered entirely respectable at places like the RAND Corporation. But it is one thing to have good aspirations, and quite another to make them come true. And the truth is that in Britain the ideal of an integrated transport system, which is to be understood strictly in the sense that the several different forms of transport should make their most economic contribution to the process of moving people and goods from one place to another, is still more of a distant goal than a reality.

The way in which energy and zeal are being lavished on the wrong problems is one of the saddest features of this sad state of affairs, as the report called *Cars for Cities* (HMSO, 21s.) illustrates all too painfully. This document is the product of two interlocking committees created three years ago by Mr Ernest Marples, a previous Minister of Transport. To begin with, the object was to see how changes in the design of road vehicles might create problems—or opportunities—in urban transport. Sensibly enough, the two committees have taken a somewhat broader view of their responsibilities than may originally have been intended, even though the handsome document they have produced studiously avoids mentioning trains and subways even as yardsticks for comparison. It is true that the more technical of the two committees, composed largely of engineers and people with practical experience of traffic problems, does have sensible things to say about the advantages of high acceleration for city cars, the potential benefits of segregating goods vehicles from others and the great advantages which may eventually come from various forms of automatic guidance in cities, but most of its effort has been spent on detailed suggestions for the design of small cars capable of carrying up to four people at a time and

usable only within cities. Most of its energy has been lavished on an elaborate scheme, which some will think a fantasy, for constructing in central London networks of elevated roads reserved exclusively for these tiny vehicles. But this is not even a poor solution to urban traffic problems. It is simply a red herring.

All this is evident from the way in which the technical committee, like the still more distinguished steering group which seems to have served in an avuncular capacity, insists that the benefits of tiny motor cars will be marginal unless they are provided with a segregated road system all to themselves. The argument is an elaboration of the simple view that to the road builders, a traffic lane must be at least as wide as the widest vehicle legally allowed to travel on it. But what would be the cost of a segregated road network for tiny cars? And if segregated road networks are within the bounds of possibility, are the tiny cars a more deserving cause than goods vehicles, or buses? On questions like these, both the committees are conspicuously uninquisitive. Instead, there are the predictable and even platitudinous exhortations. The potential benefits of "an integrated design of car, roadway and parking space" are so great that "it is vital to make a real effort" to achieve them. And "a co-ordinated research and development effort directed towards new forms of storage battery, fuel cells and advanced gas turbine engines and indeed any other prime moving device with low noise level and in-obnoxious exhaust should be mounted on a much more ambitious scale than at present". While all this is going on, nobody should be surprised if road traffic in London is found one day to have congealed entirely.

What Mrs Castle's committees should have told her, of course, is that technology as such cannot contribute directly to the solution of urban traffic problems except in comparatively unimportant ways. By now there is plenty of evidence that real improvements in the quality of surface transport for individuals are almost certain to stimulate the use made of roads. As Mr Parkinson would say—and may indeed have said—the number of cars on the roads will increase until the roads are jammed. More accurately, private individuals will tend to use their own cars in cities unless it is cheaper, quicker or more pleasant to use some other form of transport. It does not follow from this, of course, that traffic engineers should abandon as self-defeating their attempts to improve the efficiency of networks of urban roads. On the contrary, they should push on with schemes such as those which now abound for complexes of traffic intersections controlled by computers, for this is the best way of exploiting expensive capital assets

to the full. But if the real objective is a radical recasting of the pattern of urban transport, the solution is much more likely to be found in sociology and economics than in technical innovation pure and simple.

Another of the committees at the Ministry of Transport is, in this sense, a little nearer the mark. On the same day as the appearance of the report on tiny motor cars, the ministry published a report of a study of how the flow of road traffic in British cities, particularly London, might be controlled by the impositions of charges of various kinds (*Better Use of Town Roads*, HMSO, 7s. 6d.). The starting point for this argument is the calculation, not entirely above reproach, that the restraint of private motor cars in cities like London would allow other traffic to move much more quickly and economically. On one version of the argument, for example, a daily charge of 6s. for each private car entering central London would bring about a saving of £5 million a year from improved efficiency—and a cash revenue of the same amount. Even though these arguments are grossly optimistic, they are probably worth more detailed consideration. It is, after all, something to be grateful for that the ministry's committee has retreated from the old impractical idea that all vehicles might be charged an amount identical with the economic value of the use made of the roads. But if the goal is really the "elimination" of the motor car commuter, the ministry has to acknowledge that there will be a serious economic disadvantage in a system in which the grossly inadequate public transport systems now operating in British cities are the best means of transport accessible to all but millionaires.

Here too the ministry has been dazzled by oversimple considerations, most of them arithmetical. It is, however, entirely possible that much greater improvements in the over-all quality of urban transport could be obtained by comparatively easy administrative actions. Recognizing, for example, that most city transport systems are entirely adequate except at the morning and evening periods of peak travel, cheap fares for off-peak travel on public transport could bring great benefits. Comparatively small amounts of money spent on the speed and even the comfort of bus services and subways could bring important gains. Pricing policies which would cheapen the cost of travel within cities along trunk routes could help enormously to put commuter cars at a disadvantage, as could a sharper distinction between trunk routes (usually subway lines) and more local distribution networks (based more often on buses and taxis). Keeping public transport systems running late at night could be an economic gain if, on paper, a monetary loss. Using one part of the transport system to subsidize another is entirely respectable.

But which of the many possible solutions should the administrators try out? On the face of things, this is a daunting question. In reality, however, there is plenty to be done. A much more thorough understanding of how people use city transport of all kinds is an urgent need. Most of the British surveys so far have

been designed to measure the volume of traffic and not to understand why it exists, but only a sensitive appreciation of the function of urban transport will make it easy to divert it into more convenient patterns. Then there is also a need for experiment with administrative measures such as unusual pricing policies for public transport. Certainly there is more reason to hope that intelligent management of public transport policies could do more to rationalize the pattern of city transport than any artificial network of roads for tiny motor cars. In the long run, imaginative policies on transport in cities could help to shape cities into more efficient communities in which urban transport would be obviously a convenience and not a nuisance as well. But this will require that the Ministry of Transport should hold much more rigorously than it does to the view that city transport, public and private, must be dealt with as an integrated service designed to satisfy the needs of real people. In cities, if not elsewhere, there is no truth in the epigram that to travel is better than to arrive.

## NO COMPARISON

THE report of the OECD on the state of science in the United Kingdom and West Germany (see page 9) is something of a disappointment, coming as it does in a series of public documents which has done much to direct the attention of governments to important problems in the administration of science. A part of the trouble seems to have been that the decision to compare the two countries has led to an artificial emphasis on such tangible differences as may be found to exist. The danger that this would happen was appreciated before the study was begun, but foresight has not prevented the two groups of examiners from giving far too much importance, for example, to the structural differences in the machinery for the administration of science in Germany and in the United Kingdom. At the same time the report is curiously unhelpful about differences which are at once striking and potentially important. Why, for example, does German industry contribute more towards scientific research than its counterpart in the United Kingdom? The examiners of the OECD would have had a friendly reception from the British Ministry of Technology if they had been able to supply a pithy answer. No doubt there are some at least in West Germany who would have been equally receptive of a sympathetic explanation why expenditure on university research seems occasionally to have been less productive than it might have been. By paying less attention than it should have done to matters like these, the OECD report has tended to exaggerate the similarities as well as the differences between the two countries.

The study of science policy in Germany and in Britain was also, however, complicated by the importance the two governments attach to science and technology. Both of them are earnest seekers after better science policies, especially just now. The OECD



is quite right in pointing out that each of them gives a different emphasis to the objectives of policy—the British Government, for example, is most of all concerned to win some cash return from science and technology. The two governments have in common, however, the need to grapple with exceedingly complicated problems. Science policy runs into educational policy at one end of the spectrum and into taxation policy at the other. In circumstances like these, broad generalizations are unlikely to be convincing. Thus the way in which the OECD report rightly points to the cramping influence of specialization at British secondary schools will bring comfort to some of those who are engaged in battles to see things changed, but a report like this must necessarily seem somewhat remote and even oracular. Much the same is true of the chief recommendation of change in the administration of science in Britain—the claim that there should be a closer link between the empires maintained respectively by the Department of Education and Science and by the Ministry of Technology. A few years from now there may be a strong case for an amalgamation of these interests, but in spite of the enthusiasm of the House of Lords for this cause two weeks ago, it would be wrong to reunite science and technology under the Ministry of Technology until that recent creation knows much more clearly than at present what kinds of policies to pursue. Indeed, if the Ministry should accept the logic of its recent discovery that Keynes has as much to say as Rutherford about the translation of technology into money, it may grow to be more closely connected with the Department of Economic Affairs than with the Department of Education and Science. Then nobody knows what function the Central Advisory Council on Science and Technology will be able to work out for itself. Will Sir Solly Zuokerman be a co-ordinator or simply a harassed referee? These are fascinating questions, but they cannot unfortunately be answered by appealing to what may be described as recorded science policy. In this sense OECD has probably learned as much from its examinations as have the two countries most concerned.

## DOES CHINA EXIST?

Few people are well placed to know what is happening in mainland China, and one result is that even quite simple travellers' tales are eagerly sought after by those who have been compelled to stay at home. The article by Dr K. Mendelssohn on page 10 is more than a mere anecdote, of course, but it also serves to add another morsel of flesh to the crude skeleton which must at present serve as an appreciation by outsiders of the present condition of science in China. No doubt the Chinese themselves would be alarmed to know how strangely many of their recent policies have seemed from overseas. On the face of things, for example, it is hard to reconcile the policy centred on the communes in the early sixties with the character of technology as it is known in the West. By the same test, it is not

easy to see how the interests of working scientists in China will have been affected by the social phenomenon called the Cultural Revolution. Will they now be better placed to work effectively? Or will they find that too much energy must be spent in the pursuit of orthodoxy? These are important questions, for it is only a matter of time, though perhaps a long time, before Chinese scientists are fully integrated within the international community. It is disappointing that these questions are so hard to answer.

Who is to blame? The most obvious thing to say is that there is no obvious reason why the mainland Chinese should at this stage pay close attention to the international interests of their scientists. They have other fish to fry. Yet there is plenty of evidence in the past few years of a wish somehow to demonstrate that science and technology are flourishing in mainland China. There was, for example, quite open boasting a year ago about the way in which a group of Chinese chemists had been able to synthesize insulin, and, in retrospect, there was plainly plenty to boast about. Although the synthesis of insulin was completed almost at the same time in the United States, it looks as if the Chinese group had to contend with more serious difficulties; a good many of the natural intermediates in the synthesis of insulin were not easily obtainable, for example. It is also understandable that the government at Peking should be eager to point out the cleverness with which technical people have been able to make thermonuclear weapons a mere decade after what seemed at the time to be a great step forward in nuclear technology—the opening of a modest research reactor in 1958. Yet the scientific literature which is now increasingly available, often in translation, shows that these events are not strictly occasions for surprise. There is plenty of talent in the universities and institutes, though there is a long way to go before the research is being carried out on a scale which matches the size of China and the energy of its population.

In circumstances like these, patience is evidently the greatest need. It is too soon to expect that Chinese scientists should mix more willingly with the rest of the world. There is, however, good reason why they should be less suspicious of a continuing relationship with institutions elsewhere. After all, the Chinese themselves must know how much they gained in the fifties from their ability to move comparatively easily to institutes like that at Dubna in the Soviet Union. By now they should also have discovered that even in Western Europe, scientists do not always have horns growing out of their heads. Such exchange agreements as there have been, however, have so far been discouraging. Chinese visitors to laboratories in Europe have kept themselves in isolation. Visitors to Peking have sometimes found themselves kicking their heels in hotels. Language is an obvious difficulty, but only half the story. Is it too much to hope that exchange agreements will function more generously now that the first thermonuclear weapon has been exploded? That would be a curious irony.

## NEWS AND VIEWS

### Uranium In Plenty

THE United States Atomic Energy Commission has now made public the production capacity of the gaseous diffusion plants at which it manufactures enriched uranium, chiefly as a demonstration to industrial interests in the United States and elsewhere that nobody needs to worry about the availability of enriched uranium for power production, at least for some time to come. Inevitably, the AEC has had to provide information about its capacity to manufacture nuclear explosives which has only previously been guessed at. There is, however, at this stage no prospect that the technology of gaseous diffusion will become public knowledge, and the AEC has emphasized its intention that classification should persist.

The way in which the gaseous diffusion plants have been run down in the past six years is also apparent from the statement by the AEC. Electricity consumption at the three plants now operating is running at about 3,000 MW, compared with a peak consumption of nearly twice as much in the early sixties. In its statement, the AEC says that it is at present intended to reduce the operation still further until electricity consumption is running at roughly 2,000 MW by the end of 1968. Even then, when production will be roughly a third of full capacity, the AEC will be able to produce more than 3,500 metric tons of uranium metal enriched to 2 per cent in uranium-235. The corresponding production of uranium sufficiently enriched for the manufacture of weapons would be more than 30 metric tons a year.

The production capacity of the gaseous diffusion plants has been described in terms of the number of units of separative work performed on the isotopes of uranium by different quantities of electrical power (see Table 1). The non-linear character of this relationship no doubt reflects the way in which production is most efficient when the diffusion plants are working below their full capacity. The separative work done in separating isotopes is really a measure of the entropy of mixing of the two products, and in the operation of a diffusion plant the work done to produce a given quantity of enriched uranium will depend not merely on the degree of enrichment but also on the concentration of uranium-235 in the depleted uranium rejected from the plant as tailings. At present the AEC is operating its plants in such a way that tailings contain 0.2531 per cent of uranium-235, which implies that annual production (with electricity consumption running at 3,000 MW) would be equivalent to 2,615 metric tons of uranium enriched 3 per cent or 5,215 tons of uranium enriched 2 per cent.

Table 1. SEPARATIVE CAPACITY OF US GASEOUS DIFFUSION PLANTS AS A FUNCTION OF POWER CONSUMPTION

Megawatts	Annual production (millions of kilogram units of separative work)
2,000	8.9
3,000	5.9
6,000	17.0

In its statement the AEC says that the US government will need "only a small per cent" of the production of the gaseous diffusion plants during the seventies. Now that private operators of power stations in the United States may own their own uranium, the AEC is going to undertake to enrich this at its diffusion plants beginning in 1969.

### British Diffusion

INTEREST in gaseous diffusion for the separation of uranium isotopes has also recently been awakened in Britain. For one thing, work is well in hand with the scheme to prepare the diffusion plant at Capenhurst for the more efficient production of enriched reactor fuel. This work will cost a total of £14 million, and is intended to put the UK Atomic Energy Authority in a position to supply fuel for the advanced gas cooled reactors now being built in Britain—but not yet elsewhere. But there is also talk of the further extension of the plant to provide enriched fuel for power reactors being built in Europe. These schemes raise a number of problems.

The chance that the United Kingdom might be able to sell enriched uranium cheaply enough to compete with fuel from the United States is clearly sufficiently real for meaningful discussions on the subject to be held, but there is also talk of how it would be even more advantageous if an enlarged diffusion plant could be coupled more or less directly to an AGR reactor so as to take the fullest advantage of the high load factors of both types of machines. But if Capenhurst is to be extended so as to produce enriched fuel for reactors in Europe, who is to pay the initial cost? Obviously the AEA would like to see some at least of the capital coming from Europe, although it is hard to see how that could happen without compromising the authority's right—even duty—to keep the technology of gaseous diffusion to itself. But it is also known that the Government as a whole is uneasy about some of the political implications of the scheme. The possibility that export sales of reactors may eventually be determined by the capacity of the potential vender to offer continuing supplies of enriched uranium is never far beneath the surface.

### Computers In Concert

THE British computer industry will be permanently transformed by the proposed merger of two of the principal companies in the field—English Electric and Elliott Automation. On its own merits, the scheme is likely to appeal to shareholders of Elliott Automation, who have not done particularly well in recent years and who will be flattered to know that English Electric has valued their business at more than £41 million. Then everybody concerned will be cheered to know that the Industrial Reorganization Corporation has agreed to lend the joint enterprise £15 million in return for a modest rate of interest and the right to buy English Electric shares six or eight years hence. Evidently the marriage is well blessed, although it seems also to be an open secret that without this handsome dowry from public funds it would have been much less attractive to the proposed partners—both of them have found in recent years that good ideas

are easier to come by than the capital with which to finance development.

The technical advantages of the merger are easily appreciated. English Electric has recently won renown for itself in the design of time sharing computers. It is well placed in Europe and, like Elliott Automation, has established valuable outlets in Eastern Europe. It will be at least a formidable contender for the role of the largest computer manufacturer in Britain—a part now played by International Computers and Tabulators, which has computers worth £40 million on order. But the technical diversity of the merged company is in many ways even more remarkable. English Electric is traditionally strong in power machinery, from diesel engines to electrical generators of all kinds, and may thus provide a ready-made use for many of the process control devices which have been developed by Elliott Automation. At the same time, the experience of Elliott Automation with micro-circuits and with smaller computers may be useful to English Electric. Probably the biggest danger in the new arrangement is that the merged company may repeat the error of Elliott Automation in recent years of biting off more than it can chew.

## Plasma Instabilities

THE Science Research Council has set in motion a study of plasma physics in Britain. According to the official notice issued by the Plasma Physics Panel, its aim is "to provide a factual basis for forecasting research needs in this subject, as indicated both by university departments and by actual and potential applications to industry and technology". The review is being conducted by Dr P. A. Davenport of the Culham Laboratory of the UKAEA.

Dr Davenport has started by writing to universities to determine what courses of training in plasma physics are available. Most of these are well known to the SRC, but Dr Davenport is hoping that any he has missed will come forward to be counted. The inquiry also covers research in universities and government laboratories, and was originally initiated by the SRC to enable it to determine what areas of plasma physics should be fertilized with grants. The survey will also include an assessment of the applications of plasma physics in industry.

The review follows another rather different review of plasma physics. This was carried out by the Ministry of Technology, to determine the future of the AEA fusion research programme. The minister, Mr Wedgwood Benn, recently hinted at the results of the survey: "... this is a very difficult problem... the AEA work is excellent in every way, but prospects of fusion producing power are less hopeful than before. Fusion is not really likely to meet our needs in the near future... the AEA was right to establish Culham Laboratory, but the problems have got worse, and with the fast reactor coming along..." No decision has been announced, but it is clear that Culham sits somewhat unhappily beneath the Mintech umbrella, large though it may be. Pay-off in three years is the ministry war-cry these days.

That Culham finds itself in this position is something of a historical accident, a result of the separation of science from technology in Whitehall. It hardly needs a wild stretch of the imagination to see Culham

under SRC control, and then at least the pressure to produce a fusion reactor next week would be lessened. The change might well be more a book-keeping operation than anything else, and need cause little disturbance to personnel. By a nice irony, the notice setting up the SRC review is issued from Culham Laboratory, and is signed by the director, Dr R. S. Pease. In another guise, he is chairman of the Plasma Physics Panel of the SRC.

## Another Look at Southern Skies

THE agreement between President Johnson and President Frei of Chile to build a large optical telescope in the Chilean Andes was clearly no empty promise inspired by the camaraderie of the Punta Del Este conference. Further details now available indicate a firm commitment to go ahead with the project which will make the Cerro Tololo observatory one of the few major observatories in the Southern Hemisphere. Thirty-six and sixty inch instruments are already under construction there. Thanks to the lack of city lights, dust and industrial contamination, the site is perfect for an observatory, and the absence of man-made contamination is aided by particularly favourable meteorological conditions.

The new telescope is to be paid for jointly by the National Science Foundation and the Ford Foundation, through the Association of Universities for Research in Astronomy, Inc. The instrument will be essentially the same as that being designed for Kitt Peak National Observatory, near Tucson, Arizona. Both are to be 150 inch instruments, and it is expected that design duplication will greatly reduce costs. This means that no fewer than three 150 inch instruments will be built to virtually the same design, for it was recently announced that the Southern Hemisphere Telescope to be built in Australia would be based on the Kitt Peak design. Costs for that instrument, to be paid jointly by the SRC and the Australian Government, are estimated at £4.4 million (\$12.3 million), while the Chile telescope is to cost \$10 million.

## Turtles come to Britain

TURTLES are animals of tropical and sub-tropical waters, but sometimes they are stranded on British beaches. The first record of a landing is due to Sibbald who, in 1684, was told by a worthy man of Orkney that turtles were sometimes captured there; between September 1938 and February 1939 eleven turtles were stranded in Great Britain and Ireland. Since then receivers of wrecks and HM coastguards have been keeping records for the British Museum (Natural History), which is anxious to collect as much information as possible about these strandings. To help turtle catchers identify finds, the museum produced a booklet *Stranded Whales and Turtles* which is now superseded by *British Turtles* by L. D. Brongersma, director of the Leiden Museum. Anybody finding a turtle is requested to inform the British Museum by telegram immediately, and if sending the specimen itself to send it alive—turtles are extremely difficult to kill humanely except by hypodermic injection.

Turtles are known to travel great distances—for example, from the Brazilian Coast to the island of

Ascension—between feeding and breeding areas, but such migrations take place within one climatic zone. It is remarkable, as Mr Brongersma points out, that the animals should reach Northern Europe where the climate is unsuitable for a long stay, and where they cannot breed (the temperature is far too low).

Most of the animals found in British waters seem to come from the Western Atlantic Ocean and the Gulf of Mexico. Kemp's Ridley, *Lepidochelys Kempi* (Garman), one of the five species described in *British Turtles*, breeds only in the Gulf of Mexico, and may pass through the Florida Strait to the east coast of America. It sometimes continues its voyage from America to Europe, partly carried along by the Gulf Stream. The age of some of the specimens found suggests that hurricanes in the Caribbean area could sweep some very young turtles out into the ocean, where they lose their bearings and then just follow the current. Some specimens have lacked one of their front flippers, which implies that they would only have been able to swim in circles—clearly they must have been swept along by the current. The journey to Europe seems to take between thirteen and seventeen months. Although protected by their shells, the turtles are still in danger of having flippers bitten off by sharks or sliced off by propellers of ships, so that the specimens which finally reach the British Museum are not always complete.

## Structure and Reactivity of Ribonuclease

from Professor E. A. Barnard

THE recent determinations of the three-dimensional structure of ribonuclease offer an unusual opportunity for insights into the molecular basis of enzyme action, and were the background for the international symposium held at the State University of New York at Buffalo between May 31 and June 1.

To begin with, there was a correlation of the determinations of the structure itself. Models and maps of the 2 Å structure of ribonuclease A due to G. Kartha, J. Bello and D. Harker and that of ribonuclease S due to H. W. Wyckoff and F. M. Richards (at 3.5 Å) were compared side by side. The very high degree of agreement between the structures was remarkable, considering the use of two protein forms in different heavy atom derivatives and in very different solvents. Most of the differences occur, as expected, in the regions on both sides of the bond (20–21) that is cleaved to form the ribonuclease S derivative. C. H. Carlisle who, using quite different derivatives, has obtained a map of ribonuclease A at 5.5 Å, showed that this was compatible with the Kartha's map with a transposition to a corresponding origin of co-ordinates: this map should lead to the same structure when taken to higher resolution.

A good deal of attention was paid to evidence on binding properties. Both the Roswell Park group (Kartha and colleagues) and the Yale group (Wyckoff, Richards and colleagues) showed that one bound ligand, defining the active centre regions, can be located in a cleft. The Yale workers specified the binding of inhibitors containing iodouracil. Several participants related the specific binding of di-anion inhibitors to interactions at a cluster of histidine and lysine side chains. G. G. Hammes interpreted evidence of relaxa-

tion rate to show discrete steps in the binding, involving histidines and (by presumption) a carboxyl.

There was much less agreement on the mechanism of catalysis. Different hypotheses by B. R. Rabin, G. G. Hammes and H. Witzel were separately put forward as consistent with the structure. It seems not to be possible to specify from the X-ray evidence the type and the extent of structural change which occurs on binding, chiefly because a ligand bound to the active site has so far always been necessary for crystallization. This, in itself, suggests a more motile structure for the free enzyme.

Some new directions of work became clear at this conference. H. A. Scheraga forecast the total computation of the structure of proteins such as ribonuclease and lysozyme, using their sequences and the minimization of energy. This bold approach has been applied so far to gramicidin S, oxytocin and, now, to two regions of ribonuclease. The method should yield the structure the proteins would possess if isolated, so that its validity has not yet been fully tested. In comparative studies, C. H. W. Hirs showed that pig pancreatic ribonuclease is a series of glycoproteins. E. A. Barnard, M. H. Gold and E. N. Zendzian reported a new study of the distribution of pancreatic ribonucleases through the vertebrates, and presented evidence of reactivity and specificity which shows that the active centre is similar. The active centre specific labellings they obtained point the way to phylogenetic sequence comparisons, but the time is clearly still distant when it will be possible to make a full comparison of three-dimensional structures in this series.

## Circular DNA

from our Correspondent in Molecular Biology

MUCH interest has in recent years been generated by the discovery of circular DNA in viruses, and more recently in mitochondria from a variety of cells. This DNA is fully double stranded, and has been shown to be covalently cyclic. The properties innate in such a structure have been investigated by Vinograd and his associates. They arise from the topological restriction that, for a given molecular weight, the number of turns of the two-stranded helix is fixed. This confers on the molecule an enhanced resistance to denaturation, and hydrodynamic properties which differ from those of linear native DNA.

Good evidence was obtained earlier that the normal form of mitochondrial circular DNA is supercoiled, and shapes were observed in the electron microscope which were best described as being like pretzels. Vinograd and his group showed that when a single break is introduced in one strand, or when a small degree of denaturation is provoked, the supercoil is able to unwind and the molecule reverts to the untwisted circular form.

Radloff, Bauer and Vinograd (*Proc. US Nat. Acad. Sci.*, **57**, 1514; 1967) have now devised a striking method for the separation of intact circular DNA from the linear and the damaged circular species with which it is extracted from the cell. The principle involves the binding of a dye, ethidium bromide, which belongs to a class of planar conjugated molecules now generally held to be capable of intercalating, or sliding between adjacent bases, in a helical DNA. There is

both *a priori* and experimental evidence that such an insertion must stretch the molecule and give rise to some degree of unwinding of the helix. When the dye is added to the circular DNA, the superhelix untwists, in order that the number of turns in the primary helix may remain invariant. With addition of further dye, a superhelix of the opposite sense begins to form, so as to take up more slack, until a rather rigid structure is produced which cannot deform sufficiently to accommodate more dye. The circular form thus binds substantially less dye than the linear form. But when a dye is attached to DNA, there is a decrease in the density of the molecule; consequently the circular DNA saturated with ethidium has a greater buoyant density than saturated linear DNA which contains more dye, and is widely separated from it by sedimentation in a caesium chloride density gradient. Excellent fractionations are achieved, which must exclude even molecules with a single break in one strand.

Electron microscopy on preparations from HeLa cells reveals, in addition to the circular molecules previously encountered in mitochondria (mean length 4.8 microns), some large supercoils which are multiples of these, and also some very small and polydisperse circles (0.2–3.5 microns). These could code only for between 200 and 3,500 amino-acids, and their function is unknown. It will be interesting to establish whether they are universal constituents of mammalian cells.

## Genome Transcription

from our Correspondent In Cell Biology

In bacteria, unlike eucells, the replication of the DNA genome goes on throughout virtually the whole of the cell division cycle. This implies that transcription and replication of DNA occur simultaneously. Indeed, it has been suggested that the basal level of enzyme synthesis could be controlled by a coupling of these processes, with transcription occurring only at or near the replication point where stretches of single stranded DNA might be expected. This is not a universal mechanism, however, for, as Maaloe and Kjelgaard (1966) showed, ribosomal, transfer and some species of messenger RNA are made throughout the whole cell cycle.

Several important features of the pattern of DNA transcription and replication during the division cycle of *Escherichia coli* have recently been determined by Cutler and Evans (*J. Mol. Biol.*, **26**, 91; 1967) using an ingenious technique they devised (*ibid.*, p. 81) for the isolation of segments of the *E. coli* genome. Synchronously dividing cultures of *E. coli* were fed pulses of 5-bromouracil at different times throughout the division cycle. If the population is sufficiently well synchronized, all cells will have the same region of DNA labelled during any one pulse. The labelled cells are then collected, their DNA extracted and sedimented in a density gradient which separates the denser 5-bromouracil labelled DNA from the rest. The various DNA fractions labelled at different times in the cell cycle fail to hybridize with each other to any great extent; thus they appear to be unique segments of the genome.

Cutler and Evans have been able to measure the amount of RNA which is transcribed off each of these segments at particular times in the cell cycle by the determination—using a  $P^{32}$  pulse labelling technique—

of the amount of RNA synthesized during the cell cycle of a synchronous culture that will hybridize with each DNA segment and which is therefore homologous with it. They find that the entire genome is being transcribed continuously but that the levels of transcription of a particular segment show characteristic fluctuations during the cell cycle.

It is interesting that a high level of transcription of one region is usually accompanied by high levels for all the other segments. How this co-ordination of transcription of the whole genome is achieved remains to be seen. There is some evidence that control of ribosomal and/or transfer RNA transcription may be related to the position of the DNA replicating point, but this is not the case for most of the genome.

One most interesting observation is that two separate segments of the genome are complementary to 16S and 23S ribosomal RNA and most species of transfer RNA. This may mean there are two distinct sites containing the genes for these RNA species although the inherent limitations of hybridization experiments make this interpretation equivocal.

## Can Algae utilize Methane?

from our Correspondent In Microbiology

REVIEWING hydrocarbon fermentations and their industrial application, A. E. Humphrey (*Biotech. Bioeng.*, **IX**, 3; 1967) writes, "Until recently, the primary industrial concern with hydrocarbon microbiology was in connexion with oil prospecting, corrosion problems and formation of a microbial sludge in jet fuel tanks". The scope of hydrocarbon microbiology has expanded rapidly in the meantime and currently the quest is for means of exploiting this microbial activity commercially. Propitious reports of the use of microbes in the partial refining of crude oil fractions and in the production of edible yeast and several biochemicals from hydrocarbons provide a great stimulus for continued research and development.

A report from Dr Lennart Enebo working at the Royal Institute of Technology in Stockholm (*Acta Chem. Scand.*, **21**, 625; 1967) may charge the interest in these matters still further. While studying the growth of photosynthetic sulphur bacteria in different gaseous environments Dr Enebo isolated the green alga *Chlorella* from highly reducing enrichment media in which carbonate and methane provided the carbon sources. Passage of the alga on a glucose medium led to an almost complete, but not total, elimination of the associated microflora. The addition of high quality methane to the carbonate medium culminated in a 35–45 per cent enhancement of algal growth. The grounds for believing that the alga assimilates the hydrocarbon hinge largely on two points. First, the alga excretes various metabolites which are likely to inhibit the growth of methane oxidizing bacteria. Second, free oxygen is produced by algal cultures growing in an atmosphere of 100 per cent methane and it seems reasonable to suppose that this may act in the oxidation of the paraffin to methanol. The subsequent assimilation of  $C_1$  units as carbon dioxide or their incorporation into sugar phosphates has been postulated but not proved. Enebo's claim for methane utilization by *Chlorella* is supported by the fact that he was unsuccessful in demonstrating other methane

oxidizers in his algal cultures. It is possible, however, that the small residual contamination may exercise an effect on algal growth by oxidizing extracellular metabolites produced by the alga. This process would depend on the oxygen derived from algal photosynthesis and would produce carbon dioxide for fixation. Other investigators have found that methane fermenting bacteria will grow on methane alone, although here also carbon dioxide may function as a "primer" for the system (Wolnak *et al.*, *Biotech. Bioeng.*, IX, 57; 1967). Unfortunately, Enebo does not record the absolute carbon requirement of his *Chlorella* strains.

It would be premature to dwell on the technical value of this newly discovered methane assimilating system, but, with growth optima established and the selection of suitable strains, several possibilities might be explored. Enebo indicates an application for gas exchange in closed systems where oxygen enrichment is needed and the production of algal mass on a methane-carbonate basis, even under anaerobic conditions. Although sound economically, few attempts have been made to produce food by fermenting methane and in part, to quote Humphrey further, "The arguments against such a process stem from toxicology and consumer-acceptance problems". The algal system may prove to be an attractive proposition to those interested in this technology.

## Cows as Subjects

THE National Institute for Research in Dairying at Shinfield near Reading is one of the largest research establishments financed by the Agricultural Research Council, with a staff of about 450, more than 800 acres of farmland and 500 cattle. Since the foundation of the institute in 1912, it has been closely associated with the University of Reading, which provides its governing body.

Although the institute is concerned primarily with helping farmers and dairymen to solve their practical problems, a surprising amount of fundamental research is done there. The chemical microbiology department, for example, is working on the antibiotic nisin, discovered at the institute, in the hope of relating the structure and molecular size of nisin to its antibiotic properties. The molecule of nisin seems to polymerize readily, and it may be that the monomer is the active form. The department has also carried out some work on the activity of rennin, including preliminary work on its structure.

The more practical side of the work of the institute includes fundamental studies of cheese and the cheese-making process. The institute has one of the two sterile cheese vats in the world, and these have made it possible to produce cheese using only rennin and a pure "starter" organism. It seems that the character of a cheese is determined chiefly by the nature of the bacterium used to start the process of fermentation. The other organisms which contaminate the bacteria used commercially are not necessary to the process and probably give rise to the "off-odours" which can mar the quality of cheese.

The two farms belonging to the institute are used for studies of the growth and feeding of cows raised under various controlled regimes. The institute also maintains a herd of pigs, some of which are raised under germ-free conditions.

## Parliament in Britain

LORD WINTERBOTTOM, Parliamentary Secretary, Ministry of Public Building and Works, said that progress on the scheme for a National Reference Library for Science and Innovation had suffered a severe set-back because the Greater London Council had discontinued negotiations on the South Bank site for the library, which the council now required for an urgent purpose of their own (undisclosed). The minister was now seeking for a suitable alternative site for the library. The present situation was entirely unsatisfactory, since that part of the library which is in Whiteley's building at Bayswater is thoroughly unsuitable for the work. One or two possibilities in London were being investigated and Lord Winterbottom agreed that the matter is urgent and that good access by public transport and, if possible, a central location of the site selected are important. (Question, House of Lords, June 22.)

LORD BESWICK, the Parliamentary Under-Secretary of State for Commonwealth Affairs, said that there is no evidence, either from research carried out in the United Kingdom or from the study of the results of tests held in the United States, of damage to health from sonic bangs. Damage to buildings varies with the intensity of the bang, but soundly constructed buildings are not affected by bangs of the kind which a supersonic transport aircraft might be expected to make. For operational reasons, the Concord civil aircraft will not reach supersonic speed until at least 100 miles after take-off. (Question, House of Lords, June 19.)

THE Iron Casting Industry (Scientific Research Levy) Order 1967 was approved by the House of Lords on June 19. It imposes levies on the industry to finance scientific research carried out by the British Cast Iron Research Association when the statutory levy introduced under the Iron and Steel Act 1953 ceases with the repeal of that Act on July 28. The levies are expected to yield about £200,000 a year and this will enable the research association to qualify for a grant from the Ministry of Technology of about £80,000 a year. Lord Shackleton, Minister without Portfolio, referred to the initiation by the association of the research which led to the production of spheroidal graphite cast iron, but said that further economic advantages were expected to arise from increased efficiency rather than from innovation, particularly in melting, moulding materials, methods of automatic control, and improvement of working conditions in the foundries.

THE Prime Minister, Mr H. Wilson, declined to appoint a Minister of State in the Ministry of Technology solely concerned with the development of the technology of the sea bed. He did not think that a full-time ministerial appointment could be justified, but agreed that a major marine science programme could be sustained on its own economic merits. Mr Wilson agreed that the time had come for a much wider investigation of marine technology and science and this was one reason for establishing the co-ordinating committee for scientific research. (Question, House of Commons, June 22.)



# Tale of Two Countries

The OECD has already produced reports on the condition of science and technology in several member countries as part of a systematic review of science and science policy. Its most recent venture is a comparative study of science policy in the United Kingdom and in West Germany.

THE possibility that science policy in European countries may be changing more quickly than the Organization for Economic Co-operation and Development can produce reports is one of the impressions created by the latest in the series of reviews of national science policies—this time a comparative study of affairs in the United Kingdom and in West Germany (OECD, \$5.50, 32s., 22 francs). But the document is also valuable as a proof that the two countries have in common not merely size and economic prosperity, but problems as well. There will also be great, if recondite, interest in the evidence given by officials of the two governments at the "confrontation" arranged in Paris at the beginning of March 1966. Many of the hopes and justifications then presented have since been falsified by events.

The notion of a comparative study seems to have been prompted by the statistical similarity of the two countries. In area, for example, they differ by 1.6 per cent and in population (in 1964) by 6.8 per cent (with West Germany slightly the larger on both counts). In 1962 and 1964, the GNP of West Germany was roughly 10 per cent greater than that of the United Kingdom, but the two countries differed sharply in how they spent their wealth—the United Kingdom spent more than West Germany on private consumption (65.5 per cent and 57.6 per cent of the GNP respectively) and less on investment in buildings and machinery (16.2 per cent and 25.4 per cent respectively). The United Kingdom spent more than West Germany on research and development in 1962 (see Table 1), but the difference is not substantial when the cost of military research and development is left out of account. The contribution of the two governments to civil research and development was substantially the same, but British industrial companies seem to have been spending rather more of their own funds on research and development than their counterparts in West Germany. There is a similar pattern in the distribution of effort, measured in money terms, in 1964 and 1965, for which the OECD quotes figures which have been compiled from its own surveys. The report points out that research and development expenditure is increasing rapidly in both countries, and that the figures it quotes may differ because of differences in interpretation—the British figures for university research and development do not, for example, include expenditure by the research councils on units which are closely associated with universities.

The report points out that government support for civil research and development, in both countries the source of more than a third of all spending, is divided among a number of agencies—in Germany between the Federal Government (which maintains a Ministry of Scientific Research) and the Länder governments (which support the universities), and in Britain between the Ministry for Education and Science and the Ministry of

Technology. In the circumstances it is not surprising that the two teams of examiners which undertook the separate study of the two countries should have given a good deal of their attention to problems of co-ordination. On the whole, the report takes the view that co-ordination comes more naturally in West Germany than in the United Kingdom. The report is particularly pleased with the way in which organizations such as the Deutsche Forschungsgemeinschaft and the Max Planck Gesellschaft zur Förderung der Wissenschaften, supported as they are by public funds, are nevertheless able to function effectively in all fields of science.

Yet "co-ordination at government level" is "equally urgent in both countries", in Britain because of the way in which responsibility is divided between two ministries, one of which (the Department of Education and Science) is responsible not merely for research but also for the still larger expenditure on education of all kinds. One possibility going through the minds of the examiners was that the Council for Scientific Policy might be given a more powerful part to play, outside the narrow confines of the Department of Education and Science, but Sir Frank Turnbull, then Deputy Secretary at the Department of Education and Science, argued eloquently against such an arrangement on the grounds that "we believe that in our system of Government it is best to have scientific advice tendered directly to the minister who is responsible for a given field". He was speaking, of course, before the formation of the Central Advisory Council on Science and Technology under Sir Solly Zuckerman.

In West Germany, according to the OECD report, the existence of a central policy making body (the Wissenschaftsrat), formally required to give advice to the Federal Government as well as to the governments of the Länder, is a logical arrangement. Nevertheless, the argument goes, the way in which funds for the support of research are channelled through outside bodies means that the German Government can rarely initiate new developments. Instead, it must function as a "responsive" agency. Things are complicated by the way in which the Wissenschaftsrat cannot be sure that its recommendations on policies which must be implemented by the Länder governments will actually be carried out. Nevertheless the committee has embarked on an attempt to draw up a comprehensive plan for the development of science and technology in West Germany, and the OECD examiners are clearly delighted with the way that project is going.

By comparison with the Government of West Germany, the British Government seemed to the examiners to be preoccupied with efforts to increase productivity and to apply the results of research industrially. German interests were more often concerned to "catch up with the international development of science in certain fields and to implement a nationally co-ordinated development of the educational system".

On education, the OECD examiners have come down heavily against specialization in the schools, which seems to have been most marked in Britain. Although the examiners were impressed with what they describe as the "efficiency" of university education in Britain, they argued in favour of a lengthening of the university course from three to four years. In Germany, too, there

Table 1. RESEARCH AND DEVELOPMENT IN THE UNITED KINGDOM AND WEST GERMANY (£1 = DM. 11.20)

	United Kingdom	West Germany
Total (£ million)	657.7	404
Percentage of GNP	1.3	2.3
Total civil (£ million)	412	367
Government spending (total)	378	185
Government civil spending	183	148
Private sector	290	219
Total spent in universities	22	78

is a need for longer courses of study. The German engineer schools are held out by the examiners as a good example. The OECD report emphasizes that in both countries, problems of scale and of rapidly increasing size are only now becoming apparent, so that changes of structure and of policy will have to be continuous and rapid in the years ahead.

In general, the examiners decided that "the relationship between the scientists and government, the degree of common thinking and reciprocal understanding", are more highly developed in Britain than in Germany. On the other hand, there is a better appreciation of "the importance of science and scientific training in industry and in the general public".

## Science in China

by

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In the last few years the development of science and technology in China has gone on apace, and seems to be linked with political development. Dr Mendelsohn has been there three times in the past seven years.

WHEN at the turn of the century the tottering Manchu Dynasty disintegrated, China had seen remarkably little change for six centuries. One of the main reasons was the extreme conservatism of her rulers. The Manchus, being strangers and, in Chinese eyes, semi-barbarians, had done everything to fit in with the accepted mode of Chinese life and, in fact, held on to tradition more tenaciously than the Chinese themselves. The educational system, too, remained firmly based on the Chinese classics which in the public examination system provided the only passport to the mandarinship. Coupled with this attitude was a pronounced xenophobia and a strict prohibition on Chinese seeking to leave their country. Only after the inroads made by Western powers, beginning with the opium war and with the establishment of the Treaty Ports, did knowledge of Western technology start to seep gradually into the country.

After the establishment of the republic in 1911, some well-to-do Chinese began to send their sons to Europe, America and, above all, to Japan to acquire some technological and scientific training. In China itself, university study in science commenced only in 1920. From the very beginning it was hampered, however, by the ever increasing unrest and the incessant campaigns which the various war-lords fought against each other. At the same time the Japanese occupation of Manchuria was gradually increasing, leading in the end to the full scale invasion of China.

When at last in 1949 the present regime extended a unified rule over China, a large proportion of the few scientifically trained Chinese had either perished or left the country. In a much devastated China with a population which had hardly emerged from a medieval feudalistic society, technological training had to start from scratch. In the beginning the Russians rendered a good deal of help by sending experts into China and by accepting Chinese students into their own universities and technical training institutes. When visiting Moscow in 1957, I learned that my Soviet colleagues were indeed much impressed with the ability and single-minded devotion to work of their Chinese students.

At the time of my first visit to China in 1960, the Russian experts were just beginning to leave. Then, as well as in later years, my Chinese colleagues always acknowledged fully the help which the Russians had given to them, although there were other aspects of the withdrawal, such as leaving factories half finished, which were a severe blow to China.

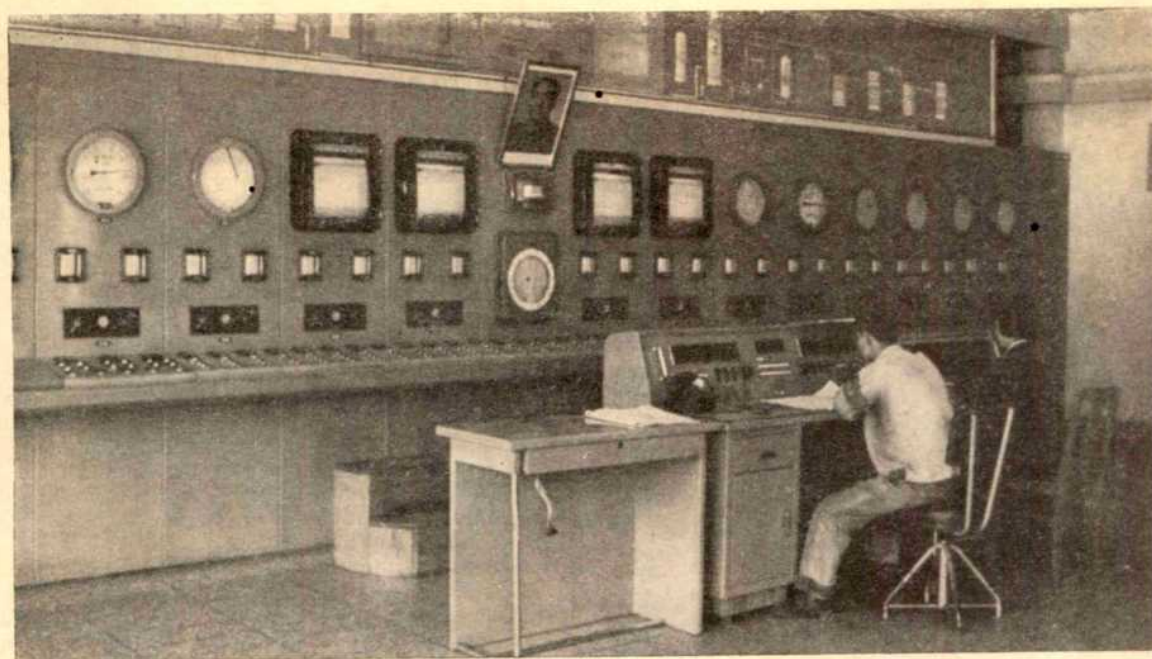
Russian influence also left its mark on the organization of education and research, resulting in a pattern which remained largely unchanged until the cultural revolution of last summer. While certain features of this pattern were modelled on the Soviet example the Chinese departed

right from the beginning in a number of important aspects. Comparatively little is known about the detailed plans for the impending changes in scientific training, but I was given a certain amount of information by Chinese scientists visiting this country in the course of the last year and particularly on my third visit to China in September and October 1966. Something about these future developments will be said later, after dealing with the state of affairs which has obtained until now.

The Chinese student enters his university after a general education at a school which has given him no specialized training. The university science course takes from 5 to 6 years, and the first 3 years are spent on a very thorough general study of the student's subject. There is a certain amount of specialization in the remaining years with a research project in the last one. The course ends with a final examination but no degree is awarded. The student then lists ten choices of employment arranged in order of preference, and the authorities, when placing him in a job, will take this into account as well as his ability and performance at study. One of the difficulties which arose was that the older universities with an established reputation were naturally inclined under this system to secure the best pupils for their own staff positions, leaving the lesser grades for the many newly established centres of learning. In the end a new scheme was introduced which was designed to overcome this drawback. Each of the older universities was made responsible for a number of new ones and the success of each of these groups was then assessed together. This forced them to send some of their good students to these new establishments in order to give them a good start. My Chinese friends assured me that this method had proved very successful.

Another important feature of the Chinese system of higher education is that technology, medicine and practical law are not included in university studies. Here the reason is that in a rapidly developing country an enormous number of engineers, doctors and legally trained administrators will be required, and that inclusion of all these students would have produced a severe imbalance of the university faculties. Instead, all these people are educated at specialized institutions. Taking, for instance, the case of a physicist, we would find the student at Peking University if his aim is to study the fundamental aspects of the subject. If, on the other hand, he is more interested in applied physics, he would turn to one of the technical universities, such as Tsinghua or Hangchow, for his training. Finally, the specialist will study at one of the institutions devoted to a strictly limited field, as, for instance, electronics or hydraulics. In these the specialist subject is not only taught but all the applied research going on in the field is also carried out. Generally speaking, for all studies, except the most fundamental aspects,





A Red Guard at the control desk of a fully automated nitrogen fixation plant near Shanghai (1966).

much stress is laid on close co-ordination between basic research, development and production. Great care is taken that the technical managers of factories are in frequent consultation with these research institutes and they are kept informed, through frequent conferences and visits to other establishments, of the current state of their field throughout the country.

Much of the fundamental research is carried out in the institutes of the Academia Sinica which, at least in its original pattern, is modelled on the Soviet Academy of Sciences. Like the latter, the Academia Sinica comprises, apart from the natural sciences, arts subjects such as literature, history and economics. The scientific institutes are by now well equipped for advanced research, and last October at the Institute for Solid State Physics I was shown superconducting solenoids of niobium-zirconium and of niobium-tin made of wires manufactured in China. There was also equipment for making artificial diamonds which are of similar size and quality as those produced in Western countries.

Staffing still presents a serious problem although now a new generation of scientists and technologists trained in China is becoming available. In the early years, at the time of my first visit in 1960, the whole teaching burden was carried by a small number of men who had been trained between 1920 and 1940, mostly abroad. They were getting old and were, in addition, terribly overworked. A few years later these men had largely moved into administrative positions and their place was taken by the next generation trained in China as well as in the Soviet Union. I was much impressed not only by their degree of competence and ability but also by their excellent knowledge of current research outside China. It frequently happened in discussion after my lectures that they would make reference to results or theories published in the *Physical Review* in the last few months.

There have often been reports in the West of the detrimental effect which insistence on political studies or time spent on parades or demonstrations have had on scientific and technological progress in China. Of this I have seen little. If, indeed, my Chinese colleagues have spent some of their time in this way, they seem to have shouldered this burden in their stride. In fact, judged by the state of affairs in October 1966, scientific and technological progress has been very remarkable

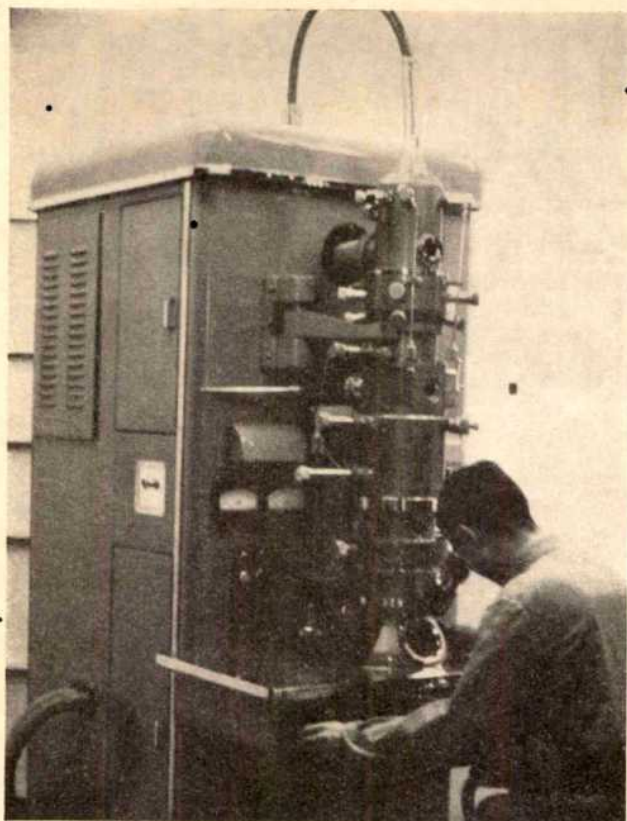
since I saw it first, 6 years earlier. Technological products are usually a pretty good guide and the stage has now been reached when Chinese industry can manufacture practically anything that the West can produce.

In 1960 there existed little in the way of modern factories. It was the period of the much ridiculed backyard industry when I saw girls winding with their bare hands transformers for the agricultural grid. This operation was carried out in an agricultural commune where hardly the simplest machine tools were available. In the open, among horses, ducks and chickens, boys and girls were sitting on the ground, cutting out the transformer housing from sheet metal with hammer and chisel. Miraculously, in the end the finished transformers appeared, complete with cooling coils, and ready for dispatch. Admittedly, productivity was miserably low, but it has to be remembered that it was greater than no productivity at all, which would have been the case without these small plants. A rather more important aspect, and possibly the salient one, is the industrial training which these places provided in an otherwise completely agricultural society. The boys and girls who learned their trade in this hard and exacting school in the end provided the foremen for the factories which were being built at the same time.

Two and a half years later I visited the first of these new factories, one of which made excellent precision grinding machines for a budding machine tool industry. Some of the new factories were equipped with Russian machinery, but by now this has been supplemented or even replaced by excellent Chinese made equipment. The early sixties saw the rise of Shanghai to one of the largest manufacturing cities in the world. Its present population is somewhere between seven and ten million. It is now surrounded by a number of new satellite towns, all with modern blocks of flats and shopping centres, to accommodate the rising industrial labour forces.

By now Chinese manufacturing capacity has shifted into high gear. There are, first of all, the standard products of an industrial power such as ocean going ships, air conditioned and diesel driven trains, cars, buses, trucks, electric generators and gas turbines. Beyond this there is the manufacturing equipment; machine tools of every description and a lot of ancillary plant such as arc melting furnaces, vacuum deposition units and electronic gear,





Using an electron microscope made in China.

to mention just a few. Even more impressive are the highly sophisticated products which can only be found in a fairly advanced technological society. These are things like electron microscopes, industrial spectrographs, precision balances, single lens reflex cameras and computers.

I have mentioned these things because they seem to provide a better appreciation of the underlying scientific and technical training than educational statistics. They also show that this training must be of high standard and broadly based to be able to account for the great variety of products. The most remarkable aspect of this technological advance is the fantastic speed with which it has been achieved. The complete change of the technological scene which I witnessed in the interval of only a few years is almost unbelievable.

One of the main springs of the development clearly is the unbounded enthusiasm with which the whole population has set its mind on the rapid reconstruction of the country. When in 1949, after a century of chaos and humiliation, China emerged again as a sovereign power, the Chinese could hardly believe that the age of war-lords, invasion and famine was over and had given way to stability and security. Once they had realized this unbelievable good fortune, they threw themselves wholeheartedly into the task of perpetuating and improving this new state of affairs. They were told that the creation of a modern technology was the most important way in which the country could be strengthened and the standard of living could be raised. On my second visit my colleagues introduced me to Mao Tse-tung and Foreign Minister Chen Yi. Both of them, in their welcome to me as a foreign scientist, emphasized the need for China's scientific and technological development. When I mentioned that they were progressing rapidly, Chen Yi brushed this aside, saying that China was technologically still backward and must learn quickly. This attitude of not being content with the present achievements and of spurring on to still more rapid advance is the

note met in all educational establishments as well as in the factories.

This is not the place to discuss in detail the meaning of the cultural revolution, but it is quite apparent that the pace is not slackening but, wherever possible, is increasing. This emphasis on rapid development is particularly held by the young generation, who cannot remember the bad old days and who have grown up in security and relative comfort. The call for "continuous revolution" means, above all, the resolve to continue the effort of the last seventeen years. That no break in the progress of science and technology is envisaged is clearly shown by a special provision in the programme of the cultural revolution, the famous decision of the Central Committee of the Chinese Communist Party of August 8, 1966. In point 12 it says: "Special care should be taken of those scientists and scientific and technical personnel who have made contributions. Efforts should be made to help them gradually transform their world outlook and their style of work". Clearly the emphasis is on the term 'gradually' which is a firm directive not to interfere with the development of science and technology.

In the early summer of 1966 a physics colloquium was held in Peking, and while other papers were published under the authors' names we find one coming from the research group on the theory of elementary particles, Peking. This styling is in full accord with the tenets of the cultural revolution which deprecates striving for personal fame. The title of the paper is *Research on the theory of elementary particles carried out under the brilliant illumination of Mao Tse-tung's thought*. It contains a very serious and quite impressive attempt at solving the internal structure of elementary particles in which the successive steps are copiously underlined by reference to Mao's works. The essential feature is an internal wave function describing the structure of the hadron and the advantage of this approach is that correlation of the theory with experimental results can be made without having to make assumptions on the unknown dynamics operating inside a particle. In true Maoist language the relevant chapter is headed "How can you catch tiger cubs without entering the tiger's lair?".

New and far reaching plans for a change of the educational system were, in fact, the very first sign of the cultural revolution. In 1965 already Chinese colleagues visiting Britain told me about this impending move and we can expect that, when the dust has settled after the parades and wall posters, these proposed reforms may be implemented. The basic feature will be a much closer link between study and production. In the scientific field it will probably result in alternating the student's activities between classroom and factory. This is, of course, similar to our own sandwich courses which have yielded very good results. Secondly, efforts will be made to shorten the present course of study which is based on the Soviet system. The Chinese are confident, from the experience gained in the past years, that their courses can be shortened by more intensive work without degrading the syllabus. Finally, more attention is to be paid to ability than memory. All university examinations are to be of the open book type, but the questions asked will be much harder than hitherto. All these seem to be very reasonable proposals designed to educate fairly rapidly a large number of competent scientists and technologists.

There is a curious tendency in Western countries to underrate the scientific and technological potential of the communist states. This may be largely because of the preoccupation of the daily press with ideological issues. It has caught the West unprepared when the sputniks went up after having been told that Russian science was relying largely on spying. It seems that we may be heading for a similar situation with regard to China when we are inundated by doubtful reports of internal unrest and impending civil war at a time when that country's scientific and technological potential is rising at this high rate.



# Principles of Classification and Nomenclature of Viruses

by  
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Gibbs, Harrison, Watson and Wildy criticized the use of a binominal nomenclature for viruses (*Nature*, 209, 450; 1966) because, they said, equal weight cannot be given to all characters according to genuine Adansonian principles. Dr. Lwoff denies that this is so, and argues in favour of a binominal nomenclature.

In July 1966, in Moscow, the International Committee for the Nomenclature of Viruses (ICNV) met for the first time. Their discussions revealed that a number of virologists seemed to be unaware of the principles and significance of classification and also of the use and usefulness of a binominal nomenclature. A few virologists made use of a binominal nomenclature without knowing it, but it was obvious that systematic and nomenclature were sometimes confused. For example, Gibbs, Harrison, Watson and Wildy (from now on G.H.W.W.) write: "The idea of a code has recently been considered both for viruses and bacteria". Lwoff, Horne and Tournier dealt with viruses<sup>1,2</sup>, but discussed only classification, and did not mention the nomenclature which G.H.W.W. were concerned with.

The time has come when a discussion of the principles of classification and of nomenclature would be useful for both medical and plant virologists.

Specific things must have specific names which designate a category. In order to be recognized and identified, the category to which a given name applies should be defined accurately. For a category to be defined criteria have to be selected. How should we deal with the definition and nomenclature of viral categories? A few viruses, although different, exhibit a certain number of common characters. These related viruses must be placed in groups named in such a way as to avoid confusion, disorder, and changes of nomenclature.

Since the time of Linnaeus binominal nomenclature has been used extensively. As I have said, some virologists seem to ignore binominal nomenclature and others use it unknowingly. For example, three types of poliovirus have been recognized: I, II and III. These types are related; they share a certain number of properties which transcend their differences. Because of these common properties virologists have decided to give them the common name, Poliovirus. In a binominal nomenclature poliovirus would be the genus and I, II, III the species. When we speak of Poliovirus I, II or III we make use of a binominal system.

To group species under a common generic name is an act of classification; it means that the common traits as well as the differences of the species have been recognized. In this respect, but in this respect only, binominal nomenclature cannot be separated from systematics.

At the congress in Moscow the species was defined as a collection of viruses with like characters, and the genus as a group of species showing certain common characters. Both the species and the genus have to be named, and this implies an acceptance of binominal nomenclature, which is, of course, essential if nomenclature is to express the affinities of the viral species. Furthermore, the ICNV decided that "an effort should be made towards a latinized binominal nomenclature" and has, already, adopted some rules for the latinization of names. That latinization is necessary in order to confer an international status to a nomenclature has long been recognized by zoologists, botanists and bacteriologists.

When speaking of the virus of smallpox we use common

language, which also applies to pox virus. But, if we speak of *Poxvirus variolae*, we have a latinized binominal nomenclature. When G.H.W.W. speak of "Herpes virus simplex" they are very close to the latinized binominal nomenclature, *Herpesvirus simplex*. Is this latinized binominal nomenclature really a catastrophe, as some virologists seem to think?

Among the active opponents to the binominal system are G.H.W.W., who object that "the latin binominal system is rigidly based on the characters chosen to determine the form of the hierarchy of taxa: divisions, order, families, etc. . . ." A taxon is a specific group of organisms, such as mammals, diptera, mycobacteria, and a category is a rank in a categorical hierarchy. Thus the divisions, orders and families to which G.H.W.W. are referring to as taxa are categories.

If the virus of variola major is named *Poxvirus variolae*, and if it is the type species of the genus *Poxvirus*, then the name *Poxvirus variolae* will not depend on the nature of the characters used in the definition of, for example, families, orders and classes. In this respect, binominal nomenclature is unrelated to the form and hierarchy of categories of higher rank. Whatever the hierarchy of characters selected for the definition of families, orders, and other categories the end result would be the same. The second objection of G.H.W.W. to binominal nomenclature is that if one of the characters should be found unsatisfactory, then the whole hierarchy might collapse and new names would have to be found. It will not be because somebody finds the criteria of a given classification of viruses unsatisfactory that the classification will collapse, for the names of the classes and orders are completely independent of the names of the families, genera and species. The final objection that viral nomenclature should be based on a general classification of viruses is incomprehensible. Giving a name to a species means that its discriminative characters have been recognized, and to unite species into a genus is to recognize their common characters. To name a species or a genus is an act of nomenclature, and to group species into a genus is an act of classification. Nomenclature is not, however, based on a "general" classification of viruses. The name *Herpesvirus simplex* will be the same whatever the general classification of viruses. The herpes simplex virus of G.H.W.W., in a latinized binominal nomenclature, would be *Herpesvirus simplex*. The two names have exactly the same content of information and one cannot be bad if the other is good.

Thus, while criticizing classification in general, G.H.W.W. have started to define groups and to propose names. The groups are defined by a series of signs. For example, *Herpes simplex virus* is:  $\frac{D}{2} \frac{74}{X} \frac{S}{S} \frac{V}{0}$ , where

*D* stands for DNA and 2 is the number of strands; 74 is the molecular weight of the DNA and *X* is the percentage of DNA; *S* is the spherical shape of the "particle" and the spherical shape of the nucleocapsid; *V* is the vertebrate (host) and 0 means no vector is known.

The authors call this formula a cryptogram. As they point out, the cryptogram is part of the name and "a name should be simple, easily understood and usable by people speaking different languages". Yet most of the signs of the cryptogram correspond to the first letter of English words. And sigla have been forbidden by the ICNV. I should like to be sure that virologists really prefer a cryptogram to a name. Names like Herpesvirus or Poxvirus or Myxovirus are really more easily memorized than a formula with eight terms, and furthermore, in the list given by G.H.W.W., different characters are designated by the same letter. This is not going to clarify anything. Finally, most alphabets have only twenty-five letters and we have to label sixty characters.

When defining categories, should all characters or a selected few be considered? G.H.W.W. state: "We can think of no satisfactory way of estimating the relative importance of different characters and therefore consider it essential to follow the principles of Adanson, who suggested that all known information should be used and that all characters should be considered equally important". Mayr<sup>4</sup>, however, said that Adanson did advocate the weighting of characters, a process which, Mayr says, is essential to numerical methods.

Why, then, do pheneticists insist on non-weighting? One of many answers is that they do not know how to weight or that they do not like doing it. The other is that the programming of information fed to a computer is easier if all the characters have equal weight, that is the same information content.

G.H.W.W. consider that classification of viruses on Adansonian principles is now possible with the help of a computer. This might be true if genuine Adansonian principles are put to work and not G.H.W.W.'s misinterpretation. The question immediately arises: where does the computer lead? Sokal and Sneath, the main supporters of the computer, admit that "about the only thing it can do is the estimate of similarities between taxa. The rest of the taxonomic task needs the experience and the judgment of the specialist in the field."

The only operationally definable category is recognized to be the species, which can be pragmatically defined as a group of entities with identical characters, with the exception of phenotypic and genotypic alterations. It is the least arbitrary category. The genus is a group of different species. The family, a group of genera, the order, a group of families, and so on. All these categories are arbitrary, and even if the viral world was monophyletic, even if the complete phylogeny of viruses was known, a classification based on phylogeny would be unnatural and arbitrary, for many features of the viruses have certainly evolved independently. In order to classify, one must select characters, those characters which are fitted for a harmonious and logical system of order. Yet natural groups do exist in nature as a result of evolution. Viruses which show similarities have probably derived from a common ancestor. Because they share a certain number of characters they are united into the same category.

One category is necessarily different from another. Aristotle recognized that categories exist only by virtue of a definition, and, in order to define a category, discriminative characters have to be put to work. A category can be defined only by a character or a group of characters which are absent in other categories. If a family, for example, was defined by all the characters of one species there would be one family of each species. According to genuine Adansonian principles, a category should be defined by a minimum of characters. An array of other traits will serve to define the various different members of the category. Uniting a series of viruses under one name means that their useful discriminative characteristics have been recognized. The higher the rank of a category, the fewer must the characters be. If this elementary principle is ignored, no classification is possible.

Our system<sup>2</sup> makes use of four characters: the nature of the DNA or RNA; the symmetry of the nucleocapsid (helical or cubical); the nature of the nucleocapsid (naked or enveloped); the diameter of the nucleocapsid for virions with helical symmetry, or the number of capsomeres for virions with cubical symmetry. These four characters when used give categories which are recognized as valid by most virologists, for example, Poxviruses, Myxoviruses, Herpesviruses. So why add more characters to those four which are not only sufficient but also necessary? A system of classification which neglects the symmetry of the nucleocapsid would end up with categories including widely different unrelated species: viruses with a cubical and with a helical nucleocapsid would be in the same family.

If a computer had to deal with two viruses with fifty-nine like characters, the two viruses differing by the nature of the host: bacterium or vertebrate. In view of the fifty-nine like characters, the computer would place the two viruses in the same family, and would do exactly the same if the two viruses differed only by the nature of the genetic material or the symmetry of the nucleocapsid. The computer has no right to decide that a character should transcend another and has no way of selecting the characters according to their importance.

I suppose that most virologists will agree that two RNA viruses with cubical symmetry, a naked nucleocapsid and thirty-two capsomeres should belong to the same family, although one thrives in bacteria and the other in vertebrates. It is clear that the genetic material and the host should not have the same value so far as a classification is concerned.

Gibbs, Harrison, Watson and Wildy state that a classification of viruses should be based on at least sixty, equally weighted independent characters. When, however, they tried to define viral "groups", they selected eight characters.

For obvious reasons, practice has been different from theory, but either the theory or the practice should have led to a classification. Universally accepted, viral groups should have been "rediscovered", but this was not the case. Even the revised and amended principles have failed. A classification should be based on a minimal number of characters, rationally selected for their discriminative value. What virologists need is a real classification—and not unapplicable principles.

According to G.H.W.W., viruses cannot be classified objectively and rationally, but this should be possible soon. They consider that the existing classifications are irrational and subjective, but my colleagues have started naming "groups". It is recognized that the coining and application of any collective or generic term is an act of classification. Thus, when G.H.W.W. select characters, define and name groups, they are on dangerous ground; they are classifying, without knowing it.

A selection of characters is necessarily arbitrary, and it has the same degree of arbitrariness whoever makes the selection. The rationality of the selection should be judged objectively by asserting the usefulness of the characters for the classification.

The reader might ask why we want a classification. A classification is useful because it starts discussions, has a heuristic value, allows predictions, is useful for teaching purposes, and allows related viruses to be united into one and the same category. A negative attitude leading to an absence of classification has no value.

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<sup>1</sup> Gibbs, A. J., Harrison, B. D., Watson, D. H., and Wildy, P., *Nature*, **209**, 450 (1966).

<sup>2</sup> Iwoff, A., Horne, R., and Tournier, P., *Cold Spr. Harb. Symp. Quant. Biol.*, **37**, 51 (1962).

<sup>3</sup> Iwoff, A., and Tournier, P., *Ann. Rev. Microbiol.* (1966).

<sup>4</sup> *Ann. Inst. Pasteur*, **159**, 625 (1965).

<sup>5</sup> Mayr, E., *Systematic Zool.*, **14**, 78 (1965).

<sup>6</sup> Mayr, E., *Amer. Zoologist*, **5**, 165 (1965).



# Oxygen Isotope Analyses and Pleistocene Temperatures Re-assessed

by

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Oxygen isotope analyses of foraminiferal tests in deep-sea cores have been interpreted as showing that the temperature of seawater in the Caribbean and equatorial Atlantic varied by as much as 6° C during glacial cycles. This evidence has now been reinterpreted, and changes in oxygen isotope composition are now said to correspond with the extraction of large amounts of water from the oceans during glacial periods and the recirculation of this water during periods when glaciers were at their present levels.

THE method whereby palaeotemperatures are obtained by determining the oxygen isotope composition of carbonate fossils is now well known<sup>1-3</sup>. In recent years Emiliani has used this technique to elucidate the climatic record of the Pleistocene. To do this, he has made many hundreds of oxygen isotope analyses of the tests of planktonic foraminifera, taken from deep sea cores. Typically rather smooth curves representing the change in isotopic composition with depth in the cores (that is, with time) are obtained; the wavelength of the oscillations is very roughly 40,000 years, and the amplitude is about 1.65 parts per thousand in the Caribbean and the Equatorial Atlantic<sup>4</sup>. On the assumption that the isotopic composition of the oceans may have changed by about 0.5 parts per thousand, these measurements are interpreted as showing that the surface temperature of the Caribbean Sea and the Equatorial Atlantic fluctuated by about 6° C during the glacial-interglacial cycles.

From the beginning the results showed a satisfactory measure of agreement between the climatic record which Emiliani thus obtained, and the climatic record inferred by micropalaeontologists investigating faunal changes represented in the same deep-sea cores<sup>5,6</sup>. More recently a very detailed faunal analysis of a single core<sup>7</sup> has provided a climatic record which is almost identical with that obtained by Emiliani<sup>4</sup>.

Despite this striking agreement, many workers have doubted the validity of Emiliani's work<sup>8,9</sup> on the grounds that, although Emiliani has made allowance for the changing isotopic composition of the oceans during the waxing and waning of the continental ice sheets, his correction factor is not altogether well founded. This change in ocean isotopic composition is due to the fact that the isotopic composition of atmospheric precipitation, and hence that of a continental ice sheet, is different from that of the ocean; to give an extreme example, the isotopic composition of snow accumulating near the Pole of Inaccessibility in Antarctica is some 60 parts per thousand deficient in oxygen-18 compared with ocean water<sup>10</sup>. The factors governing the isotopic composition of atmospheric precipitation are well known<sup>11</sup>, and the likely average isotopic composition of a Pleistocene ice sheet can be estimated from information regarding its location, thickness and surface temperature distribution. Olafsson<sup>9</sup> has made such an estimate for the larger Pleistocene ice sheets, making an essentially two-dimensional approximation; that is, he assumes that the average isotopic composition taken over the surface of an ice sheet is a good approximation to its three-dimensional average. Having made this calculation, Olafsson suggests that the entire record of isotopic change recorded by planktonic foraminifera may be ascribed to changes in the isotopic composition of the oceans, after abstraction to the ice fields of about 2.5 per cent of the water now present in the oceans.

I have recently recalculated this effect, taking into account the flow of ice in three dimensions. This is quite

straightforward because the gross features of ice flow in a glacier are well known<sup>12</sup>. Even without assuming any decrease in air temperature during glacial stages, this calculation yields a result close to that obtained by Olafsson. (His calculation was criticized by Emiliani<sup>13</sup> because of the arbitrary nature of the air temperatures which he assumed.)

If such large changes in ocean isotopic composition have taken place, analyses of benthonic foraminifera, living in an environment of rather constant temperature, should yield exactly the same record as do analyses of planktonic foraminifera. I have analysed a selection of benthonic foraminifera from Lamont core A 179-4. Planktonic foraminifera from this core were analysed isotopically by Emiliani<sup>14</sup> and faunally by Ericson and Wollin<sup>5,6</sup>. The core was taken in the Caribbean Sea, south-east of Jamaica (16° 36' N., 74° 48' W., 2,965 m depth) and was described by Ericson and Wollin<sup>5</sup>. The samples analysed cover the last two "glacial" stages, and the intervening "interglacial"<sup>14</sup>, or "interstadial"<sup>15</sup>.

In Fig. 1 the benthonic analyses are plotted against Emiliani's determinations for the planktonic species *Globigerinoides sacculifer* from the same levels. Numbers denote the depth of each sample in cm below the top of the core. Point O (circled) represents the calculated isotopic composition of benthonic foraminifera living today, assuming a temperature of 4° C (ref. 15) and an isotopic composition for the water of -0.2 parts per thousand (ref. 16).

Bottom water in the Caribbean is derived from the Atlantic, being selected from about the 1,700 m depth level by the sill in the Virgin Island Passage<sup>17</sup>. The temperature crudely represents the bulk temperature of Atlantic water<sup>18</sup>, and would be expected to change with time to a small extent compared with changes in surface temperature.

If the glacial-interglacial variation in ocean isotopic composition had been 0.5 parts per thousand (compare Emiliani<sup>4</sup>, page 120), points on Fig. 1 should lie on line A. Similarly, if the variation had been 1.0 or 1.5 parts per thousand, points should lie on line B or C respectively. If there were no systematic change in temperature with changing isotopic composition, the points should lie on line D.

The fact that the points lie closest to line D shows that changing ocean isotopic composition is certainly the primary cause of the isotopic changes observed in planktonic foraminifera. I believe that the deviations from line D in the present series of measurements are likely to have been caused by random errors in measurement and random uncertainties in sampling. The latter occur because the benthonic foraminifera analysed may not have lived at exactly the same time as the planktonic individuals analysed by Emiliani. It is known that bottom mixing takes place on the sea floor; Arrhenius<sup>19</sup> quotes a mean mixing depth of 3-5 cm. Emiliani's measurements represent the average isotopic composition of some 100 to

400 foraminifera, and should be relatively independent of uncertainty caused by this effect. On the other hand, the benthonic determinations are based on between 0.3 and 0.4 mg calcium carbonate<sup>20,21</sup>, which may be obtained from three to ten individual foraminifera, and may be subject to some uncertainty on this account. The extent of the uncertainty obviously depends on the rate of change of isotopic composition; at most levels a small mixing would scarcely affect the result obtained, but at a point such as the 10 cm level, just above the sediment representing the rapid melting of the last continental ice sheets, a small mixing uncertainty would produce a large isotopic uncertainty.

The measurements made by Emiliani<sup>14,22</sup> on benthonic foraminifera from core 234 may be plotted and interpreted in a similar fashion. Emiliani has previously had to invoke the presence of excess glacial meltwater in the bottom water in order to explain these measurements. The presence of excess meltwater in the surface water has also been deduced<sup>9</sup>.

Emiliani has refuted the possibility that large changes in ocean isotopic composition have taken place<sup>13</sup> by referring to his own measurements of the isotopic composition of benthonic foraminifera from the Pacific Ocean<sup>23</sup>. These analyses are supposed to show that the maximum glacial-interglacial change in ocean isotopic composition was only 0.5 parts per thousand. Unfortunately the samples were selected according to Arrhenius's interpretation of the cores concerned, and their detailed relation to climatic changes is, in fact, far from proved<sup>24</sup>.

Until recently, satisfactory climatic records had not been obtained from Pacific cores by faunal analysis, but work by Blackman and Somayajulu<sup>24</sup> shows that suitably chosen cores display faunal changes of the same order as those observed in the Atlantic; these changes are presumed to have occurred synchronously in the two oceans. If this was the case, samples of benthonic foraminifera from the cores investigated by these workers, selected on the assumption that the faunal changes are indeed synchronous, should show the same change in isotopic composition as has been shown to have taken place in the Atlantic. I have analysed two samples from core DWBG 114 (taken at 18° 20' S., 79° 21' W., in a depth of 2,300 m), taken from 3-6 cm and from 15-17 cm (ref. 25). They

were selected to bracket the most recent transition from high latitude fauna to equatorial fauna; this should be the best period over which to test whether or not a significant change in isotopic composition took place in the Pacific, because we can feel very confident in correlating it with the similar transition in the Atlantic.

The results are given in Table 1. At present, the bottom temperature is about 1.5° C (ref. 26) and the bottom water has an isotopic composition near -0.17 parts per thousand (ref. 27), so that benthonic foraminifera living today will have an isotopic composition of about +3.8 parts per thousand; it appears that the large *Pyrgo* fragment which made up the bulk of sample 1 must be derived from a few cm lower, or elsewhere. Ignoring this measurement, the average for the 3-6 cm level is +3.7 parts per thousand, while the average for the 15-17 cm level is +5.1 parts per thousand, giving a difference of about 1.4 parts per thousand.

Table 1. ANALYSES OF BENTHONIC FORAMINIFERA FROM PACIFIC CORE DWBG 114

No.	Depth	Description	$\delta^{18}O$ (parts per thousand)
1	3-6	Large corroded <i>Pyrgo</i> fragment, and a few smaller fragments of <i>Pyrgo</i> sp.	+5.02
2	"	Several <i>Planulina</i> sp.	+3.57
3	"	Three <i>Orbulina</i> sp.	+3.82
4	"	Mixture of small benthonic spp. (no <i>Miliolidae</i> )	+3.73
5	15-17	Several <i>Uvulgerina</i> sp.	+5.03
6	"	Several small individuals <i>Pyrgo</i> spp., and fragments	+5.18

Emiliani has made one pair of analyses of benthonic foraminifera bracketing this short period; they are from a mixture of levels 43.5 and 53.5 cm in core 234, and from 2-3 cm in the pilot core 234.1A (ref. 22) (taken in the Eastern Equatorial Atlantic), and the isotopic difference is 1.61 parts per thousand.

We therefore have clear evidence that Atlantic and Caribbean surface waters, Atlantic bottom water, Pacific bottom water, and Atlantic deep water as represented by Caribbean bottom water, all registered a glacial-interglacial change in isotopic composition of about 1.4-1.6 parts per thousand, as predicted by realistic calculations.

It may still appear that this body of information could be incorporated into an explanation in terms of a pattern of temperature change. Here there are two restrictions. First, the change in isotopic composition is clearly required by calculations based on the fact that ice is known to have built up on the continents during certain stages of the Pleistocene. Second, in the circulating body of the oceans, we cannot postulate any reduction in the temperature at the heat sink in the system, because it is already near freezing point; this means that it is very difficult to postulate a large reduction in temperature in ocean bottom water, only two or three degrees higher than freezing at present, unless it is caused by an enormous drop in the heat put into the system. If this had been the case, surface temperatures would clearly have had to fall enormously. The isotopic evidence does not suggest any greater change at the surface than in the coldest depths. As regards changes in surface temperature of the order of a degree or so, this work only shows that there is no reason to restrict hypotheses to patterns of temperature change in which temperatures at the ocean surface drop in proportion to the extent of glaciation.

It was pointed out by Broecker<sup>28</sup> that the well documented movements in marine planktonic faunal provinces during the Upper Pleistocene could have been caused by changes in salinity rather than by changes in temperature. The evidence that has been presented shows that this was probably the case. An interesting consequence of this conclusion is that all the evidence which was previously interpreted as suggesting a rapid amelioration in climate close to 11,000 years ago, obtained from the investigation of deep-sea cores<sup>29</sup>, must now be interpreted as demonstrating the rapid melting of continental ice sheets at

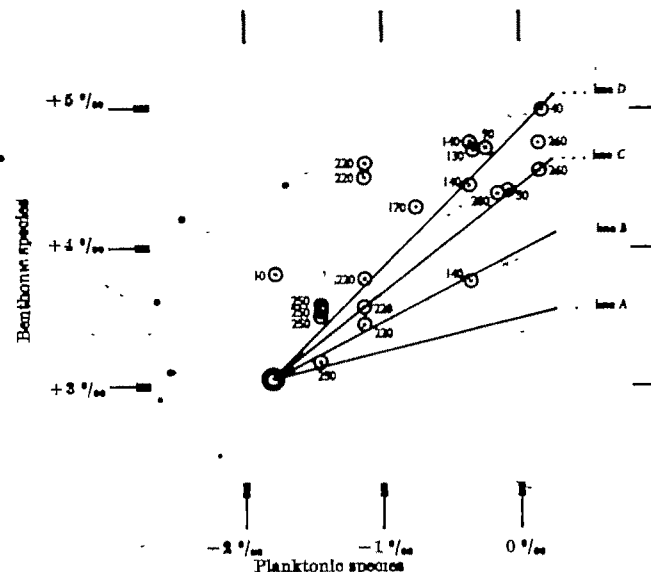


Fig. 1. Oxygen isotopic composition of benthonic foraminifera from Lamont core A 179-1, plotted against oxygen isotopic composition of the planktonic species *Globigerinoides sacculifer* (Brady) as determined by Emiliani<sup>14</sup>. All analyses are expressed as deviations in parts per thousand (‰) from the PDB standard<sup>3</sup>. Analytical precision is  $\pm 0.20$ ‰ (2σ) for benthonic analyses and  $\pm 0.20$ ‰ (2σ) for Emiliani's measurements. One analysis (250 cm, +3.55‰) is of part of a benthonic echinoid.

this time. This is in good agreement with the terrestrial evidence.

An inspection of the detailed record which Emiliani obtained from core 179-4 (ref. 30) in the light of the present work reveals an apparent paradox. If the curve representing variation in the isotopic composition of the planktonic species *Globigerinoides rubra* and *G. sacculifer* must be interpreted entirely in terms of changes in the isotopic composition of the water, taking place through the whole column of water, then the species *Globorotalia menardii*, which registers a smaller glacial-interglacial range in isotopic composition than actually occurred in the water, must have lived in warmer water during the glacial periods than during the interglacials.

Closer examination shows that this is only to be expected on the basis of the evidence available. Emiliani<sup>31</sup> showed that the depth habitat of this species, as revealed by the isotopic temperature which it records, is determined by water density. Hence, as during a glacial period the water density increased with increasing salinity, the population of this species would need to migrate upwards to warmer levels in order to inhabit water of the preferred density.

Although it has generally been assumed that the temperature changes recorded in deep-sea cores were synchronous with the principal glacial episodes in the Northern hemisphere, this could only be proved for those glacial episodes which can be dated radiometrically. On the other hand, if the faunal and isotopic changes which have been observed are the result of the extraction of large quantities of water from the oceans and their storage in the form of ice, their relation to glacial events is unquestionable. It is simply necessary that every faunal or isotopic curve be re-read, taking "cold" to mean "extensive continental glaciation" and "warm" to mean "glaciers reduced to their present level".

In conclusion, it should therefore be emphasized that the time sequence which Emiliani<sup>4</sup> has been able to obtain by the analysis and correlation of many deep-sea cores remains of inestimable value; indeed, its value is in a sense enhanced by the certainty that it is a time-sequence for terrestrial glacial events, rather than oceanographic events. His work remains a uniquely valuable contribution to geology and equally to archaeology.

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## An Antigenic Site of Sperm Whale Myoglobin

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Part of the antigenic activity of sperm whale myoglobin is known to reside in the C-terminal region. A more detailed investigation shows that the C-terminal antigenic site of the molecule corresponds to the C-terminal hexapeptide which includes the corner between a helical segment and a non-helical segment of myoglobin.

PREVIOUS attempts to define the structural features of the antigenic sites of sperm whale myoglobin showed that the C-terminal heptapeptide of myoglobin partially inhibited the precipitation of metmyoglobin (MetMb) and apomyo-

globin (ApoMb) by certain antisera to MetMb<sup>1,2</sup>. The results suggested that the heptapeptide represented at least a significant portion of one of the antigenic sites of sperm whale myoglobin. An attempt has been made to delineate more closely the structure of the C-terminal antigenic site by comparing the inhibitory activities of a

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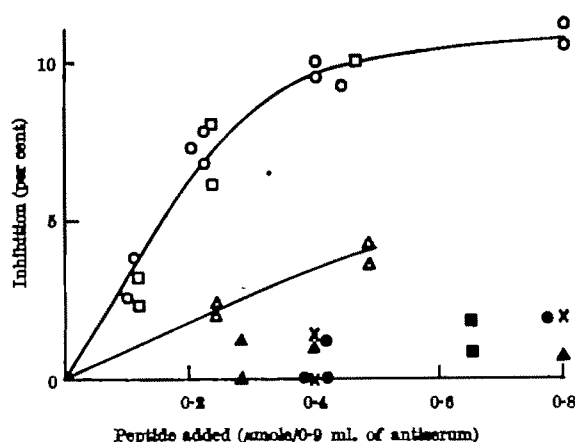


Fig. 3. Inhibition of precipitation of 20.2 µg of ApoMb with 0.9 ml. of antiserum WD by increasing amounts (dissolved in 0.2 ml.) of peptides D2 (○), D2a (△), 1T2 (□), D3 (●), D1b (▲) and 1T1 (◇), and by peptide D2 which had been incubated at 37° C for 3 h with 5 per cent by weight of nagarse (×). The washed precipitates were dissolved in 0.5 ml. of 0.1 normal sodium hydroxide and the extinctions of solutions measured at 290 mµ; the decrease in extinction due to the inhibitor was expressed as a percentage of the extinction obtained in the absence of inhibitor ( $E_{290}^{1\text{ cm}} = 0.545$ ).

measuring inhibitory activities of some of the chymotryptic and tryptic fragments.

Peptides D3, D1b, D2, D2a, 1T1 and 1T2 (0.4 to 0.8 µmole) caused not more than 2 per cent inhibition of precipitation of ApoMb by antisera WM and WK. The experimental error of the inhibition test was equivalent to about 2 per cent inhibition, so that these peptides were probably inactive under the conditions used. In contrast, the precipitation of ApoMb by antiserum WD was inhibited by peptides D2, D2a and 1T2. The inhibitory activities of the peptides with antiserum WD are summarized in Fig. 3 and Table 2. The results indicated that peptides D3, D1b and 1T1 were probably inactive. On the other hand, it can be seen (Fig. 3, Table 2) that the C-terminal hepta- and hexa-peptides (D2 and 1T2 respectively) had similar inhibitory activities, which were less than that of peptide 1 (Table 1), and that the inhibitory activity of a pentapeptide (D2a) included in D2 was less than that of D2. It was concluded that peptides D2 and 1T2 represent at least a portion of either the antigenic site or one of the sites of peptide 1. The results also suggest that, whereas the C-terminal Gly and/or Gln residues contribute to the inhibitory activity of D2 and 1T2, the N-terminal Lys residue of D2 makes no significant contribution to the activity of D2. The activity of peptide D2 was destroyed almost completely by digestion with nagarse (Fig. 3). High-voltage electrophoresis at pH 3.5 of the nagarse digest, and elution and analysis of the peptides, revealed the presence of 10 per cent of undigested D2, 80 per cent of Lys-Glu-Leu and Gly-Tyr-Gln-Gly, and 10 per cent of Lys-Glu-Leu-Gly-Tyr and Gln-Gly. These results suggest that Lys-Glu-Leu and Gly-Tyr-Gln-Gly are not inhibitory and that the sequence Leu-Gly formed a part of the C-terminal antigenic site. Results closely similar to those reported for peptide D2 were obtained earlier using a different anti-MetMb serum (see Fig. 8)<sup>1</sup>.

The antigenic sites of myoglobin would be expected to be composed of amino-acid residues whose side-chains are exposed on the surface of the molecule. Although the N-terminal Lys of the C-terminal heptapeptide (D2) is exposed<sup>4</sup>, it does not form a significant part of the anti-

genic site detected by antiserum WD. As a result, this site may not be larger than the C-terminal hexapeptide (1T2). Antigenic sites composed of five to six residues have been reported for synthetic polypeptidyl proteins<sup>10</sup> and the dimensions of the C-terminal hexapeptide in native myoglobin (15 Å × 9 Å × 11 Å) are similar to those deduced for the combining-site of antibodies to dextran (34 Å × 12 Å × 8 Å)<sup>11</sup>. It is not known whether all the amino-acid residues of the hexapeptide contribute equally to the inhibitory activity, but the sequence Leu-Gly was essential and the C-terminal Gly and/or Gln made a contribution. In contrast, it has been reported<sup>7</sup> that the C-terminal dipeptide of myoglobin is not an essential part of an antigenic site of the native protein. This disagreement may be due to the use of different antisera which contained antibodies with different specificities. The C-terminal hexapeptide gave a maximum inhibition of about 11 per cent with antiserum WD, whereas the C-terminal 22-amino-acid peptide (1) gave 30 per cent. Consequently if the hexapeptide represents a complete site, peptide 1 must contain at least two sites. The molar ratio of peptide 1 to antigen for half the maximum inhibition (2) was much smaller than that of the hexapeptide (150). This difference of 75 times in inhibitory capacity suggests, in contrast with the view given earlier, that the hexapeptide forms only a part of the C-terminal antigenic site of myoglobin<sup>12</sup>. Comparisons of the inhibitory activities of peptides 42 and 44 of myoglobin<sup>1</sup>, and of peptides 8 and 8 (-5) of tobacco mosaic virus protein<sup>13</sup>, indicate, however, that the inhibitory capacity of a peptide may depend on amino-acids which are not included in the antigenic site. In this case, the inhibitory capacity is probably a measure of how closely the conformation of the site in the peptide approximates to that in the native protein. If the conformation of the hexapeptide when part of peptide 1 is more complementary to that of the antibody combining-site than the conformation of the free hexapeptide, then the results given do not disprove the suggestion that the C-terminal hexapeptide of myoglobin represents an antigenic site of the native protein.

Examination of the space-filling myoglobin model<sup>4</sup> reveals that the antigenic site(s) of peptide 1 could be formed from the amino-acid residues H8, 9, 12, 13, 15, 16, 17, 19, 20, 21, 23 and 24, and HO1-5 (Fig. 1), because only these residues or portions of these residues are exposed on the surface of myoglobin. In all probability, the C-terminal antigenic site includes the corner between the H-helix and the HO non-helical segment, in which case it probably comprises two faces at right-angles to each other. This site could include portions of the side-chains of all the amino-acid residues of the C-terminal hexapeptide and of the peptide bonds between residues HO3-2, HO2-1 and HO1-H24. So far as the model is concerned, there appears to be no obvious reason why the ε-amino group of Lys H23 does not form a part of this antigenic site; however, the exact positions of the side-chains of residues HO3, HO4 and H23 relative to each other are not known. In addition, the ε-amino group of Lys H21 would be expected to form a part of the same site, which suggests that the complete site may be larger than the C-terminal hexapeptide.

A comparison of the results obtained with different antisera reveals that the inhibitory activities of the peptides depend on the antiserum used. This variation is to be expected because different antisera may contain antibodies against different sites, and the antibodies to any one site may differ in the sizes of their combining-sites and in their affinities<sup>14,15</sup>. Because the fragments of peptide 1 that inhibited antiserum WD did not inhibit antisera WK and WM, the antigenic site(s) of peptide 1 relative to WK and WM may be larger or occupy a different position from that detected by WD. Alternatively, if the strength of binding of the fragments by the antibodies of antisera WK and WM is very much less than that of the antigen,

Table 2. MAXIMUM INHIBITION OF PRECIPITATION OF ApoMb WITH ANTISERUM WD BY PEPTIDES D2, 1T2 AND D2a, AND THE MOLAR RATIO OF PEPTIDE TO ANTIGEN FOR HALF THE MAXIMUM INHIBITION

Peptide	Maximum inhibition (%)	Peptide : ApoMb molar ratio for 50% of maximum inhibition
D2	11	150
1T2	10-11	150
D2a	>4	—

the fragments would appear to be inactive in the competitive inhibition test.

To sum up, the results indicate that the C-terminal 22-amino-acid peptide of sperm whale myoglobin, which is predominantly helical in the native protein, contains at least one of the antigenic sites of myoglobin. The C-terminal hepta- and hexa-peptides were also immunologically active, but they were less active than the 22-amino-acid peptide. As the C-terminal hepta- and hexa-peptides had similar inhibitory activities, the N-terminal lysine residue of the heptapeptide probably did not form part of the C-terminal antigenic site of myoglobin. It was, however, not clear whether the C-terminal hexapeptide represented a complete site or part of a site. Evidence was obtained that one or both of the C-terminal amino-acid residues and the leucylglycyl sequence of the hexapeptide were included in the C-terminal antigenic site of myoglobin. A pentapeptide, which was part of the C-terminal heptapeptide and which was less active than the heptapeptide, was the smallest inhibitory fragment isolated.

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## Repopulation of Colony-forming Units in Mice

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Colony-forming unit repopulation has been studied in the mouse and compared with split dose total body radiosensitivity. This study indicates that post-irradiation changes in CFU do not correlate with changes in total body radiosensitivity.

When mice are exposed to X- or  $\gamma$ -radiation in semi-lethal doses, there is injury to the bone marrow with a marked decrease in the numbers of leucocytes and other cellular elements in the peripheral blood. The pancytopenia is ultimately attributed to radiation injury to the haematopoietic stem cell, that is, the killing of stem cells. The reaction of the animal to mid-lethal exposure—whether it survives or succumbs—is influenced by the surviving number of stem cells and the rate at which stem cells and their progeny proliferate and repopulate the haematopoietic system<sup>1</sup>.

Repopulation of a cell population from radiation injury seems to be divided into at least two phases: the first 24–48 h during which cellular or molecular repair of sub-lethal injury occurs, and then the proliferation of the surviving cells. These recuperative processes occur in irradiated cells cultured *in vitro*<sup>2–4</sup> and they may also occur in haematopoietic stem cells irradiated *in vivo*<sup>5,6</sup>.

There are now methods for determining the numbers of bone marrow or spleen cells which, after transplantation, can proliferate and form nodules or colonies in the spleens of supra-lethally irradiated recipient mice<sup>7,8</sup>. These colony-forming units (CFU) are thought to be haematopoietic stem cells although this has not been conclusively demonstrated<sup>1</sup>. If the size of the haematopoietic stem cell population is important in determining the radiosensitivity of an animal after sub-lethal irradiation, then the extent of stem cell proliferation after an initial exposure might be expected to correlate with, or even predict, the sensi-

tivity to a second radiation exposure. If the CFU is the stem cell or is indicative of the stem cell population, estimation of the number of CFU present after an initial exposure should at least qualitatively predict the sensitivity of the animal to a second radiation exposure.

An earlier report dealt with the extent to which the numbers of CFU in the marrow or the numbers of endogenous CFU in the spleens of mice could be correlated with the radiation protection produced by bacterial endotoxin<sup>9</sup>. Those data showed that in many respects the numbers of CFU did qualitatively correlate with the extent of radiation protection. By varying the time of endotoxin injection relative to irradiation, a procedure which influences both the extent of protection and the number of endogenous CFU, it was shown that radiation protection and the content of CFU in the spleen varied independently, and Smith *et al.* have reported similar findings<sup>10</sup>.

We have quantitatively transplanted bone marrow from sub-lethally irradiated donors to investigate the changes in the femoral CFU population after sub-lethal X-irradiation in mice treated with endotoxin and in control mice. We obtained values for the  $LD_{50}$  at various times where differences in CFU content predict differences in  $LD_{50}$  between the treated and control groups.

Female LAF1 mice between 3 and 4 months old were used. They were placed in 'Lucite' tubes which were placed on a rotary turntable for whole body irradiation by 250 kVp X-rays. The target skin distance was 40 in.; half value layer was 1.49 mm of copper and the dose rate



was 28–30 r./min. Endotoxin used in these experiments was 'Piromen', a highly purified *Pseudomonas* polysaccharide. In all experiments a dose of 50  $\mu$ g was given intravenously 24 h before irradiation in 0.05 ml.

Bone marrow was obtained as previously described<sup>8</sup>. The preparations were counted and appropriate dilutions were injected into supralethally irradiated (800 r.) recipients to determine CFU/10<sup>5</sup> cells. The irradiated recipients were housed singly as previously described<sup>8</sup>, and their spleens were removed 8 days after injection. Nodules greater than 0.24 mm were counted with the aid of a dissecting microscope, and from these data total nucleated cells, total femur CFU content and CFU/10<sup>5</sup> nucleated cells were determined.

Two groups of mice were used for single dose determinations of LD<sub>50/30</sub>. One group of mice received 50  $\mu$ g of endotoxin intravenously 24 h before irradiation; the other served as non-injected controls. Survival was recorded for 30 days. Median lethal doses (LD<sub>50/30</sub>) and other statistics were computed by methods previously described<sup>8</sup>.

Recovery from the initial radiation injury was investigated by the classical split-dose technique. Groups of control and treated mice received an initial or conditioning exposure of 450 r. (~ two-thirds of the control LD<sub>50/30</sub>). These animals were then divided into several groups and were re-exposed 5 or 14 days after the conditioning exposure to determine the LD<sub>50</sub>. The difference between the single exposure LD<sub>50</sub> and the redetermined LD<sub>50</sub> represents an estimate of the amount of injury remaining from the conditioning exposure at the time tested.

The bone marrow cellularity, the total CFU content of each femur, and the CFU content/10<sup>5</sup> nucleated cells were measured before and at various times after 450 r. These measurements were made in both treated (24 h before 450 r.) and control mice at the various times. Fig. 1 shows the changes in nucleated cell content and total CFU content of the femurs of treated and control mice. The nadir in nucleated cell count occurred at 3 days with the mice treated with endotoxin showing slightly less depression than the controls. Between 4 and 7 days the nucleated cell counts in the treated animals were rather larger than

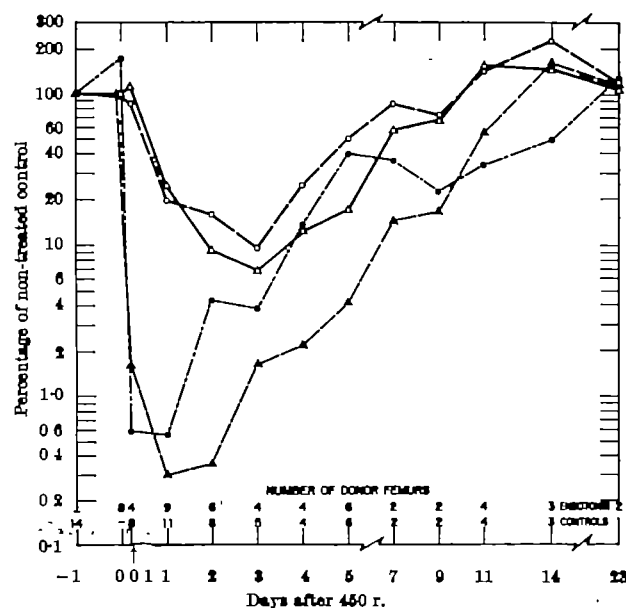


Fig. 1. Time-dependent changes in the numbers of nucleated cells and colony-forming units in the femur of control mice or mice treated with endotoxin.

Nucleated cells 100 per cent =  $1.53 \times 10^7$  CFU 100 per cent = 4,750  
Controls —○— Endotoxin —△—

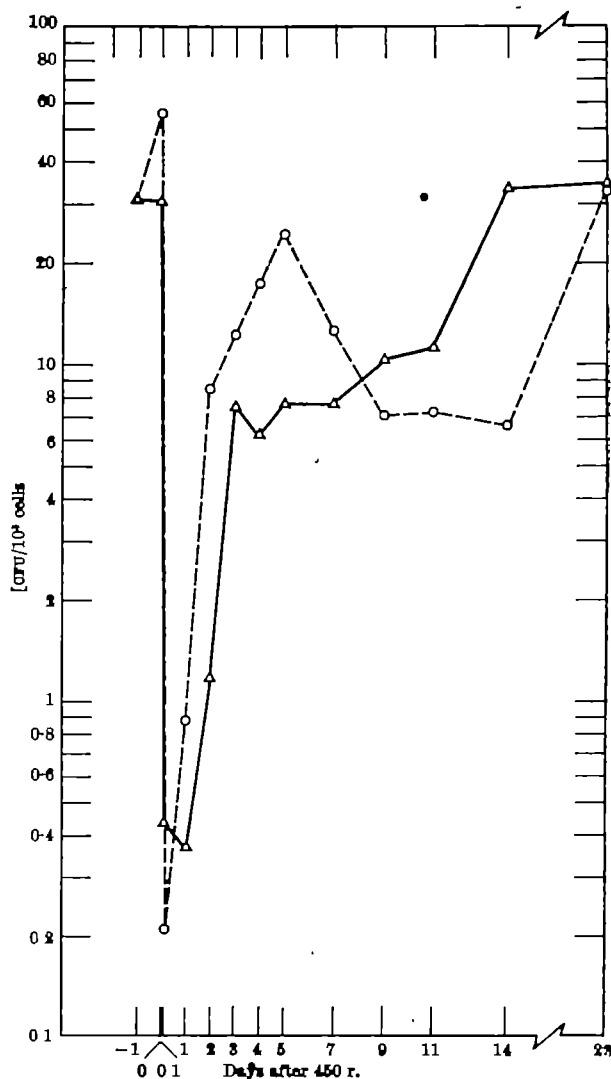


Fig. 2. Changes in femoral bone marrow CFU/10<sup>5</sup> cells after the irradiation of control mice and mice treated with endotoxin (450 r.). —○—, endotoxin; —△—, controls.

in the controls, and at 11 days the cellularity had returned to normal in both groups with some tendency to overshoot in the control animals. The femurs of mice treated with endotoxin contained about 70 per cent more CFU before exposure than did the controls. After the initial decrease in CFU the treated animals seemed to begin proliferation of CFU between 24 and 48 h after irradiation, while in control mice proliferation began after 48–72 h. The CFU content remained greater between days 1 and 7 in the treated animals, with a peak 5 days after irradiation in the endotoxin group. At that time the treated femurs contained ten times as many CFU as did control femurs (~2,000 versus ~200). After this there was a reduction in the total CFU content of the treated mice which was in contrast to the continuing proliferation in controls. The control mice later showed a 60 per cent overshoot at 14 days which was not seen in the endotoxin group, and both were near normal 23 days after irradiation.

Fig. 2 shows the changes in CFU/10<sup>5</sup> nucleated femur cells. This figure shows the relationship between the change in nucleated cell count and the change in CFU content. During any period when the CFU content increases proportionally with the nucleated cell content the curve would be flat, such as between 3 and 7 days in the controls and between 9 and 14 days in the mice treated with endotoxin. These treated mice had almost twice the concentration of CFU at the time of irradiation that the controls had, and after

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irradiation the treated animals showed an increase in concentrations of CRU 24 h before the controls showed any increase. There was a peak in the animals treated with endotoxin 5 days after irradiation, and this was succeeded by a secondary fall and a return to normal at 23 days. In controls, there was a rapid increase in concentration of CRU during days 2 and 3, followed by a plateau between days 3 and 7 at which time CRU content was about 30 per cent of the normal value. This was followed by a second increase to the normal range 14 days after irradiation.

Table 1. EFFECT OF ENDOTOXIN ON SINGLE AND SPLIT-DOSE  $LD_{50}$

Treatment	No. of mice	$LD_{50}$ (r.)
Single exposure		
Endotoxin*	79	924 (915-938)†
Controls	180	707 (691-723)
Split dose‡		
5 day endotoxin	148	696 (683-714)
Control	132	675 (651-710)
14 day endotoxin	184	731 (717-748)
Control	133	711 (697-728)

\* 50  $\mu$ g was given 24 h before irradiation.

† Brackets include 95 per cent confidence limits.

‡ Initial exposure 450 r.; 50  $\mu$ g of endotoxin was given before the initial exposure only.

Table 1 contains the single and split-dose  $LD_{50}$  determinations for treated and control mice. Twenty-four hours after treatment with endotoxin the single exposure  $LD_{50}$  was increased by 217 r. (924 r. versus 707 r.). The values of  $LD_{50}$  for treated and control mice were not different when tested 5 days or 14 days after 450 r. had been given.

In these experiments we have measured the changes in nucleated cell content and the CRU content in the femurs of sub-lethally irradiated mice. A comparison has been made between treated and untreated mice, with the object of evaluating certain aspects of haematopoietic recovery, and to attempt to relate recovery of the bone marrow CRU content to split-dose radiosensitivity.

The data show that endotoxin given 24 h before 450 r. increased the marrow CRU content at the time of irradiation without affecting marrow cellularity. This finding is in contrast to the findings of Smith *et al.*, who reported no increase in CRU content after treatment with an endotoxin derived from *S. typhosa*<sup>10</sup>. As expected, the post irradiation decrease in CRU was more rapid than the fall in marrow cellularity because the CRU assay is a viability assay. The fall in nucleated cell count is probably the combined result of reduced production, cell death, and loss of cells through maturation and release into the circulation. The extent to which release from the marrow influences the decrease in CRU content in either the treated or the control group is not known, although the units are normally released from the bone marrow at a constant rate, and endotoxins appear to increase this rate of release<sup>9,12,13</sup>.

The increase in femoral CRU content began about 24 h earlier in the animals treated with endotoxin than in the control animals, and in both groups the increase in the CRU preceded the increase in marrow cellularity by 1 or 2 days. During the first 5 days the rate of increase in CRU content may be greater in the treated than in the control animals, and this is also the case for marrow cellularity between days 3 and 7. Such an early increase in marrow cellularity in animals treated with endotoxin has been reported and these patterns of change in CRU during the first 5 days are similar to those observed in another strain of mice injected with endotoxin from *S. typhosa*<sup>11</sup>. It thus seems likely that the mice treated with endotoxin begin their post-irradiation proliferation earlier as a result of previous stimulation of the CRU compartment by the administration of endotoxin.

Between days 5 and 9 there was a marked decline in CRU content of the endotoxin-treated mice and a slight decrease in marrow cellularity. This decrease in CRU at 9 days is of considerable interest, for it suggests that the proliferative system producing CRU was exhausted by the "double" stimulation or responded to a feed-back inhibition.

At 11 and 14 days an "overshoot" in marrow cellularity was observed in both groups, and an overshoot in CRU was observed in the controls but not in the animals treated with endotoxin. During this time the CRU content in the treated animals was smaller than in the controls, as if the earlier more rapid production of CRU was at the expense of those present at 11 and 14 days.

Our data permit determination of the correlation of CRU content with split-dose radiosensitivity. If the CRU is the haematopoietic stem cell or if at any given time the CRU content is directly related to the size of the stem cell population, then animals with a greater CRU content would be expected to have a larger  $LD_{50}$ .

The present data concerning split-dose  $LD_{50}$  indicate that split-dose radiosensitivity and femoral CRU content vary independently. Five days after 450 r. had been given the  $LD_{50}$  of the animals treated with endotoxin was not significantly different from that of controls, in contrast to the 200 r. difference predicted by a ten-fold difference in the number of CRU with a  $D_{57}$  of about 90 r. At 14 days, the values for  $LD_{50}$  for the treated and control animals again were not different, although the CRU content was about three times greater in the control animals and would predict a 90 r. difference.

Five days after 450 r. had been given the CRU content was about 4 per cent of normal, while the redetermined  $LD_{50}$  indicates that there has been recuperation from 95 per cent of the initial injury (single dose  $LD_{50}$ —redetermined  $LD_{50}$ =residual injury, that is, 707 r.—675 r.=32 r. residual injury). In addition, 14 days after irradiation the bone marrow of the control animals contained about 50 per cent more CRU than before irradiation. This would predict their  $LD_{50}$  to be about 50 r. greater than that of untreated mice. This was not found, because the re-determined  $LD_{50}$  was the same as that of control mice.

The value and widespread application of the spleen CRU techniques for investigating the various radiobiological and physiological responses of this cell line are well established. A logical extension of these findings has been to equate the CRU with the bone marrow stem cell, or to assume that changes in the CRU closely reflect changes in stem cell populations. The present investigation indicates that with the methods now available, and the inherent problems in the methodology and interpretation, measurable changes in CRU content of the bone marrow do not correlate with variations in split-dose radiosensitivity, and therefore may not be closely reflecting changes in the stem cell population. The following are some of the factors which may be relevant: (a) The CRU may not be related closely enough to the "stem cell" to reflect accurately the changes in this cell population. (b) The bone marrow "stem cell" and CRU may be closely related or the same, but are not the chief factor in determining split-dose radiation sensitivity. (c) After an initial exposure the bone marrow stem cells of CRU may be particularly sensitive to trauma which could prevent accurate assessment of their total numbers by the commonly used transplantation techniques.

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# Tissue Typing in Man

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Human lymphocytes which are essentially free from other cells have been prepared from peripheral blood. Storage for long periods at  $-196^{\circ}\text{C}$  is possible without serious loss in viability. Labelling with chromium-51 enables such cells to be used in a precise serological assay with human isoantisera and rabbit complement. The procedure may well prove useful in typing human tissues.

TRANSPLANTATION of tissues between individual members of an outbred species is, with limited exceptions, followed by rejection of the grafted material. This rejection is frequently accompanied by the appearance of humoral antibodies the specificities of which reflect the histocompatibility differences between graft donor and recipient.

Many *in vitro* assay systems are available for examining the antibodies and the iso-antigens which invoke their appearance. The majority of techniques, however, involve a subjective interpretation of results, principally that of a microscopic examination of a cell population (that is, antigen) which has been damaged or agglutinated by antiserum. The introduction of precision and reproducibility into such tests would be most useful.

The release of chromium-51 from labelled lymphocytes in the presence of specific isoantisera and complement has proved a valuable objective tool in several studies on isoimmunity in mice<sup>1-4</sup>. The availability of a similar test system for man would aid histocompatibility typing and elucidation of the chemical nature of the relevant antigenic specificities.

The principal difficulty lies in the requirement for a homogeneous cell suspension in the chromium-51 assay. Inhomogeneity would introduce such variable parameters as differential labelling, fragility and isoantigen content of the various cell types. In the past few years several communications have appeared<sup>5-7</sup> describing the preparation of lymphocytes from peripheral blood. The high purity of these preparations (frequently reported as more than 95 per cent) refers, however, to contamination with other leucocytes. This ignores any contribution made by erythrocytes, which may well comprise three-fourths of the final cell population.

This report deals with the preparation of lymphocytes virtually free from any contamination, and their use, either fresh or after storage at  $-196^{\circ}\text{C}$ , in a human isoimmune chromium-51 cytolytic assay.

**Preparation of lymphocytes.** Peripheral blood (10 ml.) is defibrinated using glass balls (3 mm diameter). After separation from the clot, the supernatant fluid is mixed with one-third its volume of a fresh, warm ( $37^{\circ}\text{C}$ ), 3 per cent solution of gelatine in saline<sup>8</sup>, and erythrocytes are allowed to sediment at room temperature for 20 min. The upper layer rich in leucocytes is removed, centrifuged at 300g for 10 min at room temperature, and the pellet is resuspended in 1 ml. of 25 per cent foetal calf serum in tissue culture fluid 199 (ref. 8). (This medium is used thereafter throughout the preparation.) To the suspension is added an appropriate amount of the IgG fraction of chicken anti-human erythrocyte serum. (The production of this antiserum and the rationale of its use are discussed later.) The erythrocytes agglutinate firmly and settle out within 20 min at  $37^{\circ}\text{C}$ . The supernatant suspension

of leucocytes is carefully removed with a Pasteur pipette. This suspension is then introduced to a column (1 cm  $\times$  10 cm) of polystyrene-divinyl benzene beads<sup>9</sup> prepared in phosphate-buffered saline, and maintained at  $37^{\circ}\text{C}$  by a water-circulating jacket. Cells are washed into the body of the column with medium, followed by incubation at  $37^{\circ}\text{C}$  for 15 min. Medium is then percolated through the column. Unadsorbed cells are rapidly displaced and easily observed in the eluate; collection is usually completed in about 4 ml. of fluid following their first appearance. Centrifugation at 300g for 5 min allows collection of all cells in the eluate and also serves to pack any eluted erythrocytes firmly at the bottom of the centrifuge tube. Gentle resuspension of the pellet in a small volume of medium invariably fails to disaggregate the erythrocyte button which is thereby easily removed. Table 1 summarizes results obtained from three typical preparations of lymphocytes. The method may be scaled up to process 250 ml. of peripheral blood without difficulty. The lymphocytes so obtained are adjusted to a concentration of  $250 \times 10^6/\text{ml}$ . and to the stirred suspension is added 0.5 volumes of 30 per cent dimethyl sulphoxide in medium. 15  $\mu\text{l}$ . volumes of the mixture are sealed in haematocrit tubes, cooled at  $1^{\circ}\text{C}/\text{min}$  to  $-30^{\circ}\text{C}$  (ref. 10) and then more rapidly to  $-196^{\circ}\text{C}$ , and stored in liquid nitrogen.

Table 1. PREPARATION OF LYMPHOCYTES FROM PERIPHERAL BLOOD

Donor *	Purification stage	Erythrocytes	Leucocytes	Lymphocytes
RoCh	Original blood	59,400 †	93	37
	Defibrinated blood	40,900	43	23
	Gelatine supernatant	98	31	10
	Column eluate	0.1	0.15	7.5
ArSe	Original blood	58,500	96	36
	Defibrinated blood	45,700	65	31
	Gelatine supernatant	82	23	8.5
	Column eluate	0.1	0.14	6.4
BaFo	Original blood	61,200	61	31
	Defibrinated blood	47,000	46	21
	Gelatine supernatant	97	23	9.0
	Column eluate	0.1	0.11	7.7

\* 10 ml. blood was drawn in each case.

† All values are  $\times 10^6$ , and refer to the total cells of each type stated.

The lymphocyte suspensions are more than 99 per cent viable after trypan blue staining, and transform into blast-like cells on culture with phytohaemagglutinin<sup>11</sup>. Rapidly thawed suspensions which have been maintained at  $-196^{\circ}\text{C}$  for 2 months are more than 85 per cent viable and transform in a manner identical with freshly prepared cells. Preparation time is about 2 h, but if freezing is desired, sealing and cooling occupy a further 2 h.

**Chicken anti-human erythrocyte serum.** Chickens (White Leghorns) are immunized by multiple intravenous injections at fortnightly intervals with a 25 per cent suspension of packed, washed, human erythrocytes in phosphate-buffered saline. Birds showing high agglutinin titres are

bled 2 weeks after the final injection, serum is prepared and the IgG fraction separated on 'Sephadex G-200'. Phosphate-buffered saline at five times physiological strength is used as eluent, because it was found that lower ionic concentrations produced poor and irreproducible results<sup>12</sup>. The fractions containing IgG are concentrated and titrated using  $100 \times 10^6$  human erythrocytes in a total volume of 0.5 ml. with an incubation time of 20 min at 37° C. A strong macroscopic agglutination is used as the endpoint. The results of this titration allow simple calculation of the appropriate dilution of antiserum to be used during lymphocyte preparation.

In preliminary experiments erythrocytes were preferentially lysed (15 min at room temperature) using rabbit anti-human erythrocyte serum and guinea-pig complement. Lymphocyte preparations so obtained appeared microscopically normal, and were not agglutinated. Nevertheless when these lymphocytes were labelled and suspended in rabbit complement or guinea-pig complement alone, for 1 h at 37° C, considerable lysis was observed. The samples of complements used had been absorbed with human cells and were known to be non-toxic under the conditions used. Small amounts of the rabbit anti-erythrocyte serum appear therefore to attach to lymphocytes, presumably because of cross-reacting specificities between them and erythrocytes. Such antibodies apparently sensitized the lymphocytes, and caused lysis in the presence of complement alone during the conditions subsequently used in the isoantiserum assay, namely, 1 h at 37° C.

The use of chicken antiserum overcomes this problem, by utilizing the known<sup>13</sup> inability of chicken antibodies to cause lysis with any species of complement except their own. Now the lymphocytes so obtained may have a little chicken antibody on their surface, but because they are ultimately suspended in rabbit complement, such antibody cannot cause lysis, and therefore any cytotoxicity observed must be the result of sensitivity to the isoantiserum under test.

The agglutinins occurring in the IgG portion of chicken anti-erythrocyte serum were selected rather than using the whole serum. The simple 'Sephadex G-200' separation obviates any potential dangers caused by the presence in whole serum not only of complement but also of IgM anti-erythrocyte cytotoxins of known high lytic efficiency (unpublished data). The separated IgG, on the other hand, did not lyse human erythrocytes in the presence of chicken complement, thereby confirming the usual<sup>14</sup> poor lytic efficiency of this type of antibody.

Finally, it should be added that human lymphocytes obtained using the IgG antibody were not agglutinated by 1:10 guinea-pig anti-chicken globulin serum. The titre of IgG anti-erythrocyte agglutinins was, however, augmented by a factor of ten by the same concentration of guinea-pig antiserum. It therefore seems that the amount of chicken antibodies present on the purified lymphocytes is either nil or very little.

**The isoantiserum cytotoxic assay.** The chromium-51 assay is essentially similar to that described earlier for mouse lymphocytes<sup>1</sup>. Labelled cells are added to dilutions of isoantiserum in complement and, after an appropriate incubation, lysis is arrested. Released isotope is determined in aliquots of supernatant after centrifugal removal of cells and debris. Control tubes of cells added to diluent alone, with or without incubation, and to complement alone, are always included. Details of modifications to the reported assay are dealt with below.

(a) **Suspension media.** Cells are labelled at 37° C for 3 h at a concentration of  $20 \times 10^6$ /ml. in 10 per cent foetal calf serum containing 5 mc. chromium-51/ml. Excess isotope is washed from the cells with veronal-buffered saline containing optimum amounts of calcium and magnesium for complement activity, and 0.1 per cent of gelatine. This medium is used as the routine diluent thereafter in the lytic assay.

(b) **Cell numbers.** 3,000 lymphocytes are used per assay tube, although this by no means represents a lower limit of cell numbers; as little as 250 cells have been used. Provided satisfactory defibrination can be achieved it is therefore possible to type patients from pin-prick blood samples, which should considerably widen the applicability of the technique. Human lymphocytes appear to absorb more chromium-51 per cell than mouse lymphocytes, where 100,000 are usually necessary to achieve similar label release. This is not only sparing of target cells but may result in greater sensitivity than is found in comparable cytotoxic assays, for example, trypan blue exclusion, where larger cell numbers are necessary.

(c) **Temperature.** Human cells again possess an advantage over mouse cells. For the latter it is necessary to incubate at room temperature in order that "background" controls, that is cells with diluent only, remained low. With human cells, however, such controls are only 2 per cent greater than unincubated values after 1 h at 37° C.

(d) **Complement.** Pooled rabbit serum is adsorbed three times for 10 min at 0° C with freshly washed, packed human erythrocytes, stored at -20° C in 1 ml. volumes, and used within 2 months of preparation. Human isoantisera titrated with rabbit complement are invariably found to give higher titres than with guinea-pig complement, provided the complement units present are identical for the two cases. Similar reports on the greater efficiency of rabbit over guinea-pig complement have been made previously<sup>15</sup>. An interesting finding, however, is that when the same batches of complement from these two species are titrated with identically sensitized target cells, guinea-pig titres are much higher than rabbit. It therefore seems that guinea-pig serum may have more units of complement/ml. than rabbit serum, but the former is of a lower lytic ability per unit.

(e) **Termination of lysis.** Lysis is terminated with ethylenediamine tetraacetic acid (EDTA) in saline, as previously described<sup>1</sup>, except that trypsin is incorporated to give a final concentration of 0.08 per cent in the cell suspension. Earlier work showed that not all incorporated isotope is released during lysis. The maximum release measured probably depends on the degree of damage inflicted, and where the relevant antigen density is low, such injury could be insufficient to allow all the labelled macromolecules to escape from cells within the lytic period. Trypsin overcomes this problem by causing complete disruption of any damaged cell: undamaged cells are not affected (compare ref. 16). A purified form of trypsin is used and, because the enzyme has no metal requirement for activity, it is unlikely that EDTA causes

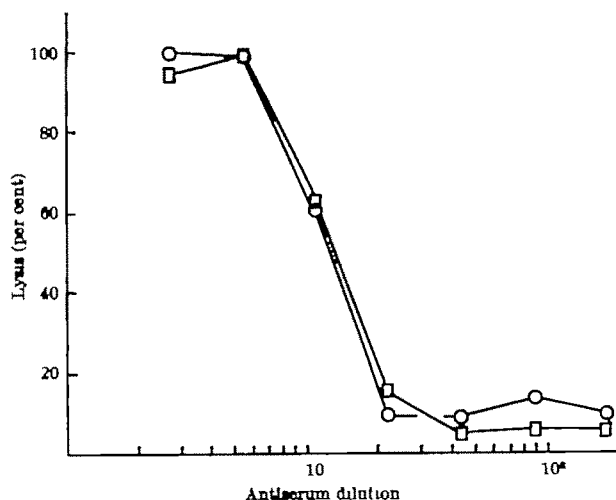


Fig. 1. Isoimmune lysis of human lymphocytes. Abscissa: antiserum dilution. Ordinate: lysis (per cent). O, Reaction terminated with EDTA-saline; □, reaction terminated with EDTA-saline containing trypsin (final concentration 0.08 per cent).

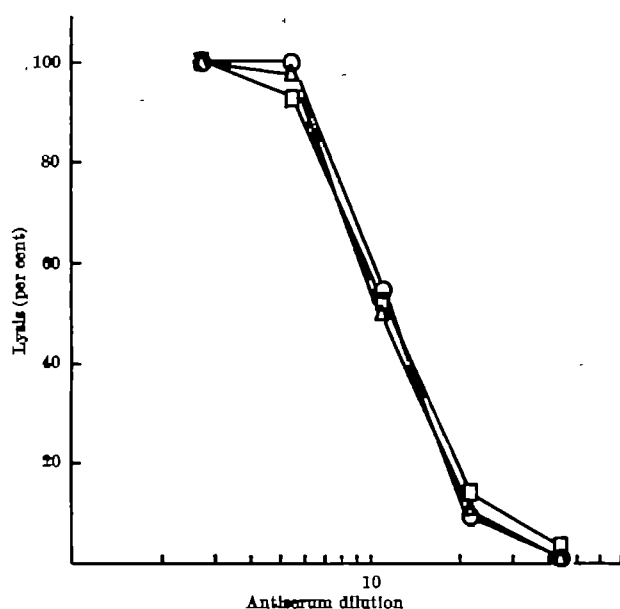


Fig. 2. Isoumune lysis of fresh and stored human lymphocytes. Abscissa: antiserum dilution. Ordinate: lysis (per cent). O, Lymphocytes freshly prepared and labelled; □, lymphocytes labelled, stored for 24 h at 4° C and used; △, lymphocytes cooled to -196° C, stored 6 weeks, thawed, labelled and used.

any inhibition. Titrations for antisera remain unchanged, as an examination of Fig. 1 will show, while sensitivity, in terms of released chromium-51, is considerably increased. Furthermore, because trypsin causes complete isotope release on complete lysis, all isoantisera are strictly comparable. When EDTA alone is used to arrest lysis, particularly after using a polyspecific isoantiserum, a maximum of about 65 per cent of incorporated label is liberated, very similar to values obtained in mouse lymphocyte lysis. It seems highly likely, however, that isoantisera will be found where this figure is much lower, as was found in mouse.

A comparison of such maxima obtained within a given lytic period and for the same cell type but with different

antisera, may indeed reveal valuable information about the density of antigenic determinants on the target surface. For present purposes of a typing test, however, increased sensitivity is considered to be of greater importance, and a trypsin containing termination reagent is routinely used.

Results of the chromium-51 assay performed on fresh or frozen-stored cell suspensions are shown in Fig. 2, which suitably indicates the reproducibility of the system. Suspensions of stored cells do give a higher background liberation of isotope, seen in the control where reaction is terminated without incubation at 37° C. This background is, however, subtracted from all values before calculation of lysis in other tubes. When this "zero time" value is expressed as percentage of label present in cells, it closely approximates the percentage of cells stained by trypan blue.

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## New Semi-synthetic Penicillin active against *Pseudomonas pyocyanea*

by

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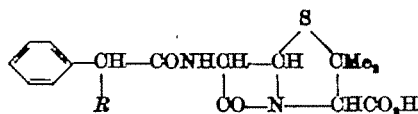
Most of the semi-synthetic penicillins developed from 6-aminopenicillanic acid have little or no activity against *Pseudomonas pyocyanea*.  $\alpha$ -Carboxybenzylpenicillin (carbenicillin: BRL 2064), on the other hand, is active against these organisms and seems to be almost completely non-toxic to man.

DURING the preparation in these laboratories of many hundreds of semi-synthetic penicillins from 6-aminopenicillanic acid, activity against *Pseudomonas pyocyanea* (*Pseudomonas aeruginosa*) has been found only infrequently. This article describes investigations of the properties and pharmacology of one compound that has been selected for

clinical trial. The clinical results (to be reported elsewhere) have shown that carbenicillin has effective anti-*Pseudomonas* activity when given to human subjects by intramuscular or intravenous injection. As with most penicillins, carbenicillin is virtually non-toxic and has no local irritant effects.

## Chemistry

The introduction of ionized substituents into the side-chain of benzylpenicillin (I) is known to lead in some cases to enhanced activity against certain Gram-negative bacteria, as in ampicillin (II) which contains a basic  $\alpha$ -substituent. We now wish to report on an analogue with an acidic  $\alpha$ -substituent, namely  $\alpha$ -carboxybenzylpenicillin (III: carbenicillin).



I:  $R=H$  (benzylpenicillin)

II:  $R=NH_2$  (ampicillin)

III:  $R=CO_2H$  (carbenicillin)

Carbenicillin (which will shortly be available under the registered trademark of 'Pyopen' from Beecham Research Laboratories) is supplied as the disodium salt and contains 5-6 per cent water. It is a white powder which dissolves freely in cold water to give a solution with a pH between 6 and 8. Carbenicillin is only moderately stable to acid, the half life at 21° C and pH 2.0 being about 140 min and at 37° C and at pH 2.0 about 80 min.

On prolonged storage, carbenicillin may decompose to some extent into benzylpenicillin and carbon dioxide. The presence of benzylpenicillin can readily be detected by paper chromatography in butanol-ethanol-water followed by development on an agar plate seeded with *B. subtilis*. Carbenicillin gives a zone of inhibition of bacterial growth near the origin ( $R_f$  about 0.03) whereas benzylpenicillin has an  $R_f$  of about 0.4. The technique is sensitive because benzylpenicillin is considerably more active than carbenicillin against Gram-positive bacteria.

## Microbiology

The antibacterial spectrum of carbenicillin is shown in Table 1. Serial dilutions of the drug were prepared in agar in Petri dishes and the surface of the agar was inoculated using one drop of undiluted overnight broth cultures. The minimum inhibitory concentrations were read after incubation overnight at 37° C. In some respects the antibacterial spectrum of carbenicillin is similar to that of ampicillin. For example, carbenicillin shows significant activity against penicillin-sensitive staphylococci and the streptococci, although this is substantially lower than that of ampicillin or penicillin G. Also, carbenicillin is not stable to staphylococcal penicillinase and is relatively inactive against penicillin-resistant strains of staphylococci. Against *Haemophilus influenzae*, *Escherichia coli*, *Salmonella* species, *Shigella* species and *Proteus mirabilis*, carbenicillin shows a similar level of activity to that of ampicillin although, in general, the activity of carbeni-

cillin is somewhat lower. Like ampicillin, carbenicillin shows only a low level of activity against most strains of *Klebsiella aerogenes*. The spectrum of carbenicillin differs from that of ampicillin, however, with regard to *Pseudomonas pyocyanea* and certain *Proteus* species and carbenicillin is primarily interesting because it is active against these organisms.

**Activity against *Pseudomonas pyocyanea*.** Compared with the activity one is accustomed to see with most antibiotics of clinical value, the activity of carbenicillin against strains of *Ps. pyocyanea* is low. Not many antibiotics are effective against *Pseudomonas*, however, and in the case of those which are active there are certain problems of toxicity. Carbenicillin, on the other hand, is remarkably free from toxic effects and this allows a dosage to be used which can result in inhibitory concentrations being reached in the body even though these levels are comparatively high.

Table 2. ACTIVITY OF CARBENICILLIN AGAINST *Pseudomonas pyocyanea*  
Minimum inhibitory concentration\* ( $\mu$ g/ml.) and number of strains

No. of strains	250	125	50	25	12.5	5.0
74		6	50	15	3	

\* Determined by serial dilution of the drug in agar; inoculum one drop of overnight broth culture diluted 1/100.

Table 3. EFFECT OF CARBENICILLIN ON THE GROWTH OF A TYPICAL STRAIN OF *Ps. pyocyanea*

Inoculum	500	250	Carbenicillin ( $\mu$ g/ml.)	125	50	25	12.5	5.0	2.5
Undiluted (about $10^7$ cells)	growth barely discernible		sparse growth with some separate colonies		confluent growth				
Diluted 1/100 (about $10^4$ cells)	no growth				semi-confluent growth				

Serial dilutions of carbenicillin prepared in agar and the surface inoculated by flooding with overnight broth culture

The activity of carbenicillin against seventy-four strains of *Ps. pyocyanea* is shown in Table 2. All the strains were recent clinical isolates. It will be seen that a concentration of 50  $\mu$ g/ml. carbenicillin was required to inhibit the majority of the strains. Some strains were inhibited by 25  $\mu$ g/ml. but other strains were only inhibited with concentrations as high as 125  $\mu$ g/ml. Determination of the minimum inhibitory concentrations of carbenicillin is made difficult by the fact that typical strains of *Ps. pyocyanea* do not show a sharp end point when the inoculum is heavy (Table 3). When a heavy inoculum is used, that is, one comprising  $10^6$  or more cells, confluent growth occurs on agar containing concentrations of carbenicillin up to about 12.5  $\mu$ g/ml., and at this concentration of drug there is no evidence of any inhibitory effect. At a concentration of 25  $\mu$ g/ml. carbenicillin, however, growth is extremely scanty compared with the control and the inhibitory effect of the drug at this concentration is very marked, although growth is by no means completely inhibited. At concentrations of carbenicillin above 25  $\mu$ g/ml., growth is extremely meagre, taking the form of a very thin film which may only just be discernible on the surface of the agar. Consequently, if the criterion of the "minimum inhibitory concentration" (MIC) is one of absolute inhibition of any trace of growth, the MIC with a very heavy inoculum may well be as high as 250  $\mu$ g/ml. or more, as a result of the scanty growth which occurs at concentrations of carbenicillin of 25  $\mu$ g/ml. and greater. When a smaller inoculum is used in the sensitivity tests, a relatively sharp end point is obtained. With an inoculum of about  $10^4$  cells a normal, pigmented, semi-confluent growth is obtained on agar with concentrations of carbenicillin up to 12.5  $\mu$ g/ml., but at 25  $\mu$ g/ml. and over there is usually no growth at all.

As might be expected, this effect of inoculum size on the MIC of carbenicillin is also seen when the tests are carried out in liquid medium. With a heavy inoculum, growth appears normal with formation of pigment and pellicle up to a concentration of about 12.5  $\mu$ g/ml. carbeni-

Table 1. ANTIBACTERIAL SPECTRUM OF CARBENICILLIN

	Minimum inhibitory concentration ( $\mu$ g/ml.)*
<i>Escherichia coli</i>	5.0
<i>Klebsiella aerogenes</i>	250.0
<i>Salmonella typhi</i>	12.5
<i>Shigella flexneri</i>	5.0
<i>Shigella sonnei</i>	5.0
<i>Pseudomonas pyocyanea</i>	50.0
<i>Proteus mirabilis</i>	2.5
<i>Proteus morganii</i>	5.0
<i>Proteus vulgaris</i>	2.5
<i>Proteus</i> sp.	5.0
<i>Haemophilus influenzae</i>	0.5
<i>Staphylococcus aureus</i> Oxford	0.5
<i>Staphylococcus aureus</i> †	50.0
$\beta$ -Haemolytic streptococcus	0.25
<i>Streptococcus faecalis</i>	25.0
<i>Streptococcus pneumoniae</i>	0.5
<i>Bacillus subtilis</i>	1.25
<i>Sarcina lutea</i>	0.5
<i>Clostridium tetani</i>	0.25
<i>Clostridium welchii</i>	0.25

\* Serial dilution in agar; inoculum, one drop of an overnight culture.

† Penicillinase-producing strain.



illin, but at 25  $\mu\text{g/ml}$ . there is no pellicle or pigment and growth is markedly diminished, although complete inhibition of growth may only be obtained with concentrations as high as 250  $\mu\text{g/ml}$ . With a smaller inoculum, a sharp end point is obtained and with most strains of *Pseudomonas* the MIC under these conditions is usually about 50  $\mu\text{g/ml}$ .

This effect of inoculum size on the MIC is outwardly similar to that seen with penicillin G and penicillinase-producing staphylococci. Carbenicillin, however, is itself highly stable to the penicillinase produced by typical strains of *Ps. pyocyanea* and the inoculum effect in this case is not due to destruction of drug, nor is the effect due to the growth of resistant mutants present in the original inoculum. When sensitivity tests are carried out on the growth which occurs in the presence of high concentrations of carbenicillin the result obtained is the same as that seen with the original culture, that is, marked suppression of growth at concentrations of 25  $\mu\text{g/ml}$ . and over, but with scanty growth nevertheless persisting up to a concentration as high as 250  $\mu\text{g/ml}$ . when the inoculum is heavy.

Carbenicillin is bactericidal and typical results with a strain of *Pseudomonas* are shown in Fig. 1. For the first 7 h a concentration of 50  $\mu\text{g/ml}$ . usually results in the killing of at least 99 per cent of the original inoculum, but some resumption of growth takes place thereafter which may even increase to a visible amount after 24 h. When this growth is used as inoculum in a repeat test, the same kill is again obtained over the first 7 h followed by a certain amount of growth. The reason for this bactericidal effect followed by growth is not known, but it does not seem to be due to the selection of resistant mutants present in the original inoculum.

**Activity against *E. coli* and *Proteus* species.** Table 4 shows the activity of carbenicillin and ampicillin against strains of *E. coli* and *Proteus* species. Against *E. coli*, carbenicillin shows activity equal to that of ampicillin when tests are carried out using dilutions of the drug in agar. An inoculum effect is also seen with *E. coli*, however, similar to that described with *Pseudomonas*. This effect is more marked with some strains of *E. coli* than others and it is also more pronounced when the tests are carried out in liquid medium. Consequently the results of sensitivity tests with *E. coli* carried out in broth frequently show carbenicillin to be markedly less active than ampicillin when the inoculum is large, but comparable with ampicillin in activity when a smaller inoculum is used.

Against *P. mirabilis*, carbenicillin shows the same pattern of activity as does ampicillin, that is, penicillinase-producing strains are resistant while non-penicillinase-producing strains are highly sensitive. Against strains of *P. morganii*, *religiosi*, and *vulgaris* carbenicillin is interesting in that many such strains which are resistant to ampicillin are relatively sensitive to carbenicillin. This would appear to be due to the greater stability of carbenicillin to the penicillinase produced by these species of *Proteus*.

**Effect of serum on activity.** Carbenicillin is not highly bound to serum proteins. Sensitivity tests carried out in

broth containing 95 per cent human serum show that the activity of carbenicillin is not significantly diminished by the presence of serum, and determinations by an ultra-filtration technique show that 53 per cent of the drug is free in human serum.

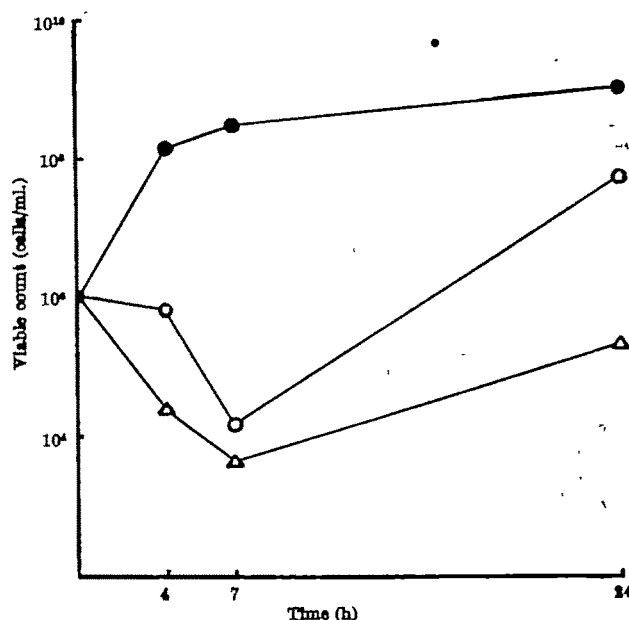


Fig. 1. Bactericidal activity of carbenicillin against *Pseudomonas pyocyanea*. ●, Control; ○, 50  $\mu\text{g/ml}$ ; △, 250  $\mu\text{g/ml}$ .

**Disk sensitivity testing with carbenicillin.** Using 6 mm paper disks containing 100  $\mu\text{g}$  carbenicillin, all strains of *Ps. pyocyanea* tested in this laboratory have given zones of inhibitions of 12–20 mm with a heavy inoculum and zones of 18–30 mm with a dilute inoculum. With a heavy inoculum a number of colonies can usually be seen within the zone of inhibition, although when these colonies are picked off and re-tested they show the same sensitivity as the original culture and again give rise to a number of colonies within the clear zone of inhibition.

In the case of Gram-negative bacilli other than *Pseudomonas*, for example, *E. coli* and *Proteus* species, a 25  $\mu\text{g}$  disk would be suitable for sensitivity testing. Using a heavy inoculum, inhibition zones of 20 mm or more are usually obtained with strains which are inhibited by 5  $\mu\text{g/ml}$ . carbenicillin.

## Animal Studies

**General pharmacology.** Carbenicillin in doses up to 500 mg/kg intravenously had no marked effects on the cardiovascular and respiratory systems and the autonomic nervous systems of cats. At 500 mg/kg there was a slight

Table 4. ACTIVITY OF CARBENICILLIN AND AMPICILLIN AGAINST *E. coli* AND *Proteus* SPECIES

Organisms	No. of strains	Compound	Minimum inhibitory concentration ( $\mu\text{g/ml}$ )* and number of strains					
			> 250	250	125	50	25	12.5
<i>E. coli</i>	51	carbenicillin	2				6	18
		ampicillin	1	1		3	35	21
<i>P. mirabilis</i> †	6	carbenicillin	5			1		7
		ampicillin	5	1				
<i>P. mirabilis</i> ‡	14	carbenicillin						3
		ampicillin						6
<i>P. morganii</i>	5	carbenicillin			2	3		4
		ampicillin						1
<i>P. religiosi</i>	5	carbenicillin					3	
		ampicillin	3	1	1	1		2
<i>P. vulgaris</i>	6	carbenicillin			1	1	2	2
		ampicillin	5		1	1		

\* Determined by serial dilution of the drug in agar; inoculum one drop of undiluted overnight broth culture.

† Penicillinase-producing strains.

‡ Non-penicillinase-producing strains.

transient hypotensive effect which disappeared within 10–20 min.

**Absorption, distribution and metabolism.** Carbenicillin is poorly absorbed orally in the rat, but after intramuscular injection the penicillin becomes distributed throughout the body in a similar manner to other penicillins. Relatively high concentrations of the penicillin appear in the alimentary tract after intramuscular administration. Chromatographic examination of intestinal contents and tissue confirms the presence of the drug in the gut. This is probably due to the excretion of bile containing carbenicillin into the intestine.

In order to determine the biliary excretion, the bile ducts of rats were cannulated with 'Polythene' tubing (0.4 mm diameter) and the animals placed in metabolism cages, after being injected with 100 mg/kg carbenicillin intramuscularly. Bile specimens were obtained at intervals of 1 h over a period of up to 6 h after dosing. A total of 15.1 per cent of the penicillin was recovered from the bile during this period and 28.8 per cent of the antibiotic was recovered from the urine over a period of 4 h.

Following intramuscular injection of carbenicillin into rabbits at a dose of 500 mg/kg, samples of cerebrospinal fluid were withdrawn from the cisterna magna at 0.5, 1, 2 and 4 h and assayed. No antibiotic was found in the cerebrospinal fluid, indicating a failure of carbenicillin to penetrate the blood-brain barrier.

When given subconjunctivally, 0.5 ml. of both 10 per cent and 25 per cent solutions of carbenicillin in xylocaine/adrenaline solution B.P. gave concentrations up to 200 µg/ml. in the aqueous humour. Detectable concentrations were present for a longer period after the injection of the 25 per cent solution.

**Prolonged toxicity studies in dogs.** Thirty pedigree beagle dogs were used in the study. They were housed singly and fed with weighed quantities of dry diet twice daily, the food intake being calculated. Unlimited supplies of water were provided and, in addition, one-third of a pint of milk daily was available.

The animals were divided into five groups of three males and three females and were dosed as follows: group 1, commencing at 2 g/kg/day subcutaneously; group 2, 500 mg/kg/day subcutaneously; group 3, 250 mg/kg/day subcutaneously; group 4, 250 mg/kg/day intravenously; group 5, controls dosed with saline subcutaneously.

Carbenicillin was dissolved in physiological saline so that 1 g of carbenicillin was contained in 1.5 ml. of saline; the solution was made up freshly each day. The dogs were weighed once a week and the dose to be given during the ensuing week was calculated. The intravenous injections were given into the veins of the forelimb. The duration of dosing was 3 months for the subcutaneous groups and 1 month for the intravenous group.

Clinical symptoms were recorded daily, body weight once a week, food intake twice daily and ophthalmoscopic examination was carried out just before the dogs were killed. Haematological, biochemical, urinalysis and serum glucose concentration tests were performed on all dogs, once before dosing began and at 4, 8 and 13 weeks after dosing had begun.

All animals which died during the experimental period were examined after death and all the remaining animals were killed at the end of the dosing period. The internal organs were examined macroscopically and the brain, pituitary, heart, lungs, liver, spleen, pancreas, thymus, prostate/uterus, kidneys, thyroids, adrenals and gonads were removed and weighed. Portions of these tissues, together with sections of aorta, trachea, cervical and mesenteric lymph nodes, salivary glands, various levels of the alimentary tract, gall bladder, skin from injection sites, sciatic nerve, optic nerve, eye, skeletal muscle and bone marrow, were fixed and stained with haematoxylin and eosin. Additional sections of the liver and kidney were specially stained for fat.

There were no deaths from drug toxicity and only one

accidental death. There were no observable changes in general physiology, apart from slight local swelling of tissues around the sites of intravenous injections. Severe local swelling, pain, "cyst formation", but little ulceration, occurred when the subcutaneous route was used for the highest dose level. At 500 mg/kg/day, moderate disturbances resulted, whereas at 250 mg/kg/day there was only minimal evidence of local irritation.

There was some suppression of weight gain associated with a suppression of appetite at the highest dose level by subcutaneous injection, the animals receiving 6–8 g/kg/day. Local irritation rather than the systemic absorption of carbenicillin may have been the chief cause of this loss of weight gain, because while at 250 mg/kg/day by intravenous injection there was no weight suppression, less weight was gained by the same dose level given by the subcutaneous route.

A slight reduction in the serum potassium concentration occurred after 4 weeks at the highest subcutaneous dose level but was not seen later. In two dogs dosed for 4 weeks by intravenous injection at 250 mg/kg, there was some reduction in the concentrating power of the kidney. No effect on the kidney was found in a repeat test at 500 mg/kg intravenously.

Only local tissue damage was found on macroscopic examination at post-mortems and only local irritant reactions at the injection sites were found on histological examination. There was no evidence of organ damage.

**Prolonged toxicity studies in rats.** Doses of 250, 500 and 1,000 mg/kg carbenicillin were given subcutaneously to groups of thirty Sprague-Dawley caesarian-derived rats from the Charles River Breeding Laboratories, Wilmington, Massachusetts, fifteen males and fifteen females to each group, over a period of 13 weeks. The rats had access to weighed quantities of autoclaved Spillers 'Laboratory Animal' diet and to tap water. The solutions of the drug were prepared freshly each day and administered in a volume of 0.4 ml./100 g. Two injection sites were chosen for each rat, each site being used on alternate days. Throughout the course of the test a record of food consumption and body weight change was kept daily and the mean weekly intake per rat calculated. Urinalysis and haematology were carried out at the beginning of dosing and at 4, 8 and 12 weeks. At the end of the test all the animals were killed by anaesthetization with ether followed by exsanguination.

After the post-mortem examination, all the essential organs were prepared for histological examination. No histological or clinical effects attributable to the drug were detected throughout the course of the dosing apart from a trend in the top group towards a slight fall in the red blood count.

**Local irritancy studies.** The local action of carbenicillin was determined in the rabbit eye after subconjunctival injection and after topical application. In the first series of experiments two animals each were allocated to groups which received 0.2 ml. of a 25 per cent, 10 per cent or 1 per cent solution of carbenicillin, respectively. The 25 per cent solution of carbenicillin was prepared in xylocaine/adrenaline solution B.P., in order to reduce the painful response to the hypertonic solution. All the animals were observed twice daily for one week after the injections and any inflammatory responses were recorded. At the end of the period the rabbits were killed and the eyes and surrounding tissues removed for histological examination. The 10 per cent and 1 per cent solutions produced very little reaction with only a very mild inflammatory response, and the histological examination showed no damage. The 25 per cent solution, however, caused inflammation with leucocytic infiltration within 3 h of the injection. Within 48 h, however, the reaction had subsided and by the end of the test period there was no evidence of inflammation.

0.2 ml. of a 25 per cent solution of carbenicillin was applied for 2 min to the eyes of rabbits after which the

antibiotic was washed out with warm saline. The eyes of the control animals were treated with saline in the same period of time. The animals were treated twice a day for 3 days. They were then killed on the fourth day and the tissues removed for histological examination. After the first application of the 25 per cent solution there was a slight inflammation of the conjunctiva in one animal which lasted 24 h, but on post-mortem examination no damage was observed; otherwise local application caused no ill effects.

Carbenicillin was also given by intramuscular injection. Twelve rabbits were closely shaven across the dorsum and two injection sites chosen in each of two sacro-spinalis muscles of the back. One site was injected with 1 ml. of a 50 per cent concentration of carbenicillin in saline and the other with 1 ml. of saline. 4 days later the animals were killed and the treated areas prepared for histological examination. At all the sites which received the drug there was fibrolysis and necrosis accompanied in five animals by oedema.

**Effects on pregnant rats.** Sprague-Dawley caesarian-derived rats from the Charles River Breeding Laboratories, Wilmington, were used. The animals were allowed to mate, the day of mating being judged by the appearance of the vaginal plug. Dosing began on day 6 of pregnancy up to and including day 15. Three groups of twenty females were taken, group 1, the control group, being dosed with saline, while groups 2 and 3 received respectively 100 and 500 mg/kg carbenicillin in physiological saline, subcutaneously. The volumes of all injections were standardized to 0.2 ml./100 g body weight. The animals were given free access to Spillers 'Laboratory Animal' diet and to tap water and were closely observed throughout pregnancy. On day 21 the animals were killed and the uterine contents examined for the number of viable young, resorption sites, litter weight and foetal abnormalities. The young were examined externally after removal from the uterus, one-third being retained for detection of visceral abnormalities and the remaining two-thirds for subsequent dissection of animals followed by clearing and staining of the skeleton by alizarin to detect skeletal abnormalities.

No obvious signs of maternal reactions were observed during the test, but treatment with carbenicillin was associated with a slight retardation in body weight gain. Conception rate, average litter weight and size were not, however, significantly affected by the treatment and there was no incidence of abnormalities due to the drug.

**Effect on pregnant mice.** Groups of twenty-two mice were mated and treated in a similar way to the rats in the rat pregnancy study except that the animals were killed on the seventeenth day of pregnancy when the uterine contents were similarly examined.

Carbenicillin had no effect on the body weight changes in the mouse, and conception rate, litter size and foetal loss were unaffected at either dosage. There was also no incidence of abnormalities and skeletal deformities due to the drug treatment.

**Peri- and post-natal development of the rat.** Tests were carried out to determine the effect of carbenicillin on male and female rats before mating and through gestation and lactation to the twenty-first day after birth. Daily doses of 100 and 500 mg/kg of carbenicillin were administered subcutaneously throughout this period.

Parent animals were unaffected, as assessed by daily observation, body weight change, conception rate, length of the gestation period, and parturition. Litter size, litter and mean pup weights and pup loss of rats receiving 500 mg/kg were unaffected at birth or after 4 or 21 days. The increased pup mortality recorded at the lower dosage of 100 mg/kg was therefore considered to be unrelated to the treatment. No abnormal pups were found.

We conclude that carbenicillin at dosages of 500 mg/kg and below does not adversely affect the peri- and post-natal development of the rat.

## Absorption and Excretion in Man

The following absorption and excretion studies were undertaken in order to determine a suitable dosage and route of administration for a proposed clinical trial.

**Assay methods.** The concentration of carbenicillin in serum and in urine was determined by the cup-plate biological assay method. Details of the technique were as described previously<sup>1</sup> except that *Pseudomonas pyocyanea* NCTC 10490 (Ellsworth strain, 1973) was used as the test organism. *Sarcina lutea* and *Staphylococcus aureus* are unsuitable as assay organisms because carbenicillin contains a small amount of benzylpenicillin and this may appear in the serum and urine together with the carbenicillin. Because *Sarcina lutea* and *Staph. aureus* are many times more sensitive to benzylpenicillin than to carbenicillin the presence of traces of benzylpenicillin may invalidate the assay. *Ps. pyocyanea* NCTC 10490, on the other hand, is sensitive to carbenicillin but is relatively resistant to benzylpenicillin. The culture is unusually sensitive to carbenicillin, showing an MIC of about 1.25 µg/ml., and it is possible to assay concentrations of carbenicillin as low as 2.5 µg/ml. using this strain, while benzylpenicillin at concentrations of up to 500 µg/ml. fails to show any zone of inhibition. Standard solutions of carbenicillin from 1–100 µg/ml. were prepared in human serum, and where necessary the serum samples were diluted with human serum to give a concentration within this range. For the assay of urine samples the standard solutions of carbenicillin were prepared in 0.05 molar phosphate buffer pH 7.0 and urine samples were also diluted as required using the same buffer.

**Oral administration.** After a dose of 500 mg carbenicillin in gelatine capsules to healthy adults in the fasting state, no carbenicillin (that is, <1.0 µg/ml.) could be detected in serum samples taken at 0.5, 1, 2, 4 and 6 h after administration.

Carbenicillin was detected in the urine, which was collected over the 6 h period after the administration of the drug, but the quantity present in the urine amounted to less than 1 per cent of the dose administered. It would appear, therefore, that carbenicillin is not absorbed to any extent when given by mouth.

**Intramuscular administration.** Carbenicillin was administered by intramuscular injection to healthy adults in doses of 250 mg, 500 mg and 1 g dissolved in water for injection. The 250 mg dose was dissolved in a volume of 1 ml. and the 500 mg and 1 g doses were administered in a volume of 2 ml. The serum concentrations obtained are shown in Table 5.

Table 5. MEAN SERUM CONCENTRATIONS OF CARBENICILLIN AFTER INTRAMUSCULAR INJECTION IN ADULT VOLUNTEERS

No. of subjects	Dose (mg)	Serum concentration (µg/ml.)				
		0.5 h	1 h	2 h	3 h	6 h
10	250	5.9	7.0	5.7	2.6*	
9	500	13.7	17.7	13.2	—	2.0
16	1000	21.6	25.3	22.1	—	10.9

It will be seen that intramuscular injection resulted in a high concentration of carbenicillin in serum with a peak at about 1 h after injection. Thereafter the serum levels fell relatively quickly and about 80 per cent of the dose appeared in the urine over the first 6 h.

Table 6 shows results for the effect of probenecid on the serum levels and the urinary excretion of carbenicillin.

Table 6. EFFECT OF PROBENECID ON SERUM LEVELS AND URINARY EXCRETION OF CARBENICILLIN IN HEALTHY ADULT VOLUNTEERS

	No. of subjects	Mean serum concentration (µg/ml.)					Percentage of dose excreted in urine over 0–6 h
		0.5 h	1 h	2 h	4 h	6 h	
With probenecid*	10	25.7	30.9	48.0	27.4	19.4	49
Without probenecid	10	26.8	29.4	23.2	10.8	3.3	84

\* Carbenicillin dose 1.0 g by intramuscular injection.

\* 1 g 1 h and 10 h before administration of carbenicillin.

Probenecid was administered to healthy adults in a dose of 1 g 10 h, and again 1 h, before the intramuscular administration of 1 g carbenicillin. It will be seen that probenecid produced a substantial increase in the peak levels of carbenicillin obtained in serum and the rate of elimination of the drug from the serum was markedly diminished.

Chromatography of human urine, obtained after intramuscular administration of carbenicillin, indicated that the drug does not undergo any substantial metabolism in the body.

**Intravenous administration.** Results are shown in Table 7 for the serum levels obtained after intravenous injection of 1 g carbenicillin. This amount was dissolved in 10 ml. water and administered over a period of 3 min. Although the drug was eliminated rapidly from the blood it will be seen that serum levels in excess of 100 µg/ml. were obtained during the first 30 min after administration of this dose.

Table 7. SERUM CONCENTRATION OF CARBENICILLIN AFTER INTRAVENOUS ADMINISTRATION OF 1 g

Subject	Serum concentration (µg/ml.)					
	0-25 h	0.5 h	1 h	2 h	4 h	6 h
A	140	127	60	28	3.7	<3.0
B	140	114	55	23	4.4	<3.0

**Carbenicillin levels in serum and in urine in relation to antibacterial activity.** As a result of the extensive elimination of the drug by way of the kidneys, relatively high concentrations of carbenicillin are obtained in the urine. In the studies involving a dose of 500 mg by intramuscular injection, the concentration in the urine collected over the first 6 h was generally 1,000-2,000 µg/ml. This is considerably in excess of the concentration of drug required to inhibit the growth of *Ps. pyocyanea*. On the other hand, it is questionable whether intramuscular injection of 1 g carbenicillin at intervals of 6 h, even in conjunction

with probenecid, would result in tissue concentrations which would be inhibitory towards typical strains of *Ps. pyocyanea*. The minimum inhibitory concentration of carbenicillin for most strains of *Ps. pyocyanea* appears to be 50-100 µg/ml., and although the drug is not highly bound to serum protein (about 50 per cent is bound) it would probably be desirable to achieve serum levels somewhat in excess of 100 µg/ml. This can readily be attained by intravenous administration.

In addition to the activity which carbenicillin shows against *Ps. pyocyanea*, the drug is active against other pathogens, including *E. coli*, and also strains of *Proteus morganii*, *P. rettgeri* and *P. vulgaris* which are generally resistant to ampicillin. On the basis of the *in vitro* sensitivity of these latter organisms to carbenicillin, the serum concentrations achieved by intramuscular administration of the drug might be adequate and the levels attained in urine are again greatly in excess of the minimum inhibitory concentrations.

The results obtained in these studies suggested that for clinical trials on the therapeutic effectiveness of carbenicillin in urinary tract infections, a suitable dosage might be 1.0 g by intramuscular injection at intervals of 6 h. The treatment of pseudomonas septicaemia, wound infections and tissue infection in general would require the intravenous infusion of carbenicillin (with or without probenecid) in order to attain inhibitory serum and tissue concentrations.

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<sup>1</sup> Knudsen, B. T., and Robinson, G. N., *Br. J. Med.*, 11, 700 (1960).

## Elimination of 5µ Particles from the Human Lung

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An apparatus is described which will provide a continuous and regular aerosol of 5µ particles of polystyrene labelled with chromium-51. The elimination of the radioactively labelled particles from the lungs after a single inhalation by human beings is followed by external gamma-ray measurements.

In order to estimate the dose of radiation to the lungs from radioactive particles in inhaled air, it is necessary to know the proportion of particles deposited in the lungs and the duration of their retention. For insoluble particles, the site of deposition is important, because the material deposited in the ciliated regions of the upper airways is rapidly removed to the pharynx and eliminated by way of the intestinal tract, whereas those particles which penetrate to the alveoli are removed much more slowly and may be permanently retained in the lymph nodes. The site of deposition depends on the size and mass of the particles and on the depth and flow rate of the breathing pattern. These variables must be controlled so far as possible to obtain meaningful experimental results.

This article reports experiments in which a monodisperse aerosol of particles 5 µm in diameter was tagged with radioactivity so that measurements could be made by external counters over the lungs. On each occasion the subjects inhaled only a single breath of the aerosol and the particles could only penetrate the airways as far as they were carried by the volume of the inhaled air, which varied between 140 and 500 ml. in the different experiments.

Spherical translucent particles of polystyrene of about unit density were continuously generated from xylene solution by a spinning disk<sup>1</sup> in a chimney which formed part of the breathing apparatus (Fig. 1). The concentration of polystyrene in the xylene was 0.2 per cent by

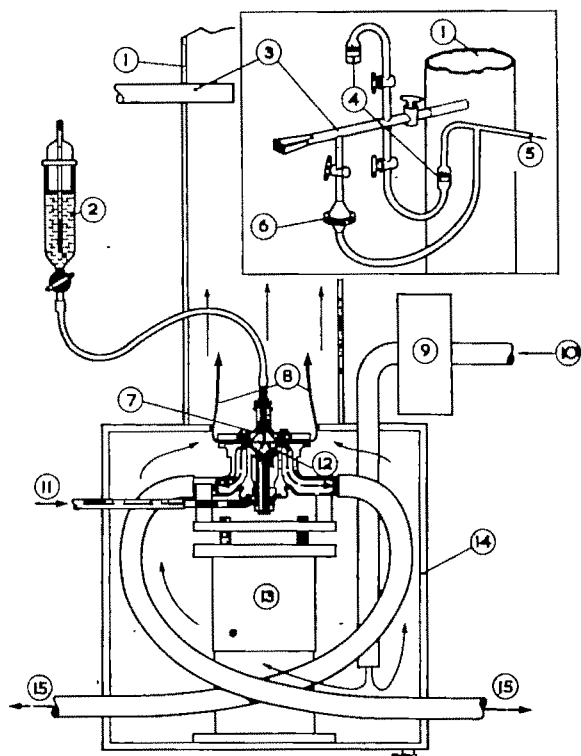


Fig. 1. Diagram of apparatus. (1) Chimney; (2) constant head feed of polystyrene solution; (3) breathing tube and mouthpiece; (4) one-way stop valve; (5) to spirometer; (6) filter for expired air; (7) hypodermic needle; (8) path of drying droplets; (9) absolute filter; (10) 40 L/min air; (11) drive air for rotor; (12) rotor; (13) shockproof mounting; (14) sealed box, (15) exhaust air from rotor drive.

weight and the rate of rotation of the disk was  $3 \times 10^4$  r.p.m. Liquid droplets of 40  $\mu$ m diameter were formed, from which the xylene rapidly evaporated leaving solid spherical polystyrene particles of 5  $\mu$ m diameter.

The particles were tagged with chromium-51, which has a radioactive half-life of 28 days, and decays by  $K$  capture, emitting a 0.323 MeV  $\gamma$ -ray in 9 per cent of its disintegrations. The chromium was dissolved in the xylene as chromium acetyl-acetonate, and was incorporated in the polystyrene particles. Not more than 1 per cent of the activity was leached out when the particles were suspended in water for 4 months.

The number of particles in the inhaled aerosol was about  $2 \times 10^4$ /l. and the activity a few  $\mu$ c./l. An initial lung burden of about 1  $\mu$ c. chromium-51 (0.09  $\mu$ c. of  $\gamma$ -ray activity) was obtained. The advantage of using a  $K$ -capture isotope was that the dose to the lung was not more than 0.5 mrad/day, which is only three times the dose rate from the natural background.

The concentration of xylene in the inhaled air was about 10 mg/l. The maximum permissible concentration<sup>2</sup> for continuous breathing is 0.9 mg/l., but the subjects inhaled a single breath only, so no toxic effects should have arisen. Nevertheless, it was desirable to exclude the possibility of some irritant or anaesthetic effect of the xylene vapour on the cilia, and an alternative method was developed for inhaling particles without xylene. The particles were generated in the usual way and collected by impaction in water containing a small quantity of surface active agent. The suspension was then sprayed through an air-blast atomizer. The disadvantage of this method was that it was wasteful of activity—the yield of aerosol being very small—and it was not possible to measure the retained activity so accurately. The results with subjects who inhaled aerosols generated by both methods, however, showed no significant differences.

It is difficult to measure the inhaled volume and aerosol concentration accurately, particularly when the particles

have appreciable settling velocities. The following technique was adopted. The spinning disk was set running, and a sample of the aerosol examined to ensure that the particle size and concentration were correct. Meanwhile, the subject inhaled air from the atmosphere at normal breathing level and exhaled into a spirometer, adjusting his tidal volume by watching the spirogram. At the end of an expiration, a tap was turned so that the next inhalation was of aerosol. This breath was held for 10 sec, to increase the deposition of particles, after which a total expiration was made through a filter (Fig. 1). The activity of the inhaled air was estimated from 500 ml. samples taken from the chimney immediately before and after the inhalation through a tube similar to the breathing tube. The activity of the exhaled air was deduced from the counting rate from the filter. In estimating the activity which entered the trachea, 60 ml. was subtracted from the inhaled volume to allow for the volume of the mouthpiece, mouth and pharynx.

Immediately after the inhalation, the subject washed out his mouth to remove any particles deposited there. Measurements of radioactivity *in vivo* were made in an enclosure with lead walls 11 cm thick. The  $\gamma$ -ray detectors were two thallium activated crystals of sodium iodide, 15.2 cm in diameter and 8.9 cm thick. They were shielded by cylindrical lead collimators with walls 1.3 cm thick, which extended 8 cm in front of the crystal faces. The detectors were placed dorsally and ventrally on the mid-line of the chest of the supine subject. The upper detector was positioned with the lower edge of the collimator over the caudal end of the sternal body and at a height such that the body just touched the collimator at inspiration. The lower detector was positioned directly below the first, with the top of the collimator 1 cm below the 1 cm thick plastic bed on which the subject lay.

The collimation was designed to minimize the response from activity in the abdomen without reducing too much the sensitivity to activity in the lung. For one subject measurements were also made with the detectors displaced 7 cm laterally and 3 cm nearer the head. The elimination rates were not significantly different from those observed with the counters positioned as normal. The geometrical arrangement of the counters minimized the changes in counting efficiency with changes in distribution of activity *in vivo*. In order to calibrate the detectors the counting rates from a standardized "point" source of chromium-51 were measured in a number of simulated distributions in the lung phantom consisting of 'Mix D' X-ray phantom wax and air spaces.

Independent estimates of the activity retained initially were obtained from the measurements of radioactivity in the lungs, from the activity of the inhaled and exhaled air and from faeces over the first 3 days (after 3 days there was insignificant activity in the abdomen). These agreed quite well, as illustrated by the results from subject D. V. B.

Activity inhaled in 440 ml.	0.92 $\mu$ c.
Activity exhaled	0.02 $\mu$ c.
Retention by difference	0.90 $\mu$ c.
Retention estimated from lung counters	0.97 $\mu$ c.
Faecal excretion in first 3 days	0.31 $\mu$ c.
Loss from lung in 3 days (Fig. 3)	25 per cent
Retention from excretion data	1.2 $\mu$ c.

In another subject the activity in urine 20 days after the inhalation was measured, and was found to be below the limit of detection (less than a twentieth of the rate of loss of activity from the lung at that time). This was considered to be evidence that the activity was fixed in the particles.

The elimination of the labelled particles from the lungs of the experimental subjects is shown in Figs. 2 and 3 on a semi-log plot. The results represent the sum of the counts from the crystals above and below the chest and are corrected for radioactive decay of chromium-51 and normalized to unit initial deposition in the lungs. Fig. 2

refers to the first series with xylene vapour present in the aerosol and Fig. 3 to the later series in which the particles were sprayed from a water suspension. It appears that the xylene vapour inhaled with the particles in the first series of experiments made little or no difference to either phase of elimination, so that the two series can be regarded as duplicates. The exhaled activity in all cases was less than 10 per cent of the inhaled activity, and the graphs therefore approximate to the proportion of the inhaled dose which is retained.

The elimination of the particles can be divided into two phases. In the first phase (10–20 h) a fraction of the initial burden was eliminated, the fraction depending on the volume of the inhalation. There was no evidence of a very rapid initial removal with a half-life of the order of 30 min as observed by Albert and Arnett<sup>3</sup> and by Morrow<sup>4</sup>. On the contrary, in several cases the removal during the first 3 h was very slow.

The present results were obtained with a monodisperse aerosol and with an artificial breathing pattern. With normal breathing and only short pauses between breaths, sedimentation of particles would be less. At high flow rates the large particles of a heterodisperse aerosol are likely to impact in the upper respiratory tract. The activity of the particles is, of course, proportional to the cube of their radius, so that measurements of activity would tend to show the fate of the larger particles. With normal breathing, a larger proportion of the particles may reach the alveoli than in the present experiments

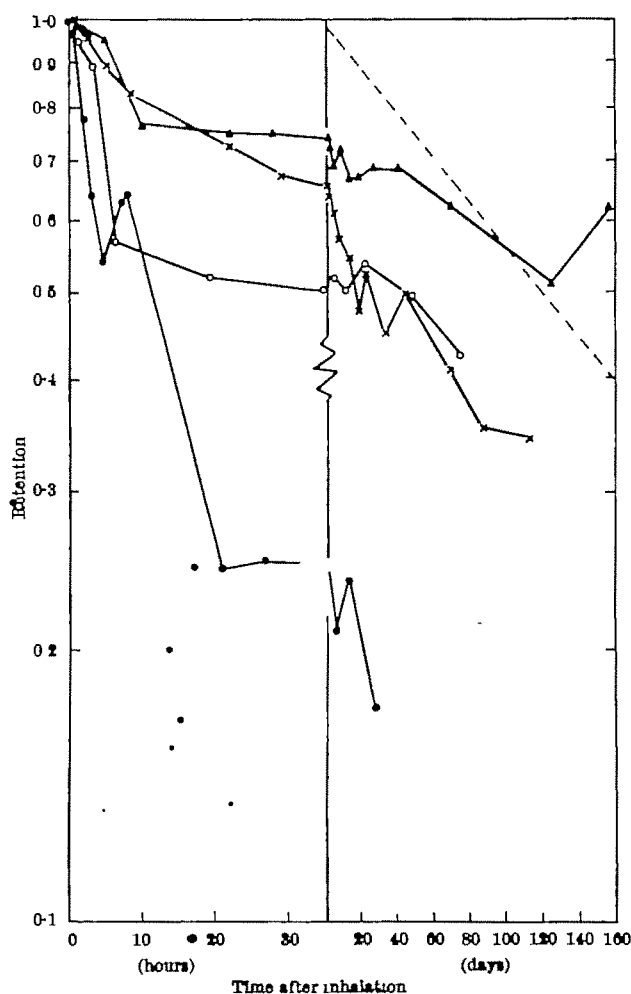


Fig. 2. Elimination of particles inhaled with xylene vapour; ----, 120 day half-period;  $\Delta$ — $\Delta$ , D.V.B. 500 ml.;  $\times$ — $\times$ , A.C.C. 500 ml.;  $\circ$ — $\circ$ , M.L.T. 250 ml.;  $\bullet$ — $\bullet$ , D.C.F.M. 140 ml.

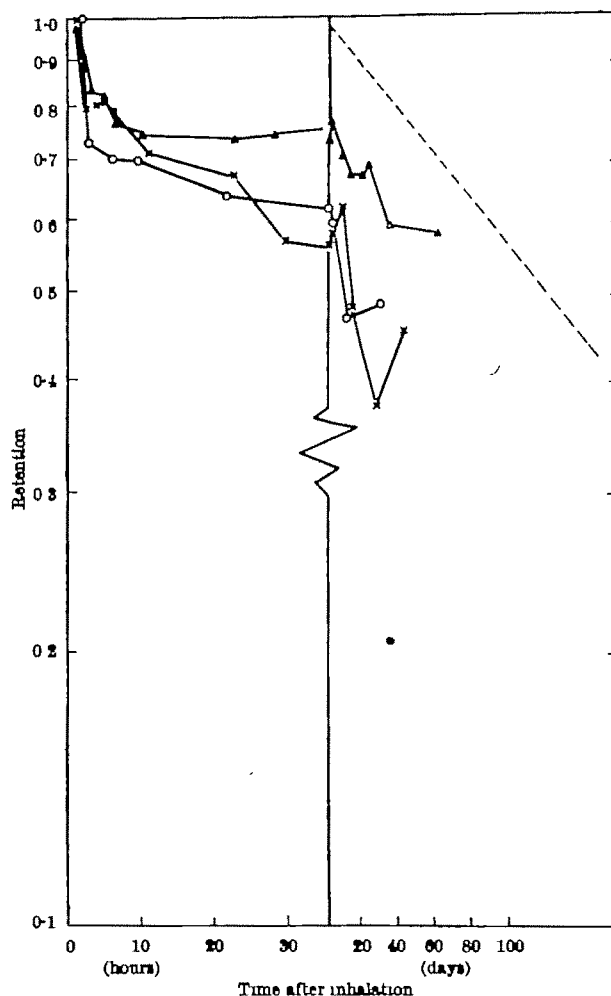


Fig. 3. Elimination of particles inhaled with water vapour; ----, 120 day half-period;  $\Delta$ — $\Delta$ , J.R. 500 ml.;  $\times$ — $\times$ , A.C.C. 500 ml.;  $\circ$ — $\circ$ , M.L.T. 250 ml.

because the dead space is not normally completely cleared at each expiration.

The slow second phase of elimination shows half-lives of 150 days–300 days in the four subjects studied as compared with 120 days adopted by the International Commission of Radiological Protection in 1959 for insoluble particles. It is assumed that this phase represents the elimination of particles that had been deposited in alveolar regions. If ciliary stasis were present in significantly large areas of the upper respiratory tract this assumption would require modification.

Because the subjects inhaled only a single breath, the particles deposited during the breath holding period must have done so on the nearest mucosal wall and thus acted as markers to show how far the air had penetrated into the lung during inspiration. Such information cannot be obtained during steady state breathing unless both the extent of air mixing and the number of particles remaining in the dead space air at the end of expiration are known. If the fraction of activity eliminated during the initial fast phase is taken to represent particles deposited from air filling the ciliated regions at the end of inspiration, and the inhaled volume is known, then an estimate of the effective volume of the ciliated airways can be made, as shown in Table 1.

If 40 ml. (volume of mouth and pharynx) are added to the figures in the last column of Table 1, estimates of the effective dead space are obtained which are very similar to measurements of effective dead space by the single breath method<sup>5</sup>. It is also clear from Table 1 that unit



Table 1

Subject	Volume of aerosol inhaled (ml.)	Volume of aerosol entering trachea (ml.)	Fraction retained with half-life > 50 days	Volume from which particles eliminated with short half-life (ml.)
D. V. B.	500	440	0.75	110
A. O. C.	500	440	0.55	180
M. L. T.	250	190	0.52	90
D. M.	140	80	0.25	60
J. R.	500	440	0.75	110

density spheres  $5\mu$  in diameter easily penetrate to the alveolar regions of the lung and are not quantitatively removed by sedimentation or by impaction in the upper airways.

It is concluded that the elimination of particles from the respiratory tract has two main phases. The rapid phase corresponds to the removal of particles initially deposited on the ciliary mucosa and the slow phase to those particles which have reached the non-ciliated

regions. The rapid phase takes 10–20 h and the slow phase has a half-period of 150–300 days. The fraction of inhaled particles deposited on the two regions depends on the inhaled volume. The effective volume of the ciliated regions can be estimated by a single breath technique, but demands an accurate measurement of the inhaled volume. Because the site of deposition is also a function of the particle size it is essential to use uniform aerosols in such experiments.

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## Dynamics of Organochlorine Insecticides in Vertebrates and Ecosystems

by

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The dynamics of the diffusion of organochlorine pesticides in ecosystems can be described by a model based on the theory of sets. The model shows that research which relies on measurement of the pesticide at different periods of time can lead to misleading conclusions.

A CONSIDERABLE amount of information is available about the concentrations of organochlorine compounds in the tissues of experimental animals that have been exposed to organochlorine insecticides under controlled conditions; the two insecticides that have been studied most intensively are DDT and dieldrin. The tissues of animals that have been exposed to DDT (either as the technical material or as *pp'*-DDT) contain *pp'*-DDT, *pp'*-TDE and *pp'*-DDE, and the hydrophilic metabolite DDA is found in the urine<sup>1</sup>. After exposing animals to dieldrin (or HEOD, the major insecticidal constituent of technical dieldrin) the tissues are found to contain HEOD and smaller amounts of polar metabolites<sup>2</sup>. The relatively slow rate of elimination of *pp'*-DDT and its congeners, and HEOD, which is reflected in the storage of these compounds in animal tissues, particularly adipose tissue, has aroused considerable interest and comment. This interest has been intensified in recent years as a consequence of reports of their widespread occurrence in the biosphere. Small amounts of these compounds are found in the adipose tissue of the general population of several countries and in terrestrial and marine wildlife.

It is desirable that this large heterogeneous collection of empirical observations should be unified, if possible, on the basis of conceptual models. If such models can be established we shall have a better understanding of the possible effects of the use of these compounds on man and his environment. The dynamics of the uptake, distribution and elimination of these compounds by animals during long-term exposure will be considered first.

The following postulates are believed to be consistent with the experimental observations.

(i) The concentration of an organochlorine insecticide in a particular tissue is a function of the daily intake

$$c_{\alpha ij} = f_{\alpha ij}(a)$$

where  $c_{\alpha ij}$  is the concentration of the  $j$ -th insecticide in the  $i$ -th tissue of an animal of species  $\alpha$ , and  $a$  is the daily intake. The results of investigations of the concentrations

of DDT in the tissues of rats<sup>1</sup>, the adipose tissue of monkeys<sup>3</sup> and of man<sup>4</sup>; of HEOD in the adipose tissue of hens, steers, hogs and lambs<sup>5</sup>, the blood of dogs, the blood and adipose tissue of man<sup>6</sup>; and of heptachlor epoxide in the adipose tissue and milk of cows<sup>7</sup>, are consistent with this postulate.

The functional relationship is apparently curvilinear when the exposure to a single insecticide is over a wide range<sup>8,9</sup>, and simultaneous exposure to more than one of the organochlorine insecticides also changes the functional relationship<sup>9</sup>.

(ii) The concentrations in different tissues are functionally related

$$c_{\alpha ij} = g_{\alpha ik}(c_{\alpha kj})$$

where  $c_{\alpha ij}$  and  $c_{\alpha kj}$  are the concentrations in the  $i$ -th and  $k$ -th tissues. Significant correlations have been found between the concentrations of DDT in the tissues of rats<sup>1</sup>, between the concentrations of HEOD in the tissues of rats and pigeons<sup>10</sup>, and in the blood and adipose tissue of man<sup>6,11</sup>, and these correlations are consistent with this postulate. Some of the correlations between the concentrations in different tissues are not significant, but this does not necessarily imply that there is no functional relationship in these cases: the experimental values include variations between individuals and are also subject to experimental error—both these variables reduce the size of the correlation coefficients and hence their apparent significance. It is further postulated that the functional relationship implies that there is a dynamic equilibrium in the distribution of pesticides in the tissues.

(iii) The concentrations of organochlorine insecticide in the tissues depend on the time of exposure

$$c_{\alpha ij} = h_{\alpha ij}(t)$$

Time-dependent concentrations have been found for DDT in the adipose tissues of rats<sup>12,13</sup>, monkeys<sup>3</sup>, cattle<sup>7</sup> and man<sup>4</sup>; for HEOD in the blood of dogs, the blood and adipose tissue of man<sup>6</sup>, and the subcutaneous fat of the quail<sup>13</sup>; for the isomers of BHC in the adipose tissue of

rats<sup>14</sup>; and for heptachlor epoxide in the adipose tissue of cows<sup>7</sup>. The relationship in all cases examined appears to be curvilinear and it is also postulated that  $h_{adj}(t)$  approaches a finite upper limit as  $t$  increases. There are indications that the concentrations may pass through a maximum and then decline<sup>11,12</sup>; the mechanism underlying this decrease of storage with continuing exposure is not clear; it may be a result of microsomal activation or a physiological change with age which varies the absolute or relative sizes of one or more of the body compartments.

(iv) When exposure ceases, the concentrations of organochlorine insecticides in the tissues decline and the rates of decrease at a given time are proportional to the concentrations in the tissues at that time

$$\frac{d}{dt}(c_{adj}) = -k_{adj}(c_{adj})$$

Decreasing concentrations in the tissues after the end of exposure have been found for DDT in the adipose tissue of cows<sup>11,12</sup>, monkeys<sup>3</sup>; for HEOD in the tissues of rats and pigeons<sup>10</sup>, in the adipose tissue of cows<sup>7</sup> and in the blood of man<sup>16</sup>; and for BHC isomers in the adipose tissue of rats<sup>14</sup>. In some cases a relationship of the semilogarithmic type appears to fit the results satisfactorily<sup>8,10</sup>, that is,  $dc/dt = -kc$ , corresponding to a pseudo-unimolecular rate of elimination. In other cases the experimental results deviate significantly from this simple relationship; some authors have transformed both the variates (concentration and time) to obtain rectilinear relationships, but these equations appear to have no theoretical basis and it is suggested that rate equations of the type

$$dc/dt = -\sum k_i c_i$$

are more appropriate. Given that the rate equations for the elimination process are of the latter form and that the experimental animal is a constant entity, then it may be shown that the accumulation curve

$$c_{adj} = h_{adj}(t)$$

will approach an upper limit (asymptote) in an exponential manner. The asymptote is a function of the daily intake (in accordance with the first postulate).

These four postulates are consistent with the compartmental models which have been used to explain the dynamics of the behaviour of drugs<sup>17</sup>, lipids<sup>18</sup>, inorganic ions<sup>19,20</sup>, etc., in animals, and it is proposed, therefore, that such a model, particularly the mamillary system with a central compartment (blood or plasma) and peripheral compartments that cannot interchange directly with each other, be used in the case of the organochlorine insecticides. The model must also include the enterohepatic circulation in view of biliary excretion of these compounds. The results available at present are not sufficient to establish the model in full detail, but experiments can easily be designed to give the information required to make appropriate tests of the model.

The symbols used above for the postulates have been used not only because of their economy, but also because they implicitly assume a basis in set theory. A compartmental model theory has been developed from set-theoretical principles<sup>21,22</sup>, and such principles are also thought to be helpful in the development of a theory of the dynamics of the behaviour of organochlorine insecticides in ecosystems. An outline of the concepts of set theory, with particular reference to biological systems, has been given by Rashevsky<sup>23</sup>. Before outlining such a model, it is appropriate to review some of the evidence, unfortunately somewhat scanty at present, obtained in studies of the distribution of residues of these compounds in the environment.

The concentration of DDT-type compounds in the adipose tissue of members of the general population of the United States has been approximately constant for some 10–15 years; the concentration of HEOD in the adipose

tissue and blood of the general population in Britain has been unchanged, within the limits of experimental error, since 1961; the concentration of HEOD in the livers of shags (from the colony in the Farne Islands) reaches a steady state at the age of about 1 year; the concentration of HEOD in the eggs of these birds has not increased over the period 1964–1966. All these observations are compatible with the concept of an upper limit of accumulation, related to the daily intake of DDT or HEOD, which has been suggested (fourth postulate). Again, the concentrations of HEOD and pp'-DDE in the eggs of the shags are significantly correlated ( $r_s = 0.71$ ,  $P < 0.001$ ), as also are the concentrations in the livers of these birds ( $r_s = 0.81$ ,  $P < 0.001$ ). These correlations are difficult to explain unless some common factors are involved in the uptake, distribution and storage of these compounds in the shags. It is also noteworthy that the concentrations of HEOD and pp'-DDE in the eggs of cormorants and shags are considerably greater than those found in nine other species of sea birds living in the same area. Some of the differences in the residues in eggs may arise from the different types of fish eaten by the birds, but it is considered plausible that the differences are caused partly, if not predominantly, by differences in the physiology of the birds, particularly in regard to the rates of elimination of these compounds. The compartmental model provides a set of consistent concepts capable of giving a unified explanation of these results of field studies.

An ecosystem may be defined as an area that includes living organisms and non-living (abiotic) components interacting to produce an exchange of materials and energy between the living and non-living components. The living organisms can be classified into three main groups: the producers (autotrophic organisms), the consumers (heterotrophic organisms), and the decomposers (saprophytes), heterotrophic organisms which break down the complex compounds of dead protoplasm and release simple substances that may be used by the autotrophic organisms. There is thus a cyclic exchange of matter and energy within the ecosystem.

The ecosystem may be regarded as a super-set with the trophic levels (corresponding to the producers, consumers and decomposers) as sets contained within the super-set. Individual species form sub-sets of the trophic levels, and individual organisms are members of the sub-sets

$$a \in S \subset T \in E$$

where  $a$  is an individual organism,  $S$  is the species to which the individual belongs,  $T$  is the trophic level and  $E$  the ecosystem; the symbols  $\in$  and  $\subset$  are the standard ones for "membership of" and "is contained in". The total annual biomass of a given trophic level is

$$\sum_{jk} m_{ijk}$$

and the total content of a particular insecticide in this trophic level is given by

$$\sum_{jk} m_{ijk} x_{ijk}$$

where  $m_{ijk}$  is the body weight of the  $k$ -th member of the  $j$ -th species in the  $i$ -th trophic level and  $x_{ijk}$  is the average concentration of the insecticide in the whole body of this individual.

At a given instant in time the total amount of the insecticide in the ecosystem is

$$(\sum_{ijk} m_{ijk} x_{ijk} + W)$$

where  $W$  is the mass of the insecticide in the non-living components (soil, water, air). In a completely isolated ecosystem the total mass of the insecticide will remain constant, although the instantaneous distribution between the trophic levels, etc., may vary, unless the insecticide is degraded either by biotransformation in the living organisms, or by chemical and/or physical processes in the abiotic components. Most ecosystems, of course, are

not isolated. The total mass of an insecticide in the  $i$ -th trophic level may increase as a result of transfer from the  $(i-1)$ th trophic level; it may decrease either by degradation of the insecticide within the  $i$ -th level or by transfer to the  $(i+1)$ th level. The total biomass in the  $i$ -th level may increase as a result of (a) migration into the ecosystem, (b) reproduction, and (c) growth of the organisms in that level. The chief factors leading to a decrease in the biomass are (a) migration from the ecosystem, (b) death of individuals, or (c) loss of weight by starvation. Precise information on these variables is not available except in a few very special systems, but this does not necessarily imply that no progress can be made in constructing a model. The system can be regarded as a black box with transfer functions that define the ratio between the input and output of mass or energy or insecticide residues. The trophic levels correspond to subdivisions within the black box; this suggested use of the black box is similar to that proposed for precursor-product relationships in the compartmental model<sup>24</sup>.

Some of the modes of change of the biomass will have concomitant effects on the total mass of an insecticide in a trophic level, for example, migration of individuals from the ecosystem decreases both the biomass and the mass of the insecticide in their trophic level. Growth of organisms will increase the biomass, but the food necessary for growth may result in a transfer of insecticide from the  $(i-1)$ th level. On the other hand, the death of an individual will decrease the biomass in the  $i$ -th level and, simultaneously, reduce the mass of insecticide. The latter will eventually be incorporated, with or without some degradation, into the saprophytic organisms, or returned to the abiotic components. The annual biomasses of the successive trophic levels become successively smaller (pyramid of mass), that is

$$\sum_{j \leq i} m_{(i-1)j} > \sum_{j \leq i} m_{ij}$$

Let the transfer of biomass from the  $(i-1)$ th trophic level in time  $\Delta t$  to the  $i$ -th level, as a result of the consumers in the latter, be

$$\sum_{j \leq i} \Delta m_{(i-1)j}$$

and let the increase of biomass in the  $i$ -th level be

$$\sum_{j \leq i} \Delta m_{ij}$$

consequent on this consumption. Then

$$\sum_{j \leq i} \Delta m_{(i-1)j} > \sum_{j \leq i} \Delta m_{ij}$$

(otherwise the pyramid of mass would not hold) and the amount of insecticide entering the  $i$ -th trophic level will be

$$(\sum_{j \leq i} \Delta m_{(i-1)j} - \sum_{j \leq i} \Delta m_{ij}) x_{(i-1)j}$$

The concentration of insecticide in the  $i$ -th level will increase unless the rate of loss of this compound from the  $i$ -th level is equal to or greater than its rate of accretion. The rate of loss of an insecticide from the  $i$ -th level is a function of the concentration in that level (because the loss depends on biochemical degradation or the death of organisms)

$$\frac{dx_{ij}}{dt} = -m(x_{ij})$$

but the relationship is probably more complex than that for the elimination from tissues (see fourth postulate). Further, this type of elimination process necessarily entails a finite upper limit of accumulation as time goes on, although, once again, the process is more complex than that in an organism because some of the insecticide transferred from the trophic level may be returned to it at a later date by way of the saprophytes and autotrophic organisms. The greater the ecological efficiency the smaller the value of the term

$$(\sum_{j \leq i} \Delta m_{(i-1)j} - \sum_{j \leq i} \Delta m_{ij})$$

The transfer functions in the model have been expressed in terms of mass, but it may be preferable to express the transfer functions in energy terms<sup>25,26</sup>, particularly if a significant proportion of the biomass of a trophic level consists of inorganic matter, for example, the shell of molluscs. The transfer of an insecticide between trophic levels would then be expressed as mass of insecticide per calorie of food consumed by the predators.

The model outlined here is also of assistance in interpreting the ecological consequences of the use of organochlorine insecticides and their consequent occurrence in the environment. The components of the environment that influence the fecundity, longevity and speed of development of an organism have been classified into five components by Browning<sup>27</sup>, one of which was "hazards". Maelzer<sup>28</sup> has criticized the term hazard as a description of a component of the environment because of the implication of general risk, and, as he points out, a particular circumstance is not always inimical in all circumstances. This distinction is also appropriate to the presence of organochlorine insecticides in the environment: the mere presence of these compounds does not necessarily entail harmful effects. There are threshold limits below which neither toxicological nor physiological effects (including behaviour) are observable.

The complete model has not yet been constructed but it is obviously complex and partially cyclic. It is clear, however, that great care is required in drawing conclusions about the behaviour of insecticides in ecosystems based on small numbers of observations, or on differences between samples from different trophic levels collected at different times. The advantage of the model, even in its present incomplete state, is that it presents a basis for assessing the validity of inferences drawn from field observations. Further, it enables sampling schemes to be devised so that the results may be used to test the validity of the model.

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# A Cosmological Model with both Radiation and Matter

by

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Previous models of the universe based on the "big bang" theory have set various times for the period when the density of matter became equal to the density of radiation. This model sets this limit at about 6.7 per cent of the present age of the universe and has an advantage because it explains what happens in the early as well as the late stages of the life of the universe.

THERE has been particular interest in recent years in the amount of radiation and matter in relativistic cosmological models. Penzias and Wilson<sup>1</sup> and Roll and Wilkinson<sup>2</sup> found an isotropic microwave background, which is believed by some<sup>3-5</sup> to be a remnant of the initial "big bang" or "fireball". Twenty years ago Gamow postulated<sup>6,7</sup> a model in which the energy density of radiation exceeds that of matter near the beginning of the universe. He says<sup>8</sup> that the matter density is less than the radiation density during about the first 10 per cent of the history of the universe and after this time exceeds the radiation density so that at present the latter can be neglected relative to the matter density. Thus different differential equations control the expanding universe for early time and for recent time. This is important because, as he shows, the thermal balance during the expansion is maintained by radiation and not by matter. According to Dicke *et al.*<sup>9</sup>, the densities become equal after about the first 0.01 per cent of the history.

Davidson<sup>9</sup> considers models in which the function  $S(t)$  of the line element

$$ds^2 = c^2 dt^2 - S^2(t) \left\{ \frac{dr^2}{1 - kr^2} + r^2 (d\theta^2 + \sin^2 \theta d\phi^2) \right\} \quad (1)$$

is given by  $S(t) = At^n$  and where the curvature  $k$  is zero and shows that, because the density  $\rho$  and pressure  $p$  are related by

$$\frac{p/c^2}{\rho} = \frac{2-3n}{3n} \quad (2)$$

the models with  $n$  slightly greater than  $\frac{1}{3}$  and slightly less than  $\frac{1}{3}$  are suitable models for early and late stages of the universe. The limits  $\frac{1}{3}$  and  $\frac{2}{3}$  give the ratio (2) as  $\frac{1}{3}$  (radiation only) and zero (matter only), respectively. His work, however, contains no mathematical way of changing from  $n = \frac{1}{3}$  to  $n = \frac{2}{3}$  as time increases. Gamow's model<sup>6-8</sup> changes from  $S \propto t$  to  $S \propto t^2$  for early and late stages.

This article proposes a cosmological model which covers both early and late stages. The model behaves like the  $n = \frac{1}{3}$  radiation model for early time and like the Einstein-de Sitter  $n = \frac{2}{3}$  matter model for late time.

Consider a model in which the function  $S(t)$  is defined by

$$\log S = \int \frac{2 dt}{3t - \frac{1}{\beta} e^{-\beta t} + \frac{1}{\beta}} + \text{const.}; \beta \text{ constant} \quad (3)$$

in a cosmologically flat space ( $k = 0$ ). Hubble's parameter and the deceleration parameter are

$$\frac{\dot{S}}{S} = \frac{2}{3t - \frac{1}{\beta} e^{-\beta t} + \frac{1}{\beta}} \quad (4)$$

and

$$q = \frac{1 + e^{-\beta t}}{2} \quad (5)$$

The density  $\rho$  and the pressure  $p$  are given by the substitution of equation (1) into Einstein's field equations so that

$$\frac{1}{3} K \rho c^4 = \left( \frac{\dot{S}}{S} \right)^2 = - \left( K p c^2 + 2 \frac{\ddot{S}}{S} \right) \quad (6)$$

Here

$$K \rho c^4 = \frac{12}{\left( 3t - \frac{1}{\beta} e^{-\beta t} + \frac{1}{\beta} \right)^2} \quad (7)$$

and

$$K p c^2 = \frac{4e^{-\beta t}}{\left( 3t - \frac{1}{\beta} e^{-\beta t} + \frac{1}{\beta} \right)^2} \quad (8)$$

giving

$$\frac{p/c^2}{\rho} = \frac{e^{-\beta t}}{3} \quad (9)$$

It will be shown later that  $\beta$  is of the order of  $10^{-17} \text{ sec}^{-1}$  so that  $\beta t$  remains small for about the first  $10^{14} \text{ sec}$  and  $e^{-\beta t}$  can be written as  $1 - \beta t$ . Also for  $t$  greater than  $10^{19} \text{ sec}$  the denominator of equation (4) is  $3t$  to a good order of accuracy. Thus for  $t < 10^{14} \text{ sec}$  and  $t > 10^{19} \text{ sec}$ ,

$$\frac{\dot{S}}{S} = \frac{1}{2t} \text{ and } \frac{2}{3t} \text{ sec}^{-1} \quad (10)$$

respectively,

$$q = \frac{2 - \beta t}{2} \rightarrow 1 \text{ and } \frac{1}{2} \quad (11)$$

respectively and

$$S \rightarrow At^{\frac{1}{2}} \text{ and } At^{\frac{2}{3}} \quad (12)$$

respectively. The term  $1/\beta$  in the denominator was included to make  $S(0) = 0$ . Also the ratio  $(p/c^2)/\rho$  in equation (9)  $\rightarrow \frac{1}{3}$  for  $t \rightarrow 0$  and  $\rightarrow 0$  as  $t \rightarrow \infty$ . Obviously there is still total conservation of energy in the model in the normal relativistic sense.

$$T^{\mu}_{;\mu} = 0 \text{ or } \frac{\partial(\rho S^3)}{\partial t} + \frac{p}{c^2} \frac{\partial S^3}{\partial t} = 0 \quad (13)$$

$\rho$  and  $p$  can be written as

$$p = p_{\text{radiation}} + p_{\text{motion}} = \frac{aT^4}{3} + p_{\text{matter}} v^2 \quad (14)$$

$$\rho = \rho_{\text{radiation}} + \rho_{\text{matter}} = \frac{aT^4}{c^2} + \rho_{\text{matter}} \quad (15)$$

Here  $p_{\text{motion}}$  is the pressure due to the random motions of the galaxies, equal to  $\rho_{\text{matter}} v^2$  (ref. 10), where  $v$  is the random radial velocity, observed by Hubble in 1936 to be less than 300 km/sec;  $a$  is Stefan's constant,  $7.6 \times 10^{-16} \text{ erg/cm}^2/\text{deg}^4$ ; and  $T$  is the radiation temperature of intergalactic space. Thus equation (9) can be rewritten as

$$\frac{p_{\text{r}}}{p_{\text{r}} + p_{\text{m}}} = e^{-\beta t} \text{ or } p_{\text{m}} = p_{\text{r}} (e^{\beta t} - 1) \quad (16)$$

where  $p_{\text{motion}}$  can be neglected as long as  $\rho_{\text{m}} < 10^7 \rho_{\text{r}}$  if  $v$  is approximately constant. As in Davidson's paper<sup>9</sup>, there must be a continual interchange of energy between matter and energy. No longer is  $\rho_{\text{m}} S^3$  a constant. Indeed, it increases with time because, defining

$$E_m(t) = \frac{1}{S^2} \left\{ \frac{d}{dt} (\rho_m S^3) + \frac{p_m}{c^2} \frac{dS^3}{dt} \right\} c^2$$

$$= \left( \dot{\rho}_m + 3 \frac{\dot{S}}{S} \rho_m \right) c^2 \quad (17)$$

this becomes (for  $p_m = 0$ )

$$E_m = \frac{12 e^{-\beta t}}{K c^2} \left( 3\beta t - 1 + e^{-\beta t} \right) \quad (18)$$

which is always greater than zero for  $t > 0$  so that  $\rho_m S^3$  increases. This  $E_m$  is, as in Davidson's paper, the total net rate of creation of material energy per unit volume over all frequencies at epoch  $t$ . Similarly

$$E_r = \left( \dot{\rho}_r + 4 \frac{\dot{S}}{S} \rho_r \right) c^2 \quad (19)$$

is the total net rate of transfer of matter energy per unit volume into radiation energy, so that

$$E_m + E_r = 0 \quad (20)$$

from equation (11) Davidson dismisses the possibility  $E_m > 0$  as "inapplicable to the universe as we observe it", but this does not seem necessarily so. That the rate of absorption of radiation per unit volume (with the resultant creation of matter) is greater than the rate of emission per unit volume at a given epoch does not mean that there will be no radiation. Indeed, the equations show that there will always be residual radiation, and that the amount can agree with that discovered.

For  $\beta t$  small and  $\beta t \gg 1$ ,

$$E_m = \frac{3\beta}{8 K c^2 t^2} \quad \text{and} \quad \frac{4\beta e^{-\beta t}}{4 K c^2 t^2} \quad (21)$$

respectively, so that  $E_m$  is quite small because  $\beta$  is of the order of  $10^{-17}$  (see later). For present  $t_0$ , the net rate of creation of matter from radiation is

$$\frac{1}{c^2} E_m = 3.2 \times 10^{-31} \text{ g/cm}^3/\text{sec} \quad (22)$$

It is interesting to compare this with the total creation rate in steady-state cosmology of  $3\rho_m H \sim 2 \times 10^{-48} \text{ g/cm}^3/\text{sec}$  (ref. 11).

It is assumed hereafter that the only contribution to  $p_{r,0}$  (the zero subscript denoting the present epoch) is that resulting from the background microwave flux already mentioned; that is, that which would be emitted by a black body at  $3.0 \pm 0.5^\circ \text{ K}$ . This is equivalent to a total radiation pressure of

$$c^2 p_r = a T^4 = 6.13 \times 10^{-13} \text{ dyne/cm}^2 \quad (23)$$

or radiation density of

$$\rho_r = 6.8 \times 10^{-34} \text{ g/cm}^3 \quad (24)$$

If the value  $(\dot{S}/S)_0 = 100 \text{ km/sec/Mpc} = 3.2 \times 10^{-18}/\text{sec}$  is used in equation (4), this together with equations (8) and (23) gives

$$e^{-\beta t_0} = 3.71 \times 10^{-4} \quad (25)$$

or

$$\beta t_0 = 10.20 \quad (26)$$

This implies that the present ratio of pressure to density is, from equation (9)

$$(p/c^2)/\rho = 1.24 \times 10^{-4} \quad (27)$$

Equations (4) and (26) now give

$$t_0 = 2.02 \times 10^{17} \text{ sec} = 6.3 \times 10^9 \text{ yr} \quad (28)$$

and

$$\beta = 5.06 \times 10^{-17}/\text{sec} \quad (29)$$

From equations (7) and (8)

$$K\rho_m c^4 = \frac{12(1 - e^{-\beta t})}{\left( 3t - \frac{1}{\beta} e^{-\beta t} + \frac{1}{\beta} \right)^2} \quad (30)$$

so that

$$\rho_{m,0} = 1.8 \times 10^{-28} \text{ g/cm}^3 \quad (31)$$

As this is two orders of magnitude higher than that of the observed density (ref. 12) of  $\rho_{m,0} \sim 7 \times 10^{-31}$  for the estimated mass of galaxies, the theory is satisfied only if there is a large preponderance of non-luminous matter.

It is interesting to compare  $\rho_m$ ,  $p_r$ , etc., for small and large  $t$ . For  $t < 10^{15} \text{ sec}$ , where  $e^{-\beta t}$  can be written as  $1 - \beta t$  to a reasonable order of accuracy, and for  $t > 10^{15} \text{ sec}$ , where  $e^{-\beta t}$  is almost zero, and  $3t_0 + 1/\beta = 3t$  to a good approximation,

$$K\rho_m c^4 = \frac{3\beta}{4t} \quad \text{and} \quad \frac{4}{3t^2} / \text{sec}^2 \quad (32)$$

respectively,

$$K\rho_r c^4 = \frac{3(1 - \beta t)}{4t^2} \quad \text{and} \quad \frac{4e^{-\beta t}}{3t^2} / \text{sec}^2 \quad (33)$$

respectively,

$$\rho_m = \beta t \rho_r \quad \text{and} \quad (e^{-\beta t} - 1) \rho_r \quad (34)$$

respectively, and

$$T = \sqrt{\left( \frac{3c^2(1 - \beta t)}{32\pi a G} \right)} \cdot \frac{1}{t} = 1.5 \times 10^{10} \frac{(1 - \beta t)^{1/2}}{t} \text{ } ^\circ\text{K}$$

$$\quad \text{and} \quad 1.7 \times 10^{10} \frac{e^{-\beta t/2}}{t^{1/2}} \text{ } ^\circ\text{K} \quad (35)$$

respectively (see also equation (10)). For  $\beta t \ll 1$ ,

$$K\rho_r c^4 = \frac{3}{4t^2} \text{ sec}^{-2} \quad \text{and} \quad T = \frac{1.5 \times 10^{10}}{t^{1/2}} \text{ } ^\circ\text{K} \quad (36)$$

None of  $\rho_m$ ,  $p_r$ ,  $\rho$  or  $\dot{S}/S$  has maximum or minimum values for finite positive  $t$ . They all decrease steadily with time.

The density of matter becomes the larger of the two densities after

$$t = 1.4 \times 10^{18} \text{ sec} \sim 6.7 \text{ per cent of present time} \quad (37)$$

that is, when

$$\rho_r = \rho_m = 1.4 \times 10^{-27} \text{ g/cm}^3 \quad (38)$$

and

$$T = 113 \text{ } ^\circ\text{K} \quad (39)$$

Notice that the value of 6.7 per cent of present time is less than Gamow's recent estimate<sup>3</sup> of about 10 per cent, his estimates for density and temperature being  $3 \times 10^{-28} \text{ g/cm}^3$  and  $50^\circ \text{ K}$ ; but the value is very much greater than that of Dicke *et al.*<sup>2</sup>.

The total density  $\rho$  is very nearly  $3 p/c^2$  (radiation) so long as  $e^{-\beta t}$  in equation (9) is very nearly 1. This depends on the order of accuracy required, but is nearly so for  $t < 10^{14} \text{ sec}$ .

The apparently *ad hoc* choice of  $S(t)$  in equation (3) was made to simplify the mathematics particularly in equations (4), (7) and (8). This choice of  $S$  changes the model from a  $n = \frac{1}{2}$  model to a  $n = \frac{2}{3}$  model as Davidson<sup>1</sup> had, and agrees with the initial radiation model required and the final model with the radiation density much smaller than the matter density, thus agreeing with present day observations. This model may provide a useful approach to the problem of building a cosmological model with both radiation and matter defined by a single metric and set of field equations.

I thank Dr N. W. Taylor for help and encouragement and for useful discussions on this work.

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# LETTERS TO THE EDITOR

## ASTRONOMY

### Observations of Quasars using Interferometer Baselines up to 3,074 km

It has previously been demonstrated by the Jodrell Bank-Malvern interferometer that some quasars have angular diameters less than 0.025 sec of arc. These measurements were made with a baseline of 120 km at a wavelength of 6 cm (ref. 1).

It is not only desirable to improve still further the resolving power at centimetre wavelengths but, in view of existing theories which predict that the observed diameter of a quasar should increase with wavelength, it is important to achieve comparable resolution at longer wavelengths. This implies the use of very long baselines. With conventional interferometers using land lines or microwave links, it is difficult to preserve the phase of the signals for distances greater than a few hundred kilometres. Furthermore, it is difficult to compensate for the large and variable time delay between reception of the signals at the two telescopes. These difficulties have been overcome by the use of wide-band magnetic tape recorders and independent local oscillators controlled by rubidium frequency standards<sup>2</sup>.

With an interferometer of this type we have observed the quasar 3C 273B at a frequency of 448 Mc/s with a baseline of 3,074 km ( $4.6 \times 10^4 \lambda$ ). Assuming a Gaussian source model the visibility of the fringes indicates that the diameter of this source is less than 0.02 sec of arc at this frequency.

Observations at 448 Mc/s have also been made at a baseline of 183 km ( $2.7 \times 10^3 \lambda$ ) and fringes of high visibility observed for the sources 3C 273, 3C 286, 3C 287, 3C 309.1, 3C 345, NRAO 530, 3C 446 and OTA 102. The maximum angular diameter of these sources is therefore about 0.3 sec of arc. Fringes of low visibility were observed for the quasar 3C 454.3.

Although some sources showed large amplitude scintillations, the fringes for all sources were extremely regular, indicating that phase scintillation was small.

The instruments used for these observations were the 46 m telescope of the Algonquin Radio Observatory, the 25.6 m telescope of the Dominion Radio Astrophysical Observatory and the 18.3 m antenna of the Defence Research Telecommunications Establishment, near Ottawa. We thank the Defence Research Telecommunications Establishment for making available the 18.3 m antenna for some of these observations.

*Notes added in proof.* Subsequently, fringes were also observed from 3C 345 using the 3,074 km baseline.

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### A Strong X-ray Source in the Vicinity of the Constellation Crux

This communication presents a preliminary report of the detection of a strong source of X-rays in the vicinity of the constellation Crux.

Two identical X-ray detection systems were included in the payloads of two Skylark rockets flown from Woomera, Australia (lat. = 30.9° S., long. = 136.5° E.), at 0032 U.T. on April 4, 1967 (Skylark SL 426), and at 2236 U.T. on April 20, 1967 (Skylark SL 425). The X-ray experiments on both flights operated successfully from ignition to re-entry 400 sec later. The data considered here will be those obtained when the rockets were at altitudes in excess of 100 km, because below this altitude the atmosphere is opaque to celestial X-rays in the energy range of interest.

The X-ray detection system on each rocket consisted of four LND proportional counters containing a xenon-methane (90 per cent to 10 per cent) mixture at 1 atm. The X-ray window of each counter was 12 cm<sup>2</sup>, and consisted of 14 mg/cm<sup>2</sup> (0.003 in.) of beryllium. The counters were calculated to have an efficiency which varied from 40 per cent at 2 keV to 98 per cent at 8 keV. The counters possessed energy resolutions of about 25 per cent full width half maximum (FWHM) when newly filled. The resolutions of some of the counters deteriorated with time, however, possibly as a result of contamination of the filling gas with electronegative material, and in the worst case were 70 per cent immediately before the flight (SL 426). Two counters used on SL 425 possessed the best energy resolution immediately before flight (~30 per cent) and for the purposes of this communication have been used to investigate photon energy spectra.

On each rocket, the counters were divided into two groups of two, each pair "looking" in one direction and having its pulse outputs combined through an equalizing network. The resultant pulse train was analysed by a two window pulse height analyser, with windows set at 2-5 keV and 5-8 keV. These windows were calibrated using the 5.9 keV X-rays from a source of iron-55. The counting rate data from the two energy windows corresponding to each pair of counters were telemetered to ground, as were the temperatures of various components. The geometrical configuration of the counters was such that about 75 per cent of the pulses due to relativistic cosmic rays were equivalent to greater than 8 keV energy deposition, and thus were rejected.

Collimators were placed in front of each of the counters to define its field of view, the angular resolutions being 10.5° FWHM in the direction of rocket spin and 35° FWHM in the plane containing the spin axis. The attitude of the rocket spin axis, and the phase of the rocket spin, were determined using optical, magnetic, and rate gyro data provided by the standard Skylark instrumentation unit. Four independent crossed-slit Sun sensors provided the angle between the solar and spin vectors, while a three-axis magnetometer permitted calculation of the angle between the magnetic and spin vectors; the two angles

Table 1. SUMMARY OF RESULTS FOR "CRUX" OBJECT

	SCO XR-1	"CRUX"
Intensity*		
SL 425 2-5 keV (o.p.s.)	200 ± 8	160 ± 7
5-8 keV (o.p.s.)	98 ± 5	33 ± 3
2-8 keV ratio "CRUX"		0.52 ± 0.04
Spectra	SCO XR-1	
SL 425 ratio 5-8 keV 2-5 keV	0.36 ± 0.03	0.20 ± 0.03
Position†		
SL 425: Right ascension	16.2 h	13.7 h
Declination	-15°	-63°
SL 426: Right ascension	16.3 h	13.5 h
Declination	-16°	-66°

\* No corrections have been applied for the efficiency of the counters. Absolute photon fluxes should not be computed from these counting rates until such corrections have been applied. (Errors shown are statistical).

† The position of SCO XR-1 is 16.28 h, 15.5 degrees (ref. 3).



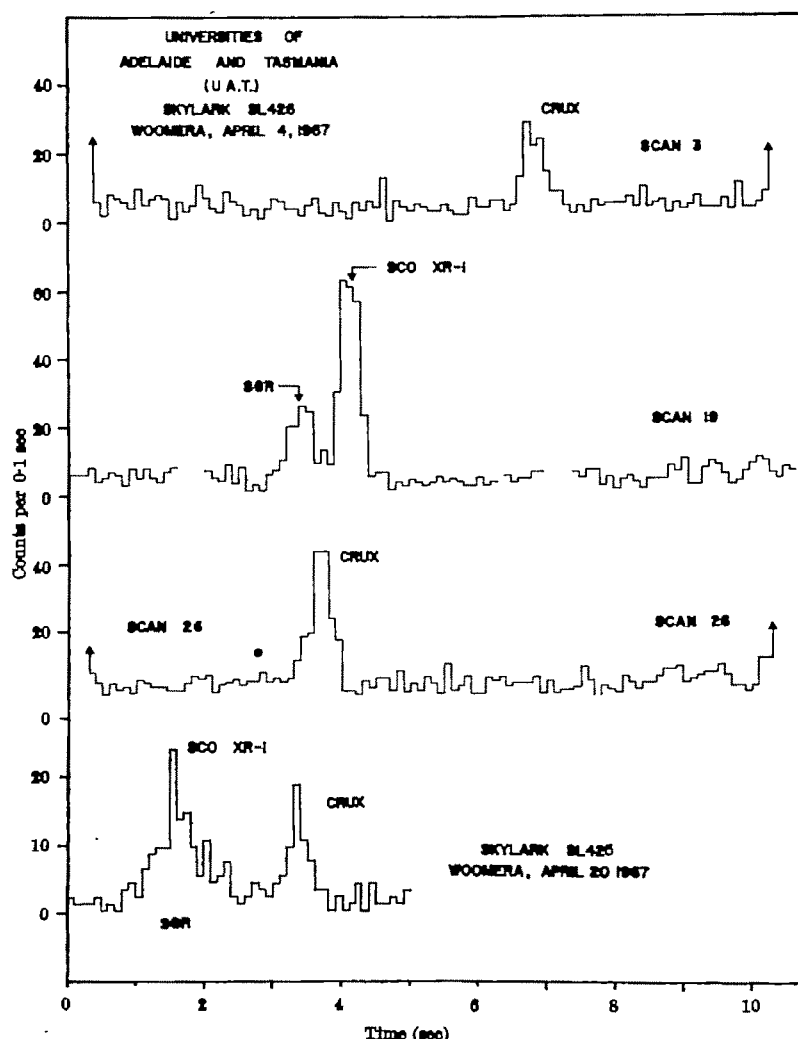


Fig. 1. The counting rates observed as the detector scanned over *SCO XR-1*, the Sagittarius group of sources and the Crux object. The *SL 425* data refer to the 2-5 keV energy window while the *SL 426* data refer to the 2-8 keV energy range.

then yield the direction of the spin vector. Using these data the direction of viewing of the two pairs of detectors was determined for all times during each flight.

Fig. 1 shows representative X-ray counting rates obtained during both flights, and Fig. 2 summarizes the motion of the centre of the field of view of the X-ray detectors on the celestial sphere for these scans. Referring to the aspect solution (for example, Fig. 2), the directions of viewing corresponding to the various counting rate maxima have been calculated, and Fig. 2 presents the positions on the celestial sphere of the various X-ray sources thus determined. It is clear from this that in addition to the observation of X-rays from the well known Sagittarius cluster of X-ray sources, and the *SCO XR-1* object, both flights detected X-rays from an object at a high negative declination. In both flights the relative positions of the Sun and the magnetic vector were such that a less accurate position could be found for the new object than for *SCO XR-1*. This is reflected in the dispersion in the positions as seen in Fig. 2 and

Table 1. While the position of the object is still somewhat uncertain, for convenience we shall refer to it as the "CRUX" object, because the positions are in the vicinity of the constellation Crux.

Table 1 presents the data from the two energy windows corresponding to the pair of counters on *SL 425* which exhibited a pre-flight energy resolution of 30 per cent FWHM. There is a significant difference between the 5-8 keV/2-5 keV ratios corresponding to *SCO XR-1* and "CRUX" which suggests that the energy spectrum of "CRUX" is softer than that of *SCO XR-1*. The two window spectra from the other counter pairs are consistent with this qualitative conclusion, after allowance for the less favourable FWHM applicable to these other counters.

Comparing *SCO XR-1* and "CRUX", both are very bright compared with the remainder of the known celestial X-ray objects, and both are objects possessing very soft spectra. These findings suggest that *SCO XR-1* and the Crux object are of a similar physical nature. In light of the recent measurement of angular extent and the tentative optical identification of the *SCO XR-1* object<sup>3</sup>, the existence of a second very similar X-ray object becomes a matter of considerable interest.

The initial X-ray detector hardware development was performed at the Southwest Center for Advanced Studies, Dallas, Texas, under an NASA contract. J. R. H. and R. J. F. acknowledge the support provided by CSIRO and Commonwealth postgraduate scholarships respectively. The assistance of the UK Science Research Council in providing the opportunity to make these flights is gratefully acknowledged, as is the help of many kinds provided by the personnel of BAC, Filton,

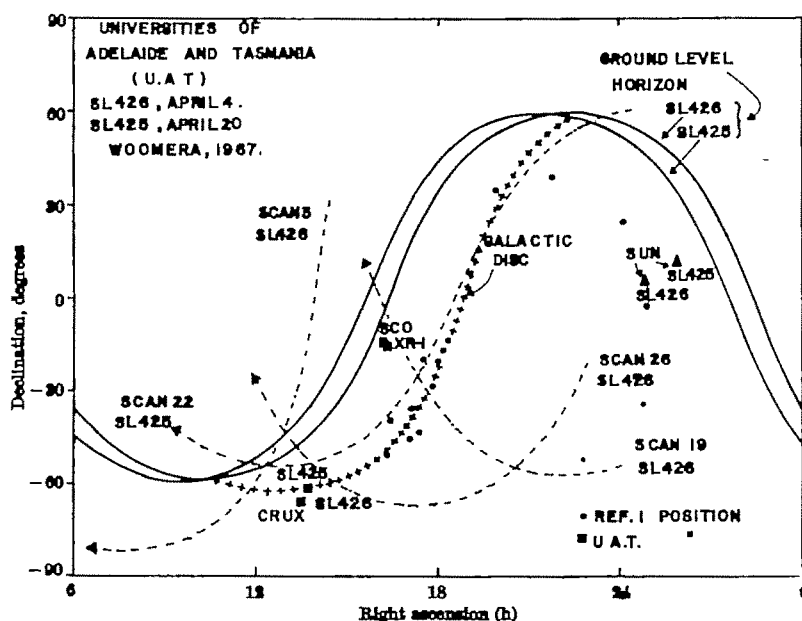


Fig. 2. Map of the celestial sphere, showing the ground level horizon for each flight and the direction the detector was looking during the scans shown in Fig. 1. The positions of known sources<sup>4</sup>, and the positions of *SCO XR-1* and "CRUX" as derived from these flights, are shown. The position of *SCO XR-1* as given in ref. 3 is obscured by the two UAT points. Note that two UAT points are given for each object, corresponding to the two flights *SL 425/426*.

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### Detection of a New Microwave Spectral Line

AFTER the radioastronomical detection<sup>1</sup> of helium in 1966 a programme to study the  $109\alpha$  transition of neutral helium was begun at the US National Radio Astronomy Observatory, and helium has now been detected in five sources (as will be described elsewhere). During the course of this work, however, unidentified microwave spectral emission has also been detected in the sources *NGC 2024* and *IC 1795*. This communication describes the observed physical characteristics of this emission, considers its possible origins and suggests further observations to identify it. In the succeeding communication, Goldberg and Dupree argue that the new line is a recombination line of carbon I.

We worked with the US National Radio Astronomy Observatory 140 ft. telescope, equipped with a cooled parametric amplifier, and the Harvard University twenty-one channel spectral line radiometer. The overall temperature of the system was about 80° K. The observations were carried out at frequencies near those of the  $109\alpha$  recombination lines of hydrogen and helium ( $\sim 5,009$  Mc/s), and at a constant polarization position angle with resolutions of 100 kc/s and 30 kc/s. Figs. 1-3 show portions of the spectra of the sources *NGC 2024* and *IC 1795*. The  $109\alpha$  recombination line of hydrogen is the strong signal on the left in Figs. 1 and 2. In *IC 1795*, there is an unidentified microwave line; on the high frequency side

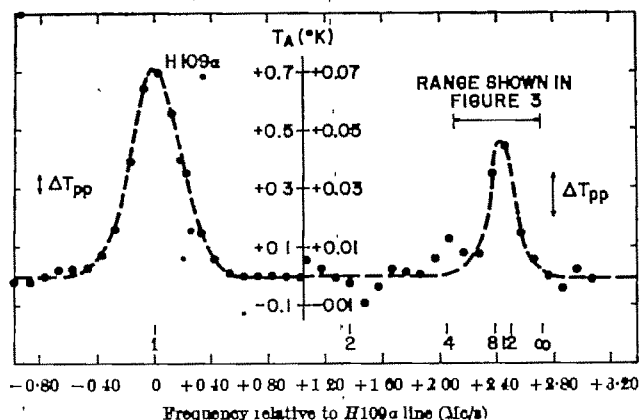


Fig. 1. Spectrum of *NGC 2024* with 100 kc/s resolution. The ordinate is antenna temperature and the abscissa is frequency relative to the  $H109\alpha$  line in Mc/s. The rest frequency of the  $H109\alpha$  line is 5,008 923 Mc/s. The radial velocity of this line is  $+7.2 \pm 0.4$  km/s. The numbers below the spectrum indicate the expected position of recombination lines of emitters of mass 1, 2, 4, 8, 12  $M_\odot$  and  $\infty$  if they have the same radial velocity as the  $H109\alpha$  line.  $\Delta T_{pp}$  is the peak to peak noise fluctuation.

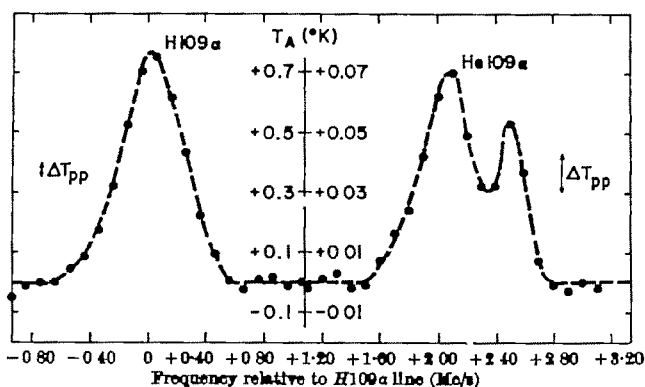


Fig. 2. Spectrum of *IC 1795* with 100 kc/s resolution. The radial velocity of the hydrogen line is  $-41.8 \pm 0.8$  km/s.

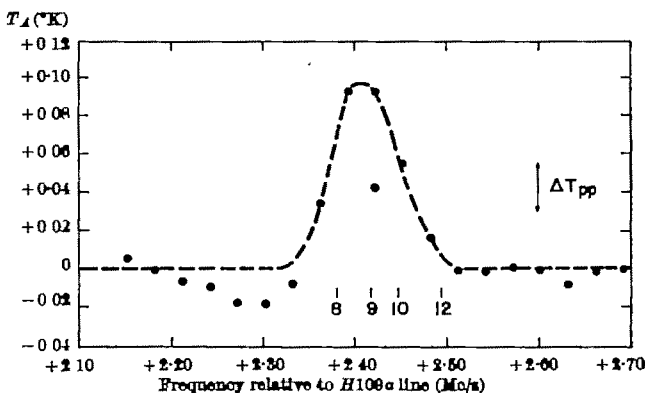


Fig. 3. Spectrum of *NGC 2024* with 30 kc/s resolution. As in Fig. 1, the numbers below the spectrum indicate the expected position of recombination lines of emitters of mass 8, 9, 10, and 12  $M_\odot$ .

of the helium profile. In *NGC 2024* an unidentified line is present, and no helium signal was observed that was significantly outside the noise.

Some characteristics of the unidentified microwave spectral lines are given in Table 1.

Source	Separation from hydrogen (Mc/s)	$v_L$ (km/s)	$T_A$ (°K)	Ratio of area under line profile to area under hydrogen profile
<i>NGC 2024</i>	$2.408 \pm 0.020$	$78^{+20}_{-10}$	$0.10 \pm 0.03$	$3.1 \times 10^{-4}$
<i>IC 1795</i>	$2.49 \pm 0.10$	$< 200$	—	—

In what follows, we shall assume that the unidentified lines have the same origin. If the hydrogen and the unidentified lines have identical radial velocities, the rest frequency of the new line in *NGC 2024* is  $5,011.33 \pm 0.02$  Mc/s. The rest frequency for *IC 1795* is slightly different but within the limits of observational error.

There are several reasons why this new emission line is unlikely to be a recombination line of hydrogen or helium. If, for example, it were a displaced hydrogen  $\alpha$  line, a large peculiar velocity ( $\geq 144$  km/sec) would be necessary to account for it. If it were a displaced helium  $\alpha$  line, the peculiar velocity required would be smaller, but because there is no corresponding feature visible on the hydrogen profile it is unlikely to be helium. If, on the other hand, the new line comes from the same region as the hydrogen  $\alpha$  line, the first higher order transition of hydrogen to lie within the error brackets would be that from  $n = 460$  to  $n = 400$ , and the intensity of that transition would be extremely small. A more decisive consideration is that the observed lines are too narrow to be attributed to hydrogen or helium in H II regions. The width of a Doppler broadened line in the region of about 5,000 Mc/s is

$$\Delta\nu = 358 \sqrt{\frac{T}{10^4} \frac{M_H}{M_S}} + 6.06 \times 10^{-3} V_T^2 \text{ kc/s} \quad (1)$$

where  $T$  is the temperature,  $M_H$  and  $M_S$  are the masses of hydrogen atoms and emitters respectively, and  $V_T$  is the turbulent velocity in km/sec. The observed upper limit to the line width for *NGC 2024* implies a maximum kinetic temperature of  $750^\circ \text{K}$  for hydrogen or  $3,000^\circ \text{K}$  for helium—temperatures which are much lower than those observed for H II regions. It is therefore natural to ask whether the new line can be a recombination line originating from atoms, ions or molecules with mass greater than those of helium.

Recent investigations of the  $137\beta$  hydrogen line in *NGC 2024* by Zuckerman *et al.*<sup>2</sup> have shown that the energy levels responsible for the radio recombination lines are not populated according to local thermodynamic equilibrium. Their results for the  $137\beta$  line imply a kinetic temperature of at least  $8,700^\circ \text{K}$  for *NGC 2024*. With this temperature, equation (1) implies that the mass of the substance emitting the new line is at least  $12 M_H$ , unless there is significant line narrowing by stimulated emission. With  $T = 8,700^\circ \text{K}$  and  $\Delta\nu = 409 \text{ kc/s}$ , equation (1) yields a turbulent velocity for the hydrogen in *NGC 2024* of about  $8.3 \text{ km/sec}$ . The width of the new line in *NGC 2024* is compatible, however, with a turbulent velocity of no more than  $3.5 \text{ km/sec}$ , no matter what the mass or temperature of the emitter may be. The new line may therefore originate from only a part of the H II region. The fact that the emission line was obvious in only two of six H II regions investigated does indeed suggest that rather specialized physical conditions must be satisfied.

If the radial velocities of the source of the new line and of the hydrogen line in *NGC 2024* are the same, the Rydberg formula implies a mass of 8 or  $9 M_H$  for the emitter. Differences of radial velocity no greater than  $5 \text{ km/sec}$  would, however, be consistent with a mass of  $12 M_H$ , corresponding to carbon. The positions of lines from emitters of several different masses are indicated in Figs. 1 and 3. We have examined several effects which would cause deviations from the Rydberg formula for complex atoms, ions, or molecules; they are all small compared with the line width. We note that  $\beta$  lines of doubly ionized emitters,  $\gamma$  lines of triply ionized emitters, and so on, fall at the same positions. A mechanism which may enhance emission lines from heavy atoms or molecules is described in the succeeding communication.

For a firm identification, more observations are needed. The lines in *NGC 2024* and *IC 1795* should be examined with greater frequency resolution in order to determine more accurately the frequency of the line relative to hydrogen and to see whether the line is resolved with  $30 \text{ kc/s}$  filters. To determine if this is a recombination line, we suggest that these observations be carried out at an adjacent transition (either  $108$  or  $110\alpha$ ).

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**Addendum (May 31, 1967).** We have now observed the  $110\alpha$  transition of the new line in *NGC 2024*, thus establishing that it is a recombination line. The observed parameters of the line at  $110\alpha$  are  $T_L = 0.10^\circ \text{K}$ ,  $\Delta\nu = 79 \pm 20 \text{ kc/s}$ , and the ratio of the area under the new line to that under the hydrogen profile was  $3.4 \times 10^{-3}$ , in very close agreement with the values obtained at  $109\alpha$ . The frequency separation of the new line from the hydrogen line was  $2.344 \pm 0.020 \text{ Mc/s}$ , which agrees with the frequency predicted from the  $109\alpha$  observations to an

accuracy of  $6 \text{ kc/s}$ . The observations were again made at the National Radio Astronomy Observatory.

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## Population of Atomic Levels by Dielectronic Recombination

IN the preceding communication, Palmer *et al.* have reported the observation of an emission line close to the expected position of the recombination line  $109\alpha$  of C I in the radiofrequency spectra of the ionized hydrogen regions *NGC 2024* and *IC 1795*. The total intensity of the newly observed line is about 3 per cent that of the neighbouring  $109\alpha$  line of H I. Let us assume that the two lines originate from the same volume of space. If the upper levels of C I and H I were populated in thermodynamic equilibrium, the relative intensities of the two lines would be roughly in the ratio of the numbers of hydrogen and carbon ions in the emitting volume. This ratio would be equivalent to that of the cosmic abundances of hydrogen and carbon if both atoms were entirely in the singly ionized condition. In the Sun, the abundance of carbon is  $5 \times 10^{-4}$  of that of hydrogen<sup>1</sup>, and there is no reason to suppose that the ratio is greatly different in H II regions. Thus, if the line found by Palmer *et al.* is indeed the  $109\alpha$  line of C I, its observed intensity relative to that of the same transition in hydrogen is at least sixty times greater than would be expected from the two assumptions of thermodynamic equilibrium and normal cosmic abundances. In this communication we point out that the apparently anomalous overpopulation of the  $n = 110$  level of C I can be explained as a result of departures from thermodynamic equilibrium, which result in the preferential population of levels of high total quantum number by dielectronic recombination.

In a low density plasma exposed to a dilute source of ionizing radiation, the populations of excited levels of atoms are fixed primarily by the balance between electronic recombination and cascade into the levels, and spontaneous transitions out of them. The populations of very high levels are also modified by collisional  $n \rightarrow n \pm 1$  transitions and by collisional ionization and three-body recombination<sup>2</sup>. These processes occur in atoms of all species. There is, however, a crucial difference between the dominant recombination process for a hydrogenic atom and that for a complex ion. The bare nucleus of a hydrogenic ion can recombine only by radiative capture, but an ion that has one or more electrons is able to capture an electron by dielectronic recombination. This process may occur at a much faster rate<sup>3</sup> and with a wholly different dependence on  $n$  and  $l$  than that of radiative capture. Burgess<sup>3</sup> has shown that the dielectronic recombination rate coefficient into level  $i, nl$ , by way of the intermediate doubly excited level  $j, n'l$ , is proportional to  $(2l+1)b(j, nl)$ . Here  $b(j, nl)$  is the factor by which the population of  $j, nl$  differs from its value in thermodynamic equilibrium. The value of  $b$  is given by

$$b(j, nl) = \frac{A_s}{A_s + A_i} \quad (1)$$

where  $A_s$  ( $\text{sec}^{-1}$ ) is the rate coefficient for autoionization from level  $j, nl$ , and  $A_i$  ( $\text{sec}^{-1}$ ) is the rate coefficient of the stabilizing transition,  $j, n'l \rightarrow i, nl$ . When  $A_s \gg A_i$ ,  $b(j, nl) =$

1 and the population has its equilibrium value. In that event the recombination rate coefficient, when summed over all  $l$  values, is proportional to  $n^2$ , whereas the corresponding rate coefficient for radiative capture is proportional to  $n^{-1}$  (ref. 4). Because the rate at which the level  $i, n$  is depopulated by spontaneous emission is approximately the same for hydrogenic and non-hydrogenic ions,  $b(i, n)$  may increase rapidly with increasing  $n$  and may become very large.

This simple picture has to be modified in two respects. First, according to Burgess<sup>3</sup>, the quantity  $b(j, n)$  is unity only for values of  $n$  smaller than about 5–10. Thereafter as  $n$  increases, the autoionization rates and the  $b(j, n)$  values decrease both with increasing  $n$  and, especially, with increasing  $l$ . For example, when  $n = 100$ ,  $b(j, n)$  is effectively zero for all values of  $l$  greater than about 6–10. Second, the populations of levels with very large  $n$  will be modified by collision. In the present context, the most important collisional process is ionization by electron impact and the inverse process of three-body recombination. These processes are certain to arrest the increase of  $b(i, n)$  at some value of  $n$  that is fixed by the electron density, and thereafter to cause it to decrease to unity at the limit when  $n \rightarrow \infty$ .

As an illustration of the points made in the preceding paragraphs, we have made approximate calculations of  $b(4s, n)$  for Ca I in which we assumed that the levels are populated both by dielectronic recombination and cascade and by three-body electronic recombination, and that they are depopulated by spontaneous transitions to lower levels and by ionization by electron impact. We used approximate hydrogenic values for the collisional and radiative cross-sections and carried out the calculations for a temperature of  $10^4$  °K and for two values of the electron density, equal to  $10^3$  and  $10^4$  cm<sup>-3</sup>. The results of the calculations are shown in Fig. 1, in which  $\log b(4s, n)$  is plotted against  $\log n$  for  $n = 10, 20, 50, 100$  and 200, the values of  $n$  for which Burgess<sup>3</sup> has published autoionizing rates in the  $4pnl$  series of calcium. When  $n$  is relatively small,  $b(4s, n)$  increases rapidly with  $n$  until collisional ionization becomes important;  $b(4s, n)$  then passes through a maximum and decreases sharply to

unity at the series limit. The maximum value occurs at  $n = 50$ –100 for  $N_e = 10^4$  cm<sup>-3</sup> and at  $n = 100$ –200 for  $N_e = 10^3$  cm<sup>-3</sup>.

Both the absolute value of  $b(i, n)$  and its rate of change with  $n$  are important in causing the line intensity to deviate from its value in thermodynamic equilibrium. First, the line should appear either in emission or absorption according to the value of both  $db(n)/dn$  and the optical depth of the continuum. Second, the transition rates are proportional to the  $b$ -value of the initial level. Third, to allow for stimulated emission the absorption coefficient in the line must be multiplied by the factor

$$1 - \frac{b(n)}{b(n')} \exp(-h\nu/kT_e) \\ \approx \frac{b(n') - b(n)}{b(n')} + \frac{b(n)}{b(n')} \frac{h\nu}{kT_e} \quad (2)$$

where  $n$  and  $n'$  are the upper and lower levels of the transition, respectively<sup>4</sup>. When  $b(n') > b(n)$ , the factor for stimulated emission is larger than the equilibrium value  $h\nu/kT_e$  and approaches unity in the limit when  $b(n') - b(n) \rightarrow b(n')$ . Conversely, when  $b(n) > b(n')$ , the stimulated emission factor is smaller than the equilibrium value and the emission intensity may be amplified by quasi-maser action.

It would be unwise to regard the preceding calculations as anything but illustrative because they do not include the effects of collisional transitions to and from neighbouring levels, nor do they take account of stimulated emission as a significant factor in populating the levels. Both effects will tend to equalize the  $b(i, n)$  values of adjacent levels and thus to decrease the values of  $db(n)/dn$ . Their introduction is unlikely, however, to alter the principal conclusions reached here, namely: (1) in conditions found in ionized hydrogen regions in the interstellar medium, the capture of electrons by dielectronic recombination can greatly increase the populations of highly excited levels as compared with those occurring in thermodynamic equilibrium; (2) there will be a value of the total quantum number  $n$  for which  $b(i, n)$ , the ratio of the actual population to that in thermodynamic equilibrium, has a maximum value; and (3) recombination lines from complex atoms should be found in emission and, with the appropriate conditions, in absorption.

Returning now to the question of the suspected 109α line of C I in the spectra of IC 1795 and NGC 2024, we have seen that if the carbon is all singly ionized and its abundance relative to hydrogen is the same as that found in the Sun, the observed line intensity requires that  $b(i, 110) \sim 60$ , neglecting stimulated emission. The autoionizing probabilities for the  $j, nl$  levels of C I are not known at present. As a rough first approximation we assume that the  $b(j, nl)$  factors for C I are the same as for Ca I. Our calculations give a value of  $b(i, 100)$  for C I equal to 10 at  $T_e = 10^4$  °K and  $N_e = 10^3$  cm<sup>-3</sup>.

We see that this estimated value is apparently too small by about a factor of six, which, nevertheless, is well within the error of estimation. Furthermore, we have not properly allowed for stimulated emission, although the value  $n = 110$  falls near the maximum of the  $b(i, n)$  versus  $n$  relationship. More exact calculations of the  $b$ 's for C I which are in progress should provide a closer comparison with the observations.

We have also examined the possibility that the carbon line could be formed in an H I region surrounding an H II region. At temperatures of  $10^4$  °K, ordinary radiative recombination<sup>4</sup> proceeds at a much faster rate than dielectronic recombination. The electron density is, however, so low in the H I region<sup>5</sup> that the rate at which the level  $b(i, 100)$  is populated is two orders of magnitude slower than for an H II region. An unreasonably large volume of H I would then be required to produce the observed emission.

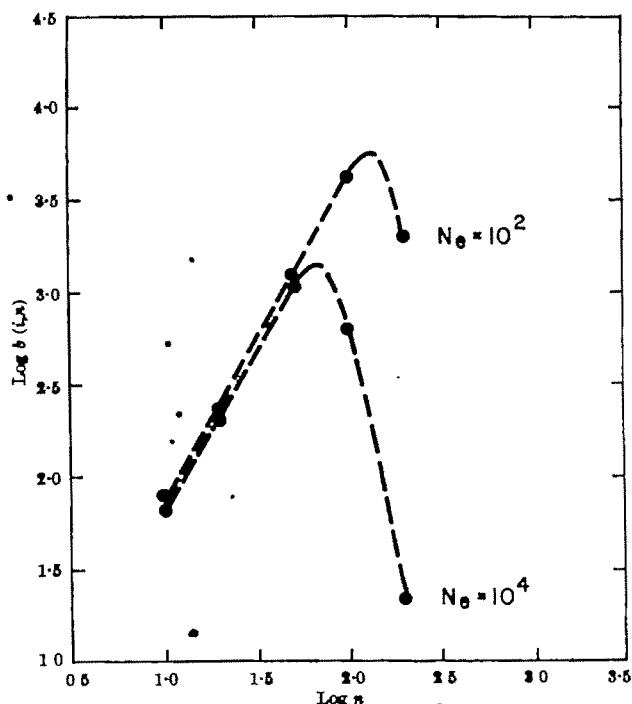


Fig. 1. Values of  $b(4s, n)$  of Ca I calculated for an electron density equal to  $10^3$  and  $10^4$  cm<sup>-3</sup>.

Finally, we should ask whether recombination lines of C are more likely to be observed than those of other elements of comparable abundance, such as N I, O I, Mg I and Si I. To provide a rough answer to this question we have calculated for each of these elements the product of the solar abundance  $A_i$  relative to hydrogen<sup>1</sup> and the total dielectronic recombination rate,  $\alpha_i$  (tot) for  $T_e = 10^4$  °K as given by Burgess<sup>7</sup>. The results are given in Table 1.

Table 1. PRODUCT OF THE SOLAR ABUNDANCE RELATIVE TO HYDROGEN AND THE TOTAL DIELECTRONIC RECOMBINATION RATE FOR  $T_e = 10^4$  °K

Atom	$A_i \alpha_i$ (tot)
C I	$1.1 \times 10^{-17}$
N I	$1.8 \times 10^{-18}$
O I	$7.1 \times 10^{-20}$
Mg I	$2.6 \times 10^{-18}$
Si I	$1.7 \times 10^{-17}$
Ca I	$5.8 \times 10^{-17}$

We note that the product appears to be greatest for magnesium and about equally large for calcium, carbon and silicon. The ionization potentials of Ca II, Mg II and Si II, however, are not greatly different from those of H I, being 11.9, 15.0, and 16.3 eV, respectively, whereas for O II the value is 24.4 eV. Thus, of the four elements under consideration, carbon is least likely to be doubly ionized in H II regions. It is noteworthy that the ionization potential of O II is nearly equal to that of He I. Thus the ratio of intensities of the  $\alpha$  lines of O I and He I should be a sensitive indicator of the flux of ultra-violet radiation shortward of 500 Å in the spectra of the exciting stars.

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## Abundance of Helium in the Sun

THE observed abundances of elements are largely understood in terms of stellar nucleosynthesis. The origin of helium, however, remains a major problem. Indeed, Hoyle and Tayler<sup>1</sup> concluded that the chief fraction of the helium in the Galaxy was synthesized not in stellar interiors but either in the primordial fireball or in a massive object which preceded the formation of the stars. This communication presents a re-determination of the abundance of helium in the Sun and also reviews determinations of the abundance of helium in other objects.

As is well known, a spectroscopic determination of the abundance of helium in the Sun can be made only with considerable uncertainty. Biswas and Fichtel<sup>2</sup> and also Gaustad<sup>3</sup> have discussed how the abundance of helium can be derived from the combination of the abundance ratios observed in solar cosmic rays and the spectroscopic determination of the abundance for nuclei with the same charge-to-mass ratios as helium.

Revised solar abundances of carbon, nitrogen and oxygen have been obtained from a comprehensive analysis of atomic and molecular spectra (unpublished results). The spectroscopic oxygen abundance is  $\log N_O/N_H =$

$-3.23 \pm 0.05$ , which with the cosmic ray ratio  $N_{He}/N_O = 107 \pm 14$  gives the solar helium abundance as

$$\frac{N_{He}}{N_H} = 0.083 \pm 0.015$$

Furthermore, the abundance ratios given by the spectroscopic analysis

$$N_O/N_O = 0.60 \pm 0.10 \text{ and } N_N/N_O = 0.15 \pm 0.05$$

are in good agreement with the cosmic ray measurements which give<sup>2</sup>

$$N_O/N_O = 0.59 \pm 0.07 \text{ and } N_N/N_O = 0.19 \pm 0.04$$

This agreement provides support for the assumption that the cosmic ray abundances for nuclei with the same charge to mass ratio are equal to the photospheric abundances.

The solar composition expressed in terms of the mass fractions of hydrogen, helium, and the heavy elements is

$$X = 0.79, Y = 0.20 \text{ and } Z = 0.013$$

Previous estimates for the helium abundance, which were based on the same cosmic ray data, gave  $N_{He}/N_H = 0.09$  or  $Y = 0.26$ . The reduction is the result of the downward revision of the spectroscopic determinations of the abundances of carbon, nitrogen and oxygen. Both the previous and present abundances of helium are consistent with recent model calculations for the solar interior<sup>4</sup>.

The composition of the solar photosphere will be assumed to be representative of the interstellar gas at the time of formation of the Sun about  $4.5 \times 10^9$  years ago. A determination of the rate of helium enrichment in the interstellar gas is obtained from a comparison of the helium abundances in the interstellar gas and young stars with the above solar abundance.

Accurate determinations of such abundances are available for gaseous nebulae. Faulkner and Aller<sup>5</sup> find  $N_{He}/N_H = 0.12$  for the Orion and  $\eta$  Carinae nebulae. Determinations of the abundance of helium for O and B stars are reviewed by Underhill<sup>6</sup>. The results are consistent with the gaseous nebulae abundances but are subject to greater uncertainty.

The helium abundance  $N_{He}/N_H = 0.12 \pm 0.02$  is taken to be representative of the present interstellar gas. This abundance exceeds the solar abundance by about a factor of two. The increase over an interval of  $4.5 \times 10^9$  years is attributed to the enrichment of the interstellar gas with helium synthesized in stellar interiors.

The total optical emission of the galaxy can be shown to correspond to the conversion of  $5 \times 10^6 M_\odot$  of hydrogen to helium in this same interval, although the helium abundance in the interstellar gas can be doubled only if the ejection of helium from stellar interiors is about 100 per cent effective. This is a gross overestimate of the likely efficiency. It is therefore necessary to suppose that the helium deposited in the previous  $4.5 \times 10^9$  years was produced before the formation of the Sun when the rate of star formation was markedly greater than at present. This supposition is consistent with Dixon's<sup>7</sup> interpretation of metal deficient stars which shows that the rate of star formation was considerably enhanced for a short period after the collapse phase of the Galaxy. Calculations by Truran, Hansen and Cameron<sup>8</sup> of the increase in the abundances of helium and heavy elements since the formation of the Galaxy provide a certain degree of quantitative support for these ideas. In particular a calculation which corresponds to an exponential decline in the total mass of the interstellar medium with a decay constant of  $3 \times 10^9$  years reproduces the observed solar and interstellar abundances for acceptable ages for the Sun and the Galaxy. Although the observations are consistent with their assumption that the Galaxy was formed from pure hydrogen gas, determinations of helium abundance in very old stars must provide the decisive test.

Unfortunately, observations of helium in old stars are not extensive. Recent discoveries<sup>9,10</sup> of B-type halo stars with an apparently low helium abundance ( $N_{He}/N_H \approx 0.01$ )



are consistent with the prediction. Other observations of similar stars suggest an abundance close to the solar value<sup>11</sup>. A possible explanation is that in such highly evolved stars the surface layers have been contaminated with material from the interior which is rich in helium. Greenstein, Truran and Cameron<sup>12</sup>, however, suggest that the helium deficient atmospheres result from a loss of helium by downward diffusion. The correct interpretation of the helium abundances in old stars must await further observations.

It will be evident that no final conclusion on the origin of helium can be proposed with any certainty. The evidence compiled here suggests that stellar nucleosynthesis of helium was responsible for the increase in the helium abundance in the interstellar gas between the formation of the Sun and the present time. The initial helium abundance for the Galaxy before the formation of stars is uncertain. The upper limit provided by the solar abundance is  $N_{\text{He}}/N_{\text{H}} \leq 0.06$ . This limit is only slightly less than that predicted by recent calculations of helium synthesis in a primordial fireball<sup>13</sup>. The recent discoveries of old stars with an apparently low helium abundance and an extrapolation of the observed rate of helium enrichment suggest that the possibility of a very low initial helium abundance ( $N_{\text{He}}/N_{\text{H}} < 0.01$ ) should be anticipated. The cosmological significance of this result has been emphasized by Hoyle and Taylor<sup>1</sup>.

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## Surface Helium Abundance in Horizontal Branch and Halo B Stars

THE surface helium abundance of stars at the blue end of the horizontal branch in globular clusters and of halo B stars has been estimated by spectroscopists to be lower than that at the Sun's surface by factors ranging from 10 (refs. 1 and 2) to 100 (ref. 3). It is assumed that the halo B stars are the field-equivalents of horizontal branch stars, and we hereafter refer to both classes as blue horizontal branch stars.

Greenstein, Truran and Cameron<sup>4</sup> have recently suggested that diffusion in slowly rotating blue horizontal branch stars may be efficient enough to establish a pronounced stratification of elements near the surface, in such a way that the abundance of helium at the photosphere would be considerably smaller than in the interior. Spectroscopic determinations of surface helium abundance, even if they were correct, would then shed no light on what the subsurface abundance might be. Apparent variations would simply reflect differences in the relative efficiency of the diffusion process from one star to the next (whether due to different rates of rotation or surface conditions) and/or differences in the time over which diffusion has been effective in depleting the surface abundance.

Deeming and Walker<sup>5</sup> have shown that, for main sequence O and B stars and for Be stars, the weakness of helium lines is correlated with the narrowness of these lines. Because meridional circulation induced by rotation tends to counter the effects of diffusion inward, these results lend support to the argument that diffusion may have been effective in depleting the abundance of helium at the surface of sharp-lined blue horizontal branch stars.

In this communication we prefer to use the term depletion mechanism when discussing the observations, because gravitational diffusion is not the only effect which could cause a separation of elements (resonance radiation absorption<sup>6</sup> is another possibility). We wish to point out that if the helium deficiency factor ( $\sim 100$ ) given by Sargent and Searle<sup>9</sup> is correct, it is a clear indication that some mechanism is effective in depleting the surface abundance. On the other hand, the estimates given by Searle and Rodgers<sup>1</sup> and Greenstein and Münch<sup>10</sup> do not allow one to come to this same conclusion. In the latter cases, we shall show that a deficiency factor of  $\sim 10$  could be consistent with the assumptions that globular cluster stars began life with a vanishingly small helium content and that no effective depletion mechanism has been operating.

Our argument is based on investigations of stars evolving from the main sequence to the red giant tip, that is, the progenitors of horizontal branch stars. The helium content to assign to the precursors themselves is, of course, related to the point in question. We have recently computed models (ref. 7 and unpublished results) for globular cluster stars of low metal content,  $Z/Z_{\odot} \approx 1/100$ , and for values of the initial helium content (a)  $Y = 0.35$ , (b)  $Y = 0.10$  and (c)  $Y = 0.00$ . Such values span the probably acceptable range. The appropriate masses to obtain satisfactory agreement with the main-sequence turn-offs are (a)  $M/M_{\odot} = 0.65-0.75$ , (b)  $M/M_{\odot} = 1.0-1.2$ , and (c)  $M/M_{\odot} = 1.2-1.4$ .

During the long main sequence phase which precedes the climb up the giant branch, these models have relatively low central temperatures and rely essentially on the pp chain for their energy generation. As a result, an extended profile of helium content versus mass fraction is built up. As the models climb up the steep part of the giant branch, the base of a growing convective envelope extends into the interior, where it is eventually forced to retreat again in front of the advancing hydrogen burning shell. While the envelope grows, convective mixing progressively increases the abundance of helium at the surface; this reaches a maximum when the envelope is at its largest extent. Whereas the models leave the main sequence when a helium core of a certain fractional mass has been attained, the structure and position on the giant branch are determined by the actual core mass. For the massive models, helium has been produced much further out as a function of actual mass. Thus, for these models, more helium is available for potential mixing to the surface. Our results for the total helium enrichment ( $^3\text{He} + ^4\text{He}$ ) of the surface are (a)  $\Delta Y \leq 0.005$ , (b)  $\Delta Y \approx 0.015-0.018$ , and (c)  $\Delta Y \approx 0.024-0.027$ . From these figures it is clear that the enrichment figure for stars of type (c) represents the lowest envelope helium content with which stars can arrive at the red-giant tip.

As an aside, it is of interest to note the region over which the change in surface composition occurs,  $1.0 \leq \log L/L_{\odot} \leq 1.7$ . Thus the change is confined to a region somewhat less than two magnitudes in extent, and ceases at a luminosity in the neighbourhood of horizontal branch luminosities<sup>8,9</sup>. There may be evidence for this in the recent results of Sandage and Walker<sup>8</sup> in M92, where an anomalous colour effect is thought to be operating in precisely the same region. Sandage and Walker reached the conclusion that if a difference in chemical composition were required to explain the difference in the ultra-violet colours for stars of a given B-V on the giant

branch and horizontal branch, the difference would have to act along the giant branch itself. The principal surface changes to be expected from our work will be among the light elements, up to carbon and nitrogen. Although the sum of the abundances of carbon-12 and nitrogen-14 remains constant along the giant branch, the ratio of nitrogen-14 : carbon-12 is increased by about a factor of three. The increase occurs as a result of convective mixing with interior regions where carbon-12 has been converted into nitrogen-14 during the main sequence phase (see, for example, ref. 10). Although it is difficult to see how burning, or a mere shuffling among the light elements, can have the desired effect, we nevertheless feel that there may well be a connexion between this observation and our results.

Returning to the giant branch, we have shown that the convective envelope attains a helium content with lower bound  $\sim 0.1 Y_{\odot}$ , where the solar value,  $Y_{\odot} \sim 0.27$  (for example, ref. 11). It is generally accepted (E. A. Spiegel, personal communication) that the convective envelope extends virtually to the photosphere, and that overshooting will certainly mix the photosphere to the same composition. Even were this not the case, models of type (b) and (c) must lose considerable mass in order to populate the horizontal branch<sup>7,8</sup> and thus the layers which become the horizontal branch photosphere must have been inside the giant convective envelope. If the mass loss occurs sufficiently late on the giant branch, the helium enrichment will be essentially that given above. If, however, mass loss were to occur earlier, before helium enrichment has attained its maximum value, then the figure could be increased. This conclusion is reached from inspecting our models where, within each class, the base of the envelope is deeper for the models of lower total mass. Thus, by comparison with a model of constant mass, more helium is scoured out, to be diluted by less hydrogen.

In order to test the sensitivity of these results to the convection theory of the outer layers, some models were run for two choices of the ratio of mixing-length to scale-height, 0.5 and 1. While the surface temperature is sensitive to the change, conditions deep inside the models remain the same, and the helium enrichment figure is virtually unaffected.

The final conclusion, therefore, is that blue horizontal branch stars should contain, before the operation of any depletion mechanism, at least  $\sim 0.1 Y_{\odot}$  in their photospheres. Sargent has asserted (personal communication) that the deficiency factor for the stars observed with Searle is undoubtedly greater than fifty. It thus seems clear that we have here a *reductio ad absurdum*, and that a depletion mechanism must have operated. This being the case, there would be no reason from spectroscopy to prefer any particular value for the initial helium content, and observations of horizontal branch stars could not be used to argue against any picture of the synthesis of primordial elements.

If, on the other hand, the Sargent and Searle observation is not confirmed, the other existing observations<sup>1,2</sup> would be consistent with (i) no helium initially, and no effective depletion, (ii) "solar-like" helium initially, and depletion by a factor of ten or (iii) some combination of (i) and (ii). According to the choice of initial helium content, stars arrive at the blue end of the horizontal branch either (i) directly, and evolve away to the red in  $2-4 \times 10^7$  years (low helium) or (ii) after evolving from the red end for  $\sim 10^8$  years (high helium)<sup>1</sup>. These results may then be combined with the observations to yield an indication of the time scales over which, on the various hypotheses, depletion may or may not have been effective. Unfortunately, the results are not unambiguous. It becomes important to determine theoretically the final steady state distribution of elements near the photosphere, if diffusion is indeed operative. Such a question, however, lies outside the scope of the present communication.

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## PLANETARY SCIENCE

### Evidence for Velocity Dispersion in Auroral Electrons

The intensities of 10 keV and 4 keV electrons have been measured from a sounding rocket launched into a post-breakup aurora. Pulsations in electron intensity were observed with a period of about 4 sec at both energies. The 10 keV pulsations occurred about half a second earlier than those at 4 keV. These results could have been caused by temporal changes in electron intensity and by a spatial structure in the path of the rocket because the causes are indistinguishable in the data. We treat the observed pulsations as temporal changes; they are similar in shape to pulsations observed in auroral light intensity, and electrons in the range 10 keV to 4 keV are important in the production of auroral light. If the pulsations are temporal in origin, the delay can be explained as a dispersion after simultaneous modulation 55,000 km from the rocket. The source of the modulation is found to be near the point where the local magnetic field line crosses the geomagnetic equator.

The measurements were made from a Nike-Apache rocket launched from Andoya in Norway at 2358 h U.T. on March 3, 1967. The flight was made during the recovery phase of a magnetic bay recorded at the Auroral Observatory at Tromsø, 120 km from the launch site. The magnetogram is shown in Fig. 1. Heppner<sup>1</sup> and

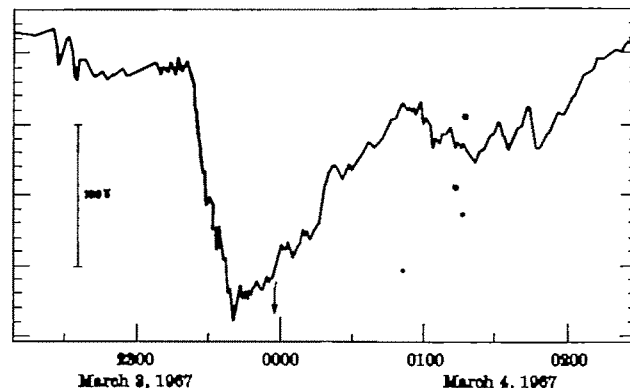


Fig. 1. Horizontal component of Earth's magnetic field measured at Tromsø, 120 km from the launch site at Andoya. The rocket was launched (arrow) 2358 h U.T. March 3, 1967. The measurements reported in this paper were taken near apogee at about 0002 h U.T. March 4, 1967. The vertical scale is given in units of  $\gamma$ , where  $1\gamma = 10^{-4}$  gauss. The magnetogram is reproduced by courtesy of the Director of the Tromsø Auroral Observatory.

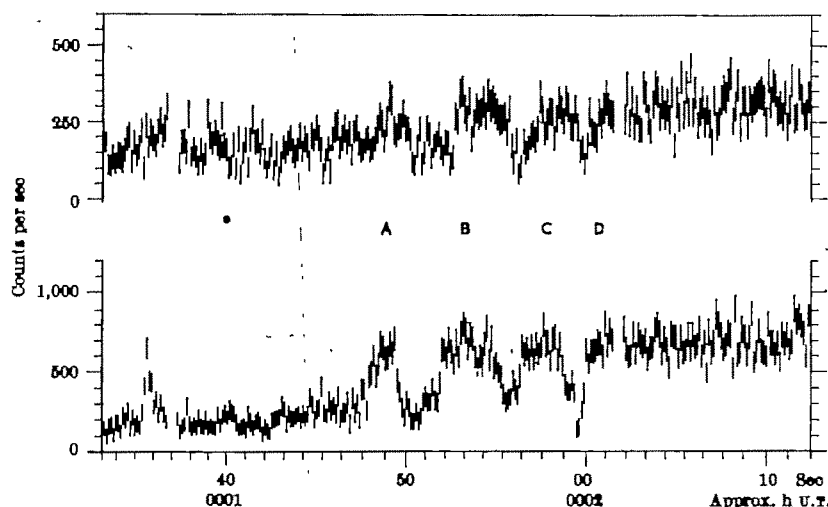


Fig. 2. Counting rates of the 4 keV (top) and 10 keV (bottom) electron detectors taken near apogee. The pulsations in electron intensity labelled A to D are discussed in the text.

Crosswell and Davis<sup>3</sup> have found that pulsating aurorae are seen most frequently at this phase of a magnetic storm. The electron detectors were channel multipliers used in conjunction with cylindrical electrostatic analysers. Two analysers selected electrons in narrow bands of energy near 10 keV and 4 keV. A third analyser with no electric field between the cylindrical plates served as a control. The measurements reported here were taken during a period of 40 sec while the rocket was near its apogee of 195 km.

Fig. 2 shows the counting rates of the 10 keV and 4 keV detectors plotted against time. The rates are represented by error bars ( $\pm$  one standard deviation) drawn through points obtained from successive rocket spin periods of 0.13 sec. The control measurement showed that the background rate was always less than 1 per cent of the rate recorded by the other detectors. In the 10 keV record (Fig. 2) a period of relatively low intensity is followed by a series of pulsations labelled A to D. The pulsations stop with the intensity at about the highest level recorded during the pulsations. These features are similar to variations in light intensity observed by Johansen and Omholt<sup>4</sup> during pulsating aurorae. The 4 keV intensity variations are similar in form but less clearly defined because the changes in intensity are smaller and the counting rates are lower. The pulsations occur about 0.5 sec earlier at 10 keV than at 4 keV. It is clear from the characteristic shapes of the pulsations that the delay does not differ from 0.5 sec by an integral number of pulsation periods. The difference in timing is illustrated in Fig. 3 in which five-point running means of the 10 keV

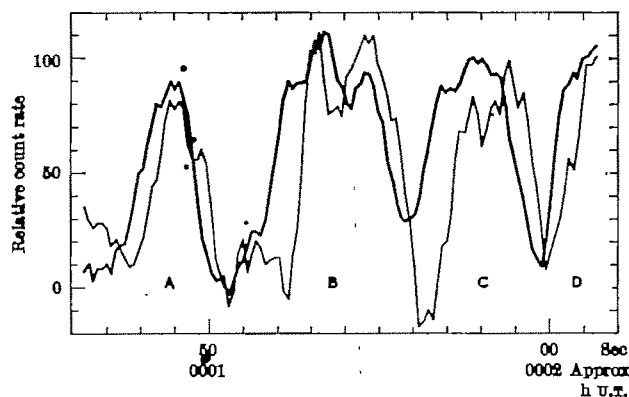


Fig. 3. Running means of the 10 keV (---) and 4 keV (—) counting rates for the pulsations A to D. The superimposed curves show that the pulsations at 10 keV occurred earlier than those at 4 keV.

and 4 keV counting rates are superimposed. Both records are normalized to an average value of zero in the 6 sec period before the pulsations, and an average value of 100 in the 6 sec period after the pulsations. Although there are differences in detail, the general features of the two records are similar with the 10 keV pulsations occurring consistently earlier than the 4 keV pulsations. A quantitative measure of the difference in timing was obtained by dividing the data of Fig. 2 into seven sections corresponding to the principal changes in counting rate, and cross correlating the data with relative delays incrementing by 0.13 sec the rocket spin period. On combining the results to obtain an average delay,  $\Delta t$ , between the 10 keV and 4 keV pulsations we obtain

$$\Delta t = 0.55 \pm 0.1 \text{ sec}$$

To find the distance of the source of the pulsations we assume that the

10 keV and 4 keV electrons were modulated simultaneously, and that their subsequent motion was adiabatic. The distance,  $s$ , between the source and the point of observation measured along the electron trajectory is then given by

$$s = \frac{V_1 V_2}{(V_1 - V_2)} \cdot \Delta t$$

where  $V_1$  is the speed of a 10 keV electron and  $V_2$  is the speed of a 4 keV electron. Substituting for  $V_1$ ,  $V_2$  and  $\Delta t$ , we find

$$s = (5.5 \pm 1.0) \times 10^4 \text{ km} \quad (1)$$

In principle the source of the pulsations can be located by tracing the electron trajectory at a distance  $s$  along the local geomagnetic field line. The flight was made at a local geomagnetic time of 0320 h during a magnetic bay on the magnetic shell  $L = 6$ . Beyond a few Earth radii this shell is distorted from the dipole form by inflation of the magnetosphere. The source, however, may be located approximately by computing electron trajectories in a dipole magnetic field. Using the value of  $s$  given in equation (1), and taking account of the range of pitch angles viewed by the detectors during each rocket spin period, we find the geomagnetic latitude,  $\theta$ , of the source to be

$$\theta = 7^\circ \text{ south} \pm 13^\circ$$

We see that an interpretation of the data based on some simplifying assumptions leads to the conclusion that electrons responsible for the production of the aurora can be significantly affected by processes occurring as far from the aurora as 55,000 km. The implication of this result is that the route taken by the auroral electrons passes close to the geomagnetic equator at a distance of six Earth radii.

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## Joint Epicentre Determination

THIS communication describes a method of determining station travel time corrections and the positions and origin times of more than one earthquake simultaneously. Application of the method to earthquakes from the same region should reveal any regional bias in travel times. Some preliminary results are presented.

Suppose the rough epicentre, depth and origin time of a seismic event are known, the equation of condition for calculating the corrections to these approximate values is<sup>1</sup>

$$\delta H + \delta h \frac{\partial T}{\partial h} + x \cos \alpha_j \frac{\partial T}{\partial \Delta_j} - y \sin \alpha_j \frac{\partial T}{\partial \Delta_j} = \delta T_j \quad (1)$$

where  $\delta T_j = A_j - H - T_j$ ;  $H$  is the approximate origin time of the event;  $h$  is the approximate depth of the event;  $\Delta_j$  is the distance from the approximate epicentre to station  $j$ ;  $\alpha_j$  is the azimuth from the approximate epicentre to station  $j$ ;  $A_j$  is the time of arrival (of the  $P$  waves) at station  $j$ ;  $T_j$  is the travel time (of the  $P$  waves) from the approximate epicentre to station  $j$ ;  $\partial T/\partial \Delta_j$  is the partial derivative of the travel time  $T (= f(\Delta_j, h))$  with respect to distance at the point  $\Delta_j, h$ ;  $\partial T/\partial h$  is the partial derivative of the travel time  $T$  with respect to depth at  $\Delta_j, h$ ;  $T$ ,  $\partial T/\partial \Delta_j$ , and  $\partial T/\partial h$  are obtained from travel time tables.

The unknowns  $x$ ,  $y$ ,  $\delta h$  and  $\delta H$ , the corrections to latitude, longitude, depth and origin time respectively, can be estimated by the method of least squares from equation (1) provided  $j > 4$ . A more accurate estimate of epicentre, depth and origin time can thus be obtained. Further corrections to this new epicentre can be calculated in the same way and the process repeated calculating successively better approximations to the (least squares) estimates of the epicentre parameters until the corrections become small enough to neglect. Convergence towards the best estimates is usually rapid.

Recent work (for example, ref. 2) has shown that equation 1 should include a term for the station correction  $S_j$ , where  $S_j$  is the difference between the observed travel time and the travel time obtained from the travel time tables. The results of several special studies have been published giving estimates of station corrections.

When these station corrections are available they can be applied to the arrival times  $A_j$  and these corrected times substituted in equation 1. The epicentre calculation can then proceed as before.

Cleary and Hales<sup>3</sup> have also shown that some station corrections are dependent on azimuth, and Herrin and Taggart<sup>4</sup> and Helterbran and Jordan<sup>5</sup> report that, at least from the LONGSHOT area, travel times vary azimuthally. Corrections for the azimuthal variations should therefore also be applied if accurate epicentres are to be estimated. But these corrections, particularly the source bias, are difficult to detect, because the usual method of estimating epicentres, by definition, shifts the position of the event until the residuals show no bias. Some of the bias can, however, be detected by estimating simultaneously the station corrections and epicentres of several events from the same region.

Equation 1 can be rewritten to include the station corrections  $S_j$ ;  $S_j$  contains the travel time bias as well as the station effect. Thus if  $\delta S_j$  is the correction to the approximate value of  $S_j$ , equation 1 becomes for the station  $j$  recording the  $i$ th event

$$\delta S_j + \delta H_i + \delta h_i \frac{\partial T}{\partial h_i} + x_i \cos \alpha_{ij} \frac{\partial T}{\partial \Delta_{ij}} - y_i \sin \alpha_{ij} \frac{\partial T}{\partial \Delta_{ij}} = \delta T_{ij} \quad (2)$$

where  $\delta T_{ij} = A_{ij} - H_i - T_{ij} - S_j$ .

As equation 2 stands,  $\delta S_j$  and  $\delta H_i$  are linearly dependent and equation 2 cannot be used to estimate the unknowns. This difficulty can be overcome by assigning a value to one  $S_j$  or, what is probably better, by assuming

that  $\sum_j S_j = 0$ , that is, it is assumed that the mean station correction is zero. With this assumption the epicentre, depth, origin time and station corrections can be estimated—at least in theory.

A computer programme has been written for joint epicentre and station correction estimation, based on equation 2. Preliminary tests using seven events from the Aleutian Islands recorded at some or all of thirty stations (each station recorded at least two events) show rapid convergence (computed corrections are usually  $< 0.01$  after three or four iterations) if the depth of at least one event is held fixed (restrained). With no depth restraints convergence is slow and the estimates tend to oscillate.

The events used include the LONGSHOT explosion: the normal method of epicentre determination gives estimates 25–30 km north of the true epicentre. The method of joint epicentre determination should give a more accurate estimate; LONGSHOT is therefore a valuable datum against which to test the joint epicentre method.

To obtain the results shown in Tables 1 and 2 all events have had their depth restrained to the depth estimated by the US Coast and Geodetic Survey; the event of October 29, 1965, LONGSHOT, has been restrained to 0 km. The arrival times used for these calculations are taken from the *Earthquake Data Reports* published by the US Coast and Geodetic Survey. The stations used have been chosen to cover as many azimuths as possible: all stations closer than  $15^\circ$  to any of the epicentres have been excluded from the analysis as have all stations for which the residuals after convergence were greater than 3 sec (the epicentres were recalculated with the stations with residuals greater than 3 sec removed).

Table 1 shows for each of the seven events: (1) the US Coast and Geodetic Survey epicentres (the LONGSHOT epicentre is the true epicentre), (2) the epicentre determined by the standard method using only those stations used in the joint determinations and (3) the results of the joint epicentre determination; Table 2 shows the station corrections. It is clear from Table 1 that the joint epicentre method gives a much closer estimate ( $\approx 1$  km) to the true LONGSHOT epicentre than the normal method ( $\approx 23$  km).

Table 1

(1) Komandorsky Isles: July 19, 1966				
USOGB epicentre	50-20 N. 164-90 E.	$A = 18$ km	$H = 1:40:53.9$	
Epicentre using thirty stations	50-43 N. 164-48 E.	$A = 18$ km*	$H = 1:40:54.9$	
Epicentre using joint method	50-28 N. 164-03 E.	$A = 18$ km*	$H = 1:40:54.8$	
(2) Rat Islands, Aleutian Islands: June 2, 1966				
USOGB epicentre	51-08 N. 175-97 E.	$A = 41$ km*	$H = 3:27:53.3$	
Epicentre using thirty stations	51-04 N. 175-89 E.	$A = 41$ km*	$H = 3:27:52.9$	
Epicentre using joint method	50-92 N. 175-08 E.	$A = 41$ km*	$H = 3:27:53.2$	
(3) Rat Islands, Aleutian Islands (LONGSHOT) October 29, 1965				
True epicentre	51-44 N. 179-18 E.	$A = 0$ km*	$H = 21:0:0.1$	
Epicentre using thirty stations	51-65 N. 179-18 E.	$A = 0$ km*	$H = 20:59:50.9$	
Epicentre using joint method	51-45 N. 179-18 E.	$A = 0$ km*	$H = 20:59:50.8$	
(4) Andreanof Islands, Aleutian Islands: July 19, 1966				
USOGB epicentre	51-73 N. 173-30 W.	$A = 47$ km*	$H = 19:20:33.4$	
Epicentre using thirty stations	51-51 N. 173-35 W.	$A = 47$ km*	$H = 19:20:33.5$	
Epicentre using joint method	51-75 N. 173-40 W.	$A = 47$ km*	$H = 19:20:33.9$	
(5) Fox Islands, Aleutian Islands: August 11, 1966				
USOGB epicentre	53-76 N. 160-74 W.	$A = 61$ km*	$H = 10:45:59.6$	
Epicentre using thirty stations	53-76 N. 160-77 W.	$A = 61$ km*	$H = 10:45:59.1$	
Epicentre using joint method	53-71 N. 160-79 W.	$A = 61$ km*	$H = 10:45:59.6$	
(6) South of Alaska: February 6, 1965				
USOGB epicentre	53-29 N. 161-81 W.	$A = 33$ km*	$H = 16:50:28.6$	
Epicentre using thirty stations	53-24 N. 161-88 W.	$A = 33$ km*	$H = 16:50:28.2$	
Epicentre using joint method	53-06 N. 161-03 W.	$A = 33$ km*	$H = 16:50:28.2$	
(7) South of Alaska: January 23, 1966				
USOGB epicentre	55-47 N. 153-59 W.	$A = 33$ km*	$H = 14:27:7.9$	
Epicentre using thirty stations	55-96 N. 153-50 W.	$A = 33$ km*	$H = 14:27:7.8$	
Epicentre using joint method	55-83 N. 153-03 W.	$A = 33$ km*	$H = 14:27:7.7$	

\* Restrained parameters.

Table 2

Station	Azimuth	Station correction	LONGSHOT residual Observed - J-B time
MBO	23.0	-0.7	Not available
OOL	41.0	-1.5	-3.6
WES	51.8	-0.9	-5.1
OTT	53.2	-1.8	-5.9
EJG	62.8	-1.0	-4.2
OPO	66.0	-1.9	-5.0
BMO	77.9	+0.0	-2.7
UBO	78.1	+0.5	-1.7
PAS	90.9	-0.3	-2.4
KIP	143.1	+2.9	-0.6
PPT	152.8	+1.9	-1.9
VUM	184.5	+2.4	-2.0
KOU	198.0	+0.8	-1.8
HNR	205.0	+1.2	-2.5
RIV	206.0	+2.2	-1.9
BRB	206.8	+0.0	-2.5
TOO	209.1	+2.0	-2.6
CTA	214.8	-1.1	-3.5
PMG	217.9	-0.4	-2.9
DAB	221.7	-0.1	-2.9
MAT	229.4	-0.0	-2.5
SHL	286.6	-1.4	-3.6
QUE	307.8	-0.1	-2.5
TNH	322.0	+0.4	-3.1
IST	340.4	-0.5	-5.4
UPP	352.0	-1.1	-5.1
TRO	353.4	-0.1	-4.0
CLL	353.5	-0.6	Not available
STU	355.7	-0.4	Not available
KON	356.3	-0.7	-4.2

The error in the LONGSHOT epicentre as determined by the standard method is caused by a regional bias in travel time in the Aleutian region. This bias can be seen in the LONGSHOT residuals—the difference between the observed travel times and J-B travel times from the true LONGSHOT epicentre (Table 2)—superimposed on a base-line shift of about -3 sec. The station corrections (Table 2) computed by considering seven Aleutian events together reveal a similar bias (but not of course the base-line shift which is taken up in adjustments to the origin time): stations to the south of the Aleutian Islands show generally positive station corrections; those to the north show generally negative corrections. The joint epicentre method takes account of the bias.

Thus in a region of known bias the method of joint epicentre determination has detected the bias and produced a more accurate estimate for a (known) explosion epicentre than can be obtained by the normal method. To do this the depths of all the events were assumed to be known. Errors in these assumed depths will tend to bias the epicentral estimates but this bias is probably small. The accuracy of the estimates of the LONGSHOT epicentre suggests either that the estimates of depth are accurate or, more probably, that only gross errors in depth have any appreciable effect on the epicentral estimates.

The joint epicentre method as outlined here breaks down if all the events used are close together—that is, if they are spread over only a few degrees of arc—because the station corrections and travel times are virtually linearly dependent. The method can, however, be adapted to obtain the relative positions of a group of closely spaced events by restraining the epicentre and time of origin of one of the events; the station corrections are then the only unknowns in the equations of condition of the restrained event. If the absolute position of the reference event is known or can be estimated by considering the reference event as one of a group of more widely spaced events—as illustrated here for the seven Aleutian Island events—epicentres determined relative to the reference event will be estimates of the absolute epicentres. A detailed picture of events on both the local and regional scale can therefore be built up. (In theory all events from a given area could be considered at once; in practice the number of events that can be considered at any one time is limited by the storage capacity of the computer available.)

More tests are required to prove the method of joint epicentre determination and work is continuing, particularly to study the value of the method for focal depth

estimation and to calculate the confidence limits on the estimates. This communication simply draws attention to the method.

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## Pulsating Radio Auroral Echoes

PULSATING auroral echoes have been described<sup>1</sup> which accompany sudden commencements, and attention has been directed to the fact that the periods of repetition of these echoes are close to those predicted for the bounce periods of hydromagnetic waves along the geomagnetic line of force terminating in the echo region. More exactly, the bounce periods considered are those for waves having angular frequencies  $\omega$  which are considerably less than  $\Omega_e$ , the minimum (equatorial plane) particle cyclotron frequency along the field line. Thus there can be an apparent agreement between echo period and hydromagnetic bounce period for  $\omega \approx 0$  up to  $\omega \approx 0.1 \Omega_e$ , beyond which the travel time increases as  $\omega \rightarrow \Omega_e$ .

Other workers<sup>2-4</sup> have tried to relate periodicities in absorption and X-ray measurements to low frequency standing-wave oscillations of the magnetic field lines and it has been suggested that the particle beam giving rise to these effects is modulated by large-scale variations in the value of the magnetospheric magnetic field. If the interpretations in ref. 1 are correct, particularly where the special event of July 15, 1959, is concerned, then the mechanism just described would be of too large a scale perpendicular to the field lines to allow the resolution in range which is obtained.

Another possibility considered here is that the pulsations arise from a resonant interaction of the type discussed by Wentzel<sup>5</sup>, and, because we require periodicities related to hydromagnetic bounce periods, we are restricted to particle beams capable of interacting with left-hand polarized waves for which  $\omega > \Omega_{ci}$ , where  $\Omega_{ci}$  is the minimum proton gyrofrequency on the path. The conditions for resonance are<sup>6</sup>

$$\left| \frac{\Omega}{\omega} \right| = \left| \frac{V_z}{U} \right| + 1 \quad (1)$$

for particles and waves of similar polarization but opposite directions along the field;

$$\left| \frac{\Omega}{\omega} \right| = \left| \frac{V_z}{U} \right| - 1 \quad (2)$$

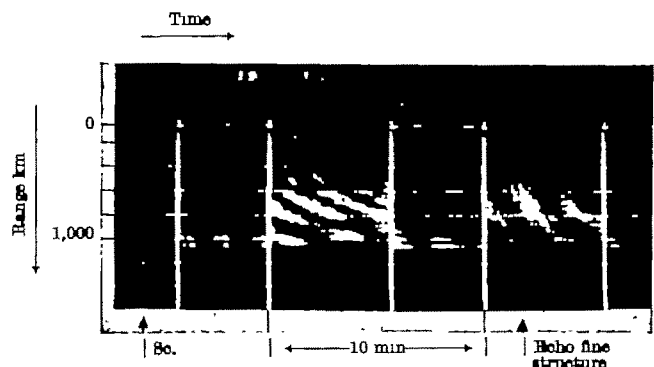


Fig. 1 Event on 8-5-60, 0421 U.T., showing fine structure in an echo pulse. (Radar operating on two beams.)

for opposite polarisations but with the same direction of travel.

Here  $V_z$  and  $U$  are the velocities in the field direction of particles and waves, respectively.

The equatorial plane region of the field line having  $L = 4$  is considered so that for protons and electrons on the model we used we have  $\Omega_{e1} = 45$  and  $\Omega_{e2} = 8 \times 10^4$  rad/sec, respectively. For a proton beam with  $\omega = 0.1 \Omega_{e1}$  and  $U = 3 \times 10^7$  cm/sec the required energy parallel to the field is 50 keV. An electron capable of interacting with the same wave would have to have an energy of several MeV. Inspection of one of the auroral echo examples (Fig. 1) suggests, in fact, that the beam may be composed of protons because one of the echoes has a fine structure period of 24 sec, corresponding to a proton energy of 150 keV (for a proton mirroring at low altitude).

The hydromagnetic wave frequency involved is 0.7 c/s and micropulsations of this frequency have been reported<sup>7</sup> after sudden commencements. Trains of pulsations were, in fact, observed with repetition periods of about 2 min for the trains. These observations can explain at least qualitatively the occurrence of pulsating auroral echoes (Fig. 2).

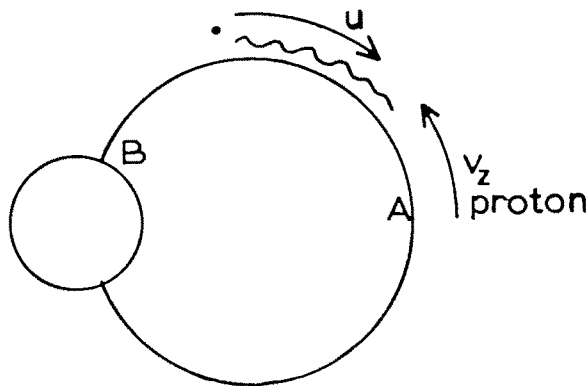


Fig. 2. Conditions under which precipitation may occur at B if a proton resonates with a wave near A.

If the resonance condition can be satisfied near A for a wave coming from B, and a proton going towards B, then the proton may be trapped<sup>8,9</sup> in the wave for at least part of its journey along the field line. Because of the resonance condition, and the fact that  $\Omega$  and  $U$  increase from A to B, it may then be accelerated along the field. If it leaves the wave or falls away from the resonance condition somewhere between A and B with a small enough pitch angle it will be precipitated. It is known<sup>8</sup> that the energy of a particle subject to trapping in a uniform steady field  $B_0$  will vary with a certain period  $T$ . If the particle is to remain trapped when  $B_0$  varies with distance, then the variation of  $B_0$  (and  $\Omega$ ) in one period  $T$  should be small<sup>8</sup>. A proton for which the low initial energy approximation<sup>8</sup> holds, putting  $B_0 = 455\gamma$ ,  $B = 1\gamma$  (wave amplitude) and  $\Omega_{e1} = 45$  rad/sec, would have a period of 8.4 sec. A velocity of  $3 \times 10^8$  cm/sec parallel to  $B_0$  has been assumed and it can be seen that in this case  $B_0$  varies too rapidly to allow trapping. Precipitation may still occur, however, as a result of the impulse given to the particle during its passage through the wave in the equatorial plane resonance region. In a frame of reference fixed to the wave<sup>8</sup>,

$$V'_z = -\beta V_0 \cos p$$

where  $\beta = eB/mc$ ,  $B$  is the wave amplitude, subscript 0 refers to the direction perpendicular to  $B_0$ ,  $p = (k \cdot \vec{V}_z - \Omega) t$ , and  $k$  is the hydromagnetic wave number.

The maximum value of the acceleration in the  $z$  direction is  $3 \times 10^8$  cm/sec<sup>2</sup> taking  $V_0$  as  $3 \times 10^7$  cm/sec and  $B$  as  $1\gamma$ . If  $p$  is initially zero and changes mainly because of the variation of  $\Omega$  with  $z$ , which will be the case if  $V_z$  is  $3 \times 10^8$  cm/sec as above ( $\Omega \approx \Omega_{e1} + bz^2$ , say near the equatorial plane), then  $p$  increases to  $\pi/2$  in about 1 sec because  $b = 2.6 \times 10^{-17}$  rad/cm<sup>2</sup>/sec for  $L = 4$ .

The change of pitch angle in traversing the whole wave can be estimated from

$$\alpha' = -\frac{V'_z}{V_0} = \beta \cos p \quad (3)$$

which neglects any change in  $\alpha$  due to  $dB_0/dz$ , except where this is involved in  $p$ . This time putting  $\Omega_1 = \Omega_{e1} + b'z$ , and integrating equation 3 gives, assuming the  $V_z$  variation is small compared with the  $\Omega_1$  variation,

$$\begin{aligned} \delta\alpha &= \beta \int_{-\infty}^{\infty} \cos b' V_z t^2 dt \\ &\approx \beta \int_0^{\infty} \cos b' V_z t^2 dt \\ &= \frac{\beta}{2} \sqrt{\left( \frac{\pi}{2 V_z b'} \right)} \end{aligned}$$

Again with  $B = 1\gamma$ ,  $V_z = 3 \times 10^8$  cm/sec and  $b' = 1.5 \times 10^{-8}$  rad/sec/cm,  $\delta\alpha$  is of order  $1^\circ$ . This probably underestimates  $\delta\alpha$  since if the proton encounters the wave on both sides of the resonance region we can double  $\delta\alpha$  and also the value of  $b'$  in the resonance region has been overestimated because it has been assumed that  $\Omega_1$  varies linearly with distance.

Thus for protons already mirroring at relatively low altitudes (for the foregoing example, the initial  $\alpha$  is  $5.7^\circ$ ) significant changes in pitch angle can occur. These may be enough to cause precipitation after only one traverse of the wave train provided that the wave amplitude is about  $1\gamma$  or greater and the proton has the correct phase  $p$  in the resonance region.

The radar echoes<sup>1</sup> are generally confined to the range interval from 400 to 1,200 km which involves that part of the magnetosphere between  $L = 3$  and  $L = 4.5$ . To explain the observed echo "dispersion" in time against range<sup>1</sup> we need only invoke the differing travel times of a particular wave frequency along different field lines. Thus it is suggested that at some stage during the sudden commencement, a particular hydromagnetic wave frequency appears on field lines in the region mentioned previously with, possibly, a delay not greater than a minute between its occurrence at small  $L$  and later at larger  $L$  values. An echo pulse will appear if the wave (or waves) is moving in the correct direction and there may be a small "dispersion" in range. Later pulses would appear each time the wave crossed the equatorial plane in the correct direction and because of the varying travel times the "dispersion" would increase. Without knowing the wave frequencies involved in actual events it is not possible to say how much the "dispersion" is influenced by  $\omega$  approaching  $\Omega_{e1}$ . The few examples studied so far suggest that  $\omega$  is not much greater than one-tenth of the minimum proton gyro frequency in the region  $L = 3$  to  $L = 4.5$ .

Current theories of micropulsations<sup>10</sup> suggest that the precipitating wave may be generated or amplified by protons with velocities parallel to  $B_0$  similar to those considered here but with large pitch angles in the equatorial plane.

This hydromagnetic mechanism appears to explain satisfactorily the production of pulsating auroral echoes, but it cannot account directly for the pulsating electron precipitation (with energies in the region of 100 keV) observed during commencements<sup>8</sup>. It is possible, how-



ever, that these electrons are accelerated into the atmosphere by a space charge electric field set up when positive charge in the form of protons is removed from the magnetosphere.

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## THE SOLID STATE

### Formation of Voids in Creep and Competition from Sintering Processes

IN the mode of fracture at elevated temperatures and low stresses which involves the nucleation and growth of voids on grain boundaries<sup>1</sup>, the initial stage of formation of the voids is generally considered to depend on a mechanism whereby close competition takes place between the rate of separation of interfaces and the simultaneous tendency to closure by sintering processes. If a void can be opened to a critical size, which depends on the stress and surface tension of the material, then its continued growth can be accounted for by vacancy condensation<sup>2</sup>, but there is little information about the important parameters which may govern conditions before this stage is reached. Proposed mechanisms for void nucleation usually envisage that the most favourable circumstances for nucleation arise when the deformation, particularly the contribution due to grain boundary sliding, is rapid. In such cases it is often considered the void has a higher probability of opening sufficiently before a competing sintering process can be effective in preventing the critical size being reached<sup>3</sup>. This letter presents initial results on experiments with the specific aim of determining the variation of nucleation rate with the rate of deformation.

In the experiments, tensile stresses between 415 and 1,400 lb./in.<sup>2</sup> (derived from weights acting on a cam which reduced the force on the specimen in proportion to the reduction in area) were applied to magnesium specimens of grain size 0.4 mm and having principal impurities in p.p.m. by weight as follows: iron 20, hydrogen 15, manganese 15, nitrogen 20, tin 10, zinc 35. The resulting creep rates of the specimens ranged from 10<sup>-3</sup> to 10<sup>-2</sup>/h at 300° C. An important additional feature was that the tests were made inside a pressure vessel containing argon at pressures between 15 and 1,000 lb./in.<sup>2</sup>, the value for each test being such that the resultant tensile stress component in the specimens was always kept at the same value of 400 lb./in.<sup>2</sup>. Under these conditions it is known that there is a constant driving force for void growth by the condensation of vacancies which diffuse from grain boundaries which is independent of the creep rate<sup>4,5</sup>. Because of this maintaining constant of the process which governed the growth of voids above a critical size, it was possible to investigate the dependence of void nucleation on deformation rate without the usual complicating conditions of different void growth rates.

Changes in the density of specimens were measured at varying stages of creep and the specimens were examined metallographically to determine the number of voids present after given conditions of test. Both these methods indicated that the number of voids was approximately

proportional to the total creep strain and that void nucleation was not strongly sensitive to the rate of deformation. It is implied from this that competing sintering processes are ineffective<sup>6</sup> or that voids are created as a result of deformation at a size sufficiently great for subsequent vacancy condensation under the applied stress and that the time must be short for voids to reach this critical size. Furthermore, there would seem to be no evidence that diffusion processes compete seriously in tending to close the voids and prevent their reaching this critical size over a wide range of strain rates where cavitation is prevalent.

If these results are assumed to be more widely applicable than in the context of the present work, then they make it possible to account for some of the principal features of creep cavitation phenomena which have been generally observed. In particular, the results substantiate the rather common feature that for a given material the elongation to fracture without superimposed hydrostatic pressure in the range where cavitation occurs is not strongly sensitive to the test conditions<sup>7</sup>. By considering that cavities are nucleated progressively with strain and by a mechanism which is not strongly sensitive to strain rate, it is possible to develop a more quantitative approach in interpreting the cavitation process.

A full account of this work will be published elsewhere.

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## BIOPHYSICS

### Decrease in Hydrothermal Stability of Collagen on Ageing in the Human Intervertebral Disk

THE shrinkage temperature of the collagen of dura mater or fascia lata has been shown to increase with age<sup>1</sup>. Shrinkage of these tissues and tendon or skin can be watched and a micro-melting point apparatus was used for their determinations. The transition can also be detected from force-temperature curves, and use of this method has shown an increase of shrinkage temperature with age for tendon collagen, except when measurements were made at pH less than 4.5. If the fibres were maintained at constant length during the heating, the shrinkage temperature showed little variation with age (or pH)<sup>2-4</sup>.

It has been claimed that measurements of the tension developed by collagen fibres during heating, the weight required to inhibit contraction and the amount of hydroxyproline or total material dissolved are more sensitive criteria than the shrinkage temperature for assessing changes in hydrothermal stability<sup>5,6</sup>. Unfortunately, the first two methods are difficult to apply to collagen which is not well orientated, as in the nucleus pulposus, the gel-like central portion of the intervertebral disk. Hallén<sup>7</sup> could not detect shrinkage of this tissue under the microscope and we also found difficulty, especially with old specimens. Differential thermal analysis<sup>8</sup> offered a more sensitive means of detecting the transition, and the technique has been applied in our

laboratories in investigations of the nucleus pulposus and the surrounding annulus fibrosus of human disks<sup>8</sup>.

Samples of specimens obtained at autopsy were soaked overnight in water at 2° C. Differential thermal analysis was carried out on the aqueous suspensions using a Dupont 900 analyser and glass beads as reference material. Thermograms were recorded with a  $\Delta T$  scale of 0.1° C/in. for maximum sensitivity and results obtained using heating rates of 5° and 10° C/min were compared. The greater heating rate usually gave larger peaks, at a somewhat greater temperature (curves 2 and 3 in Fig. 1), and so it was used in the age investigation. The nucleus pulposus generally gave broader peaks than those obtained with the annulus fibrosus, but before the age of about 65 they were fairly well defined and there was little difficulty in measuring the parameters shown in Fig. 1. At greater ages an endothermic change was almost impossible to detect in most thermograms of the nucleus pulposus, but a definite increase in the base line slope was often observed, indicating that a small transition had occurred (curve 5 in Fig. 1). A few of the oldest specimens of the annulus fibrosus gave similar results.

The values obtained for the extrapolated onset temperature,  $T_1$  (Fig. 1), for specimens of different ages are given in Figs. 2 and 3 and also regression curves corresponding to quadratic equations, which seemed to fit the data better than linear regression lines, especially for the nucleus pulposus. A statistical comparison was also made of the various age groups indicated by horizontal lines in Figs. 2 and 3. A very significant difference (0.1 per cent level) was found between the 0-10 yr group mean of the nucleus pulposus and the mean of any other group of that tissue. This was also true of the annulus fibrosus except for the 30-40 yr group, but this was significantly lower than the 0-10 yr mean at the 1 per cent level. Significant differences were also found between the 30-40 and 61-71 yr group of the nucleus pulposus (2 per cent level) and between these age groups of the annulus fibrosus (1 per cent level). There was no doubt, therefore, that  $T_1$  of both tissues tended to decrease with age, up to the sixth decade at least, and the other parameters,  $T_2$ ,  $T_3$  and  $T_4$  (Fig. 1), showed a similar decrease. The regression curves, however, indicated a marked reduction in  $T_1$  of the nucleus pulposus during the first 3 or 4 decades, followed by a more gradual change, whereas  $T_1$  of the annulus fibrosus decreased almost linearly for the whole age range.

Differences in technique may partly account for the fact that the results of our differential thermal analysis seem

to conflict with the findings of other workers<sup>1-4</sup>, but a more important factor almost certainly is the nature of the tissues used in the investigation. The annulus fibrosus and to a greater extent the nucleus pulposus contain much greater quantities of glycosaminoglycans and non-collagenous proteins (of several different types)<sup>10</sup> than the collagenous tissues investigated previously. In both areas of the disk the total amount of glycosaminoglycans, on a dry weight basis, decreases with age and also the chondroitin sulphates: keratan sulphate ratio<sup>7</sup>. On the other hand, the amounts of non-collagenous proteins increase greatly with age (ref. 9 and succeeding communication).

Conclusive evidence of the stabilizing role of the polysaccharides or other carbohydrates is difficult to obtain<sup>1</sup>, and the possible influence of non-collagenous proteins on collagen has received very little attention. There seems to be, however, little doubt that such compounds can have a significant effect on the mechanical properties of collagen<sup>11,12</sup>. It should be emphasized that pure collagen has not been isolated from the disk and that much larger quantities of sugars and non-collagenous proteins seem to be firmly bound to the fibres<sup>7,13</sup> than in other tissues, with the possible exception of cartilage. These firmly bound components probably have a greater effect on hydrothermal stability than the remainder of the ground substance and their complexes with collagen may be particularly important in the young nucleus pulposus, the collagen of which is very poorly crystalline. It is suggested that the collagen present in older specimens of nucleus pulposus has a lower hydrothermal stability than such complexes, even though it is more crystalline and, presumably, has a greater degree of crosslinking. This interpretation is supported by the relative values of  $T_1$  obtained for the annulus fibrosus and nucleus pulposus. The greater crystallinity of the annulus fibrosus at all ages is not reflected in the results until after the age of about 10 (compare Figs. 2 and 3), when the hydrothermal stability becomes increasingly dependent on the integral structure of the collagen molecules, rather than on the presence of other compounds. More information is required about the effect of ageing on the association of collagen and other tissue components before this hypothesis can be tested further.

Another factor which may be relevant is that different mechanical treatments can raise or lower the shrinkage temperature of tendon collagen<sup>14</sup>. The mechanical forces on the disk *in vivo* are quite different from those to which other tissues are subjected and could be partly responsible for the unusual effects of ageing which we have observed.

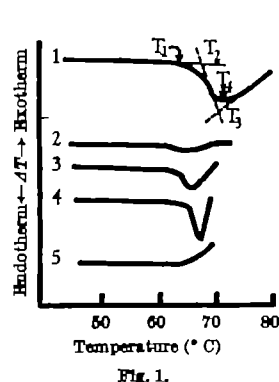


Fig. 1.

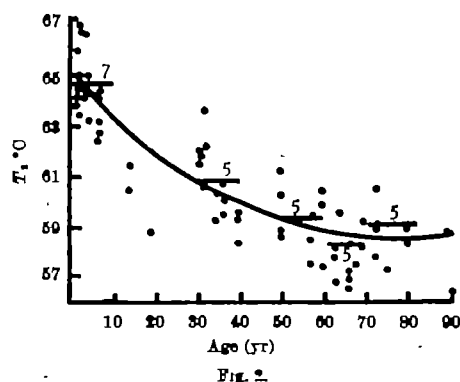


Fig. 2.

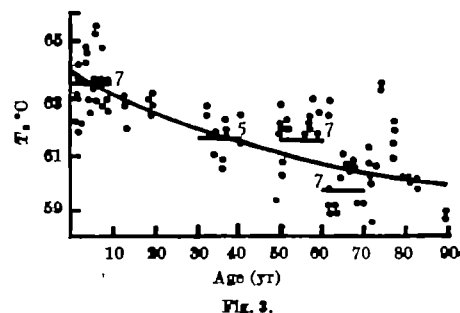


Fig. 3.

Fig. 1. Thermograms of nucleus pulposus (N.P.) and annulus fibrosus (A.F.). (1) N.P., 35 yr;  $T_1$ , onset temperature;  $T_2$ , extrapolated onset temperature;  $T_3$ , extrapolated peak temperature; and  $T_4$ , peak temperature (10° C/min). (2) N.P., 50 yr; heating rate, 5° C/min. (3) As (2) but heating rate was 10° C/min. (4) A.F., 50 yr with heating rate of 10° C/min. (5) N.P., 70 yr with heating rate of 10° C/min.

Fig. 2. Variation of  $T_1$  with age of nucleus pulposus. Two or three determinations were carried out on most specimens. The regression curve corresponding to  $T_1 = 64.92 - 0.169s + 0.0011s^2$  ( $s$  = age in years), was calculated from all observations. Standard deviation from the line = 1.17° C. The horizontal lines represent the mean values for different age groups and the number of specimens in each group is shown.

Fig. 3. Variation of  $T_1$  with age of annulus fibrosus. The regression curve, corresponding to  $T_1 = 63.77 - 0.0615s + 0.00021s^2$  ( $s$  = age in years), was calculated from all observations. Standard deviation from the line = 1.10° C. Other comments as for Fig. 2.

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## Variations in the Protein Components of Human Intervertebral Disk with Age

CHANGES in the glycosaminoglycans of the human intervertebral disk with ageing have been reported by a number of authors (see Antonopoulos<sup>1</sup> for recent work). On the other hand, there have been few investigations of the protein components, although they make up the larger part of the tissue. The collagen content has been determined in the central portion of the disk<sup>2</sup> (the nucleus pulposus) at different ages. Similar analyses have been made on the nucleus pulposus and on the surrounding annulus fibrosus<sup>3</sup>. X-ray diffraction<sup>4</sup> has indicated that at least one of the non-collagenous proteins present in the nucleus pulposus increases with age, but except for a brief reference by Hallén<sup>5</sup>, chemical data on these components are lacking. In the present investigation the effects of ageing on the non-collagenous protein nitrogen and on the tyrosine, most of which also derives from the non-collagenous proteins<sup>6</sup>, were investigated in addition to the collagen.

Specimens of apparently normal lumbar disks were obtained at autopsy and the nucleus pulposus and annulus fibrosus dissected out, discarding the intermediate zone. The material was homogenized and simultaneously dehydrated in ethanol, and allowed to equilibrate with the atmosphere before analysis. Tyrosine was determined using the 1-nitroso-2-naphthol reagent<sup>7</sup>, after dissolving the sample by a short treatment with 50 per cent hydrochloric acid at 100° C, as previously described<sup>8</sup>. Hydrolysis was carried out for 24 h before the determination of hydroxyproline by the Woessner method<sup>9</sup>.

The results for four different age groups are shown in Table 1. Differences between mean values, discussed below, were considered to be significant only when statistical analysis indicated that this was true at the 5 per cent level ( $P = 0.05$ ) at least. The percentage of hydroxyproline, and thus the collagen content, did not show a significant trend in the first three groups of the nucleus pulposus, but a lower result was obtained for group 4. In the annulus fibrosus hydroxyproline decreased from group 1 to 3 and then remained constant. These results contrasted sharply with those obtained for total nitrogen (Kjeldahl method). In the nucleus pulposus group 3 gave

a much larger value than group 1, whereas these groups of the annulus fibrosus showed almost identical values. It therefore seemed certain that appreciable changes had occurred in the amounts of the other proteins and the results calculated for non-collagenous protein nitrogen indicated a very significant increase from group 1 to 3 in both regions of the tissue.

A decrease in the absolute amount of collagen is feasible, for example, by an enzyme digestion similar to that which has been demonstrated in cartilage<sup>10</sup>. Collagen, however, usually shows very little metabolic turnover and it seems probable that the decrease in the percentage value of this protein in the annulus fibrosus was a dilution effect, caused by the large increase in the amount of non-collagenous proteins. In the first three age groups of the nucleus pulposus this effect would be at least partly nullified by the decrease in the glycosaminoglycan content, which is appreciable up to the age of about 60 yr<sup>1</sup>. A small increase in the absolute amount of collagen may actually have occurred. It should be pointed out that the dry weight of the tissue increases with age<sup>1</sup>, but the proportionate increase in analytical values, when expressed on a volume basis, will be the same for each component.

The results for tyrosine generally confirmed the age trend shown by the non-collagenous protein nitrogen, but the relative values obtained in the two determinations require some comment. Assuming a nitrogen content of about 16 per cent for the non-collagenous proteins, which have not yet been isolated in a pure form from the disk, the amounts of these components varied from approximately 20 per cent to 45 per cent in the nucleus pulposus and from 5 per cent to 25 per cent in the annulus fibrosus. Even when allowance was made on the basis of a tyrosine content of 1 per cent for human collagen<sup>11</sup>, although the true value is probably smaller, the other proteins of the nucleus pulposus seemed to contain 6 per cent to 9 per cent of this amino-acid. These values tended to increase from group 1 to 3, but the opposite effect was found in the annulus fibrosus, indicating that different non-collagenous proteins are deposited in the two regions of the disk. The tyrosine-rich protein that accumulates in the nucleus pulposus may account for the increased  $\beta$  protein type of X-ray diffraction reflexion on ageing<sup>4</sup>. A similar reflexion has not been established for the annulus fibrosus.

Ageing appears to have a much smaller effect on the non-collagenous proteins of human cartilage<sup>12</sup>, and analyses of similar proteins present in bovine cartilage<sup>13</sup> have given smaller values for tyrosine than those discussed above. The indirect method of calculation used here can only be approximate, but the results for the total dry tissues and for aqueous extracts<sup>8</sup> certainly suggest that unusually large amounts of tyrosine are present in the disk. The small amount of positive interference reported recently<sup>14</sup> for certain aromatic compounds in the 1-nitroso-2-naphthol

Table 1. COMPOSITION OF LUMBAR DISKS AT DIFFERENT AGES

	Nucleus pulposus			
	1 3-10 yr	2 20-50 yr	3 54-62 yr	4 65-80 yr
% Total nitrogen	9.6 ± 0.60 (5)	9.0 ± 1.2 (5)	11.1 ± 0.57 (6)	11.0 ± 0.45 (5)
% Hydroxyproline	3.2 ± 0.39 (5)	3.1 ± 0.60 (4)	3.9 ± 0.74 (7)	2.8 ± 0.55 (4)
% Tyrosine	1.5 ± 0.19 (5)	2.2 ± 0.54 (5)	2.3 ± 0.29 (7)	3.8 ± 0.25 (7)
% N.O. protein nitrogen*	3.2 ± 0.30 (5)	4.3 ± 0.88 (4)	5.6 ± 0.85 (6)	7.4 ± 0.82 (3)
	Annulus fibrosus			
	1	2	3	4
% Total nitrogen	13.1 ± 0.81 (5)	11.4 ± 0.82 (5)	13.0 ± 0.50 (7)	12.7 ± 0.72 (7)
% Hydroxyproline	9.1 ± 0.67 (5)	7.5 ± 1.2 (5)	6.4 ± 0.84 (7)	6.3 ± 0.79 (8)
% Tyrosine	1.8 ± 0.14 (5)	1.7 ± 0.22 (4)	2.3 ± 0.20 (7)	2.6 ± 0.27 (7)
% N.O. protein nitrogen*	0.9 ± 0.51 (5)	1.3 ± 1.1 (5)	4.2 ± 0.56 (7)	4.2 ± 0.78 (7)

Results are expressed as percentages of oven-dry tissues. The mean values for each age group are shown ± one standard deviation. Numbers of specimens are in parentheses. Hexosamine nitrogen was calculated from Hallén's data<sup>5</sup>.

\* Non-collagenous protein nitrogen = total nitrogen - (collagen nitrogen + hexosamine nitrogen).

Collagen nitrogen = hydroxyproline ×  $\frac{18.0}{13.7}$

colorimetric technique, was not likely to be significant in the present context, but further investigations on the specificity of this procedure are needed.

On the other hand, losses of tyrosine can occur during acid hydrolysis<sup>14</sup>, perhaps particularly when appreciable quantities of polysaccharides are present<sup>8,15,16</sup>. This difficulty was avoided in the present work, because complete acid hydrolysis is not necessary when the 1-nitroso-2-naphthol reagent is employed<sup>8,7,18</sup>.

The results obtained for hydroxyproline by the Woessner method were contrary to those reported by Mitchell *et al.*<sup>3</sup> and Rodighiero *et al.*<sup>3</sup>, who observed an increase with age. These workers employed the Neuman and Logan method<sup>17</sup>, which is liable to give large results in the presence of tyrosine<sup>8</sup> or other tissue components<sup>18</sup>, and the errors probably increased with the age of the disk. It would be surprising if this factor alone was responsible for the complete lack of agreement with our findings, but it is worth noting that some of the hydroxyproline values given by Rodighiero *et al.*<sup>3</sup> for the annulus fibrosus were close to that expected for pure mammalian collagen, although their results for total nitrogen and hexosamine indicated that quite large amounts of non-collagenous substances were present. The differences between our results and those of Hallén<sup>8</sup> for the nucleus pulposus are less disturbing, especially when the difficulty in distinguishing the two regions of the tissue in the older specimens is considered.

There can be little doubt that the large changes in the ratio of non-collagenous proteins to collagen which we have observed will affect the mechanical properties of the nucleus pulposus and annulus fibrosus, as suggested for other tissues<sup>19</sup>. The variations in the amount and composition of the glycosaminoglycan fraction have similar implications<sup>20</sup> and the presence of protein-polysaccharide complexes must also be considered. The nitrogen content of such complexes increases with the age of the disk<sup>21</sup>, which is interesting in relation to our results for non-collagenous protein nitrogen, but a more comprehensive investigation of these macromolecules in relation to age is now required.

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## Interlocked Stresses in Cartilage

SPECIFICALLY aligned tensile stresses have been demonstrated in human costal<sup>1</sup> and nasal septal cartilage<sup>2</sup>. The outer layers of the tissue are maintained in tension so that the intact cartilage has a balanced system of forces the resultant of which is zero<sup>3</sup>. The nasal septal cartilage exists as a plate dividing the nose into two cavities in front and may become distorted in shape *in vivo* or *in vitro* if this balance of forces is altered. Breaching the surface layers on one side will partially release the interlocked stresses of the opposite intact side and because the cartilage is not rigid it will curl towards the intact side. The degree of deformation by curling has been shown to depend on the thickness of the cartilage and the cellular alignment which actually determines the alignment of the tensile stresses within the tissue<sup>4</sup>.

Relevant to the organization of forces within cartilage is the capacity of collagen and protein-polysaccharides in combination to bind large quantities of water. Sufficient external pressure will squeeze out fluid from cartilage and in the recovery of this or similar fluid the cartilage exhibits "swelling pressure". The importance of this and the rapid movement of water within cartilage under external stress have been shown in articular cartilage<sup>5</sup>. The appearance of the collagen network in nasal septal cartilage shows it to be under stress from an expansile force<sup>6</sup>. All available evidence suggests that the tensile stresses referred to must be set up against the "swelling pressure" of the cartilage. The present investigation is concerned with the role of the major components of cartilage in the organization of the tensile stresses. An experimental technique was evolved which demonstrated against a control situation the effect of selective destruction of each of the major components on the capacity of the cartilage to curl after artificial release of the tensile stresses of one surface.

Any two adjacent strips cut from the same septal cartilage will deform about equally if the same surface of each strip is interrupted by parallel cuts 1 mm apart. These parallel cuts are made about half way through the cartilage so as to breach the continuity of one surface only. If a whole septal cartilage is cut into strips of equal width, the alternate strips can be taken as a control group. If the interlocked stresses are abolished or reduced in the experimental strips, those strips show absent or reduced deformation after incision as compared with their controls. This technique was used in the investigation to study the role of the principal components of cartilage, cells, protein-polysaccharides and collagen in the system of interlocked stresses.

Nasal septal cartilage obtained from neonatal and infant cadavers within 24 h of death was cut into strips of equal width. Alternate strips were taken for the experimental and control groups. The cartilage was tested for deformation by curling while kept moist in air with Krebs-Ringer phosphate. Dry weights of the strips were obtained at the conclusion of the experiment.

The cells were killed or their metabolism inhibited by two methods. In the first, thirty-five matched pairs of cartilage strips were taken and the experimental group was frozen with liquid nitrogen and thawed three times. In the second, thirty matched pairs were taken and the experimental group incubated in 1 per cent sodium cyanide in Krebs-Ringer phosphate for 12 h. In both experiments, the deformation was equal in treated and control groups after the release of stress at room tem-

perature. The results indicate that the interlocked stresses were not maintained by cellular activity but were rather a property of the tissue already laid down.

The protein core of the protein-polysaccharide complex was hydrolysed in one experimental group by incubating the strips with inactivated mercuri-papain. Control strips were incubated in medium without enzyme for corresponding periods of time. Thirty-one matched pairs of cartilage strips were used for these experiments. Incubation with 5  $\mu$ g/ml. papain for 1 h or with 0.5 mg/ml. hyaluronidase for 48 h completely abolished interlocked stresses. Lower concentrations of enzymes partially abolished stresses. Thirty-one matched pairs of cartilage strips were used in these experiments. Other physical properties of the cartilage and its elasticity and shape were virtually unaffected by either enzyme at these concentrations. Strips treated with either enzyme lost a major part of their chondroitin sulphate (sulphated glycosaminoglycans) to the medium, but lost practically none of their collagen (hydroxyproline). The average loss of weight was 15 per cent.

The collagen fibrillar network was degraded by incubating the experimental groups with 0.25 mg/ml. collagenase for variable periods (2–48 h). Eighteen matched pairs of cartilage strips were used in these experiments. The earliest observed effect of collagenase was to make it more plastic. Interlocked stresses were not, however, abolished until the elasticity had completely disappeared and the cartilage had become an amorphous mucoid mass. By this time much of the cartilage had gone into solution (68 per cent weight loss) and, with continued incubation, dissolved completely. Analysis of residual cartilage at several times during enzyme incubation indicated that as the collagen was degraded the whole cartilage went into solution—the hydroxyproline and protein polysaccharide concentrations in the cartilage remaining fairly constant.

When heated to 60° C, collagen fibres contract sharply. Incisions were made on one surface of the experimental and control groups of twelve matched cartilage strips producing deformation. The experimental group was incubated at 60° C for 10 min and the deformation was



Fig. 1. Illustration of the experimental technique used in this investigation. Right matched pairs of nasal septal cartilage strips are shown, the control and experimental groups being marked. • the experimental group on the right was incubated with 5  $\mu$ g/ml. of papain for 1 h. The control group on the left was incubated in medium without enzyme.



Fig. 2. Photograph taken 10 min after multiple incisions on one surface in both groups as described in the text. The control group shows the expected curl; the experimental group shows no curl because the interlocked stresses have been abolished.

no different from that of control strips. This suggested that the shrinkage of collagen did not affect interlocked stresses, indicating that specific arrangement of collagen did not determine stress alignment.

These results suggest that the protein-polysaccharides of cartilage are intimately involved in establishing differentially aligned interlocked stresses. Collagen appears to play no part in the orientation, but rather seems to act as a skeleton forming point of anchorage for the protein-polysaccharides. Other evidence suggests that these tensile stresses are set up against the swelling pressure of the cartilage.

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## PHYSIOLOGY

### A System Linking the Third Ventricle with the Pars Tuberalis of the Rhesus Monkey

IN the course of studies of the hypothalamus of the rhesus monkey, we have investigated a restricted area of specialized ependymal cells. This area lies anterolaterally in the tuber cinereum (see Fig. 1), and is distinguished by the long processes of the ependymal cells which extend to the region of the pars tuberalis; neighbouring

ependymal cells are cuboid, without processes. The elongate ependymal cells stain deeply with the Gomori chrome-alum-haematoxylin technique and may be followed with ease using the optical microscope. They are not evident after silver impregnation techniques (Bodian's silver protargol method or Bielschowsky's technique), nor are they detectable after performic acid-alcian blue staining; they can, however, be demonstrated by cresol fuchsin and aldehyde fuchsin and the periodic acid-Schiff (PAS) method.

Under the electron microscope the ependymal cells are seen to have, in addition to a few cilia, a great number of fine microvilli. The prolongations of these cells branch and appear to terminate at the junction of the pars tuberalis and the median eminence either on the walls of the hypothalamo-hypophyseal blood vascular system or in such a way as to make direct contacts with cells of the pars tuberalis. The fine branches of the ependymal processes are densely packed with membrane-bound electron-dense granules measuring about 600 Å in diameter; the terminal regions of these branches contain in addition electron-lucent vesicles about 250 Å in diameter. The contacts between the terminals and cells of the pars tuberalis are synaptoid, inasmuch as they contain clusters of electron-lucent vesicles, but no clear signs of additional electron density along the region of contact between the terminals and the pars tuberalis cells could be seen.

Some observations made on animals treated with 17- $\beta$  oestradiol indicated a possible role of the specialized periventricular ependymal system described here in the regulation of pituitary function, and some preliminary experiments were therefore undertaken to investigate this possibility.

Twelve hours after an intramuscular injection of tritium-labelled 17- $\beta$  oestradiol, samples of the cerebrospinal fluid and blood plasma were tested for radioactivity in a scintillation counter. Radioactivity was found in both samples, that of the cerebrospinal fluid being approximately one-eighth of that found in the plasma. Twelve hours after a similar injection of labelled oestrogen to an ovariectomized female monkey, its hypothalamus was sectioned by freezing microtomy and examined by autoradiography. A strong concentration of silver grains was found in the area of the specialized ependyma described, but not elsewhere.

Under the electron microscope, the specialized ependymal cells of animals injected with oestrogen were seen to contain irregular globular electron-dense masses, but these were not found in control animals.

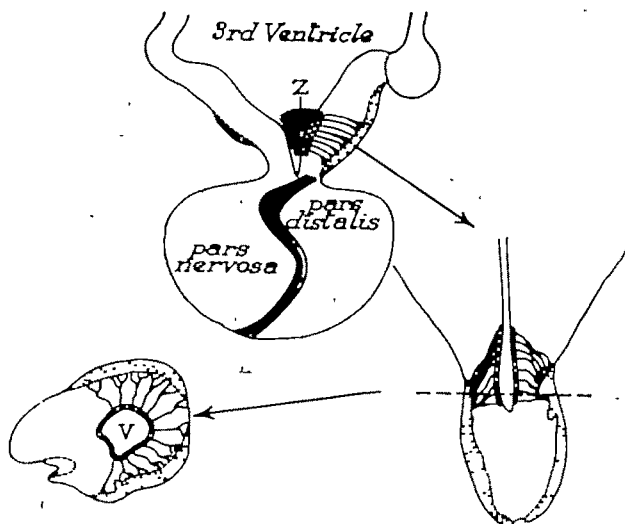


Fig. 1. Relationship of a specialized ependymal region to the hypothalamus and pituitary of a rhesus monkey. Sagittal, transverse and horizontal sections are shown above and at the right and left respectively. V, Ventricle; Z, zone of specialized ependymal cells.

The results of these preliminary experiments therefore suggest that the cerebrospinal fluid acts as a vehicle for oestrogen or its metabolites and that these may be selectively absorbed by certain specialized ependymal elements. It is interesting to note this relationship between circulating oestrogen and ependyma of the infundibular recess, in view of the known physiological relationship between this area of the hypothalamus and the anterior pituitary.

A possible role of modified ependymal elements in the regulation of anterior pituitary function has already been suggested<sup>1</sup> and a possible role of the cerebrospinal fluid in neuro-endocrine regulation has been postulated<sup>2,3</sup>. The studies described here support this postulate by demonstrating a link between the third ventricle and the pituitary in the rhesus monkey.

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### Observations on the Pars Intermedia of *Xenopus laevis*

THERE have been conflicting reports of the innervation of the pars intermedia of the amphibian pituitary. Dawson<sup>1</sup> found material coloured by "neurosecretory" stains in the pars intermedia of *Rana pipiens*, but Iturriza<sup>2</sup> could find no neurosecretory fibres with typical elementary neurosecretory vesicles at the level of ultrastructure in the pars intermedia of *Bufo arenarum*. Instead, fibres containing smaller vesicles were noted and subsequent investigations have indicated that these fibres contain monoamines, and may inhibit secretion of melanocyte stimulating hormone (MSH) from the pars intermedia<sup>3,4</sup>.

Electron microscope studies of the pituitary of *Xenopus laevis* support the view that in amphibians the pars intermedia is penetrated by fibres of the monoamine-containing type. These fibres stained with silver stains but not with chrome-alum-haematoxylin or alcian blue techniques. Under the electron microscope they were seen to contain two kinds of inclusion: (a) vesicles about 800 Å in diameter, irregular in form, each containing an electron-dense granule about 550 Å in diameter separated from the vesicular membrane by a clear electron lucent space; (b) smaller electron lucent inclusions about 400 Å in diameter. In many respects these fibres therefore resemble the type B fibres described by Knowles in the elasmobranch pituitary<sup>5</sup>; it seems probable that this form of innervation of the adenohypophysis is a consistent feature of the vertebrate pituitary<sup>6</sup> and should be termed neurosecretory<sup>7</sup>.

No direct synaptoid contacts between these type B fibres and pars intermedia cells, as have been noted in hippocampus<sup>8</sup>, were seen, but in many instances the fibres were enveloped by pars intermedia cells and it seems likely that functional contacts are made.

A diligent search did not reveal any deep penetration of the pars intermedia by type A fibres though some were seen to traverse the boundary between the pars nervosa and pars intermedia and others appeared to discharge into the vascular spaces which lie between the pars nervosa and pars intermedia. A dual control of pars intermedia function by type A and type B fibres, as in the dogfish pituitary<sup>9</sup>, cannot be excluded.



Experimental data lend support to the view that MSH synthesis and MSH release in *Xenopus* may be separately controlled. Under dim illumination and in darkness the melanophores assumed an intermediate condition (M.I. 2.5-3.5); in bright illumination with the animal on a white background they concentrated maximally (M.I. 1-2); and on an illuminated black background they were maximally dispersed (M.I. 4-5). A study of the ultrastructure of the pars intermedia cells did not reveal corresponding changes. The cells of animals adapted to a black background contained an extensive endoplasmic reticulum but few secretory vesicles, indicating active hormone synthesis and release. On an illuminated white background endoplasmic reticulum formation was also prominent though less abundant, and secretory vesicles were numerous: this indicates synthesis but little or no release, a finding supported by bioassay<sup>9</sup>. Organized endoplasmic reticulum was almost absent from animals adapted to dim light or darkness. The changes in ultrastructure indicate that illumination promotes MSH synthesis but that release only takes place when the animal is on a black background.

These observations, when compared with those reported by other workers, argue in favour of a dual control of pars intermedia function in amphibians in which hormone synthesis and release are independently regulated. The electron microscope findings indicate that two kinds of neurosecretory fibre may be concerned.

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### Reflex Withdrawal of the Eyecup in the Crab *Carcinus*

In the crab *Carcinus*, mechanical stimulation of an anterior area of the carapace supplied by the tegumentary nerve results in a rapid withdrawal of the eyecup on that side<sup>1</sup>. Sensory pathways in any of the other ipsilateral brain nerves can also elicit the downward and slightly backward reflex movement of the eyecup. The motor axons mediating the response are known to be in the optic tract<sup>2</sup>, but their peripheral connexions are unknown. The action of the eyecup muscles in the reflex has been studied here in the wider context of a survey of several eyecup movements<sup>3</sup>.

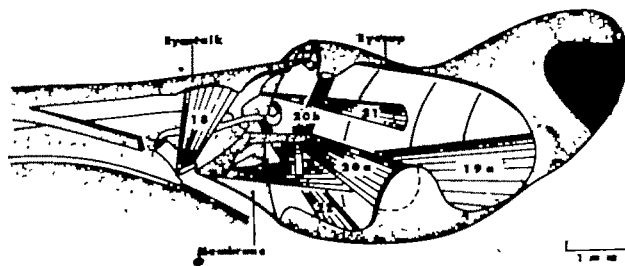


Fig. 1. Diagram of a right eye joint dissected from the lateral side to show the muscles involved in the withdrawal reflex. The flexible joint enables movement of the eyecup, to some extent, in all three planes. The nine eyecup muscles attach to internal projections of the eyestalk while muscle 18 rotates the eyestalk relative to a sclerite on the main body skeleton. The numbering of the muscles follows that of Cochran<sup>4</sup>.

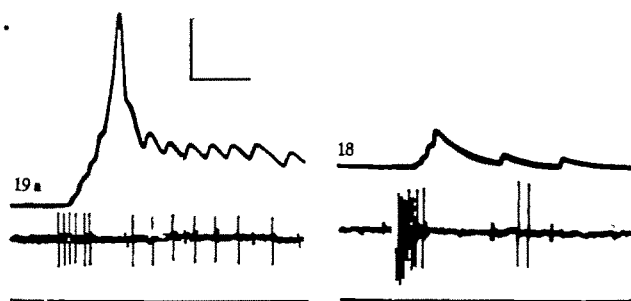


Fig. 2. Responses recorded from the optic tract (middle traces) and from muscles 19a and 18 (upper traces) to a single shock applied to the tegumentary nerve (lower traces). Records are from different preparations. Scale: 20 mV, 40 msec.

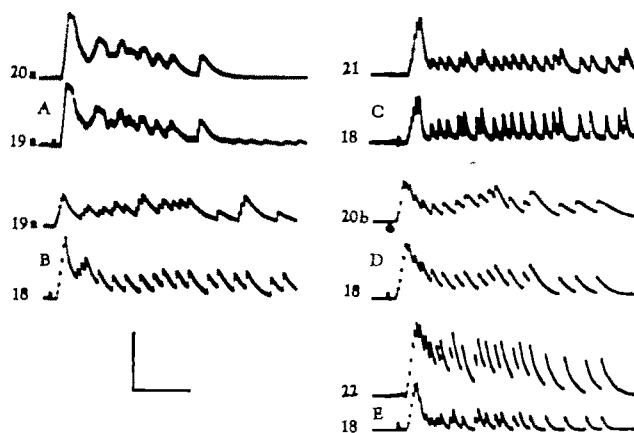


Fig. 3. Recordings from pairs of muscles in different preparations. A, The junction potentials of 19a and 20a are in phase but those of 19a and 18 are not (B). 18 is, however, in phase with 21 (C), with 20b (D) and with 22 (E). This indicates the presence of two axons, one supplying 19a and 20a, the other 18, 20b, 21 and 22. Scale: 20 mV, 100 msec.

The normal pattern of nerve impulses which cause the eyecup withdrawal can be recorded from the optic tract when the eyecup is clamped, withdrawn or even absent altogether, and numerous experiments show that proprioception is of no importance. For convenience, therefore, the eyecup of a limbless crab was cemented into its socket and conventional intracellular electrodes recorded the muscle potentials through a small hole in the eyecup exoskeleton. The reflex was elicited by applying shocks of 1 msec duration to the tegumentary nerve. Motor impulses in the optic tract were recorded with the bared tip of a stainless steel insulated wire electrode.

During a withdrawal a train of efferent impulses of two different amplitudes can be recorded in the optic tract and this pattern cannot be broken down, even in a fatigued preparation, to more than two axons. The larger spike is correlated with junction potentials recorded simultaneously in eyecup muscle 19a, while the smaller is correlated with junction potentials in eyestalk muscle 18 (Figs. 1 and 2). These axons also branch to other muscles and junction potentials recorded in muscle 19a are in phase with those in a few fibres on the dorsal surface of muscle 20a (Fig. 3A). Similarly, junction potentials recorded in muscle 21 on the medial side of the eyecup, muscle 20b, a small vertically placed muscle, and muscle 22 are in phase with those recorded in muscle 18 (Fig. 3C-E). The junction potentials of muscles 19a and 18 are never in phase (Fig. 3B). Despite many penetrations of other eyecup muscles, phasic activity associated with eyecup withdrawal has not been recorded, but until it is certain that all the fibres have been penetrated these muscles cannot be completely excluded. Thus the axon with the large spike innervates muscles 19a and 20a, while the axon with the smaller spike innervates muscles 18, 20b, 21 and 22. Although the two muscle groups show the

same latency, the form of their response differs. The junction potentials in 19a and 20a rapidly facilitate and summate and graded active responses occur which sometimes overshoot zero. The responses in the second group are at a lower frequency and without active membrane events.

During optokinetic movements of the eyecup only muscle 19a is inactive. Fibres in the other muscles tend to fall into two classes: phasic ones which are active only during an eyecup movement and tonic ones which are also responsible for maintaining any eyecup position. This difference has a structural correlate in that phasic fibres have sarcomere lengths of 3–4  $\mu$ m, while those of tonic fibres are 10–12  $\mu$ m (ref. 5). Muscle 19a, however, is histologically uniform in that the fibres all have sarcomere lengths of 3–4  $\mu$ m. These fibres also showed similar activity during a withdrawal, differing only in density of innervation by a slow axon from the oculomotor nerve responsible for holding the eyecup in its socket, once withdrawn. In muscles 18, 20a, 21 and 22 only those fibres which respond phasically in optokinetic movements are innervated by the withdrawal axons, but in 20b some tonic fibres are also innervated.

During a withdrawal the tonic activity, which normally locates the eyecup in space, is modified, but not inhibited peripherally. Only the tonic activity in muscle 23a, on the dorsal surface of the eyecup, is inhibited centrally. The remaining tonic activity is presumably overridden mechanically by the strong phasic action of the withdrawal muscles.

When a statocyst is removed and other sensory input is kept at a constant low level, withdrawal of the ipsilateral eyecup occurs "spontaneously" about every 13 sec. This indicates the presence of a pacemaker in the brain which is usually counteracted by normal sensory input<sup>6</sup>. Up to 500 msec before such a "spontaneous" withdrawal the tonic activity in all the eyecup muscles is centrally inhibited (Fig. 4) and there is a slight slowing of the tonic activity in the contralateral eyecup (Fig. 4B). After this inhibitory period, slowly facilitating and summing junction potentials can be recorded in muscles 18, 20b, 21 and 22, that is the group supplied by the axon with the smaller spike recorded in the optic tract. These cause a depolarization plateau which may last for up to 500 msec, during which a high frequency burst of junction potentials can be recorded in muscles 19a and 20a, that is that group supplied by the axon with the larger spike recorded in the optic tract (Fig. 4A). These junction potentials facilitate and summate rapidly and active membrane responses occur. Although the firing sequence of the two axons differs from that in a reflex withdrawal, the overall movement of the eyecup as seen in an unclamped eyecup is similar.

Of the six muscles involved in the withdrawal reflex, all but 19a are active in optokinetic movements. For example, when muscle 21 is active with muscles 18, 19a, 20a, 20b and 22, a withdrawal movement downwards and

away from the midline occurs. During optokinetic movements, however, the same fibres in muscle 21 together with a specific pattern of activity in the other muscles are most active in a horizontal movement toward the midline. It is thus possible for one muscle, in conjunction with different patterns of activity in other muscles, to be involved in opposite movements. Interpretation of eyecup muscle activity therefore becomes intelligible only if a group of muscles, rather than the individual muscles themselves, are regarded as functional units.

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### Variation in Physiological Properties of Crustacean Motor Synapses

THE properties of individual synapses of the motor axon which innervates the opener (dactylopodite abductor) muscle in the crayfish walking leg have been investigated by Dudel and Kuffler<sup>1</sup>, who placed an external micro-electrode close to the synaptic region to record the flow of current through the post-synaptic muscle fibre membrane during the action of the neuromuscular transmitter<sup>2</sup>. With low frequencies of stimulation of the motor axon individual synapses showed frequent failures of transmission, and the synaptic currents generated by successful transmission were small, often equal in size to a single externally recorded spontaneous miniature potential. The latter event was found to equal one quantal unit of transmitter action. At higher frequencies of stimulation, the probability of failure of transmission decreased, and the synaptic currents attained a larger average size, indicating that the output of transmitter substance from the presynaptic terminals had increased. The post-synaptic potentials recorded internally from the muscle fibres were correspondingly larger at the higher frequencies of stimulation. These observations indicate that facilitation at the crayfish neuromuscular junction is the result of some process which gives rise to a higher probability of release of transmitter substance at the higher frequencies of stimulation or during the course of a train of closely spaced stimuli.

Not all post-synaptic potentials set up in crustacean muscle fibres by a single motor axon show properties of facilitation. Some of the fibres in a muscle may show non-facilitating or poorly facilitating potentials, whereas others may show the more common facilitating potentials<sup>3</sup>. In the opener muscle of the crab *Pachygrapsus crassipes* Randall, facilitating and non-facilitating potentials have been observed in adjacent muscle fibres (Fig. 1). The question arises whether synapses of crustacean motor axons are variable in their behaviour, thus generating different types of post-synaptic potentials.

The properties of the synapses of the open-stretcher motor axon of *Pachygrapsus* were investigated by means of the technique of focal recording with an external microelectrode and it was found that some of the synapses behaved as described

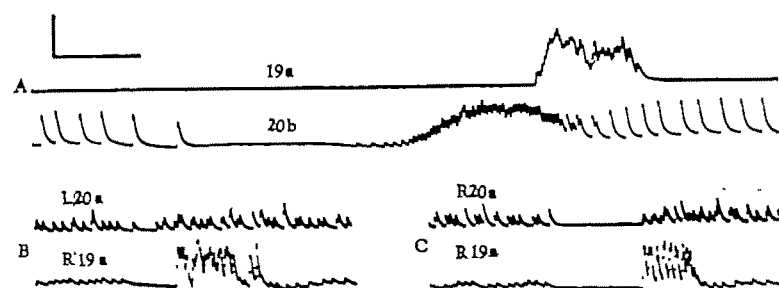


Fig. 4. A, Recordings from muscle 19a (upper) and 20b (lower) during a "spontaneous" withdrawal. Note the preceding inhibition of tonic activity and that phasic activity in 20b precedes that in 19a. B, The effect on the contralateral tonic activity compared with that in the ipsilateral eyecup (O), during a "spontaneous" withdrawal. Scale: 20 mV, 500 msec (A), 500 msec (B and C).

by Dudel and Kuffler<sup>1</sup>. Failure of transmission was common at low frequencies of stimulation, and synaptic currents were small, usually only one or two quantal units in magnitude (Fig. 2A and B). The number of transmission failures decreased at higher frequencies of stimulation, and the average amplitude of the synaptic currents increased. In other endings, however, especially those on muscle fibres showing non-facilitating post-synaptic potentials, there were no failures of transmission at low frequencies of stimulation (1/sec or 0.5/sec). Synaptic currents were several times the size of the quantal unit (Fig. 2C and D). Often these currents varied little from one stimulus to the next (Fig. 2D). As the frequency of stimulation was increased, the currents showed no marked increase in amplitude. Apparently the powers of facilitation were rather limited in such endings.

It was often possible to record rather large nerve terminal potentials at the non-facilitating synapses (Fig. 2D). This suggests that the synapses are located on large-diameter axon branches, which may be capable of supporting a propagated action potential. The nerve terminal potentials recorded at the facilitating synapses were generally smaller. Possibly these synapses are located mostly on fine axon terminals, which may not conduct a propagated action potential, but rather a decremental response<sup>4</sup>.

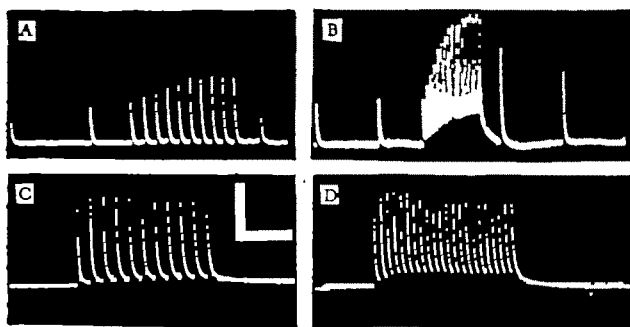


Fig. 1 Facilitating (A and B) and poorly facilitating (C and D) post-synaptic potentials from two fibres in the opener muscle of *Paedopygus*. Calibration: voltage, 10 mV, time, 1 sec.

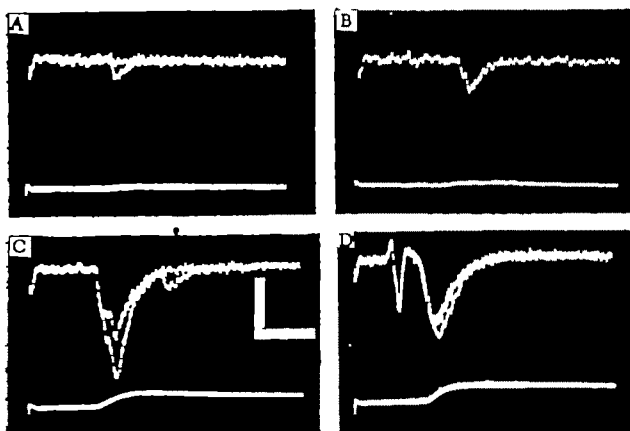


Fig. 2 Synaptic currents (top records) set up by 1/sec stimulation of the opener-stretcher motor axon of *Paedopygus*. Lower traces show the internally recorded post-synaptic potentials of the muscle fibres. Records were from a facilitating synapse (A and B) and from two poorly facilitating synapses (C and D). In the former, transmission failures were frequent (A) and currents small; A and B show currents of one and two quantal units, respectively. In C and D, currents were several times the quantal unit (the size of which is comparable with the spontaneous potential in C). A large nerve terminal potential precedes the synaptic current in D. Calibration: voltage 400  $\mu$ V (top traces) and 20 mV (bottom traces), time, 4 msec.

The above observations suggest that there are different types of synapse associated with a single motor axon, but that the ultimate basis for the differences has yet to be determined.

The ratio of facilitating to non-facilitating synapses supplied to a muscle fibre by a particular axon probably determines in large measure the properties of the post-synaptic potentials evoked by that axon. Different motor axons (for example, the "fast" and "slow" axons of certain leg muscles) may differ in the relative numbers of the various types of synapse they possess.

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### Secretion of a Chromaffin Granule Protein, Chromogranin, from the Adrenal Gland after Splanchnic Stimulation

Banks and Helle<sup>1</sup> have reported that stimulation of the isolated bovine adrenal gland, during retrograde perfusion, releases not only catecholamines but also a soluble protein present in the chromaffin granules; this finding has since been confirmed<sup>2,3</sup>. We propose the term "chromogranin(s)" as suitable for the soluble protein fractions of the chromaffin granules of the adrenal medulla.

In order to determine whether the secretion of chromogranin from the adrenal medulla occurs in the living animal, we have looked at the adrenal glands of calves. We collected adrenal venous blood from a calf (aged 293 days) under pentobarbitone anaesthesia during and between periods of stimulation of the splanchnic nerve. The experimental conditions were as described by Comline and Silver<sup>4</sup> except that adrenal effluent blood was collected from a catheter inserted through the adrenal vein into the venous sinus of the gland. The main component of the chromogranins was purified by the method of Smith and Winkler<sup>5</sup> and a rabbit antiserum was prepared against the purified protein. This chromogranin was assayed by a complement fixation method that was accurate within the limits of 0.5–2.0 times the observed value.

The catecholamine and chromogranin contents of samples of adrenal venous plasma collected during, and after, three periods of nerve stimulation are given in Fig. 1. The secretion of the protein accompanied and continued after that of the catecholamines. The ratios of catecholamines ( $\mu$ moles) to chromogranin (mg) in the venous plasma during each of the 5 min periods were 3.3, 11.3 and 4.2; these ratios are of the same order of magnitude as that found for isolated chromaffin granules (unpublished work of Schneider, Smith and Winkler) which was  $10.1 \pm 3.9$  ( $n=10$ ). The results of this experiment are supported by observations on two calves under chloralose anaesthesia, in which the adrenal venous plasma was tested with the rabbit antiserum to the purified main component of the chromogranins; the plasma from one calf was tested by the Ouchterlony immunodiffusion method, and that from the other calf was examined by complement fixation. In both experiments the plasma collected during periods of stimulation contained much more chromogranin than did the plasma collected during control periods.

We conclude from these observations that the secretion of a soluble protein from chromaffin granules accompanies that of the catecholamines on stimulation of the splanchnic nerve *in vivo*. This adds further significance to the *in vitro* investigations which have shown that the entire

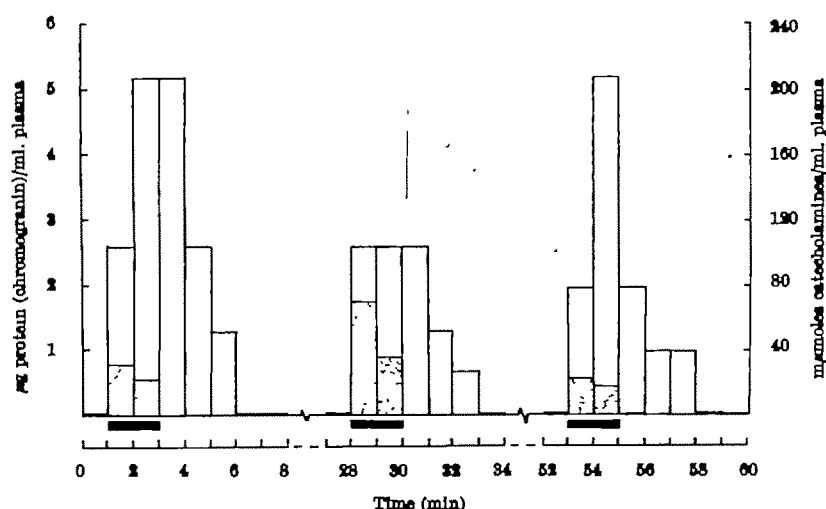


Fig. 1. Catecholamine and chromogranin content of adrenal venous plasma. The heights of open and stippled columns represent respectively the concentrations of chromogranin and of catecholamines in the plasma. The black horizontal bars represent periods of stimulation (30/sec, 20 V) of the splanchnic nerve.

soluble constituents of the chromaffin granules are secreted<sup>1-3,6</sup>.

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### Inhibition of Gastric Acid Secretion by a Purified Bacterial Lipopolysaccharide

LIPOLYSAOCHARIDES from Gram negative bacteria produce many systemic toxic effects when injected into experimental animals and are thought to institute the processes referred to in humans as endotoxic shock<sup>1</sup>. They act by altering regional blood flow and leading to ischaemia in critical areas of the body, especially the mid-gut<sup>2</sup>.

A direct relationship between gastric mucosal blood flow and gastric acid secretion has been demonstrated<sup>3</sup>, and injections of a purified lipopolysaccharide into dogs have reduced total gastric blood flow<sup>4</sup>, apparently through a mediating substance. We have tried to link the two sets

of observations and to investigate the effect of a bacterial lipopolysaccharide on the secretion of gastric acid.

A purified lipopolysaccharide, prepared as described before<sup>5</sup> from the lysine-dependent mutant *Escherichia coli* ATCC 12408, was supplied by Dr K. W. Knox. The preparation was diluted before injection with sterile phosphate buffer (0.1 molar, pH 7.4) to give the concentrations shown in Table 1. Secretion of gastric acid in sixty-five Sprague-Dawley rats weighing 85–180 g was measured by pylorus ligation<sup>6</sup>. Groups of six rats were injected intravenously with increasing concentrations of lipopolysaccharide at the time of pylorus ligation; they then rested for 4 h after which gastric juice was aspirated and the acid content was measured. Mean acid secretion by each group of rats was determined and the dose-response curve shown in Fig. 1 was constructed.

The results (Table 1, Fig. 1) show that the purified *E. coli* lipopolysaccharide was a powerful inhibitor of rat gastric acid secretion. Acid secretion was almost abolished by all doses of lipopolysaccharide greater than 50 µg/kg while secretion was reduced to 50 per cent of control values by a dose of 1.25 µg/kg (about 0.15 µg/animal). In agreement with earlier experiments we found that larger doses were toxic, the LD<sub>50</sub> in this experiment being 2 mg/kg.

It is probable that the reduced total gastric blood flow after injection of lipopolysaccharide is associated with reduced gastric mucosal blood flow which is the cause of the reduced secretion of acid. It is interesting to note that this effect on total gastric blood flow seems to be mediated by a separate substance produced in the body,

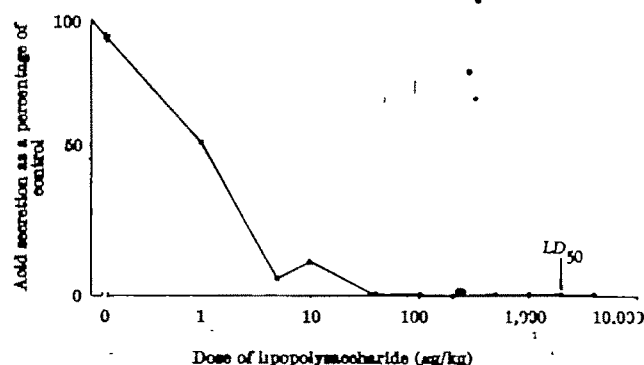


Fig. 1. Mean acid secretion by groups of rats given *E. coli* lipopolysaccharide expressed as a percentage of control.

Table 1. MORTALITY AND ACID SECRETION IN GROUPS OF RATS GIVEN INTRAVENOUS INJECTIONS OF *E. coli* LIPOLYSAOCHARIDE

No.	Dose (µg/kg)	Volume of buffer (ml/kg)	Mortality (per cent)	Acid secretion (mean)	m.equiv./100 g/4 h (standard error)
6	0	5	0	339	±40
5	1	5	0	191	±84
6	5	5	0	24	±4
6	10	5	0	45	±22
6	50	5	0	3	±2
6	100	5	0	3	±2
6	200	5	17	0	±0
6	500	5	17	3	±1
6	1,000	5	33	3	±3
6	2,000	5	50	0	±2
6	4,000	5	66	3	±3

probably in the intestine, because it cannot be produced by lipopolysaccharide injected directly into the gastric vessels<sup>4</sup>.

Gastric acid secretion is very sensitive to intravenous injections of lipopolysaccharide and this agent seems to be one of the most potent of all inhibitors of acid secretion yet described. Other endotoxins are at present being prepared from a variety of Gram negative bacteria and their effects on gastric acid secretion will be assessed.

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### Transmission from Excitatory Nerves to the Smooth Muscle Cells of the Rat Seminal Vesicles

THIS communication reports some investigations of transmission from excitatory nerves to the smooth muscle cells of the seminal vesicle of the rat. The seminal vesicles were dissected from adult rats and the preparation inserted between ring electrodes and bathed in a Hukovic solution<sup>1</sup>. The method used to stimulate the intramural nerves and the technique involved in recording with microelectrodes from the smooth muscle cells were the same as previously described<sup>2</sup>.

Stimulation of the intramural nerves with single pulses lasting 1 msec gave rise, after a latent period of about 8 msec, to a transient depolarization, the excitatory

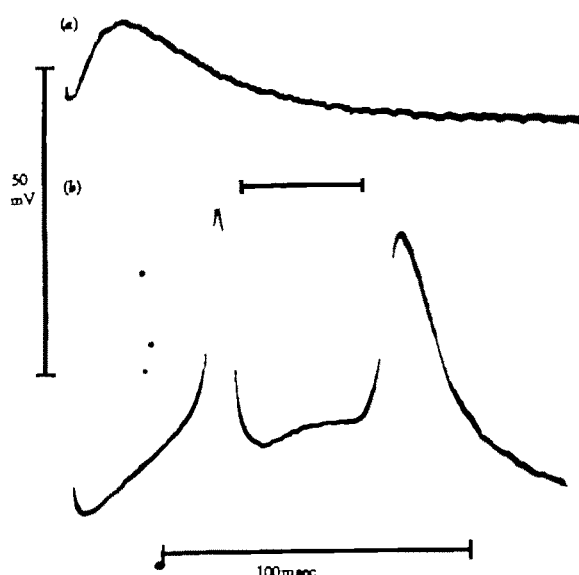


Fig. 1. Effect of stimulating the intramural excitatory nerves to the seminal vesicles with single pulses. *a*, Excitatory junction potential arising from stimulating with a pulse of sub-maximal strength. *b*, Junction potential arising from stimulation with a supra-maximal pulse, leading to the firing of two action potentials. (Note different time scale in *a* and *b*.)

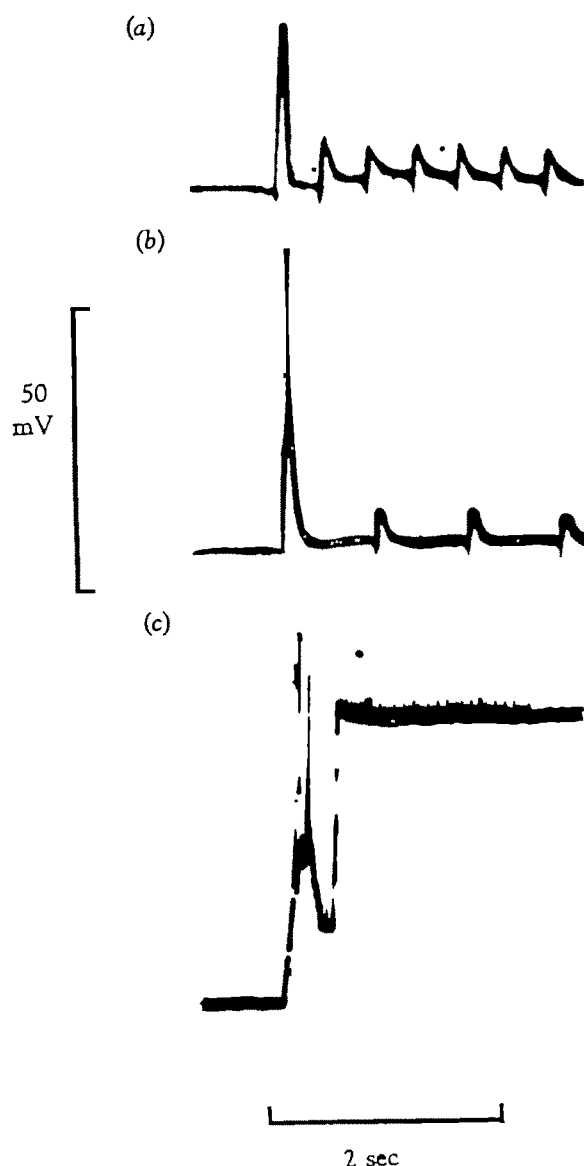


Fig. 2. Effect of repetitively stimulating the intramural excitatory nerves to the seminal vesicles. *a*, Successive junction potentials after the first are much smaller in amplitude, stimulation frequency 2 c/s. *b*, First junction potential of a train fires an action potential, but succeeding junction potentials do not, stimulation frequency 1 c/s. *c*, High frequency of stimulation (20 c/s) only transiently depolarizes the muscle cells because of the decrease in amplitude of successive junction potentials. Note repetitive firing of action potentials during depolarization. The microelectrode became completely dislodged by muscle contraction before the membrane repolarized during stimulation.

junction potential, which decayed exponentially with a time constant of about 150 msec (Fig. 1*a*). It was possible to depolarize the smooth muscle cells by passing a current through them by means of an intracellular electrode. I found that the membrane time constant was only about 2–3 msec, which is much less than the time constant of the excitatory junction potential.

When the stimulation applied to the intramural nerves was increased, the amplitude of the excitatory junction potential increased in at least five steps. This suggests that each smooth muscle cell is affected by at least five axons. When the excitatory junction potential reached an amplitude of about 25 mV, an action potential was initiated with an overshoot of 15 mV and a maximum rate of increase of 12 V/sec (Fig. 1*b*). Successive excitatory junction potentials arising from repetitive stimulation of the intramural nerves at 1 c/s with pulses of supra-threshold strength decreased in size until a steady

amplitude was reached (Fig. 2a and b). At high frequencies of stimulation the membrane was only transiently depolarized (Fig. 2c). When the interval between the pulses was greater than 30 sec, however, it was no longer possible to detect decreases in the amplitude of the excitatory junction potential.

The very short latency of 8 msec indicates that the time between stimulus and the beginning of the release of transmitter from autonomic axons is less than this. The large latencies found for transmission from intramural nerves to the smooth muscle cells of the guinea-pig taenia coli<sup>2</sup> and the rabbit colon<sup>4</sup> should therefore be attributed to other factors in the transmission link. Although transmissions from the hypogastric nerves to the vas deferens<sup>4</sup> and from the pelvic nerves to the distal colon<sup>4</sup> are known to be enhanced by facilitation, no facilitation has been observed from intramural inhibiting nerves to the taenia coli<sup>2</sup>. The present investigation indicates that there may be a depression of transmission from some autonomic nerves to smooth muscle with successive stimuli.

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### Isolated Double Perfusion of the Liver in the Study of the Reticulo-endothelial System

THERE is still disagreement about whether reticulo-endothelial function depends on a serum factor<sup>1-3</sup>. There is substantial evidence that in certain mammals, especially the rat, "reticulo-endothelial phagocytosis" requires the participation of a serum factor for certain colloids. In this species, blockade of the reticulo-endothelial system can be attributed to depletion of a serum factor, perhaps antibody or a complement component. By contrast, in the rabbit, reticulo-endothelial blockade can occur independent of serum factors<sup>4</sup>, and experimental agammaglobulinaemia in the chicken is not associated with any deficiency of reticulo-endothelial clearance *in vivo*<sup>5</sup>. Until now, however, no critical evidence has been presented about the exact nature of the serum factor operating in the rat system or the nature of the blockade in animals not requiring the serum factor.

We have tried to simplify the experimental system and isolate for study the factors involved. We chose organs primarily involved in reticulo-endothelial function and wanted to be able to manipulate the contributions of serum and colloid in a system more easily controlled than the *in vivo* systems generally used. We therefore used the double liver perfusion system shown in Fig. 1. This system is a modification of the system described by us in 1961, which was a simplification of the methods used by Miller<sup>6</sup>. It makes it possible to test protein synthesis and bile formation<sup>7,8</sup> in the perfused liver.

The single perfusion apparatus was modified so that a single isolated liver could have its entire perfusate changed without interrupting the circulation through the liver. This "double perfusion" apparatus is simply two single systems combined. The perfusate used was a mixture of total fresh blood taken from Sprague-Dawley rats and heparinized (500 u/ml.) with Tyrode solution (pH 7.6-7.4) in the ratio of 3:1.

20 mg of aureomycin and 800 mg of dextrose were also added. The total volume of perfusate was 100 ml. The liver was isolated from a Sprague-Dawley rat anaesthetized with 'Nembutal' (3 mg/100 g body weight).

After mid-line laparotomy, the bile duct was cannulated with a polyethylene catheter. Then the stomach was removed to reveal the liver and the portal vein cannulated with a cannula 1 mm in diameter. Another cannula was placed in the inferior vena cava at the level of the thorax. The liver was rapidly removed and connected to the apparatus; the period of anoxia was less than 3 min. The flow through the liver was kept constant at  $15 \pm 1$  ml./min.

To measure the speed of the clearance by the liver, we chose radioactive 'Auroclod' (Abbott) containing gold-198. The disappearance of the gold in the perfusate was followed by analysing the radioactivity of serial samples taken every 5 min. The activity of the samples was counted in a solid scintillation counter and reported as c.p.m. after correction for background.

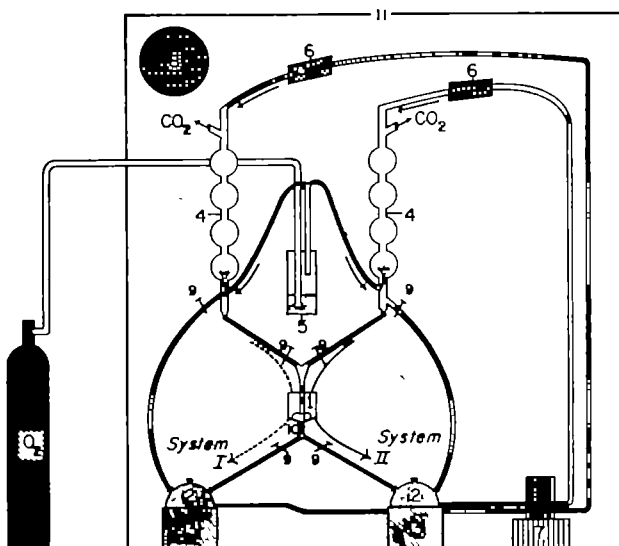


Fig. 1. Double perfusion apparatus for the isolated liver. (1) Liver in plastic chamber. (2) Reservoir for perfusate. (3) 'Magnestar' providing homogeneous mixing of the perfusate. (4) Glass 'lung' in which perfusate is oxygenated. (5) Humidifier for oxygen. (6) Filter. (7) Pump. (8) Heater with thermostat maintaining temperature at 96° F. (9) Stopcocks. (10) Flowmeter. (11) Cabinet containing the system.

In our first series of experiments, we found that the clearance of gold in a liver perfusate decreased with time. Also, when a second or third dose of 'Auroclod' was added, the clearance was similar, although less rapid and less complete than that of the preceding identical dose of colloid. Our results show that this phenomenon is reversible in the isolated liver preparation. When 2.5 mg 'Auroclod' was added 3 min after the start of perfusion, half the gold was cleared from the perfusate 10 min later (Fig. 2). The same dose was again added at 40 min; 50 per cent clearance took 14 min. After the third dose, the time taken for 50 per cent clearance was 23 min. When we then changed the entire perfusate at 155 min, thus using rat blood fresh to the liver, and added another similar dose of 'Auroclod', the time required to clear 50 per cent of the circulating gold was again short, 8 min; after another dose only 14 min, and a final dose 21 min. It is clear from these experiments that diminution of the rate of clearance of gold may be increased simply by changing the perfusate for fresh, previously unperfused, rat blood. We interpret these observations as indicating that rat serum contains a factor which may be exhausted by the initial repeated perfusion of gold, and which is renewed by adding fresh rat blood to the perfusing system. We think that the decreasing rate of clearance observed initially is due to exhaustion of the serum factor and not to functional blockade of the reticulo-endothelial cells or to a shift in site of phagocytosis. In



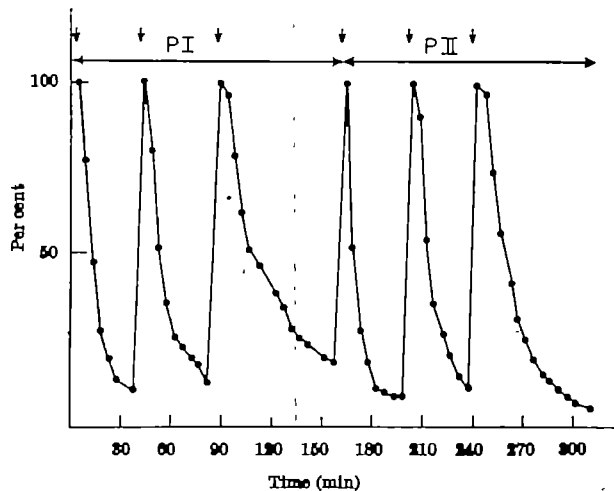


Fig. 2. Demonstration by double-perfusion technique of the dependence of reticulo-endothelial blockade on blood. Ordinate, percentage of radioactivity present in the perfusate taking the 3 min sample as 100 per cent. At the top of the Figure, the arrows indicate addition of 'Aurocolloid', 2-5 mg. Abscissa, time in min.

our experiments, careful monitoring of the outflow of blood and the controlled circulation allowed us specifically to exclude outflow circulatory block as a basis for the decrease in function. Furthermore, varying the timing of the point of administration of the colloidal gold after the beginning of perfusion indicated clearly that the circulating factor was not being used non-specifically or metabolized by the liver.

Numerous repetitions of this type of experiment in this system have established that the method used is reproducible. Comparison of rabbit with rat livers, rat blood with rabbit blood or perfusates lacking serum factors, and the critical effects of dosage and continued presence of added colloid can be evaluated, as can the critical effects of specific antibody, complement or other serum factors. Indeed, this method should be valuable in obtaining critical analysis of reticulo-endothelial function in the absence of many factors difficult to control in the intact animal. In experiments similar to those described, but comparing non-serum containing perfusate and rat serum, it was found that serum factor is not required for phagocytosis, but that rat serum increases the rate of clearance of different circulating particles during perfusion of the isolated liver.

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## Nystagmus induced by Unilateral Labyrinthectomy affected by Sleep-Wakefulness Cycle

It has been reported that post-rotatory nystagmus is replaced by slow ocular movements during behavioural sleep in children<sup>1</sup>. Sleeping infants are also said to show no nystagmus<sup>2</sup>. In cats a decrease in wakefulness as revealed by the electroencephalogram has been recently shown to be paralleled by a reduction of rotatory nystagmus<sup>3,4</sup>. We have investigated the modifications induced by the sleep-wakefulness cycle in "spontaneous" nystagmus after unilateral labyrinthectomy<sup>5</sup>.

The experiments were performed on cats. Screw type electrodes were implanted, under 'Nembutal' anaesthesia, in the skull for electroencephalogram recording and in the superior wall of the orbital cavity of each side, after the frontal sinuses had been opened, for recording ocular movements. Stainless steel electrodes were inserted into the posterior cervical muscles for electromyogram recording. During the same session the animal had one labyrinth destroyed through the bulla. On the day after the operation the cat was placed in a sound-proof room and records were made by an ink-writer electroencephalograph for several hours during the day.

Unilateral labyrinthectomy induced a regular horizontal nystagmus with the quick component toward the normal side. During wakefulness with desynchronized electroencephalogram and high cervical electromyogram the nystagmus was recorded on the electro-oculogram as regular high amplitude oscillations at a frequency of about 1/sec (Fig. 1A). These deflexions were not generally modified when the animal closed its eyes as long as it was awake. As the cat became more relaxed with lower cervical electromyogram and electroencephalogram synchronization the nystagmus seemed to be irregular, reduced in amplitude and it often disappeared synchronously with the cortical spindles (Fig. 1B). Nystagmic oscillations comparable with those found in wakefulness could be present during the interspindle hulls (Fig. 1B). When

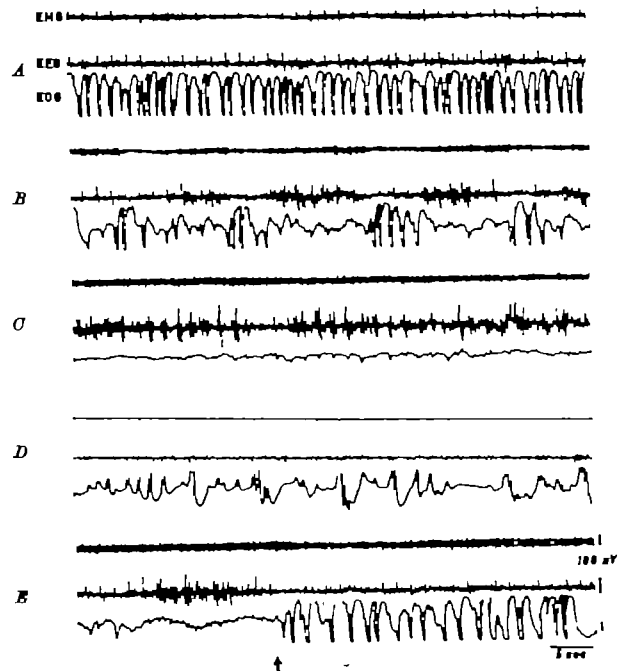


Fig. 1. Cat one day after unilateral labyrinthectomy. Posterior cervical electromyogram (EMG), bifrontal electroencephalogram (EEG) and ocular movements, measured by electro-oculogram (EOG). Note the regularity of "spontaneous" nystagmus during wakefulness (A) and its modifications during synchronized (B and C) and desynchronized sleep (D). The nystagmus reappears at the moment of arousal (E) induced by a natural stimulus (at the arrow).

the electroencephalogram became highly and persistently synchronized, particularly in the phase immediately preceding a period of desynchronized sleep, the nystagmus disappeared and was replaced by low voltage irregular oscillations (Fig. 1C).

The appearance of a spontaneous episode of desynchronized sleep was characterized by deep changes in the electro-oculogram. Slow irregular ocular movements were present, at times interrupted by high voltage rapid eye movements typical of this phase of sleep\* (Fig. 1D). Sometimes ocular movements which resembled the nystagmic oscillations of wakefulness could be recorded independently of the rapid eye movements.

Any natural stimulation able to yield an electroencephalographic and behavioural arousal either from synchronized or desynchronized sleep invariably induced the reappearance of high voltage regular nystagmic movements (Fig. 1E).

The effects of the sleep-wakefulness cycle on nystagmus after unilateral labyrinthectomy could be observed only for 4-5 days because from then on the "spontaneous" nystagmus faded away confirming previous findings\*.

The results suggest that the mechanism of nystagmus which follows unilateral labyrinthectomy in cats may be actively inhibited by the nervous structures involved in the synchronization and desynchronization of the electroencephalogram during natural sleep.

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### Multimodal Interneurons in Cockroach Cerebrum

By means of stainless steel microelectrodes, we have recorded multimodal interneurons in the protocerebrum of the cockroach, *Periplaneta americana*. At least two basic types of interneurons were recorded; one was excited by visual and a variety of mechanical stimuli, while the other was excited by visual stimuli and inhibited when abdominal movements occurred. Interneurons similar to one or the other of these two types have previously been reported in the optic lobe and protocerebrum of the migratory locust, where they also showed auditory responses<sup>1</sup>, in the optic nerve of decapod crustaceans<sup>2</sup>, and in moth protocerebrum<sup>3</sup>. In spite of these similarities, however, the cockroach units also showed certain distinct properties of their own.

Cockroaches were first anaesthetized with ether and then fixed in wax by pouring liquid paraffin of low melting point over them. The wax was then scraped away to expose the head, abdominal spiracles, and other body regions as necessary. Sufficient cuticle was removed to reveal the brain. The brain was desheathed before the electrodes were inserted. Electrodes were made from stainless steel insect pins etched with acid to a tip diameter of 1-2  $\mu$  and insulated to the tip with lacquer<sup>4</sup>. The light source was a standard low voltage microscope lamp, while tactile stimuli were delivered with a fine glass rod.

Most of the multimodal interneurons observed had a fairly slow somewhat irregular spontaneous discharge rate of 1-10/sec and were presumably large, because they

produced spikes nearly twice the amplitude of other units recorded. They responded with a brief burst of spikes when the light was turned off, to gentle touches on abdomen or pterothorax, and to gentle touches or brief displacements of cerci and tarsal segments. The duration of the bursts varied roughly from 0.1 to 1 sec, depending on the stimulus parameters. The first response of a series was usually the most marked; this was especially true when the light was turned off, where bursts following 30 sec or more in light contained more spikes and had shorter latency than those following briefer periods of light adaptation. Some units occasionally also responded when the light was turned on and when the ipsilateral antenna was displaced. These responses were, however, somewhat ambiguous. Only antennal responses depended on unilateral stimuli; all the others were elicited more or less equally effectively by stimuli applied either on the same or on the other side (Fig. 1).

The precise anatomical location of these units was not determined, but the approximate position was ascertained from surface contours and depth of electrode penetration. They occurred in the protocerebrum ventral and lateral to the mushroom bodies in a region of several large fibre tracts<sup>5</sup>. Insertion of the electrode in the appropriate area yielded reliable recordings from all healthy preparations.

These units differed from the multimodal interneurons reported in other arthropods<sup>1-3</sup> in that the most effective visual stimulus was to turn the light off. Turning the light on only occasionally, and not always unambiguously, elicited bursts of spikes. We found no auditory responses, as seen in the locust, nor did we find any small field tactile responses such as are seen in decapods. Rather, touch stimuli to a wide area including body and legs gave con-

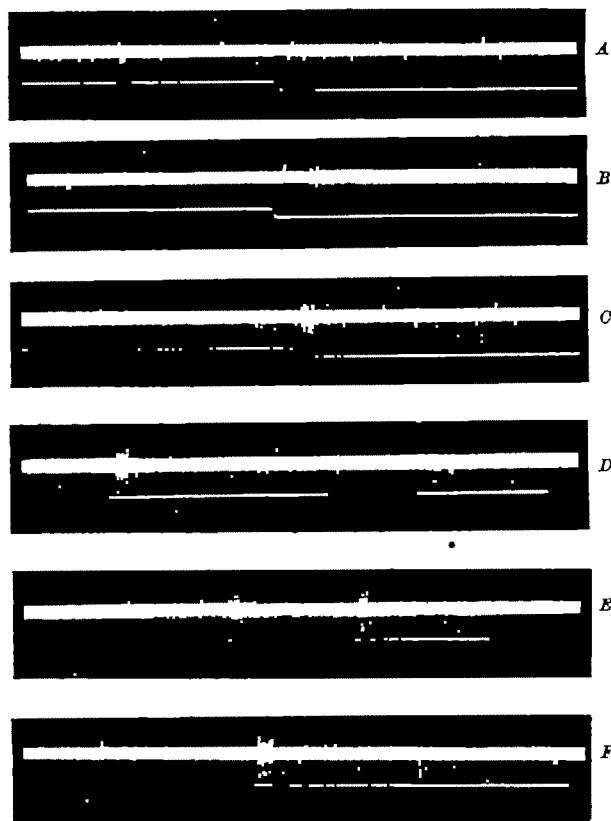


Fig. 1. Responses of a multimodal interneuron. A-C, Responses to light off after 5, 10, and 30 sec, respectively, of light adaptation; downward deflection of lower beam indicates stimulus. D, Two responses to touch of ipsilateral cercus. E, Two responses to touch of contralateral cercus. F, Response to touch of ipsilateral mesothoracic tarsus. In D-F the lower beam indicates the approximate duration of stimulus, each sweep 2 sec long. Note that unit also fires spontaneously.

sistent results. We were unable to detect differentially coded responses to spatially separated somatic stimuli.

The function of this type of multimodal interneurone is not clear, although these units are by implication involved in "startle" responses<sup>1</sup>. Tactile stimuli which yield bursts of spikes, such as tarsal or cercal touch, cause intact animals to jump. Tarsal touch is more effective than cercal, although we saw no consistent difference in the spike train; by contrast the animal is markedly sensitive to even the gentlest puff of air directed at the cerci. Abdominal and cercal stimuli elicit impulses in the giant fibre system<sup>2</sup>, and it would seem that the activity of giant fibres is transmitted to the brain where it is presumably integrated with other inputs.

Unlike touch, turning the light off caused no discernible behavioural response in our intact animals; if multimodal units are involved in startle reflexes, this is somewhat surprising because these neurones showed consistent bursts when the light was turned off which matched those following touch. Animals did, however, show pronounced responses to an approaching hand. The integration by other units of light off (dimming) with movement may therefore be necessary before impulses are transmitted to lower centres controlling locomotion. In the case of tactile inputs, the lower centres would be stimulated before the multimodal units<sup>3</sup>. In the case of touch, these units are probably activated after a motor response has been triggered, whatever that might mean. An "arousal" system may be indicated, but we have no idea of the nature or location of whatever would be "aroused". The synchronized response to antennal shock seen in cockroach mushroom body<sup>4</sup> may also be relevant to such a system; its latency was considerably longer than the response elicited in giant fibres by the same stimulus.

The second type of multimodal interneurone observed was excited by light on and inhibited by light off and abdominal movements (Fig. 2). Similar units have been reported only from moth protocerebrum<sup>5</sup>. The light responses of these neurones, however, duplicated those of the "sustaining units" reported from cricket brain<sup>6</sup>. These responses were the following: (i) a transient rise in frequency as the light intensity increased by increments, (ii) a maintained steady frequency positively correlated with light intensity, (iii) a transient decrease in frequency when light was dimmed, and (iv) total cessation of activity when the light was turned off. In addition, the cockroach multimodal units were inhibited when the animal raised and extended the abdomen; other abdominal movements and all tactile stimuli were ineffective except when the

stimuli caused the abdominal movements described. These multimodal interneurones are undoubtedly homologous with the sustaining units of the cricket, for they are similar both in their response and in their anatomical location in tracts running from the optic nerve toward the midline of the brain. Inhibition thus appears to take place somewhere in the optic stalk.

Units detecting static and transient functions of light intensity are an obvious necessity for visual systems, and it is not surprising that they have been found in the cockroach. What is not obvious *a priori*, however, is the inhibition of such units by movements of the abdomen.

We are not sure what the function of this inhibition is. It is, however, well known that in arthropods impulses arising from somatic proprio- and mechano-receptors enter the optic stalk<sup>1,7</sup>. Our results are indirect evidence, at least, that some of the fibres involved may have an inhibitory function.

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### Daily and Tidal Components in the Persistent Rhythmic Activity of the Crab, *Sesarma*

In intertidal organisms the rates at which various physiological functions proceed are often related to the state of the tide. In a habitat in which environmental conditions change so drastically with the ebb and flow of the tidal waters, this is not particularly surprising. What is remarkable is that the rate of many of these functions continues to vacillate in approximate synchrony with the tide when organisms are removed to non-tidal, constant conditions in the laboratory. These persistent vacillations are referred to as tidal or bimodal lunar day rhythms. By way of example, persistent tidal rhythms have been described for oxygen consumption in crabs<sup>1</sup>; vertical migrations of planarians<sup>2</sup> and diatoms<sup>3</sup>; colour change in crabs<sup>4</sup>; spontaneous activity in crabs<sup>5,6</sup>, amphipods<sup>7,8</sup>, and fish<sup>9</sup>; and filtration rate in mussels<sup>10</sup>.

Some investigators have reported that in addition to a lunar day rhythmic component in a particular activity of the organism, a 24 h component was present simultaneously<sup>11,12</sup>. Enright<sup>13,14</sup> discredited these earlier claims of dual rhythmic components and stated that there are "... no convincing demonstrations that any organism simultaneously shows both an endogenous tidal and an endogenous circadian rhythm". Since then, other investigators, working on a variety of organisms, have reported what appear to be dual rhythmic components in their results<sup>15-18</sup>, while others, who have specifically looked for such a feature, have found no period which approximates 24 h associated with the tidal rhythms they were investigating<sup>7-10</sup>. In the work reported here, the spontaneous locomotor activity of the intertidal grapeoid

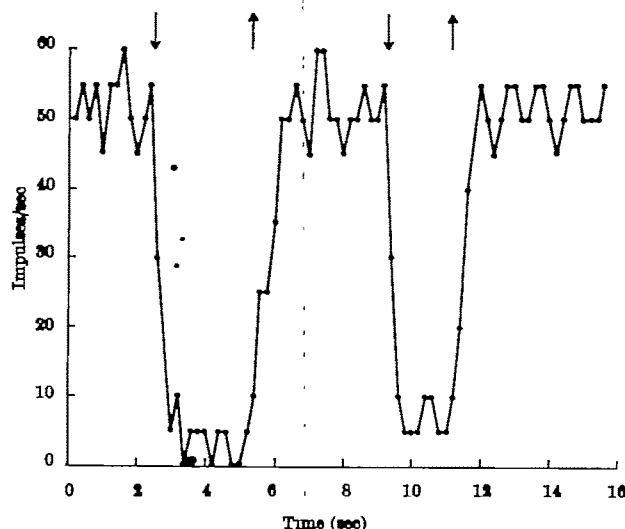


Fig. 2. Continuous record of multimodal interneurone inhibited by abdominal movements. Downward arrow indicates beginning and upward arrow cessation of movement.

crab, *Sesarma reticulatum*, was examined, and clear out rhythmic components of approximately 24 and 24.8 h were found in the locomotor activity.

Crabs were collected from their burrows in the region of the Marine Biological Laboratory, Woods Hole, Massachusetts, and placed in individual actographs, in constant darkness, and a constant temperature of 20° C. After 10–15 days in the actographs, the conditions became foul and the crabs became abruptly inactive or died. Entire series of crabs were therefore replaced at intervals of about 2 weeks. Actograph movements were recorded on an Esterline-Angus event recorder, and the activity of as many as ten crabs could be monitored simultaneously. More than 12,500 "crab-hours" of data went into the investigation reported here.

Over a period of 2 months, five groups of crabs were investigated, and a population average obtained for each hour. As a means of graphic presentation, a method used previously<sup>14</sup> was again utilized. In brief, an average hourly activity rate was calculated for each day of the investigation. Each day was then represented on a graph as an unshaded horizontal bar, and all hourly activity values equal to, or greater than, the daily mean were represented by black squares and plotted with respect to their proper temporal position within the bar. The net result was that minor fluctuations in the activity pattern were "filtered out" and do not appear on the graph, while the times of maximal activity are emphasized by the black squares dispersed along an otherwise unshaded bar. Results on consecutive days were plotted one beneath the other; and the completed graph is shown in Fig. 1. (It should be emphasized that this method of presentation is used only to produce a graph which summarizes and condenses a great deal of data and brings out major trends in the behaviour of the population.) It is quite clear from

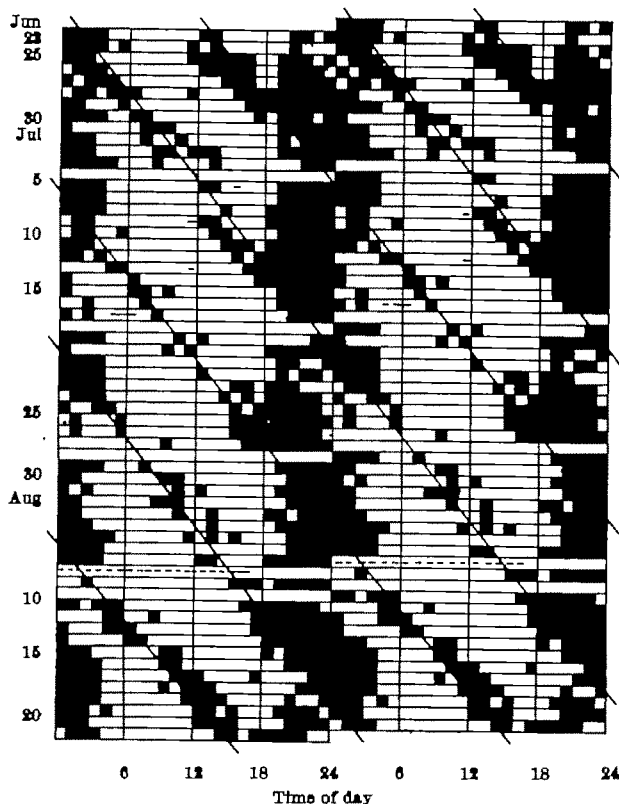


Fig. 1. Consecutive daily activity patterns of a population of *Sesarma reticulatum*. The graph has been duplicated, and the right-hand graph displaced upward by one day, in order to facilitate the visualization of the movement of the tidal peaks across the solar day. Parallel, oblique lines superimposed over the figure represent the mid-point of daily high tides. Dashed lines represent mechanical failures of the recording system. The entire crab populations were replaced on July 5, 18 and 29, and August 10.

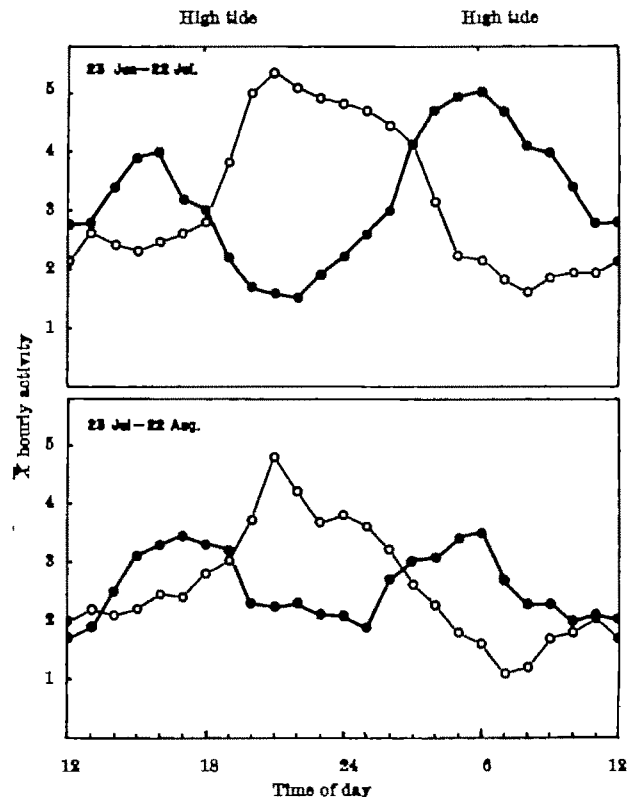


Fig. 2. Dual components in the persistent rhythmic activity of *Sesarma reticulatum*. Mean solar day rhythm is indicated by open circles; the time scale on the abscissa is pertinent only for this rhythmic component. Solid circles represent the bimodal lunar day rhythmic component. Maxima in this rhythm correspond to the times of high tide (mid-points of high tide are indicated at the top of the figure).

Fig. 1 that most of the daily activity was concentrated between 1800 and 0400 h, there being a strong tendency to activity between 1800 and 2400 h—indicating the presence of a persistent nocturnal, 24 h rhythm in the results.

There are also short "bursts" of activity which occur progressively later each day. They tend to scan across the figure at a rate of roughly 50 min/day, providing visible confirmation of the presence of a tidal component in the activity pattern. The parallel oblique lines superimposed over the figure represent the midpoint of high tides in the natural habitat of the crab, and show that the crabs continue to time their running activity so that a relatively constant fraction of it corresponds with the times of high tide in nature.

The same graphical method was used with the records of each individual crab used during the 2 month investigation, and the same general dual component rhythmic pattern was apparent in each, though usually not as distinctly as when the results were lumped together. It was therefore felt best to present the overall trend of the population in a single figure.

Fig. 1 emphasizes the stability of the period length of both the rhythmic components in the results. Given this criterion—as outlined by Enright<sup>14</sup> and Mercer<sup>17</sup>—one can justifiably construct "form-estimate curves" for the 24 h and 24.8 h components. All the results collected were included in the construction of these curves.

A mean solar day curve was obtained by averaging all the values obtained, in each hour, for the 30 day period from June 23 to July 22, and again—separately—for the second 30 day period between July 23 and August 22. When a 30 day period is used, every phase of the lunar cycle has scanned the hours of the solar cycle once, therefore randomizing the contribution the former makes to the latter. The two average daily curves are seen in Fig. 2. The forms of the two curves are slightly different, but the amplitudes are identical; both emphasize a strong peak of

nocturnal activity. It must be remembered that these curves were obtained by summing five separate groups of crabs. While this is not ideal, it is inevitable because the crabs do not survive for more than a fortnight.

To construct a mean lunar day curve the hourly relationship of consecutive days was realigned so that the hour of lunar zenith on one day was lined up directly beneath the hour of lunar zenith on the next (that is, the results were organized into a lunar day relationship) and corresponding hours of the lunar day summed and averaged for the two monthly periods. With each 30 days of results realigned in this manner, every day of the solar day rhythm is made to scan the hours of the lunar day cycle once, now randomizing the effect of the solar day. The resulting mean lunar day curves are seen in Fig. 2. The phase relationship and form of the two curves are virtually the same, but the amplitude of the curve for June 23 to August 22 is only 58 per cent that of the previous months. The average hourly activity for the second 30 day period was also reduced, dropping from 3.21 to 2.59 major movements/h.

It is quite obvious, therefore, that at least in some intertidal organisms a single rhythmic process may be a montage of approximate 24 h and 24.8 h components. From an ecological viewpoint, one might logically expect to find these two elements co-existing in the rhythms of truly intertidal organisms, because the major environmental factors effecting changes in the physiology of such organisms are imposed on them in these two frequencies. While it is quite clear that some organisms with tidal rhythms lack an associated approximate 24 h component, it may well be that, when more detailed investigations are made of the tidal rhythms of other organisms, they too will demonstrate the presence of the additional second frequency.

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## IMMUNOLOGY

### Amino-terminal Amino-acid Sequences of Human Plasma 3S $\gamma_1$ -Globulin and Carbonic Anhydrase B

THE 3S  $\gamma_1$ -globulin of normal human plasma<sup>1</sup> is known to possess chemical and physicochemical properties that are very similar to those of carbonic anhydrase B of human erythrocytes<sup>2,3</sup>. The amino(N)-terminal amino groups of both proteins appear substituted and that of carbonic anhydrase B was reported to be acetylated<sup>4</sup>. To obtain evidence for the identity of these two proteins and to elucidate further their primary structure, the N-terminal

amino-acid sequence of human 3S  $\gamma_1$ -globulin and carbonic anhydrase B was investigated.

Both proteins were analysed by the same procedure and yielded essentially identical results. Protein (105 mg) was dissolved in 20 ml. of water, the pH adjusted to 8.0 and the resulting solution incubated at 70° C for 20 min to denature the protein. After digestion with pronase, the N-terminal acidic peptide fraction which formed was isolated as reported before<sup>4</sup>. The amino-acid composition of this fraction (Table 1) suggested that the N-terminal peptides of both proteins probably consist of 1 mole each of aspartic acid, serine, proline and alanine and that pyroglutamic acid can be excluded as N-terminal amino-acid.

The peptide fraction was resolved into six components by high voltage paper electrophoresis in pyridine-acetate buffer, pH 3.5 (ref. 5). The chief component seemed to be a homogeneous tetrapeptide considering its amino-acid composition (1 mole each of aspartic acid, serine, proline and alanine), and was recovered in an amount corresponding to 0.6 mole/mole of 3S  $\gamma_1$ -globulin and 0.7 mole/mole of carbonic anhydrase B, respectively. The smaller recovery of the tetrapeptide as well as the relatively larger quantities of impurities in the acidic peptide fraction (Table 1) derived from the 3S  $\gamma_1$ -globulin are the result of the presence in this protein preparation of  $\kappa$ - and  $\lambda$ -determinants<sup>6</sup>.

The presence of an acetyl residue as the blocking group of the N-terminus of the tetrapeptide was established by hydrazinolysis<sup>7</sup> (100° C for 17 h) and, after removal<sup>8</sup> of excess hydrazine *in vacuo* at 0° C, by chromatography of the residue. Several appropriate reference substances (Fig. 1a) were used to identify this residue, the amount of which was estimated by comparing the intensity and the size of the spot obtained with those of known amounts of the pure acetylhydrazine and was found to be approximately 1 mole/mole of protein.

The C-terminal amino-acid of the tetrapeptide (0.24  $\mu$ mole) was determined by hydrazinolysis according to Bradbury<sup>9</sup>. After dinitrophenylation the formed dinitrophenol-amino-acid was extracted by Akabori's method<sup>10</sup> and subjected to paper chromatography. Aspartic acid (0.6 mole/mole of peptide) was the sole DNP-amino-acid found.

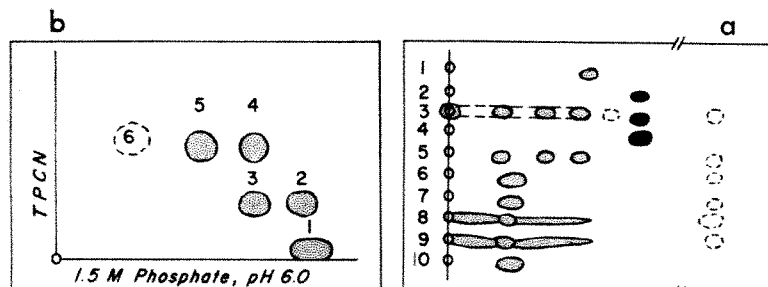
Table 1. AMINO-ACID COMPOSITION OF THE N-TERMINAL PEPTIDE FRACTION DERIVED FROM 3S  $\gamma_1$ -GLOBULIN AND CARBONIC ANHYDRASE B

Amino-acid	3S $\gamma_1$ -Globulin (mole/mole of protein)	Anhydrase B (mole/mole of protein)
Aspartic acid	0.80	0.95
Serine	0.70	0.82
Threonine	0.13	—
Glutamic acid	0.20	0.10
Proline	0.63	0.95
Glycine	0.25	0.16
Alanine	0.76	0.96

For the complete elucidation of its amino-acid sequence, the peptide (0.2  $\mu$ mole) was partially hydrolysed<sup>1</sup> with 1 normal sodium hydroxide at 30° C and for 40 h, neutralized and dinitrophenylated. The DNP-derivatives were chromatographed to yield five compounds (Fig. 1b). The relative position on the chromatogram, the  $OD_{254}/OD_{280}$  ratio and absorption maximum of each DNP-derivative (Table 2) indicated that compounds 3, 4 and 5 were serine, proline and alanine, respectively, that compound 1 was a mixture of DNP-aspartic acid and a DNP-prolylpeptide in equimolar ratio and that compound 2 was a DNP-peptide without N-terminal proline<sup>11</sup>. Further, the N-terminal amino-acid of compounds 1 and 2 proved to be aspartic acid (DNP-proline is very unstable) and serine, and the non-N-terminal amino-acids aspartic acid and proline, respectively. It might be concluded from these data that compound 1 consisted of DNP-aspartic acid and DNP-proline-aspartic acid and compound 2 was DNP-serine-proline. The great stability of N-acetyl-alanine and certain other N-acetyl amino-acids<sup>12</sup> to mild alkaline hydrolysis explained the small recovery of free

Table 2. SOME PROPERTIES OF THE DNP-AMINO-ACIDS AND DNP-PEPTIDES OBTAINED BY PARTIAL ALKALINE HYDROLYSIS OF THE TETRAPEPTIDES OF 3S  $\gamma_1$ -GLOBULIN AND CARBONIC ANHYDRASE B

DNP-compounds	Maximum absorption (m $\mu$ )	OD <sub>290</sub> OD <sub>280</sub>	Recovery (mole/mole of tetrapeptide)	Relative positions of the amino-acids and peptides in the tetrapeptide	Comments
1	368	0.81	0.40	Aspartic acid	C-terminus
4	385	1.15	0.40	Proline-aspartic acid	The tetrapeptide contained 1 mole of aspartic acid, proline, serine and alanine
2	353	0.56	0.04	Proline	
3	360	0.61	0.36	Serine-proline	
5	360	0.59	0.40	Serine	Substituting amino group of N-terminus
Additional constituent	—	—	0.08	Alanine	
			1.0	Acetyl	

Fig. 1. The amino-terminal amino-acid sequence of human plasma 3S  $\gamma_1$ -globulin and carbonic anhydrase B.

alanine. These findings showed that acetyl-alanine is located at the N-terminus of the tetrapeptides.

This investigation reveals that the amino-acid sequence of the tetrapeptide isolated from both the 3S  $\gamma_1$ -globulin and carbonic anhydrase B is acetyl-ala-ser-pro-asp, and that this peptide represents the N-terminus of both proteins. Thus, the chief component of the plasma 3S  $\gamma_1$ -globulin appears identical with erythrocyte carbonic anhydrase B.

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### Purification and Characterization of Carcino-embryonic Antigens of the Human Digestive System

In previous studies from this laboratory, at least two common tumour-specific antigens, or antigenic determinants, were demonstrated to be present in adenocarcinoma of the human colon<sup>1</sup>. Subsequent investigations demonstrated identical antigens in all malignant tumours of the endodermally derived epithelium of the gastrointestinal tract, liver and pancreas, as well as in

foetal gut, liver and pancreas obtained between two and six months of gestation<sup>2</sup>. These carcino-embryonic antigens (CEA) were absent from all other normal, malignant or otherwise diseased adult tissues and all other foetal tissues tested. It was suggested that the carcino-embryonic antigens may represent cellular constituents which are repressed during the course of differentiation of the normal epithelium of the digestive system and reappear in the corresponding malignant cells by a process of derepressive dedifferentiation. Preliminary investigations with tumour tissue extraction in 0.6 molar perchloric acid led to the conclusion that the CEA were protein-polysaccharide

complexes, and immunological data suggested that the tumour-specific antigenic determinants contained carbohydrate components<sup>1,3</sup>.

The purpose of the present investigation was to attempt to purify and characterize the chemical composition of the CEA found in a single autopsy specimen of a histologically confirmed colonic carcinoma which had metastasized to the ovary. A metastatic rather than a primary tumour was selected in order to obtain a large mass of tumour material from a single source.

The presence of CEA activity in the initial homogenate and at each stage of the purification procedure was determined by precipitin-inhibition and direct Ouchterlony testing against specific anti-CEA antiserum<sup>2</sup>.

The 500 g specimen of tumour tissue was homogenized and then extracted in an equal volume of 1.2 molar perchloric acid. After dialysis against distilled water, the active supernatant was lyophilized and further purified by paper block electrophoresis and then column chromatography on 'Sephadex G-200'. Ultra-violet spectrophotometry at 254 m $\mu$  during the chromatographic procedure gave rise to three zones (Fig. 1). Lyophilization of the antigenically active eluate, which was confined to the first zone, yielded 25 mg of powdered material, representing 0.005 per cent of the initial wet weight of tissue.

Initial ultracentrifugation analysis of the purified CEA was complicated by the presence of a soluble, nitrogen-free, glucose polymer contaminant from the 'Sephadex' gel which sedimented at 2.4S. Complete removal of the contaminant was accomplished only by washing the 'Sephadex G-200' with large volumes of buffer under suction in a sintered glass funnel. Repeat ultracentrifugation

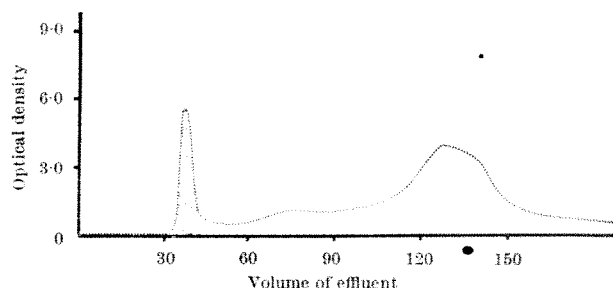


Fig. 1. 'Sephadex G-200' chromatography of the CEA preparation following extraction in 0.6 M perchloric acid and separation by paper block electrophoresis. The shaded area indicates the zone containing the CEA activity.



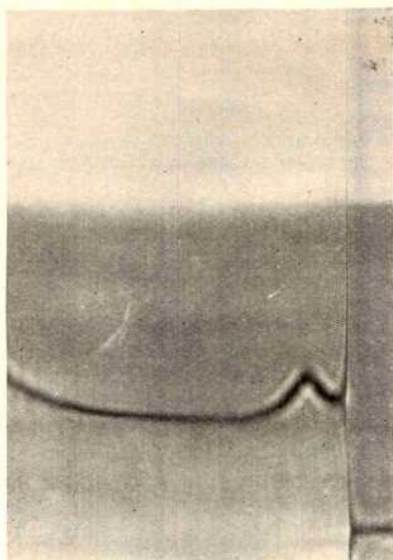


Fig. 2. Ultracentrifugal analysis of the purified CEA preparation. A single peak was obtained at 5.1S.

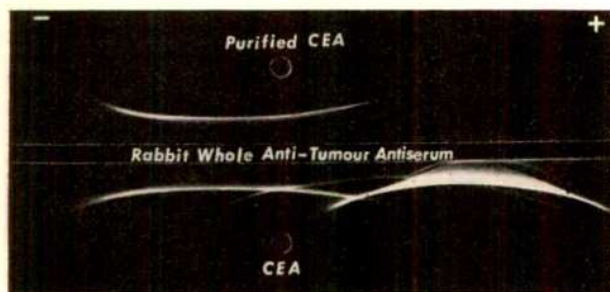


Fig. 3. Immunoelectrophoretic pattern of the perchloric acid extract of the tumour homogenate (CEA), and this extract further purified by paper block electrophoresis and column chromatography on 'Sephadex G-200' (purified CEA), against untreated rabbit anti-human colonic cancer antiserum.

gation studies following removal of the contaminant revealed the presence of a single peak at 5.1S which contained all the antigenic activity (Fig. 2).

Immunoelectrophoresis of the purified CEA preparation was performed against untreated rabbit anti-human colonic cancer antiserum<sup>1</sup>. The result obtained was that of a single precipitin band in the  $\beta$ -globulin region (Fig. 3). This finding indicates the relative immunogenic purity of the preparation, but does not completely exclude the presence of additional weak antigens in the purified CEA preparation.

A 10 mg sample of purified CEA was prepared for qualitative carbohydrate analysis by hydrolysis in a sealed 'Pyrex' tube for 16 h in 0.5 normal hydrochloric acid at 100° C. Analysis of the hydrolysate by descending paper chromatography revealed the presence of galactose, glucose, mannose, fucose and glucosamine. (Some or all of the glucose may have represented contamination from the 'Sephadex' column.) The presence of sialic acid in the purified CEA was demonstrated by the use of Ehrlich reagent according to the technique described by Werner and Odén<sup>2</sup>.

A 5 mg sample of purified CEA was prepared for amino-acid analysis by a modification of the technique described by Lea and Schon<sup>3</sup>. Analysis of the hydrolysate was performed in an amino-acid analyser. The purified CEA was found to contain measurable quantities of eight amino-acids. There were relatively high proportions of tyrosine and lysine with respect to the other six amino-acid residues. If the tyrosine content of CEA is given a

relative value of 1.0, the relative values of the remaining amino-acid residues are approximately as follows: lysine 0.8, aspartic acid 0.3, threonine 0.25, serine 0.3, glutamine 0.3, glycine 0.3, and alanine 0.15.

Further investigations now in progress in our laboratory are designed to compare the quantitative chemical composition of the CEA from a number of different human digestive system cancers with comparable fractions from a variety of other human tissues, both normal and cancerous.

This work was supported by research grants from the National Cancer Institute of Canada, Toronto, Ontario; and the Cancer Research Society, Inc., Montreal, Quebec. The analysis of the amino-acid preparation and the ultracentrifugation studies were performed with the co-operation of Dr A. H. Schon, Department of Chemistry, McGill University.

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### Detection of Genetic Antigens utilizing Gamma Globulins coupled to Red Blood Cells

THE genetic antigens of the human gamma globulin system have been detected primarily by a haemagglutination inhibition reaction<sup>1</sup>. Anti-Gm or Inv antibodies from rare human sera are generally utilized in the test system together with Rh positive red cells coated by specific anti-Rh antibodies. This method has proved of considerable value but also has a number of important limitations. The finding that primate and rabbit antisera appropriately absorbed can replace the rare human sera eliminated one major defect in the system<sup>2,3</sup>. A second major problem has been the dependence on selected anti-Rh antibodies containing the specific genetic antigen required for red cell coating. This problem has been brought to the fore in recent attempts to discover new genetic markers for the subgroups of IgG as well as for IgM and IgA. In many instances incomplete anti-Rh coats could not be found which contained the specific type of protein required. For example, the Gm (n) antigen<sup>4</sup>, which was delineated recently for the IgG2 class<sup>5</sup> of proteins, could only be determined by relatively insensitive precipitation procedures because no suitable anti-Rh coat could be found.

The present investigations were undertaken in an attempt to develop a typing system using red cells coated directly with isolated globulins containing the required genetic antigen. Some efforts in this direction have been made previously utilizing the tanned cell technique<sup>6</sup>. This work was confirmed, but the tanned cell procedure proved inferior for these delicate genetic systems. Considerably greater success was achieved through direct coupling of myeloma proteins to the red cells by the bis-diazotized benzidine (BDB) technique.

Isolation of myeloma proteins and macroglobulins by zone electrophoresis, pepsin-digestion of gamma globulin and isolation of heavy and light chains were performed as previously described from this laboratory<sup>6</sup>. Anti-Gm antisera were obtained by immunizing rabbits, monkeys

\* The nomenclature used for the heavy chain subgroups of IgG is that recently adopted by a subcommittee of WHO: IgG1 (We or  $\gamma$ 2b); IgG2 (Ne or  $\gamma$ 2a); IgG3 (Vi or  $\gamma$ 2c); IgG4 (Ge or  $\gamma$ 2d).

and baboons with isolated myeloma proteins or macroglobulins or respective heavy chains. After absorption with human sera negative for the corresponding genetic factor, the antisera showed specific anti-Gm reactions in agglutination and inhibition tests with reference anti-Rh coated red cells<sup>2</sup>. Anti-Gm (g) 3978<sup>7</sup> and anti-Gm (a) 3070 of human origin were also used.

A stock BDB solution was prepared, and stored as described by Gordon *et al.*<sup>8</sup>. The optimal proportion of BDB and antigen used for coating of a certain amount of red cells was determined for each new batch of BDB stock solution by titrations. The following procedure was generally adopted. To 0.06 ml. of three-times washed and packed<sup>9</sup> human O red cells, 0.06 ml. BDB solution (stock solution diluted 1/15 in 0.15 molar phosphate buffer pH 7.3) was mixed. After 2 min, 1–1.5 mg of isolated gamma globulin (for example, 1 c.c. of a 0.1 per cent solution) was added and the mixture left at room temperature for 15 min. The cells were then washed four times in phosphate buffered saline pH 7.3, resuspended to a 1 per cent dilution, and used the same day that they were coated.

Tests and readings with the coated red cells were routinely done by tube technique, but the slide modification could also be used, as described for the anti-Rh system<sup>6</sup>. When properly coated, the red cells showed no agglutination in saline or in normal human or rabbit serum diluted 1/5, but gave a high titre against standard anti-Fr II antiserum. The coating by this BDB method was performed in excess of antigen. The supernate fluid could be used again for coating of red cells; it retained specific inhibitory capacity, and further addition of antigen did not strengthen the reaction of the coated cells. On the other hand, decreasing amounts of antigen for coating quickly lead to weaker or non-reacting red cells. These findings also compared well with those using reference anti-Rh antibodies<sup>9</sup>.

Table 1. TEST SYSTEMS USING RED CELLS COATED WITH MYELOMA PROTEINS OF DIFFERENT GENETIC TYPES AND ABSORBED PRIMATE AND RABBIT ANTISERA GIVING SPECIFIC AGGLUTINATION

Red cells coated with:	Specificity and titre of absorbed antiserum*
Myel. Ke Gm (a+)	Anti-Gm (a)† 1/300
Myel. Fe Gm (b+)	Anti-Gm (b) 1/32
Myel. Gr Gm (f+)	Anti-Gm (f) 1/50
Myel. Vi Gm (g+)	Anti-Gm (g)† 1/128
Myel. Ne Gm (n+)	Anti-Gm (n)† 1/64

\* The titre given is the working titre utilized and not the highest titre showing specificity.

† Primate antisera.

For Gm testing, myeloma proteins of Gm (a), Gm (g), Gm (f), Gm (b) and Gm (n) type were coupled to red cells by BDB and used with respective anti-Gm antisera (Table 1). In the Gm (a), Gm (f) and Gm (b) tests different myeloma proteins were selected for coating than those used for immunization, thus eliminating the problem of individual specific determinants<sup>6</sup>. For Gm (g) and Gm (n) typing the homologous system was used and the individual specific antibodies were absorbed out by the respective myeloma proteins digested by pepsin (refs. 4 and 7, and results to be published).

Table 2 illustrates typical inhibition reactions for the different systems as utilized for the Gm typing of sera. Gm (a+) or Gm (f+) sera inhibited the Gm (a) and Gm (f) systems in dilutions of 1/640. Gm (g), Gm (b) and Gm (n) inhibition was in the range of 1/40 to 1/160. In contrast, negative sera diluted 1/2 did not inhibit. Inhibition with myeloma proteins also gave identical results and showed similar inhibitory capacity whether anti-Rh coated or myeloma protein coated cells were used. A protein, positive for a certain Gm factor, showed inhibition in concentrations down to 4–16 µg/ml. Gm typing of human sera was done at the dilution 1/10 and gave clear readings with negative controls. Fifty selected Caucasian and Negro sera were studied by the BDB technique and in all instances complete concordance with the anti-Rh

Table 2. INHIBITION REACTIONS FOR Gm TYPING USING RABBIT AND PRIMATE ANTIBODIES AND RED CELLS COATED BY MYELOMA PROTEINS

Typing system	Phenotype of inhibitor serum*	Degree of agglutination at progressive dilutions of inhibitor						No inhibitor
		2	10	40	160	640	2,560	
Gm (a)	a + g + f - b - n -	0	0	0	0	0	2	2
	a - g - f + b + n +	2	2	2	2	2	2	
Gm (f)	a - g - f + b + n +	0	0	0	0	0	2	3
	a + g + f - b - n -	2	3	3	3	3	3	
Gm (b)	a - g - f + b + n +	0	0	0	2	3	3	3
	a + g + f - b - n -	3	3	3	3	3	3	
Gm (g)	a + g + f - b - n -	0	0	1	3	3	3	3
	a - g - f + b + n +	3	3	3	3	3	3	
Gm (n)	a - g - f + b + n +	0	0	0	Tr	3	3	3
	a + g + f - b - n -	2	3	3	3	3	3	
	a - g - f + b + n +	2	3	3	3	3	3	
	a + g + f - b - n -	2	3	3	3	3	3	

\* The underlined letters designate the specific antigen tested.

system was obtained. The Gm (n) system was studied in particular detail. Seven Gm (a + g + f - b -) sera were Gm (n-). Of fifteen Gm (a + g + f + b +) sera, six were Gm (n+) and nine Gm (n-). Of eighteen Gm (a - g - f + b +) sera, sixteen were Gm (n+) and two Gm (n-). The Negro sera were Gm (a + g - f - b +) and Gm (n-). The several Gm (f + n -) sera were of special interest and were tested at least twice. They gave a clear negative pattern in the Gm (n) test. All the results were the same as for the precipitation system<sup>4</sup>.

Red cells coated with pooled gamma globulin were also used for the test systems involving the principal subgroup of gamma globulin and worked well for Gm (a) and Gm (f). Gamma globulin fragments and chains could be utilized as coating agents and isolated heavy chains of the γG3 myeloma protein Vi were advantageously used for red cell coating and typing for Gm (g). Anti-Gm factors from human sera specifically agglutinated the myeloma protein coated red cells. Red cells coated with Gm (g) myeloma protein Vi were agglutinated by anti-Gm (g) 3978 which was used at the dilution 1/32 for typing both myeloma proteins and human sera. Corresponding results were obtained with the Gm (a) myeloma protein Ke and anti-Gm (a) 3070. The system was also utilized for Inv (1).

The myeloma proteins thus coupled to red cells by BDB showed a specificity and sensitivity very similar to the system utilizing anti-Rh antibodies. Although the agglutinates dispersed somewhat more readily than those with anti-Rh coated cells, the agglutination was strong and could be read after centrifugation within minutes. This new method offers a valuable supplement to the anti-Rh antibodies used for coating. The main importance is the extension to globulins of classes or subclasses where the anti-Rh system cannot be used. More than one hundred high titre incomplete anti-Rh sera were tested without finding anti-Rh antibodies which revealed IgG2, IgG4, IgA or IgM antigenic characters. All these classes of immune globulins could be coated, however, on red cells which then gave agglutination reactions with rabbit and primate antisera which were class specific. In the case of the IgG2 class, the additional Gm (n) specificity was readily demonstrated and utilized for genetic typing.

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# Autotransplantation and Homotransplantation of Thyroid Gland in the Hamster Cheek Pouch

EXPERIMENTAL transplantation of the thyroid gland has been widely investigated<sup>1,2</sup>. As a rule autotransplantation of the adult thyroid is easily attained, but positive results with homotransplantation in laboratory animals (mammals) are sometimes achieved only after additional treatment with corticosteroids<sup>3,4</sup> or after implantation in the anterior lye chamber<sup>5,6</sup>, brain or, especially in the case of the guinea-pig<sup>7</sup>, in the testes.

In 1951, Lutz *et al.*<sup>8</sup> first used the hamster cheek pouch for transplantation of tumours. The cheek pouch is a favourable immunologically tolerant site not only for homo and heterotransplantation of tumours, but also for such normal tissues as skin<sup>9</sup>.

Here we report the survival of thyroid tissue implanted in the cheek pouch. This indicates the acceptability at this site of a highly specialized organ.

Fifty-eight male and female non-inbred Syrian hamsters (*Mesocricetus auratus* Waterhouse, 1839) were used. The donors and recipients were of the same sex and about 3 months old. Table 1 shows the experimental conditions and the results. The cheek pouches of the animals with transplants were checked weekly, and the animals were killed between the forty-fifth and ninety-third days after transplantation. Transplants were only considered to have survived if certain features were present: viable transplants are commonly reddish and surrounded by a set of vessels. They have a typical appearance under the microscope (Fig. 1) and neither degenerative parenchymal changes nor inflammatory reactions are evident.

Table 1. EXPERIMENTAL GROUPS AND RESULTS AFTER TRANSPLANTATION OF A HALF THYROID GLAND IN CHEEK POUCH OF THE SYRIAN HAMSTER

Group	No. of animals	Treatment before transplantation	Mode of transplantation	No. of takes Positive	Negative
I	13	Thyroidectomy (surgical only)	auto	12	1
II	16	Thyroidectomy (surgical or after 500 $\mu$ c. <sup>131</sup> I treatment)	homo	13	3
III	19	Non-treated	homo	4	15

The follicles of the transplants from groups I and II were smaller than those of group III, while in the normal gland *in situ* the larger follicles were distributed at the periphery. The cells of the first two groups were cuboidal or cylindrical but in group III they were lower. The size and appearance of the follicles and cells depend to some extent on the



Fig. 1. Microscopic structure of a gland 45 days after homotransplantation. (Haematoxylin and eosin,  $\times c. 40$ .)

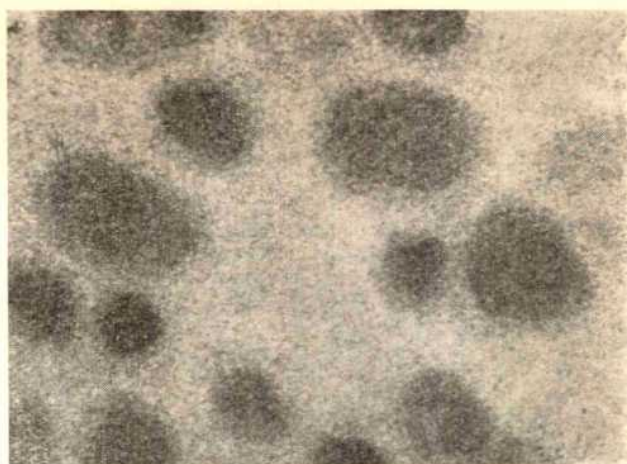


Fig. 2. Autoradiograph from the same gland ( $\times c. 70$ ).

mechanical pressure exerted by walls of the pouch. A colloid was abundantly present in the follicles from group III, while in groups I and II it was not homogeneous after staining with eosin and periodic acid-Schiff, and many vacuoles were visible at the internal cell apex. The nuclei of the cells were dark and had a coarse chromatin network. About 10–20 per cent of the follicular and inter-follicular cells have a clearer cytoplasm and nucleus.

We thyroidectomized some of the animals from group III several days before killing them in order to verify that pituitary extract stimulated the transplants. It was found that the latter are more like the transplants of groups I and II than others.

The animals examined had been injected intraperitoneally with 2  $\mu$ c. of <sup>131</sup>I sodium iodide 6 h before they were killed. After fixation paraffin slides of their thyroid glands were mounted on strips of Kodak 'AK 10' stripping films.

Under the experimental conditions used, inorganic iodine-131 was taken up only by the tissues from groups I and II, that is, in the animals deprived of thyroids *in situ*. Accumulation of radioactive iodine was high and irregular in different follicles (Fig. 2).

These results show that the cheek pouch of the Syrian hamster is a convenient site for thyroid transplantation. A much better survival of homotransplants was observed in thyroidectomized animals. This supports Halstead's views<sup>10</sup>, which have been contested in recent years by some authors<sup>11</sup>.

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## PATHOLOGY

**Effect of Cyclophosphamide, 6-Mercaptopurine or Methotrexate on the Furth Rat Leukaemia**

In the course of experiments designed to test the effect of new cytotoxic chemicals on the Furth leukaemia in the rat, we decided to examine some clinically established cancer chemotherapeutic agents. Because we believed that the action of the agents might have an immunological basis, each was tested alone and in combination with Freund's complete adjuvant.

The Furth leukaemia used came, in 1964, from the Chester Beatty Research Institute which, in turn, received it from Dr Jacob Furth in 1957. The special strain of Wistar rats had the same history and have been maintained as a closed colony since they were received in Leeds. The leukaemia was passed through male rats by the intraperitoneal injection of 750,000 leukaemia cells in citrate saline. This leukaemia kills its hosts 9–16 days after inoculation. The total white cell count immediately before death always exceeds 100,000 and is usually 150,000 to 200,000 cells/mm<sup>3</sup>. The liver is haemorrhagic and enlarged, often with a ruptured capsule. The spleen is enlarged and friable, with prominent Malpighian bodies. The mediastinal, para-aortic, lumbar and mesenteric lymph nodes are massively, and the axillary and inguinal glands slightly, enlarged. The killing time of this leukaemia has previously been delayed by treatment with *o*-merphalan<sup>1</sup> and, to a lesser extent, with CB1718 (hydantoin-5-spiro-2'-(*x*-di-2'-chloroethylamino)-1', 2', 3', 4'-tetrahydronaphthalene)<sup>2</sup>.

Young adult rats (120–150 g) of either sex were maintained on 'Oxo' diet 41B and water *ad libitum*. Freund's complete adjuvant, 'Endoxana' cyclophosphamide, 6-mercaptopurine and methotrexate were administered as shown in Table 1.

The results (Table 1) show that Freund's complete adjuvant induced no increase in survival, compared with

the control animals, and that 6-mercaptopurine or methotrexate increased the survival time by about 60 per cent, which is of marginal significance. The animals treated with cyclophosphamide, however, showed, when the experiment was terminated, a mean survival of 112 days, while two animals were still alive at 210 days. Freund's complete adjuvant increased this survival to a mean of 170 days, with eight rats still alive at 210 days ( $P=0.012$ ) (ref. 3).

A further four experiments were carried out to confirm and amplify this result. In four of the five experiments, cyclophosphamide induced a considerable lengthening of life. Freund's complete adjuvant, by itself, had no effect on the leukaemia. When the cyclophosphamide was injected shortly after the rats were inoculated with the leukaemia (Table 2, experiments 1–3) a further marked increase in survival resulted with Freund's complete adjuvant. On the other hand, when the cyclophosphamide treatment was delayed until the total white cell count was beginning to rise, that is, until the position approached that of a clinically detectable leukaemia, Freund's complete adjuvant had no significant effect (Table 2, experiments 4, 5).

The fact that cyclophosphamide alone, or, more often, in combination with Freund's complete adjuvant enabled rats to survive for more than 200 days, instead of the normal 9–16 after inoculation, led us to attempt to determine whether or not they still carried the leukaemia. To this end, the ten survivors in experiment 1 (Table 2) were treated as follows: three (one of which had originally received cyclophosphamide alone) were killed and examined; a similar group was re-inoculated with 750,000 cells from the Furth leukaemia, and the remaining four were re-inoculated and treated with cyclophosphamide on days 1 and 4, and Freund's complete adjuvant on days 8, 11 and 12 thereafter.

Two of the three rats killed at 210 days after the initial treatment had total white cells counts of 22,300 and 18,100 cells/mm<sup>3</sup> respectively. No abnormality apart from

Table 1. EFFECT OF THREE CHEMOTHERAPEUTIC AGENTS, WITH AND WITHOUT FREUND'S COMPLETE ADJUVANT, ON THE FURTH LEUKAEMIA IN RATS DERIVED FROM THE CHESTER BEATTY RESEARCH INSTITUTE

Group	Treatment	No. of rats	Mean survival $\pm$ standard deviation (days)	Survivors	Notes
1	None	5 M 5 F	11.1 $\pm$ 0.5		
2	FCA* on days 1, 4, 7, 8 (subcutaneously)	5 M 5 F	11.1 $\pm$ 0.3		
3a	Cyclophosphamide (5 mg/100 g rat) on days 1, 4 (intraperitoneally)	5 M 5 F	111.7	2 at 210 days	From survivors at 150 days $P=0.012$ (exact method for 2 $\times$ 2 tables) <sup>3</sup>
b	Cyclophosphamide (5 mg/100 g rat) on days 1, 4 (intraperitoneally) + FCA on days 8, 11, 13 (subcutaneously)	5 M 5 F	173.0	8 at 210 days	
4a	6-mercaptopurine (5 mg/100 g rat) on days 1, 5, 11, 13 (intraperitoneally)	5 M 5 F	17.7 $\pm$ 1		
b	6-mercaptopurine (5 mg/100 g rat) on days 1, 5, 11, 13 (intraperitoneally) + FCA on days 8, 11, 13 (subcutaneously)	5 M 5 F	17.2 $\pm$ 1		
5a	Methotrexate (5 mg/100 g rat) on day 1 (intraperitoneally)	5 M 5 F	17.8 $\pm$ 2		
b	Methotrexate (5 mg/100 g rat) on day 1 (intraperitoneally) + FCA on days 8, 11, 13 (subcutaneously)	5 M 5 F	16.3 $\pm$ 2		

\* FCA, Freund's complete adjuvant (0.5 ml./100 g rat).

Table 2. ACTION OF CYCLOPHOSPHAMIDE AND FREUND'S COMPLETE ADJUVANT ON THE FURTH LEUKAEMIA IN RATS DERIVED FROM THE CHESTER BEATTY RESEARCH INSTITUTE

Experiment	Treatment and days after inoculation	No. and sex of rats	Survival (days)	Mean survival (days)
	Cyclophosphamide (5 mg/100 g rat) intraperitoneally			
	Freund's complete adjuvant (0.5 ml./100 g rat) subcutaneously			
1†	1, 4	5 M, 5 F	10, 11, 11, 11, 11, 11, 11, 11, 12, 12	11.1
	1, 4	5 M, 5 F	28, 28, 69†, 75, 113, 127, 128, 129, 210*, 210*	111.7
	1, 4	5 M, 5 F	11, 11, 11, 11, 11, 11, 11, 11, 11, 12	11.1
	1, 4	5 M, 5 F	22†, 28†, 210*, 210*, 210*, 210*, 210*, 210*, 210*, 210*	173.0
2	3, 5	3 F	10, 10, 10	10.0
	3, 5	6 F	13, 13, 13, 19, 20, 20	16.3
	3, 5	5 F	9, 9, 9, 10, 10	9.4
	3, 5	6 F	59, 148, 178, 201, 205, 254*	174.2
3	2, 5	6 M	10, 10, 10, 10, 10, 10	10
	2, 5	6 M	19, 28, 30, 41, 44, 86	41.3
	2, 5	6 M	31, 41, 46, 300*, 300*, 300*	169.7
4	8, 12	6 F	12, 13, 13, 13, 13, 14	13.0
	8, 12	6 F	40, 42, 43, 44, 47, 50	44.3
	8, 12	6 F	39, 42, 42, 47, 55, 68	48.8
5	8, 11	6 M	13, 13, 13, 14, 14, 14	13.5
	8, 11	6 M	43, 45, 45, 48, 68*, 68*	46.2
	8, 11	6 M	27, 42, 42, 42, 43, 43	40.0

\* Rat surviving. † Died without leukaemia on naked eye examination. ‡ From Table 1.



bronchiectasis was seen at post-mortem examination. Histological examination confirmed the bronchiectasis and showed reactive hyperplasia and mild fibrosis in the associated mediastinal lymph nodes. There was no evidence of leukaemia. The third rat had a slightly raised total white cell count of 32,000 cells/mm<sup>3</sup> (polymorphs 35 per cent, lymphocytes 55 per cent, monocytes 5 per cent, eosinophils 3 per cent and basophils 2 per cent) and post-mortem examination showed severe bronchiectasis and a large pulmonary abscess, together with slight enlargement of the para-aortic and mediastinal lymph nodes. Histological examination of these nodes showed, in addition to inflammatory changes, areas in which the normal lymph node structure was replaced by sheets of leukaemic cells, indicating that this animal still had a residual leukaemia. At post-mortem, blood from these three rats was withdrawn and, in each case, an amount containing 750,000 white cells was injected into two further rats. These six animals are still surviving 69 days afterwards. The group of three rats which were reinoculated with the Furth leukaemia died at 13, 14 and 14 days—a survival which is similar to that of a group of untreated rats inoculated with the same blood (15 days). The reinoculated rats which received both cyclophosphamide and Freund's complete adjuvant are still alive 69 days after the introduction of the leukaemia.

The fact that one of the three rats which were killed 210 days after inoculation showed microscopic evidence of leukaemia is clear evidence that treatment with cyclophosphamide and Freund's complete adjuvant does not completely eradicate the leukaemia. This is supported by the observation in experiment 2 (Table 2) that rats treated in the same way died of leukaemia after 201 and 205 days. Their leukaemia was demonstrated by examination with the naked eye. Nevertheless, the increase in survival brought about by the treatment is impressive. The effect of the antimetabolites 6-mercaptopurine and methotrexate is in no way changed by the use of Freund's complete adjuvant. On the other hand, the effect of the cytotoxic cyclophosphamide is enhanced by the use of the adjuvant.

These results are difficult to explain. They could be accounted for by the suggestion that the cyclophosphamide destroys a high proportion of the leukaemic cells by direct cytotoxic action and by stimulating an immunological attack against the products of the chemical reaction between it and various cellular constituents. The action of Freund's complete adjuvant would then be to heighten this immunological attack and to reduce further the number of fully viable leukaemic cells. This property of the adjuvant is effective only when the number of leukaemic cells is small for a period shortly after inoculation but is, presumably, swamped when the leukaemia begins to develop. The alternative explanation, that Freund's complete adjuvant acts by enhancing an immunological attack on the cellular constituents of the Furth leukaemia itself, is rendered less likely by the failure to demonstrate a second set response when rats which had survived for more than 200 days after the first inoculation were reinoculated.

We thank Dr Jean A. S. Pringle for the histological examinations, Mr G. Littlewood for maintaining and treating the animals and Dr D. B. Clayson for help in the preparation of this paper.

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## "Enteque Seco", Calcification and Wasting in Grazing Animals in the Argentine

"ENTEQUE SECO" is the name given to a wasting disease of grazing animals frequently encountered in the central eastern and south-eastern areas of the province of Buenos Aires and which is a cause of considerable economic loss to livestock producers.

The disease was first reported in 1898 by Lignières<sup>1,2</sup>, who thought it might result from chronic pasteurellosis. Eckell *et al.*<sup>3</sup> have discussed in some detail the various factors involved in its aetiology, and Arnold and Bras<sup>4</sup> drew attention to the similarity between enteque seco and Naalehu disease in Hawaii and Manchester wasting disease in Jamaica.

Enteque seco seems to be confined to the poorer, swamper areas of the province of Buenos Aires on well defined soil types<sup>5</sup>. It is most often seen in Aberdeen Angus, Shorthorn and Hereford breeding cows, because these are the animals most commonly run in the affected areas. Bulls, heifers, and steers can also be affected, however, and other breeds of cattle such as the Friesian are known to be susceptible. Sheep and horses are not often found with the disease, but lesions have been found at necropsy in both species<sup>2,6</sup>.

The incidence of the disease is difficult to assess accurately, but some herds have been found with as many as 10 per cent of animals affected. The incidence of the disease is known to vary with the year, the season and the site.

The earliest symptoms are a stiffened and painful gait with a progressive loss of weight and symptoms of ill-thrift and emaciation. If the animals are removed in the early stages from the affected areas they recover quickly, but they may eventually die if they are left.

In advanced cases the radiocarpal joints thicken and become stiff and the distal joints become very straight. In more advanced cases the joints cannot be extended completely, the thoracic limbs bow outwards and the animals tend to "buckle over" and walk with an arched back, carrying the weight on the forepart of the hooves (Fig. 1). The shoulders appear stiff and deformed and the articulation of the forequarters is distinctly abnormal. The head is usually carried very erect.

Such animals are often excitable and tire easily if they are made to exercise. They may show symptoms of acute cardiac and pulmonary insufficiency. If they are removed from the affected areas at this stage the severe stiffness and deformity are not lessened, but the wasting is usually stopped and the animals may gain weight if moved to good pasture. Practical experience has shown that these animals are not suitable breeders and they are usually culled and slaughtered.

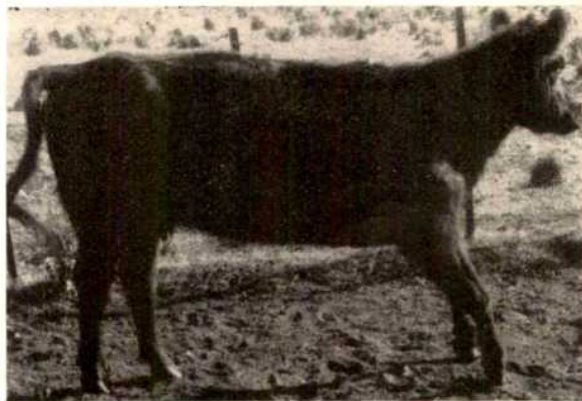


Fig. 1. A young Aberdeen Angus cow with advanced enteque seco showing typical stance of severely affected animals. The stiffened, bowed forelimbs and deformed shoulder with the weight taken on the forepart of the hooves are characteristic. Note also erect carriage of head and dull, wasted appearance.



At necropsy the animals show a widespread metastatic calcification of the vascular system and the soft tissues. The heart and aorta invariably show the most marked effects (Fig. 2). The left auricle and ventricle are invariably more affected than the right parts of the heart.

Calcareous deposits are often found in the lungs, particularly around the periphery of the diaphragmatic lobes (Fig. 3). These lesions appear to develop later than those in the vascular system: the heart and the vessels are frequently found damaged without any effect on the lungs, but lung damage is never found alone.

The cartilage of the appendicular skeleton is always eroded in advanced cases, and the bearing surfaces of the leg joints take on a very worn appearance, being almost completely devoid of cartilage. The bearing surfaces are also often eroded.

The liver and spleen appear to remain unaffected. The kidneys are sometimes affected by diffuse damage, with some calcification of the pelvic region<sup>7</sup>.

Ruksan *et al.* have shown that animals pasturing in areas known to produce the disease have high concentrations of calcium and inorganic phosphorus in the blood<sup>8</sup>. Concentrations of these two elements ranging from 25 to 50 per cent above normal are often found. These blood concentrations change within 48–72 h when the affected animals are moved to good pasture. We think that these high concentrations of calcium and phosphate on the blood are the underlying cause of the calcification found in affected animals. No infectious processes appear to be involved, and the symptoms are similar to those found in vitamin D intoxication<sup>7</sup>.

There is no evidence from soil analyses to throw light on the change of blood calcium and inorganic phosphate<sup>8,10</sup>. Also, the concentrations of calcium and phosphorus in bulk forage samples from affected areas seem to be essentially normal<sup>9</sup>.

A mixed forage sample from an area prone to entequ seco was recently shown to be able to raise the concentration of calcium and phosphorus in the blood of 2 year Aberdeen Angus heifers when given in the form of air-dried and milled material by way of rumen fistulae. As little as 1 kg of the material, given as a single dose, increased the calcium and phosphorus concentrations for 4–5 days<sup>11</sup>. When the samples of the original plant material were ashed, the activity was lost. Much of the activity could be recovered in the aqueous fraction after extraction with water, and remained in the supernatant after extraction with 80 per cent alcohol<sup>12</sup>. On the basis of these findings, it was thought that a specific principle or principles was implicated which was soluble in water and organic, non-protein and non-allergenic in nature.

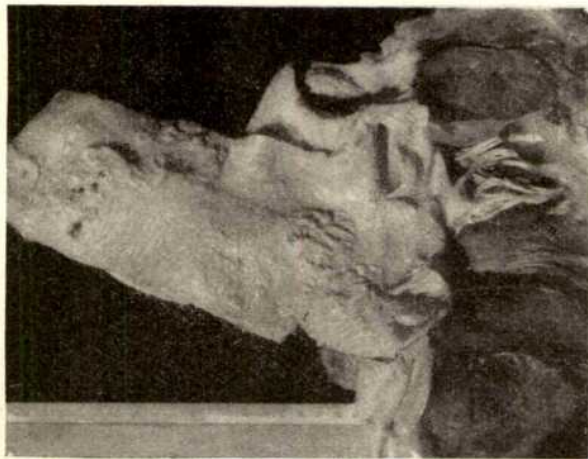


Fig. 2. Massive calcification of the aorta and heart from an affected animal. The enlarged and heavily mineralized chordae tendineae are a quite typical feature.



Fig. 3. The cut edge of a diseased lung showing advanced calcification. The latter is usually most prominent around the periphery of the diaphragmatic lobes.



Fig. 4. *Solanum malacoxylon* Sendtner. Balcarce, Province of Buenos Aires.

Thirty-six species were found in the sample<sup>13</sup>, and seven were collected for further investigation. *Solanum malacoxylon* Sendtner (probably conspecific with *Solanum glaucum* Dun.<sup>14,15</sup>) was later found to be capable of reproducing the results found in fistulated animals dosed with the whole sample. Extracts of *Solanum malacoxylon* in water are also active. As little as 5 g of dry matter given as a single dose by stomach tube can produce changes in the calcium and phosphorus in the blood of 900 lb. heifers for several days. A similar effect can be produced in 100 lb. Romney ewes by as little as 0.5 g<sup>11,12</sup>. These experiments were carried out using leaves and stems of *S. malacoxylon* (about one-third leaf, two-thirds stem, on a dry matter basis). Later work has shown that almost all the toxin is contained in the leaves, so that the amount of leaf material alone required to produce the effect can be assumed to be even less.

*S. malacoxylon* is distributed over the area in which the disease is known to occur, such anomalies as may exist being explicable on the basis of the transfer of animals between areas (which may occur frequently), variations in animal management or in the toxicity and palatability of the plant. The plant is generally fairly unpalatable, but this does not preclude its ingestion in small amounts by animals grazing freely, as has been found in the field<sup>16</sup>.

Although many questions remain to be answered, some points are clear. The majority of plant species growing in entequ seco areas are common species with wide distributions in areas outside those in which the disease is known



to occur. A number of indigenous species which might be thought to contribute to the disease have been shown to be unable to raise the concentrations of inorganic phosphorus and calcium in the blood. The fact that the careful removal of all traces of *S. malacozylon* from representative samples of mixed herbage results in the complete inactivation of the sample would seem to show that only *S. malacozylon* is involved in causing the disease<sup>12</sup>.

The hypothesis that *S. malacozylon* is the only species involved in causing enteque seco is being tested further in experiments that are now in progress.

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### Demonstration of Copper and Acid Phosphatase Activity in Hepatocyte Lysosomes in Experimental Copper Toxicity

EXCESSIVE amounts of copper accumulate within the tissues in Wilson's disease (hepatolenticular degeneration). Cirrhosis is believed to be an inevitable consequence of chronic copper toxicity<sup>1</sup>. Attempts to produce equivalent hepatic dysfunction and cirrhosis in experimental animals by copper poisoning have been unsuccessful, however<sup>2</sup>.

In Wilson's disease, high concentrations of copper have been localized to acid phosphatase-rich hepatocyte lysosomes by combining electron probe microanalysis with enzyme cytochemistry<sup>3</sup>. In rats poisoned with copper the metal has been demonstrated by light microscopy staining methods<sup>4-6</sup> in pericanalicular granules which resemble, in appearance and cytoplasmic location, lysosomes visualized in acid phosphatase preparations. In experimental copper toxicity, however, acid hydrolase activity and copper have not been visualized in the same preparation and in the same granules. It seemed worth while to find out whether the copper granules in these animals also showed acid hydrolase activity and were in fact lysosomes<sup>7,8</sup>.

This was accomplished by staining for both copper<sup>9,10</sup> and acid phosphatase activity<sup>11</sup> on the same section, with fairly specific procedures.

Specimens of liver, obtained from rats given drinking water containing 0.25 per cent copper sulphate for several months, were fixed overnight in cold 4 per cent neutral formaldehyde containing 1 per cent calcium chloride. Frozen sections (10 $\mu$ ) were incubated for acid phosphatase activity in the naphthol AS-TR phosphate-hexazotized pararosaniline medium of Barka and Anderson<sup>12</sup>, washed

thoroughly and then immersed very briefly in Timm's silver nitrate-hydroquinone solution<sup>9,10</sup>. Timm<sup>9,10</sup> has shown that in tissues with very high concentrations of copper the metal may be stained black by simply immersing a section in a solution of silver nitrate and hydroquinone. This reaction is less sensitive but more specific than the more common silver sulphide procedure for visualizing copper<sup>3,5,9,10,12</sup>. In the latter procedure the metal is first sulphated by exposing the tissue to hydrogen sulphide. When the sulphide step is omitted the silver solution does not make iron visible, iron being the only other metal likely to be present in liver in large enough quantities to stain. This solution, prepared just before use, is made up of one part of 5 per cent silver nitrate and 5 parts of a mixture of 2 g hydroquinone and 5 g citric acid in copper-free distilled water. The doubly stained sections were dehydrated and mounted in 'Permount'.

Black grains of silver, marking the sites of copper, were deposited on pericanalicular cytoplasmic granules which had been stained red by virtue of their acid phosphatase activity, identifying the granules as lysosomes<sup>7,8</sup> (Fig. 1). Difficult to demonstrate in a black and white photomicrograph, the combination of red and black was striking when viewed in the microscope. Not all lysosomes contained stainable copper. The silver solution did not alter the acid phosphatase reaction product, but fewer copper granules could be seen in sections that had been incubated for enzyme activity. Similar cytoplasmic granules were made visible by staining unincubated sections with rubeanic acid, considered to be a specific stain for copper<sup>13</sup>. Normal rat liver did not stain with the silver solution and rubeanic acid.

Although copper, in high concentrations, is localized to hepatic lysosomes in Wilson's disease<sup>3</sup> and in animals intoxicated with copper, the absence of cellular necrosis and cirrhosis in experimental copper poisoning<sup>2,4,5</sup> suggests that an additional factor is responsible for liver damage in the human disorder. The possibility must be considered that some unknown factor in Wilson's disease may damage the lysosomal membrane, leading to release

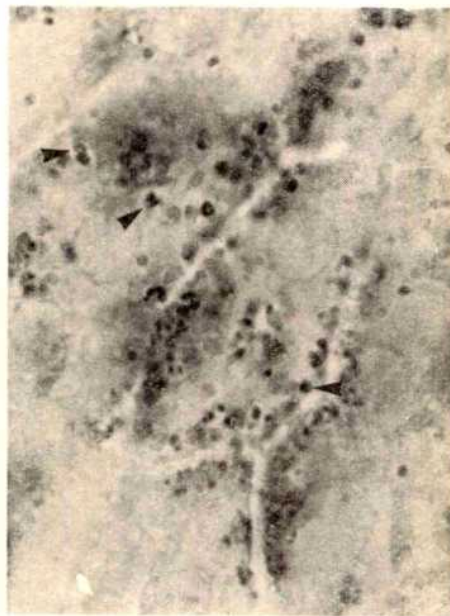


Fig. 1. Frozen section of rat liver poisoned with copper. This specimen contained, 1245 $\gamma$  of copper/g wet weight of liver. Normal concentration is approximately 10 $\gamma$ . The section was incubated for acid phosphatase activity<sup>11</sup> and then stained for copper. The black stain marks the site of copper and is deposited on many of the less darkly stained (red on the slide) lysosomes made visible by the acid phosphatase procedure. This is difficult to demonstrate in a black and white micrograph, but both stains are evident in some granules (arrows). ( $\times$  2,000.)



of copper into the cytoplasm and cell death. At present, we have no evidence that such a release takes place.

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## BIOCHEMISTRY

### Dietary Starch, Dietary Sucrose and Hepatic Pyruvate Kinase in Rat

It has been pointed out<sup>1</sup> that a low activity of hepatic pyruvate kinase (ATP-phosphotransferase *EC* 2.7.1.40) favours gluconeogenesis, and a high activity favours lipogenesis from oxidizable substances. These experiments with rats showed that the activity of the enzyme was

Table 2. PYRUVATE KINASE ACTIVITY OF RAT LIVER

Activity as $\mu\text{moles/min/g}$ fresh weight ( $\pm S.E.$ )						
Diet	No. of rats	Days on diet	Age at start (days)	Weight at start (g)	Weight at end (g)	Pyruvate kinase activity
60 per cent sucrose	6	10	39	93	134	102.7 $\pm$ 15.4
60 per cent starch	6	10	39	95	134	20.2 $\pm$ 2.1
Carbohydrate-free, I	5	10	39	96	142	10.4 $\pm$ 1.3
Carbohydrate-free, II	4	10	39	97	149	7.6 $\pm$ 0.9
Total carbohydrate-free	9	10	39	97	145	9.2 $\pm$ 0.9
72 per cent sucrose	5	98	28	42	283	63.0 $\pm$ 5.8
72 per cent starch	5	98	28	41	281	28.1 $\pm$ 3.8

rapidly affected by alterations in the amount of carbohydrate in the diet, being highest when the diet contained 80 per cent carbohydrate (as sucrose), less when it contained 55 per cent carbohydrate (mostly as starch) and least when it contained no carbohydrate.

In view of recent findings that levels of blood lipids and amounts of adipose tissue in rats are changed when the dietary carbohydrate is changed from starch to sucrose<sup>2</sup>, we have measured the activity of hepatic pyruvate kinase in rats given diets with the one or the other carbohydrate at the same levels.

Male hooded rats of the Lister strain were used for the experiments. The composition of the diets is shown in Table 1. The enzyme activity was estimated by the method of Bücher and Pfeleiderer<sup>3</sup>, except that the concentration of adenosine diphosphate was increased to  $1.3 \times 10^{-3}$  molar. Preliminary experiments with weanling rats showed that purified diets with 60 per cent sucrose gave an enzyme activity three or four times higher than that given by diets with 60 per cent starch. The effect was seen after 3 days on the diet, and there was only a

Table 1. COMPOSITION OF DIETS  
Male hooded rats were given these diets, and water, *ad libitum*. Quantities are in g/km

	Carbo- hydrate- free, I	Carbo- hydrate- free, II	Sucrose 60 per cent	Starch 60 per cent	Sucrose 72 per cent	Starch 72 per cent
Sucrose	0	0	600	0	720	0
Starch	0	0	0	600	0	720
Casein	700	500	240	240	180	180
Malze oil	0	0	0	0	20	20
Butter	0	0	0	0	30	30
Arachis oil	0	0	80	80	0	0
Margarine	220	420	0	0	0	0
Salt mixture	50	50	50	50	50	50
Vitamin mixture	0.058	0.058	0.058	0.058	0.058	0.058
Choline chloride	1	1	1	1	1	1
Solka floc	30	30	30	30	0	0

slight increase in the enzyme activity on either diet after another seven days.

The results of two further experiments are shown in Table 2. In each instance, the difference in activity of pyruvate kinase between the dietary treatments is highly significant ( $p < 0.001$ ). The highest level of activity was found in rats that were put on the experiment when they were 39 days old, and fed the purified diet with 60 per cent sucrose for 10 days. The results of this experiment parallel closely those<sup>1</sup> in which the diet with 80 per cent sucrose gave an enzyme activity about ten times that given by carbohydrate-free diets. Our results with the starch-containing diet suggest, however, that the intermediate level found by these authors with diets containing 55 per cent carbohydrate as starch was due more to the type of carbohydrate than to its quantity.

The difference in pyruvate kinase activity in rats fed sucrose or starch for 98 days, though still highly significant, was less than in rats fed for only 10 days. It is possible that this was caused by the differences in the type of dietary fat, but it may be that the animals gradually adapt to the differences in carbohydrate.

Differences in enzyme activity produced by the different levels of fat in the two diets free of carbohydrates were not significant at a level of  $P = 0.05$ . We might expect significant differences with greater differences in fat content; as it is, the differences are in the expected direction in view of the higher ketogenic potential of the diet higher in fat.

The observation<sup>1</sup> was confirmed that a diet with carbohydrate produces a higher activity of hepatic pyruvate kinase in rats than a diet free from carbohydrate. We must add, however, that the activity of the enzyme also depends on the type of dietary carbohydrate, as sucrose produces a considerably higher activity than does starch.

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### Milk and Serum Iron and Iron-binding Capacity in the Rabbit

IN the course of a study of protein metabolism during lactation in the rabbit it was found that iron added to the whey fraction of rabbit milk changed the colour from yellow to bright pink. The colour change was similar to that observed when iron is added to the serum iron-binding protein, transferrin. It suggested that rabbit milk contains a high concentration of similar protein. This would be a most unusual finding, and it prompted an investigation of the iron concentration and iron-binding capacity of rabbit whey and rabbit serum during lactation.

Samples of blood and milk were obtained at intervals during lactation from the first to about the thirtieth day of lactation. The blood, obtained by incision of the marginal ear vein, was allowed to clot and the serum was used for measurements of iron and total iron-binding capacity (TIBC)<sup>1</sup>. Milk was expressed with care from the mammary glands to avoid contamination with iron. Part was used to estimate the whole-milk iron concentration<sup>2</sup>. The remainder was centrifuged at 4° C for 1 h at 25,000*g*, and the clear layer between fat and casein removed and recentrifuged as before. The resulting clear whey was used to determine the total iron-binding capacity by the same method as used for serum. Six non-lactating mature female rabbits were used to obtain control serum iron and iron-binding capacity values.

The results are summarized in Fig. 1. The serum iron concentration was high in the first few days of lactation, but after about the fourth day it fell to the same level as in the non-lactating rabbits, and remained at this level for the rest of lactation. The serum TIBC remained relatively constant during lactation at a significantly higher level than in the non-lactating animals. In the milk the iron concentration was similar to that of serum for the first 3 weeks of lactation, but thereafter it increased and was much higher than in serum in samples collected between the twenty-fifth and thirty-second days of lactation. The milk TIBC was about four times as high as the serum value for the first 3 weeks of lactation, and increased after that to even higher levels.

When the proteins of rabbit whey were compared with those of serum by paper electrophoresis<sup>3</sup> it was found that the principal whey protein had a similar electrophoretic mobility to the  $\beta$ -globulin of serum (Fig. 2). Quantitative

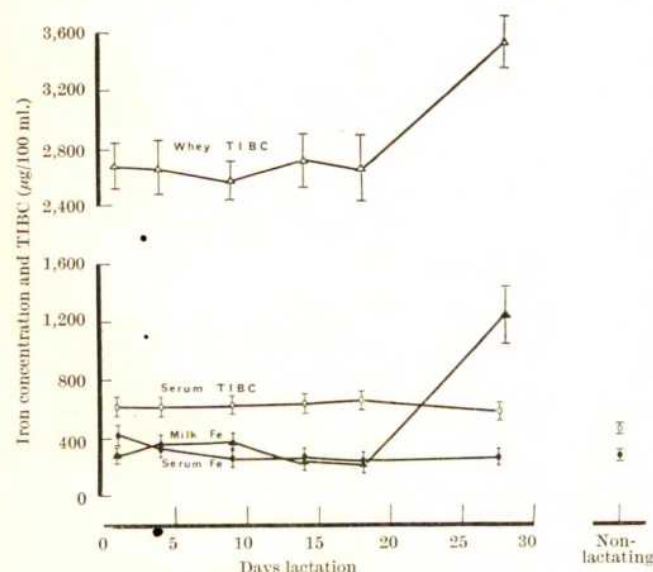


Fig. 1. Serum and whey iron concentration (Fe) and total iron-binding capacity (TIBC) during lactation in the rabbit. Each point is the mean of six rabbits at the time indicated except for the last point which is the mean of twelve rabbits sampled between days 25 and 32 of lactation. The mean serum values of six non-lactating rabbits are also shown. The vertical lines represent  $\pm$  one standard error of the mean.

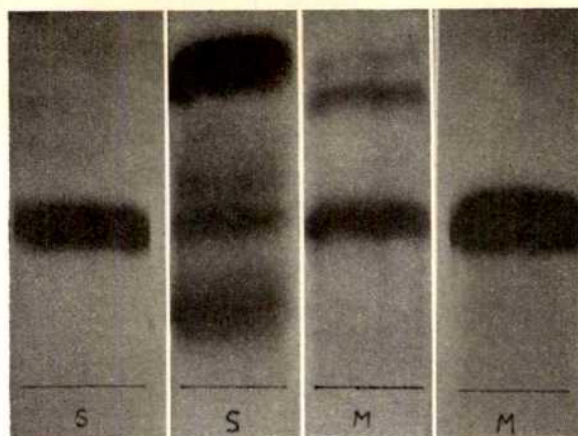


Fig. 2. Paper electrophoresis of rabbit serum (S) and rabbit whey (M), and autoradiographs obtained by the addition of iron-59 to the serum and whey.

analysis of the electrophoretic strips showed that the  $\beta$ -mobility component represented about 50 per cent of all the whey protein. The fact that the major whey protein fraction was indeed an iron-binding protein was shown by autoradiography. Serum and whey to which iron-59 chloride had been added in amounts less than the latent iron-binding capacity were fractionated by paper electrophoresis and exposed before staining to X-ray film. The autoradiographs so obtained showed that all of the radioactive iron was bound to serum  $\beta$ -globulin and to the whey protein with similar electrophoretic properties (Fig. 2).

The high values found for serum TIBC in the latter half of pregnancy in the rabbit<sup>4</sup> continue during lactation. The high serum iron value found during the first few days of lactation results from a sudden rise at the time of parturition from the low concentration observed towards the end of pregnancy<sup>4</sup>. This rise may be the result of the sudden cessation of iron loss to the foetuses. With the onset and increase in lactation during the first few days after parturition, loss of iron into the milk may replace that to the foetuses, so that the serum iron concentration falls somewhat. Although the milk iron concentration is not high, it represents a daily loss of about 500  $\mu$ g iron, because it has been found that rabbits in this colony usually secrete 150–200 ml. of milk per day. This is a considerable amount of iron to a small animal like the rabbit and must result in greatly accelerated plasma iron turnover and iron absorption.

The milk TIBC and electrophoresis results show that a major protein component of rabbit milk whey is an iron-binding protein with similar properties to those of serum transferrin. The significance of this finding is uncertain. It seems unlikely that the milk protein is necessary for the transfer of iron to the milk. Other animals with much lower concentrations of iron-binding protein in the milk secrete iron into the milk in higher concentrations than does the rabbit. In the rat<sup>5</sup> and a marsupial, the Rottneest Island quokka<sup>6</sup>, iron is secreted into the milk in concentrations greatly exceeding the serum iron concentration and the whey iron-binding capacity. In both these species, whey iron-binding capacity values are much lower than in the rabbit, although the iron-binding capacity of the rat whey rises towards the end of lactation as it does in the rabbit. The milk iron-binding protein of the rabbit may correspond with the red protein, or lactoferrin<sup>7–11</sup> of human and bovine milk, but is present in much higher concentrations. Indeed, if it is assumed that the rabbit protein has a molecular weight similar to transferrin (90,000) and binds two atoms of iron per molecule, the whey concentration may be calculated as 2 g/100 ml. earlier in lactation, later rising to 2.5 g/100 ml. With the secretion of 150–200 ml. milk of which about two-thirds is whey,



2 g or more of the iron-binding protein may be secreted each day.

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### Effect of Actinomycin on Protein Synthesis by Lymphocytes

WHEN human lymphocytes are incubated with phytohaemagglutinin (PHA), an extract of *Phaseolus vulgaris*, they transform into blast cells, which enter cell division<sup>1,2</sup>. This process is accompanied by a rise in the rate of synthesis of RNA and protein<sup>3,4</sup> and by changes in the types of RNA synthesized<sup>5,6</sup>. Actinomycin, which acts primarily by inhibiting RNA synthesis, is known to inhibit protein synthesis secondarily in many tissues, but the synthesis of some proteins has been found to be resistant to actinomycin<sup>7</sup>. This communication reports the effect of actinomycin on protein synthesis by lymphocytes.

DL-1-<sup>14</sup>C-leucine (32 mc./mmole) was used in these experiments. Solid PHA was reconstituted in tissue culture medium TC199, and used at a concentration of 0.015 ml./ml. of lymphocyte suspension.

Lymphocytes were prepared from human blood by sedimentation with gelatine<sup>8</sup>. The supernatant was diluted with 2 vol. of medium TC199 to give a suspension containing  $0.6-1.2 \times 10^6$  lymphocytes/ml. Cultures of 0.5 ml. were prepared and incubated as previously described<sup>4</sup>. At the times indicated in Fig. 1 the culture medium was replaced by fresh medium containing <sup>14</sup>C-leucine (1  $\mu$ Ci./ml.) and with or without actinomycin D (10  $\mu$ g./ml.).

Cultures prepared in this way contained variable numbers of cells other than lymphocytes, mainly granulocytes. In some experiments granulocytes and monocytes were removed by passing the gelatine supernatant through a cotton wool column, as described by Lamvik<sup>9</sup>. The yield (50-75 per cent) and purity (95-100 per cent) of lymphocytes obtained in this way were similar to those obtained by Lamvik. The lymphocytes were collected by gentle centrifugation and suspended in medium composed of 25 per cent inactivated bovine serum and 75 per cent TC199 to give a concentration of  $0.4-0.9 \times 10^6$  lymphocytes/ml.

Incorporation of <sup>14</sup>C-leucine was assessed as described previously<sup>4</sup>. In some cases (Fig. 1b and 1d), however, the membranes on which the radioactive precipitate had been collected were washed with 2 per cent (v/v) acetic acid, placed in a vial, dried, and covered with toluene scintillator containing 2,5-diphenyloxazole (3.5 g/l.) and 1,4-bis-(5-phenyloxazol-2-yl) benzene (50 mg/l.). The radioactivity was determined in a Nuclear-Chicago scintillation spectrometer, at an efficiency of about 50 per cent. The efficiency using the original method was about 20 per cent. Four or five replicate cultures were used for each measurement.

Fig. 1a shows that the incorporation of <sup>14</sup>C-leucine by freshly prepared cultures is almost completely inhibited after incubation with actinomycin D for 3 h. Very similar

results were obtained using the purified lymphocytes prepared with a cotton wool column (Fig. 1b). After incubation with PHA for 1 or 2 days (Fig. 1c) the incorporation of <sup>14</sup>C-leucine was still markedly inhibited by actinomycin, but a proportion was found to become less sensitive to the antibiotic. Incorporation was, for example, proceeding at 15-30 per cent on the control rate 5-7 h after the addition of actinomycin. This effect was not dependent on the addition of PHA, because it was also observed when cultures were incubated without PHA for 1 or 2 days before the addition of actinomycin (Fig. 1d).

The decrease in the sensitivity of protein synthesis to actinomycin does not result from a lesser sensitivity of RNA synthesis to the action of the drug. Incubation of lymphocyte cultures alone does not result in any detectable decrease in the sensitivity of <sup>3</sup>H-uridine incorporation to actinomycin while incubation with PHA causes a significant increase. Actinomycin at this concentration (10  $\mu$ g./ml.) inhibits <sup>3</sup>H-uridine incorporation by cultures without PHA by 85-90 per cent, while <sup>3</sup>H-uridine incorporation by cultures which have been incubated with PHA for 2 days is inhibited by 97 per cent.

Actinomycin was not found to increase the rate of loss of <sup>14</sup>C-leucine which had been incorporated into protein. When lymphocytes which had been incubated with <sup>14</sup>C-leucine were transferred to a medium containing either an excess of cold leucine or cycloheximide, an inhibitor of protein synthesis, the presence of actinomycin in the second medium did not increase over a period of 7 h the slow loss of <sup>14</sup>C-leucine observed.

It has been suggested that the effect of actinomycin on protein synthesis may be affected by the glucose concentration in the incubation medium<sup>10</sup>. In these experiments

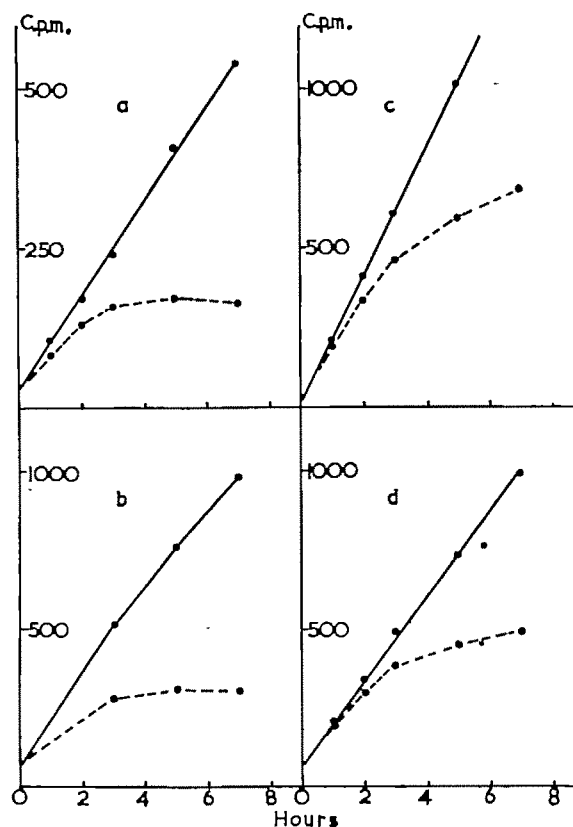


Fig. 1. Effect of actinomycin on incorporation of <sup>14</sup>C-leucine. Cultures of 0.5 ml. were preincubated with or without PHA as indicated below. The medium was then replaced by medium containing <sup>14</sup>C-leucine (1  $\mu$ Ci./ml.) and with or without actinomycin (10  $\mu$ g./ml.) and incubation continued at 37° C. ●—●, Control; ○—○, actinomycin. a, No preincubation; b, cultures prepared by the cotton wool column method; c, cultures preincubated with PHA for 22 h; d, cultures preincubated alone for 21 h.

the glucose concentration at the time of addition of actinomycin was the same in all experiments (5.5 mmolar).

Actinomycin has itself been claimed to cause the degradation<sup>11</sup> or stabilization<sup>12</sup> of RNA, but other authors have concluded that actinomycin does not affect the stability of rapidly labelled or messenger RNA (refs. 13-17). The possibility that actinomycin affects protein synthesis in some way other than through inhibition of RNA synthesis cannot be ruled out<sup>18</sup>. It seems likely, however, that most of the functional messenger RNA of freshly isolated lymphocytes is degraded within 8 h of the addition of actinomycin, while after incubation of lymphocytes *in vitro* a proportion becomes more stable.

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## Vectorial Metabolism

THEORIES of active biological transfer have been discussed in the light of the Curie principle<sup>1,2</sup>, which forbids the combination of a scalar force, such as the chemical affinity of a reaction, with a vector flux such as the diffusion flux of a solute<sup>3</sup>. In active transfer, solutes are transferred against their gradients of electrochemical potential by means of energy derived from metabolism. It has usually been stated that direct thermodynamic cross coupling between a chemical reaction and the flux of an active transfer cannot occur<sup>3</sup> and, instead, the chemical reaction is supposed to modify the electrochemical potential of the transferred solute which then diffuses spontaneously.

Active transfer systems ( $\text{Na}^+ + \text{K}^+$ -activated ATPases) have been described in which the transfer seems to be obligatorily coupled with the chemical reaction supplying the energy<sup>4</sup>, and the concept of "vectorial metabolism" has been proposed<sup>5</sup> to describe this obligatory association. The ion transfer appears to be an integral part, not a consequence, of the chemical reaction and could not therefore result from a restriction imposed by the structure of a membrane on the free diffusion of a reactant or a product (see ref. 1). This association between a chemical reaction and an ion transfer would seem to violate the Curie principle. A theory of direct cross coupling between a chemical reaction and a diffusion process has been put forward<sup>6</sup>, but has been severely criticized<sup>1,3</sup>. The present author has suggested<sup>7</sup> that direct coupling between a chemical reaction and a diffusion process is permissible if the transition-state theory of Eyring and Eyring<sup>8</sup> is employed to formalize active transfer. The relation

between this suggestion and the proposal of vectorial metabolism is discussed here.

In transition-state theory<sup>8</sup>, the course of a chemical reaction is denoted by the passage of reactants and products over a saddle-point on a potential energy surface along a single co-ordinate, the "reaction" co-ordinate; and diffusion processes also take place along a single co-ordinate. Similarly, the direct coupling between chemical reactions and diffusion processes suggested by the present author<sup>7</sup> was postulated to occur in a system described by one co-ordinate, in which reasons were given for allowing the vectors which represented the diffusion flux and force to degenerate<sup>9</sup> to scalar quantities.

According to Guggenheim<sup>10</sup> the reaction co-ordinate can be given a geometrical meaning, that is the passage of individual molecules along a reaction co-ordinate involves an actual translocation in space. In isotropic systems, these individual molecular displacements cancel each other out, so that there is no net transfer of reactants or products in any particular direction. In anisotropic systems, on the other hand, if a preponderance of the individual reaction co-ordinates is orientated in one particular direction in three-dimensional space, net fluxes of reactants and products in that direction will automatically result. It is therefore suggested that the occurrence of vectorial metabolism depends on the anatomical properties of the system rather than on the nature of the coupling between chemical reactions and translocations.

This concept can be illustrated by a discussion of some enzyme reactions. NAD and NADP-dependent dehydrogenases show stereochemical specificity and can be classified into two groups according to which side of the pyridine ring the hydrogen atom from the substrate is attached<sup>11</sup>. It has been suggested that a pyridine nucleotide molecule forming a "sandwich" between the protein apoenzymes of two dehydrogenases could transfer hydrogen atoms from the substrate of one to the substrate of the other through a molecular rearrangement without disrupting the "sandwich"<sup>12</sup>. If the pyridine nucleotides form part of a membrane, a net flux of hydrogen atoms across the membrane will occur if all the pyridine rings are facing the same way. No net flux of hydrogen atoms in any one direction will occur if the reaction is proceeding in a homogenate in which the pyridine rings are randomly orientated. Irrespective of whether the reaction is taking place in a membrane or in a homogenate, the passage of reactants along the reaction co-ordinate includes a translocation of hydrogen atoms in one particular direction relative to the pyridine ring.

The action of ( $\text{Na}^+ + \text{K}^+$ )-dependent ATPases exhibits a stereospecificity which resembles that shown by the pyridine nucleotide enzymes. ATPase activity in homogenates of membranes is dependent on sodium and potassium ions without causing a net flux of either ion in any particular direction, whereas in intact membranes the sodium and potassium ions must be on opposite sides of the membrane for hydrolysis of ATP to occur and the ions are transferred in opposite directions across the membrane<sup>13</sup>. Here, the passage of reactants along the reaction co-ordinate includes translocations of sodium and potassium ions in opposite directions relative to the active site. When a majority of the reaction co-ordinates are orientated normal to the surface of the membrane, ATP-dependent fluxes of sodium and potassium ions across the membrane will be observed. When the active sites, and therefore the reaction co-ordinates, are randomly orientated in a homogenate, no net transfer of ions in any direction will be observed.

The Curie principle, which is concerned with the symmetry or asymmetry of systems<sup>1</sup>, is not violated in vectorial metabolism because, by the principle of entropy balance<sup>14</sup>, the degree of asymmetry of the system as a whole is either unchanged or reduced at the end of the chemical reaction. The coupling between active transfer and a chemical reaction is discussed elsewhere in relation



to the irreversible entropy production within the whole system<sup>1</sup>.

The allosteric rearrangement postulated by Jardetzky<sup>12</sup>, like the change-of-state reaction postulated by Vidaver<sup>14</sup> to account for facilitated diffusion, assumes that the complex between the membrane component and the transferred species undergoes a configurational rearrangement which results in the translocation of the transferred species. As pointed out elsewhere<sup>7</sup>, this type of phenomenon is included in the entropy of activation when transition-state theory<sup>8</sup> is used to formulate a theory of active transfer. If one of the reactants is a macromolecule, the site of the chemical reaction may be separated spatially from the site of the coupled diffusion, as in the allosteric model of Jardetzky<sup>12</sup>. It would not be surprising to find that some of the reactant species were spatially separated from each other during a chemical reaction when three or more reactant species are forming a transition-state.

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## Observations on the RNA Metabolism of Leukaemic Cell Cultures

MORE than half the RNA synthesized in mammalian cells is ribosomal and transfer RNA. This RNA is rich in guanine (G) and cytosine (C) and is polymerized by a DNA-dependent RNA polymerase<sup>1,2</sup>. Conversely, the RNA synthesized in mammalian cells infected with cytotoxic single-stranded RNA viruses, such as poliovirus and B<sub>19</sub> virus, is predominantly viral RNA. This RNA is rich in adenine (A) and uracil (U) and is polymerized by virus-induced, RNA-dependent RNA polymerase<sup>3-5</sup>. Cells infected with such viruses also show repression of the synthesis of GC-rich cellular RNA<sup>6,7</sup>. Little is known about the metabolism of RNA in mammalian cells infected with leukaemia viruses. The present study was undertaken to examine some gross characteristics of RNA synthesis in leukaemia cells from

mice in which the leukaemias resulted from infection either with the Rauscher leukaemia virus or with a virus from radiation-induced myeloid leukaemia<sup>10</sup>. Recent investigations have shown that the nucleic acid of the Rauscher virus is single-stranded RNA with about 52 per cent total G+C and a low uridine content<sup>11,12</sup>. The nature of the nucleic acid of the radiation-induced myeloid leukaemia virus is not known.

Myeloid leukaemias and reticulum cell sarcomata were induced in *RF/Urf* mice by injecting the radiation myeloid leukaemia virus or the Rauscher virus, respectively. Primary cultures of normal spleen cells and cultures of the leukaemia cells from grossly infiltrated spleens were prepared by the method of Ginsburg and Sachs<sup>13</sup>. The medium used in all cases was Eagle basal medium containing a four-fold increase in the concentration of essential amino-acids and vitamins, with 20 per cent horse serum. Each of the three cultures was divided into three groups and treated as follows: 1 h after explantation ( $7 \times 10^4$  cells/ml.), actinomycin D (1  $\mu$ g/ml.) was added to one group and puromycin (50  $\mu$ g/ml.) was added to a second group. A third group in each culture remained untreated. 2 h after explantation, all cultures received phosphorus-32 (35–40  $\mu$ Ci/ml.). 5 h after the addition of phosphorus-32, the cells were collected, frozen and the labelled RNA extracted with phenol at 60° C (ref. 14). Specific activities, distribution of phosphorus-32 among the nucleotides, and fingerprint patterns of the labelled RNA were determined as previously described<sup>15,16</sup>.

The labelled RNA from leukaemic cells infected with the Rauscher virus showed a slight average increase in specific activity compared with normal spleen cells (Table 1). Extensive variation in the specific activity of labelled RNA from normal spleen cell cultures was evident, however. In spite of this variation it is obvious that the specific activity of labelled RNA from myeloid leukaemia cells was markedly increased (Table 1). To what extent these increases might reflect differences in mitotic indices has not been determined. None the less, leukaemic cells and normal spleen cells both showed a marked inhibition (85–92 per cent) of RNA synthesis in the presence of actinomycin D, indicating that the RNA synthesized in such cells was predominantly DNA-dependent. This contrasts sharply with the behaviour of cells infected with cytotoxic viruses, in which RNA synthesis is insensitive to actinomycin D (ref. 17). These results also argue against, but do not entirely exclude, the possibility that an RNA-dependent RNA polymerase was responsible for the synthesis of a large portion of the RNA in the leukaemic cells.

Whereas cultures of both normal spleen cells and leukaemic cells resulting from infection with Rauscher virus showed 60–70 per cent inhibition of RNA synthesis in the presence of puromycin, myeloid leukaemia cells under identical conditions showed more than 95 per cent inhibition (Table 1). The reason for such sensitivity to puromycin is not known. It has been reported, however, that the synthesis of ribosomal RNA is preferentially inhibited by puromycin<sup>18,19</sup>. Attempts to repress the development of myeloid leukaemia in infected mice by daily injections of puromycin (100  $\mu$ g/mouse) have been unsuccessful (Teeter and Tyndall, unpublished results). While the effects of puromycin on the synthesis of RNA in the two

Table 1. CHARACTERISTICS OF LABELLED RNA METABOLISM IN NORMAL AND LEUKAEMIC CELL CULTURES

	Untreated	Specific activity* Actinomycin- treated	Puromycin- treated	Oligonucleotide ratios†			Total‡ Gp+Cp Ap+Up
				ApUp/Up	GpApCp/Up	GpCp/Up	
Myeloid leukaemia spleen cultures	80,420 (25,000–27,000)	3,964 (3,180–6,060)	1,507 (635–2,580)	0.46 (0.42–0.54)	0.78 (0.75–0.80)	0.73 (0.70–0.77)	1.35 ( $\pm 0.06$ )
Rauscher virus-induced leukaemia spleen cultures	5,121 (4,179–6,656)	457 (231–657)	2,056 (1,040–2,630)	0.49 (0.44–0.53)	1.08 (0.88–1.30)	0.88 (0.80–0.95)	1.66 ( $\pm 0.15$ )
Normal spleen cultures	3,703 (1,600–7,900)	553 (240–1,161)	1,152 (550–2,316)	0.54 (0.45–0.64)	0.70 (0.51–0.85)	0.60 (0.55–0.75)	1.23 ( $\pm 0.18$ )

\*  $\text{CPM}^{32}\text{P-RNA}/\text{OD}_{260}$  total RNA. Average of three experiments. (Range of values in parentheses.)

† Determined by densitometric analysis of fingerprint patterns (see ref. 9). Average of three experiments.

‡ Distribution of phosphorus-32 among the nucleotides following alkaline hydrolysis. Average of two experiments.

types of leukaemia cells differ reproducibly, RNA from both types of leukaemia cells is rich in guanine and cytosine as is shown by a high content of total Gp and Cp, as well as by high contents of GpCp and GpApCp oligonucleotides (Table 1). High levels of total G and C in leukaemia spleen cells resulting from Friend virus infection have also been reported<sup>20</sup>.

The synthesis of RNA rich in total Gp and Cp and GpCp and GpApCp oligonucleotides in virus-infected leukaemic cells contrasts sharply with the repression of GC-rich RNA synthesis in cells infected with cytotoxic viruses<sup>21</sup>. The ratio of both GpCp and GpApCp to ApUp oligonucleotides in leukaemic cell RNA is equal to or greater than that in normal spleen cells (Table 1); however, the degree to which the ratio in leukaemic cells differs, if at all, from that of the corresponding normal stem cells cannot be specified. The ratio in leukaemic cells is also greater than that previously reported for HeLa cells and L cells<sup>14,21</sup>. To what degree malignant cells from various sources follow a similar pattern cannot be stated without more extensive investigation. It is noteworthy, however, that Muramatsu and Busch have shown that various cell fractions from Walker rat tumour cells have a higher content of GC-rich RNA than those from corresponding normal liver cells<sup>22</sup>. The contrast between the characteristics of the RNA synthesized in leukaemia cells and that synthesized in cells infected with cytotoxic viruses is not unexpected, considering that cells infected with cytotoxic viruses are degenerative and synthesize predominantly viral RNA, with repression of the synthesis of GC-rich cell RNA. Leukaemic cells, however, are proliferative and apparently synthesize both GC-rich cell RNA and virus RNA. It is interesting to note that polyoma tumour virus, unlike some other DNA-containing viruses, also induces virus and cell DNA synthesis on infection<sup>23</sup>.

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## DNA and RNA Synthesis and the Formation of Blastogenic Factor in Mixed Leucocyte Cultures

AFTER incubation for several days, cultures of mixed leucocytes from two individuals contain immature mononuclear cells. These cells incorporate thymidine into DNA (refs. 1 and 2). A material which stimulates blastogenesis of allogeneic leucocytes is released into the surrounding medium when human leucocytes are cultured *in vitro*<sup>3-5</sup>. More of this material is released into the medium from mixed leucocyte cultures than from cultures of leucocytes from single donors. This communication reports experiments which were designed to determine DNA and RNA synthesis and the formation of blastogenic factor in mixed leucocyte cultures after various periods of incubation.

The subjects were normal volunteers. An aseptic technique was used throughout the experiments. A leucocyte suspension was prepared as previously described<sup>1</sup>. The leucocytes were washed once with medium 199. The cells were finally resuspended in fresh plasma pooled from several healthy donors, using sufficient to adjust the leucocyte count to between 5,000 and 7,000/mm<sup>3</sup>. The cell suspensions were diluted with medium 199 so that the final plasma concentration was 20 per cent. The mixed cultures contained a 1:1 mixture of cells from two subjects. Unmixed cultures of leucocytes from one of the donors were also established. The cell suspensions were incubated in 17 × 100 mm disposable culture tubes. Mixed or unmixed leucocyte cultures were divided into three groups. The first group consisted of mixed leucocyte cultures. Cell-free culture medium and the ultracentrifuged precipitate were prepared from ten culture tubes in this group, after incubation times of 8, 18, 24, 36, 48, 120, 168 and 240 h. Smears were made from the cells in the cultures at these times and stained with Jenner-Giemsa. Blastogenic activity of these preparations from mixed cultures after these incubation times was assessed as described previously<sup>3</sup> by adding the preparations to leucocyte cultures from a single donor, incubating for 5 days, and measuring the uptake of <sup>3</sup>H-thymidine by the cultures by means of a liquid scintillation counter<sup>6</sup>. The results are expressed as c.p.m./4 × 10<sup>5</sup> cells. The second and third groups consisted of both mixed and unmixed leucocyte cultures. At the same time intervals, <sup>3</sup>H-thymidine (specific activity 5 c./mmole and a final concentration in each culture tube of 1 µc./ml.) was added to two tubes of each culture in the second group. After incubation for 2 h at 37° C, the cells were washed once in cold physiological saline, and then twice in 5 per cent trichloroacetic acid (TCA). DNA was extracted by the Schmidt-Tannhauser technique<sup>7</sup>, except that the concentration of potassium hydroxide was decreased to 0.5 normal. At the same time intervals as those of cultures in the second group, uridine-5-T (specific activity 5 mc./mmole and a final concentration in each culture tube of 1 µc./ml.) was added to two tubes from each mixed and unmixed culture in the third group. After 2 h at 37° C, their RNA was extracted by a slight modification of the Schmidt-Tannhauser technique<sup>8</sup> as previously described. The radioactive content of the DNA or RNA fraction was counted in a liquid scintillation counter. Quenching was measured by an internal standardization method<sup>9</sup> using <sup>3</sup>H-toluene as the internal standard. Quenching was nearly identical for the samples of RNA and DNA. The concentration of the DNA or RNA present in each preparation was determined by measuring the optical density at 260 mµ in a spectrophotometer with a hydrogen lamp. The results are expressed as c.p.m./10 µg of DNA or RNA.

As shown in Table 1, the ultracentrifuged precipitates from mixed leucocyte cultures which had been incubated for 18 h showed slight blastogenic activity. There was a further increase in activity of similar preparations from

Table 1. ELABORATION OF BLASTOGENIC FACTOR IN MIXED LEUCOCYTE CULTURES DURING INITIAL 48 h INCUBATION

Length of initial cultures (source of blastogenic material) (h)	<sup>3</sup> H-thymidine uptake (c.p.m./4 × 10 <sup>4</sup> cells)*	Control cultures (5-day) of unmixed leucocytes
8		
18	1,148 ± 274	1,206 ± 108
24	2,408 ± 994	1,125 ± 76
36	3,904 ± 272	1,158 ± 125
48	4,897 ± 745	1,037 ± 102
	5,878 ± 849	1,062 ± 96

\* Average value obtained from four experiments ± S.D.

Table 2. DNA AND RNA SYNTHESIS AND BLAST TRANSFORMATION IN MIXED AND UNMIXED LEUCOCYTE CULTURES DURING INITIAL 48 h INCUBATION

Length of cultures (h)	<sup>3</sup> H-uptake (c.p.m./10 μg)*		RNA synthesis†		Blast transformation‡
	Mixed cultures	Unmixed cultures	Mixed cultures	Unmixed cultures	
8	1,193 ± 144	1,251 ± 145	1,210 ± 819	1,174 ± 210	(-)
18	1,319 ± 266	1,227 ± 95	1,221 ± 85	1,182 ± 106	(-)
24	1,268 ± 169	1,168 ± 106	1,212 ± 280	1,228 ± 119	(-)
36	1,259 ± 130	1,206 ± 84	—	—	(-)
48	2,905 ± 806	1,111 ± 123	1,782 ± 46	1,036 ± 79	(+)

\* Average values obtained from four experiments ± S.D.

† DNA and RNA syntheses were determined by incorporations of <sup>3</sup>H-thymidine and uridine-5-T, respectively. See the method in the text.

‡ Blast transformation was determined in Jenner-Giemsa stained smears independently by two observers and was expressed on an arbitrary scale. —, No large basophilic blast cells were found in smears; +, blast cells were occasionally found in smears.

cultures which had been incubated for 24 h. At this time, RNA and DNA synthesis was the same in mixed leucocyte cultures as in the unmixed control cultures. No evidence of any blast transformation was found in smears from 24 h mixed cultures (Table 2).

The blastogenic activity reached a peak just after 120 h of incubation, but it decreased after 168 h (Fig. 1). The uptake of tritiated thymidine in cultures of leucocytes containing the precipitates from mixed cultures incubated for 120 h was 8,046 (mean from four experiments) ± 1,148 (S.D.) c.p.m./4 × 10<sup>4</sup> cells, while those in leucocyte cultures with the precipitates from 72 h and 168 h mixed cultures were 5,428 ± 135 and 4,302 ± 233, respectively. The blastogenic activity of the cell-free culture medium was determined at the same time intervals as those of the ultracentrifuged precipitate, and the effects of the two preparations were entirely similar. The ultracentrifuged precipitate was more active, however, than the cell-free culture medium.

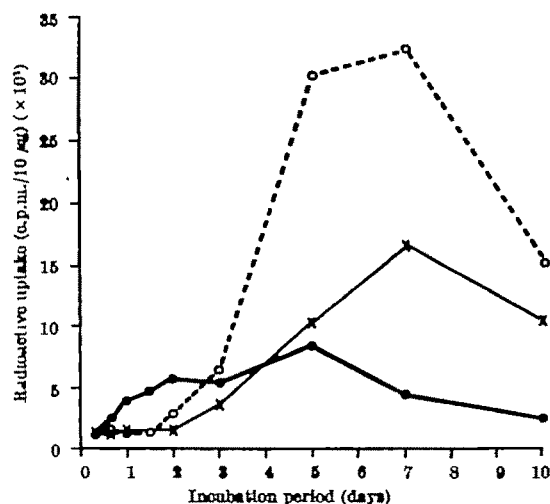


Fig. 1. DNA and RNA synthesis and formation of blastogenic factor in mixed leucocyte cultures. Each point represents the average value obtained from four experiments. The DNA (O---O) and RNA (x---x) syntheses were determined as described in the text. The activity of the blastogenic factor (●---●) is expressed as the uptake of <sup>3</sup>H-thymidine (c.p.m./4 × 10<sup>4</sup> cells) in 5-day cultures of leucocytes containing 1 ml. of the ultracentrifuged precipitate prepared from mixed cultures after various periods of incubation.

As shown in Table 2, DNA and RNA synthesis began to increase above that of the control cultures only after incubation for 48 h, at which time a few blasts were first seen in the stained smears. As the incubation time of the mixed cultures was prolonged, there was an increase in DNA and RNA synthesis (Fig. 1) and in the amount of blast transformation. DNA synthesis reached its maximum after incubation for 120 h (the uptake of <sup>3</sup>H-thymidine in these cultures was 30,079 (mean) ± 4,901 (S.D.) c.p.m./10 μg DNA), and then continued at this level until 168 h incubation (32,242 ± 7,127 c.p.m./10 μg DNA), after which it decreased (Fig. 1).

The RNA synthesis reached a maximum after incubation for 168 h (Fig. 1). The uptake of <sup>3</sup>H-uridine in mixed cultures incubated for 168 h was 16,539 (mean from four experiments) ± 4,901 (S.D.) c.p.m./10 μg RNA. The radioactive uptakes in mixed cultures incubated for 120 h and 240 h were 10,238 ± 933 and 10,420 ± 943, respectively.

It seems pertinent that significant stimulation occurred in the cell-free medium of 18 h and 24 h mixed cultures before there was any evidence of increased DNA and RNA synthesis and at a time when large basophilic blast cells could not be demonstrated morphologically. It is probable that the blastogenic factor is not produced by the lymphoblastoid cells.

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### *In vitro* Inhibition of Erythrocyte Glucose Consumption by Human Growth Hormone

We have found that human growth hormone *in vitro* markedly inhibits the glucose consumption of human erythrocytes. This effect could not be duplicated with growth hormone preparations from the pig, cow or monkey.

Erythrocytes from the heparinized blood of healthy adult males were freed from leucocytes and platelets by centrifuging at 200g for 15 min, followed by aspiration of the plasma and buffy coat. The cells were washed three times in four volumes of 0.15 molar saline and centrifuged at 1,000g after each wash. The supernatant and buffy coat were aspirated after each wash. The washed erythrocytes were incubated in a Krebs-Ringer bicarbonate buffer, pH 7.4, at an adjusted haematocrit of 33 per cent. The erythrocytes were enriched with glucose to a final concentration of 10 mmol/l. Three ml. of this erythrocyte suspension were incubated in a 25 ml. flask for 2 h at 37° C in a metabolic shaker set at 80 oscillations/min. The gas phase was 5 per cent carbon dioxide in air. One of each pair of flasks contained purified human growth hormone (Wilhelmi H.S. 612B) in a final concentration that ranged from 0.5 to 32 μg/ml. The glucose concentration of the incubation mixture was determined at 0, 1 and 2 h by the glucose oxidase method<sup>1</sup>. Duplicate glucose determinations showed variations of less than 2 per cent.

In seven experiments using purified human growth hormone (HGH) at a final concentration of 16  $\mu\text{g/ml}$ , red cell glucose consumption fell 32.9–59.0 per cent, the mean fall being 49.3 per cent (Table 1). A difference in glucose consumption between the control red blood cells and those treated with growth hormone was observed within 15 min and was evident throughout the 2 h incubation period. Glucose consumption remained linear for 2 h in the control flasks and those supplemented with growth hormone.

Table 1. EFFECT OF GROWTH HORMONE PREPARATIONS ON HUMAN RED CELL GLUCOSE CONSUMPTION

Preparation	No. of studies	Concentration ( $\mu\text{g/ml}$ )	Glucose consumption ( $\mu\text{moles/ml RBC/h}$ ) Control (mean)	Glucose consumption ( $\mu\text{moles/ml RBC/h}$ ) +HGH (mean)	Inhibition (per cent)	P
Purified human	7	16	1.61 $\pm$ 0.25	0.81 $\pm$ 0.18	49.3 $\pm$ 9.8	< 0.001
Crude human	13	128	1.56 $\pm$ 0.21	0.66 $\pm$ 0.11	56.6 $\pm$ 4.7	< 0.001
Porcine	3	128	1.71 (1.58–1.83)	1.74 (1.60–1.86)	0.0 (8.9–23.4)	> 0.5
Bovine	5	128	1.43 (1.21–1.71)	1.23 (1.10–1.44)	15.3 (7.9–24.0)	< 0.01
Monkey	3	128	1.49 (1.25–1.69)	1.24 (1.21–1.49)	11.3 (7.9–14.0)	< 0.05

As shown in Fig. 1, the depression in red cell glucose consumption varied with dose over a range from 1 to 32  $\mu\text{g/ml}$ . No effect was observed with HGH concentrations less than 1  $\mu\text{g/ml}$ .

The inhibition of glucose consumption by HGH was not affected by the simultaneous addition of Lilly glucagon-free crystalline insulin in concentrations as high as 2 U/ml.

In experiments in which fructose was used as a substrate, HGH at a final concentration of 16  $\mu\text{g/ml}$  depressed fructose consumption by an average of 44.7 per cent.

Erythrocyte glucose-6-phosphate (G-6-P) and fructose-6-phosphate (F-6-P) concentrations were determined before and after incubation of human erythrocytes with glucose in the presence of 16  $\mu\text{g/ml}$  of HGH for 2 h. In three experiments the erythrocyte G-6-P concentrations before incubation were 8.80, 9.79 and 10.20  $\mu\text{moles/100 ml}$  of erythrocytes. The concentrations of G-6-P in the red cells incubated with HGH rose to 13.70, 14.52 and 15.42  $\mu\text{moles/100 ml}$ , respectively. The concentrations of F-6-P in these same cells rose from values of 2.28, 2.48 and 2.61 to 3.44, 3.57 and 3.90  $\mu\text{moles/100 ml}$ , respectively. The concentrations of G-6-P and F-6-P were unaltered in the erythrocytes incubated without HGH.

The production of carbon-14 dioxide in the red cells remained constant in the presence of HGH, despite a drop in the total glucose consumption (Table 2). Red

Table 2. EFFECT OF HGH ON RED CELL CARBON-14 DIOXIDE PRODUCTION

	Total glucose consumption ( $\mu\text{moles}$ )	Carbon-14 dioxide ( $\mu\text{moles}$ )
Control	2.33	0.13
+HGH	1.57	0.13
Control	2.33	0.13
+HGH	1.20	0.13
Control	2.70	0.11
+HGH	1.31	0.11

About 1.5 mo. of radioactive glucose- $^{14}\text{C}$  were added with carrier glucose to each flask (specific activity 118,000 c.p.m./ $\mu\text{mole}$ ). At the end of the 2 h incubation period radioactive carbon dioxide was collected by introducing 0.8 ml. of 4 normal sodium hydroxide into the centre well of a rubber stoppered flask and terminating the reaction by introducing 0.5 ml. of 4 normal sulphuric acid into the erythrocyte suspension with a hypodermic needle through the stopper. After the carbon dioxide had been collected it was precipitated as barium carbonate, plated to infinite thickness, dried, weighed, and counted in a windowless gas-flow counter.

cell lactic acid production<sup>1</sup> was found to parallel the fall in glucose consumption.

The increase in the intracellular concentrations of G-6-P and F-6-P that accompanies the inhibition of glucose uptake produced by HGH *in vitro* suggests an effect on glycolysis at or beyond the phosphofructokinase reaction.

Table 1 shows that the concentration of crude HGH (Wilhelmi H.8. 455.4) required to produce a 50 per cent inhibition of red cell glucose consumption was eight times that of the purified HGH preparation.

When crude growth hormone from other species was used at a final concentration of 128  $\mu\text{g/ml}$ , little or no effect on glucose consumption in human erythrocytes was found (Table 1). Porcine GH (NIH-GH P 365.4) had no effect, while the inhibitory effect of bovine GH (NIH-GH B10) averaged 16.2 per cent and that of monkey GH (Wilhelmi M 732.B) averaged 11.5 per cent.

Erythrocytes from two children with short stature secondary to congenital hypopituitarism showed the same response *in vitro* to crude HGH (128  $\mu\text{g/ml}$ ) as cells from normal subjects. Inhibitions of 52.6 and 45.8 per cent were recorded. These two children later responded to growth hormone administration with retention of nitrogen and acceleration of linear growth.

By contrast, an infant with multiple congenital anomalies and a chromosomal aberration who weighed only 1,750 g at 7 months, despite normal levels of growth hormone, failed to show the normal *in vitro* response to growth hormone. Glucose consumption was inhibited by only 14.1 per cent in this patient.

This *in vitro* model may be a simple system for evaluating the activity of growth hormone preparations in man.

This *in vitro* effect of HGH on glucose consumption in human erythrocytes may reflect a physiological control mechanism. The glucose consumption of human erythrocytes in the adult is said to average 24 g/day<sup>2</sup>. A mechanism to modulate glucose consumption in the fasting state when glucose is in short supply has not previously been recognized<sup>3</sup>.

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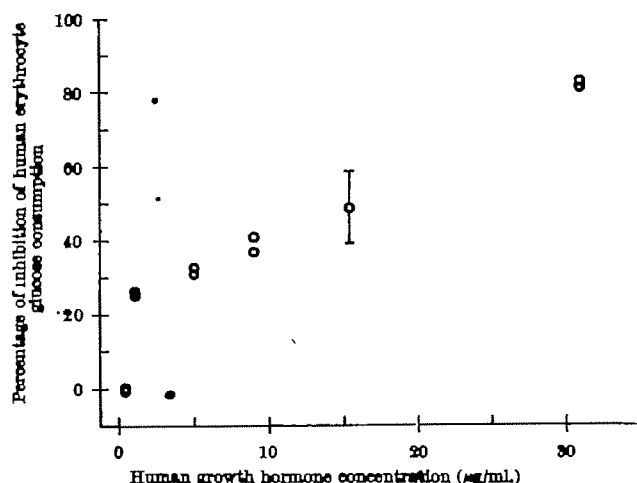


Fig. 1. Effect of variations in concentration of purified human growth hormone on human erythrocyte glucose consumption.

## Uptake of Chylomicron Lipid by Rat Liver Parenchymal Cells *In vivo*

THERE are two theories about the uptake of chylomicron triglyceride by the liver. The widely held view that chylomicrons are removed intact from the circulation<sup>1-3</sup> has been challenged by Felts and Mayes<sup>4</sup>. They propose that the triglycerides are not taken up directly by the liver but are hydrolysed elsewhere by lipoprotein lipase, the resulting non-esterified fatty acids (NEFA) being taken up by the liver cells. *In vivo* evidence that intact chylomicrons are removed by the liver has been presented earlier<sup>5</sup> but was open to two objections<sup>6,7</sup>. First, heparin was used as an anticoagulant in the collection of the chylomicrons and sufficient could have remained after washing to stimulate lipoprotein lipase activity in the circulation. Second, studies on whole liver tissue may not give a true picture of events occurring in the parenchymal cells themselves, since trapping of chylomicrons in the tissue spaces does occur both *in vivo*<sup>7</sup> and in perfusion experiments<sup>8</sup>.

To overcome both objections, the following experiments were performed. Chylomicrons were obtained after administration of glycerol tri [1-<sup>14</sup>C] palmitate to rats in which thoracic duct cannulae had been established. The only heparin employed was a small amount in the saline filling the cannula when it was inserted. The first millilitre of lymph to emerge was discarded. The washed chylomicrons (0.75–2.05 mg of lipid) were injected into the exposed femoral vein of fed rats over a 30 sec period. One minute after injection was completed, the liver was perfused with ice-cold, calcium free Locke's solution containing 0.27 molar sodium citrate, and homogenized in Hanks solution. The homogenate was filtered through nylon mesh (60 $\mu$  pore size) and the filtrate centrifuged at 1,300*g* for 1 min. The sedimented parenchymal cells were washed and the total lipid extracted for determination of <sup>14</sup>C distribution<sup>9</sup>.

Most of the <sup>14</sup>C-palmitic acid associated with the hepatic parenchymal cells is in the triglyceride, with much smaller amounts present in the phospholipids (Table 1). Thus although studies of total quantity of lipid taken up by the liver may not be valid if based on measurements of the whole tissue, the distribution of chylomicron lipid in the parenchymal cells is very similar to that in the whole tissue<sup>3,10</sup>. When tritiated palmitic acid was injected as a complex with albumin, 57.8 per cent of the radioactivity was in the phospholipids and 27.8 per cent in the triglyceride. These measurements were made on the whole perfused liver, since the distribution of the isotope shows that there is no problem of contamination by trapped fatty acid. If chylomicron triglyceride had been hydrolysed to NEFA before uptake by the liver cells, the distribution of radioactivity should have been similar in the two experiments. As they are so different, they confirm earlier findings<sup>3,10</sup> and establish that under normal physiological conditions chylomicron triglycerides are directly removed from the circulation by the liver cells.

Further evidence which counts against lipoprotein lipase filling an important role in the uptake of chylomicron lipids is given by the finding that, after inhibition of this enzyme with protamine sulphate, the pattern of uptake and metabolism of chylomicron lipids in liver is essentially the same as that seen when no inhibitor is used (compare refs. 3 and 5).

Table 1. DISTRIBUTION OF <sup>14</sup>C IN RAT LIVER PARENCHYMAL CELL LIPIDS 1 MIN AFTER INJECTION OF CHYLOMICRONS LABELED WITH <sup>14</sup>C PALMITIC ACID

Lipid class	<sup>14</sup> C distribution* in	
	Original chylomicrons	Liver cells after chylomicron injections†
Triglycerides	93.1	73.1 ± 2.4
Phospholipids	6.8	19.2 ± 3.0
Cholesterol esters	0.4	1.2 ± 0.4
NEFA	1.1	6.2 ± 1.1

\* As percentage of total lipid <sup>14</sup>C.

† Mean of 5 expts. ± standard error.

The failure of Felts and Mayes<sup>4</sup> to detect significant uptake of chylomicron lipids by perfused rat liver in the absence of lipoprotein lipase is difficult to explain. Their conclusion, however, that NEFA are the principal form in which fatty acids actually traverse the liver cell membrane is in agreement with our earlier findings<sup>3</sup>.

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## Freeze-drying and Protein Denaturation in Muscle Tissue; Losses in Protein Solubility

IN the course of a search for possible relationships between the freeze-drying procedure and the subsequent extractability of proteins from the freeze-dried tissue, it was found that freeze-drying of ox muscle does not necessarily reduce the extractability of the component proteins separable by Halander's procedure<sup>1</sup> which employs, in sequence, 0.03 molar phosphate buffer and 1.1 molar potassium iodide. These findings were, in part, a confirmation of the work of Cole and Smithies<sup>2</sup> and of Connell<sup>3</sup> and support the thesis that freeze-drying need not cause irreversible denaturation of the proteins present. Furthermore, our results explain the observations of those workers who, in experiments in which the effects of dehydration and of high temperature were combined, found that rapid freeze-drying lowers the extractability of proteins<sup>4</sup>. Thinking that the losses in extractability reported might be caused by the high temperature generally used to accelerate freeze-drying, we tried to separate the effects of freeze-drying from those of high temperature. We were thus able to show that heat applied during the course of freeze-drying to accelerate dehydration may induce insolubility in the portion already dried, while the part still to be dried remains undamaged.

Tissue samples were cut from the longissimus dorsi taken from beef carcasses, 2 to 4 days after death. Two freezing rates were employed: (1) slices of tissue 1 mm thick were immersed abruptly in well stirred isopentane maintained at -150° C; (2) a piece of tissue roughly 2.5 cm thick was allowed to freeze in the still air of a cold room at -10° C (these pieces were subsequently cut into cubes 1 cm on edge for freeze-drying).

Freeze-drying was carried out in an all-glass high vacuum apparatus<sup>5</sup> in which the specimen chamber could be (1) maintained at any desired temperature and (2) isolated from the condenser by closure of a wide-bore stopcock. In all cases we maintained the specimen chamber at -10° C with the aid of an automatically regulated external cooling bath, to the point where either (1) the specimen contained about 2 per cent residual moisture (on a dry weight basis) or (2) contained less than 0.2 per cent moisture, these arbitrary points of "completion" being measured by the method of increase of water vapour pressure to equilibrium (with the sample still at -10° C).

After some of the freeze-drying runs, the dried tissues were allowed to warm to room temperature, air was admitted to the apparatus, and the tissues were immedi-



ately submitted to the protein extraction procedure. After other freeze-drying runs, we retained the samples under vacuum in the specimen chamber, which was then warmed in an oil bath until the temperature of the tissues reached 80° C, where the batch was maintained for 24 hours. When the tissues were dehydrated to the 2 per cent moisture level, the specimen chamber was isolated from the condenser throughout the 24 h period at 80° C. When dehydration to better than 0.2 per cent was sought, we maintained the connexions from the specimen chamber to the condenser. A comparison of the effects of heat in the presence of 2 per cent water and in the almost complete absence of water was thus afforded.

Extraction of the dehydrated tissues was carried out by disintegration in 0.03 molar potassium phosphate buffer of pH 7.4 in a Waring blender, followed by exposure of the washed residue to 1.1 molar potassium iodide in 0.1 molar potassium phosphate of pH 7.4 (ref. 1). Suspended fats were removed by filtration of the 0.03 molar buffer extract on a large filter paper. Non-protein nitrogen and low-salt-soluble proteins (extracted together by the dilute buffer and separated with the aid of trichloroacetic acid), and fibrillar proteins (dissolved by the 1.1 molar potassium iodide in 0.1 molar buffer), were each determined by the biuret reaction. The insoluble residue was determined gravimetrically after washing and drying. The results are presented in Tables 1 and 2.

The tables show that there is no loss in extracted protein or any increase in the insoluble residue as a result of freeze-drying, provided that the specimen chamber is maintained at -10° C throughout drying and afterwards warmed only to room temperature. On the other hand, exposure of tissue, freeze-dried at -10° C, to 80° C for 24 h leads to losses in extractability of low-salt-soluble and of high-salt-soluble proteins of the order of 30 per cent and 65 per cent respectively. Thus the exposure to 80° C has affected the structural (high-salt-soluble) proteins much more than it has the sarcoplasmic (low-salt-soluble) proteins and the percentage reductions in extractability are to a first approximation independent of the rate at which the tissue was originally frozen and independent of the limits to which freeze-drying was subsequently carried.

In other experiments, the detailed results of which are not reported here, we found similarly that extractability is not reduced after freeze-drying in a specimen chamber maintained at room temperature where the tissue remains frozen due to the evaporative cooling involved. Further, extractability is not reduced by simple drying of tissue (to a 2 per cent moisture level) at temperatures between 2° C and 20° C in conditions controlled to prevent freezing by evaporative cooling. We observed that extractability is equally high in samples freeze-dried to 2.0 per cent and to 0.2 per cent residual moisture.

Irreversible reactions of a type leading to losses of solubility are thus not encountered as a result of freeze-drying itself. Such reactions are, however, induced to a smaller extent in the sarcoplasmic proteins and to a

greater extent in the structural proteins by exposure of the freeze-dried tissue to 80° C.

The mechanisms by which these insolubilities are induced are open to many interpretations. The sarcoplasmic protein fraction consists of many different molecular species, the distribution and stability of which in the freeze-dried tissue may vary widely. The structural proteins, on the other hand, are distributed according to an orderly arrangement and are composed of myosin, actin and tropomyosin in the ratios 54:21:15 or thereabouts\*. Since we have observed a reduction in solubility of structural proteins of about 65 per cent in each experiment involving exposure to 80° C, we make the tentative suggestion that the myosin molecules may be rendered insoluble while actin and tropomyosin retain their extractability. Furthermore, since this 65 per cent insolubility is achieved by 24 h at 80° C in the presence of comparatively little water—less than that required to complete a monolayer on the protein molecules present—it seems likely that the decreased solubility results from cross-linking reactions between side chains of adjacent undenatured protein (myosin) molecules rather than by a coprecipitation of unfolded peptide chains (that is, through a "true" denaturation). It is possible, however, that a combination of both mechanisms is involved in much the same way as that proposed by Connell<sup>2</sup> for the case of freeze-dried muscle tissues exposed to temperatures of 37° and 50° C for prolonged periods.

Further studies are envisaged in which it would seem necessary to follow separately the losses in solubility of actin and of myosin as a function of the time during which a piece of freeze-dried tissue is maintained at a given high temperature and a given relative humidity.

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## Vitamin Distribution in Human Plasma Proteins

APART from vitamin B<sub>12</sub>, very little is known about the relation of vitamins and plasma proteins<sup>1-3</sup>. By coupling the availability of pure human blood fractions with sensitive, specific microbial and chemical vitamin assays, the amount of vitamin in various fractions of human plasma was determined. Such information is needed for charting transport mechanisms for the various vitamins or, conversely, assigning transport functions to the various blood proteins.

The human plasma fractions studied were:  $\alpha$ -globulin (Cohn fraction IV-1);  $\beta$ -globulin (Cohn fraction III);  $\gamma$ -globulin (Cohn fraction II); crystalline albumin (crystallized four times); and  $\beta$ -lipoprotein. These were all homogeneous fractions prepared by the classic Cohn procedure. All fractions met the standards of the Protein Foundation, Boston, for homogeneous protein purity. The nitrogen content of each fraction was determined by the Kjeldahl procedure<sup>4</sup>.

The plasma fractions for vitamin assay were prepared as follows: (a) Thiamine; 100 mg of the fraction was suspended in 25 ml. of a filtered solution of papain (500 mg papain in 100 ml. distilled water). The suspension was incubated for 3 days at 37° C. After incubation, 25 ml. of aconitic buffer, pH 4.5 (500 mg of *trans*-aconitic acid in 100 ml. of distilled water; pH adjusted to 4.5 with

Table 1

Fr. temp. °C	Fr.-dr. temp. °C	Residual water (per cent)	80° C. 24 h	Low-salt-soluble fraction (per cent)	Non prot. nitr. (per cent)	High-salt-soluble fraction (per cent)	Insoluble residue (per cent)
-150	-10	1.8	No	6.6	1.8	10.8	0.9
-150	-10	4.0	Yes	6.1	2.0	4.6	9.8
-150	-10	0.2	Yes	4.2	0.5	3.5	10.1
-10	-10	2.6	No	6.7	0.3	12.8	1.2
-10	-10	2.0	Yes	4.1	1.6	4.0	8.0
-10	-10	0.2	Yes	4.3	0.4	3.7	9.8

Table 2

Controls				
Fr. temp. °C	Low-salt-soluble fraction (per cent)	Non prot. nitr. (per cent)	High-salt-soluble fraction (per cent)	Insoluble residue (per cent)
-150	7.4	2.5	13.5	0.5
-10	6.3	0.3	11.5	1.1

potassium hydroxide), was added and the suspension autoclaved for 30 min. The supernatant was then assayed as for blood with *Ochromonas danica*<sup>1</sup>. (b) Pantothenic acid; 50 mg of the fraction was suspended in 50 ml. of diastase solution (200 mg 'Clarase' (Fisher Scientific Co., New York), dissolved in 100 ml. distilled water and filtered free of debris). The suspension was incubated for 3 days at 37° C. After incubation, it was autoclaved for 30 min and the supernatant assayed as for blood<sup>1</sup>. (c) Biotin; 50 mg of each fraction suspended in 50 ml. of diastase solution (200 mg 'Clarase' dissolved in 100 ml. of distilled water and filtered free of debris) was incubated for 3 days at 37° C; after incubation, the suspension was autoclaved for 30 min and the supernatant assayed as for blood<sup>1</sup>. (d) Vitamin B<sub>6</sub> and riboflavin; 250 mg of each fraction suspended in 5 ml. of pH 4.5 citrate-phosphate buffer were autoclaved for 15 min. After autoclaving, 20 ml. of diastase solution (80 mg 'Clarase' dissolved in 20 ml. of distilled water). The suspension was incubated for 3 days at 37° C, then autoclaved for 15 min; 25 ml. of distilled water was then added. The supernatant was assayed as for blood<sup>1</sup>. (e) Nicotinic acid; 50 mg of each fraction suspended in 100 ml. of filtered papain solution (1 g papain in 100 ml. of distilled water). The suspension was incubated for 3 days at 37° C, and then autoclaved for 30 min. The supernatant was assayed as for blood<sup>1</sup>. (f) Vitamin B<sub>12</sub>; 200 mg of each fraction was suspended in 10 ml. of *trans*-aconitic buffer at pH 4.5 to which 5 mg of sodium metabisulphite was added. The suspension was autoclaved for 30 min; 10 ml. of distilled water was added after autoclaving. The supernatant was assayed with *Ochromonas malhamensis* as for blood<sup>1</sup>. (g) Total folate activity; 100 mg of each fraction was diluted with 10 ml. of phosphate buffer at pH 6.1<sup>16</sup> to which 10 mg of papain and 10 mg of ascorbic acid were added. The suspension was incubated at 37° C overnight, then autoclaved for 10 min and the supernatant assayed for total folate activity, including N<sup>5</sup>-methyl tetrahydrofolate (N<sup>5</sup>-methyl THF), as for serum<sup>16</sup> with *Lactobacillus casei* (ATCC 7469); the total reduced-folate activity except for N<sup>5</sup>-methyl THF was assayed in a similar way using *Pedococcus cerevisiae* (ATCC 8081); *P. cerevisiae* does not respond to total N<sup>5</sup>-methyl THF<sup>16</sup>. The difference between total folate activity and total reduced folate activity is taken to be total oxidized folate activity, including N<sup>5</sup>-methyl THF as well. (h) Vitamin A,  $\beta$ -carotene, vitamin E, and ascorbic acid; 200 mg of each fraction was suspended or dissolved in 2 ml. of distilled water. Extraction and determination of vitamin A and  $\beta$ -carotene<sup>16</sup>, vitamin E<sup>11</sup>, and total ascorbate<sup>13</sup> were carried out as for serum.

The results in Table 1 show that the  $\alpha$ - and  $\beta$ -globulins together contain the bulk of the vitamins distributed on plasma proteins. Ascorbate, biotin, and vitamin B<sub>6</sub> are found mainly in the  $\alpha$ -globulin; thiamine, pantothenate, and reduced folates occur mainly in the  $\beta$ -globulin fraction.

Table 1. PERCENTAGE OF VITAMIN IN HUMAN PLASMA PROTEINS\*

	IV-1 $\alpha$ -Globu- lin	III $\beta$ -Globu- lin	II $\gamma$ -Globu- lin	Crystal- lized albumin	III $\beta$ -Lipo- protein
Thiamine	19 (2.4)	30 (3.7)	14 (1.8)	12 (1.6)	25 (3.1)
Pantothenic acid	19 (11.0)	46 (25.4)	8 (18)	30 (164)	2 (11)
Nicotinic acid	23 (110)	23 (110)	16 (80)	18 (91)	23 (110)
Biotin	43 (1.8)	19 (0.8)	3 (0.1)	24 (1.4)	3 (0.1)
B <sub>6</sub> activity	64 (5.1)	13 (1.0)	11 (0.8)	6 (0.5)	6 (0.5)
Riboflavin	26 (2.6)	27 (2.7)	19 (2.0)	10 (1.0)	18 (1.8)
B <sub>12</sub>	35 (1.7)	43 (2.1)	0	0	23 (1.1)
Total reduced- folate activity excluding N <sup>5</sup> - methyl THF	27 (90)	40 (132)	3 (8)	2 (7)	28 (92)
Total oxidized folate activity including N <sup>5</sup> - methyl THF	19 (63)	4 (12)	6 (19)	5 (15)	66 (218)
Ascorbic acid	42 (52)	11 (13)	26 (32)	21 (26)	0
$\beta$ -Carotene	0	0	0	100 (54)	0
Vitamin A	0	50 (9)	0	0	50 (9)
Vitamin E	0	0	0	0	100 (231)

\* Numbers in parentheses indicate actual value in ng/mg nitrogen; vitamin B<sub>12</sub> and folates are in pg/mg nitrogen.

Riboflavin and vitamin B<sub>12</sub> are almost equally distributed between the  $\alpha$ - and  $\beta$ -globulins. Most of the oxidized folates and all the vitamin E are concentrated in the  $\beta$ -lipoprotein fraction; vitamin A is equally distributed between  $\beta$ -lipoprotein and  $\beta$ -globulin, whereas nicotinic acid activity is fairly evenly distributed in all the fractions. Besides carrying much of the biotin and pantothenate, albumin is the only fraction in which  $\beta$ -carotene was found.

Until now most of the work on the vitamin distribution of human plasma proteins centred around vitamin B<sub>12</sub><sup>1-3</sup>. Such studies showed that the activity of vitamin B<sub>12</sub> was mainly in the  $\alpha$ - and  $\beta$ -globulins. Our results are similar (Table 1).

It is still not clear why plasma proteins bind vitamins. Presumably, the plasma fractions act as vitamin transporters to metabolic sites, or as temporary storage depots. Vitamin binding is altered in some disease states. This is seen in pernicious anaemia and leukaemia<sup>12,14</sup>, where the ratio of vitamin B<sub>12</sub> binding by  $\alpha_1$ ,  $\alpha_2$ ,  $\beta$ -globulins is changed. During liver disease or injury, the concentration of vitamins in hepatic tissue decreases<sup>15</sup>; on the other hand, the circulating concentration of vitamin B<sub>12</sub> during an acute hepatitis infection increases<sup>14</sup>. In the latter instance, it seems that the liver has temporarily lost its capacity to bind vitamin B<sub>12</sub>, leaving to plasma binders the conservation of the vitamin B<sub>12</sub> released from the liver.

In surveying here the distribution of particular vitamins among particular plasma fractions, using the same assay methods for all the fractions surveyed, the differences in the vitamin distribution (Table 1) between these fractions acquired more significance than the comparison of absolute values obtained by other methods in other laboratories. Although we found that differences in vitamin distribution among human plasma fractions do exist (Table 1) we can now only speculate about the reasons for them.

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## MICROBIOLOGY

# Lack of Correlation between Production of Interferon and Protection of Temperature in Mice Infected with Sindbis Virus

Most authors believe that interferon is important in the defence against virus infection<sup>1,2</sup>, but other non-specific factors may also be involved, such as hyperthermia, the importance of which has often been pointed out<sup>3-5</sup>. Some authors think that there may be a relationship between the production of interferon and the variations of the body temperature, with hyperthermia causing an increase<sup>7</sup> and hypothermia a decrease<sup>8</sup> in interferon production.

We have pointed out before that the inhibition of the development of vaccinia virus in the brains of mice, kept at 35° C, is connected with the thermal sensitivity of the development of the viral strain and not with the interferon production<sup>6</sup>. Vaccinia virus did not induce interferon in any significant amount, and so we conducted the same experiments with Sindbis virus.

Inbred albino mice (of the I.C. strain from the Institut de Recherches Scientifiques sur le Cancer, Villejuif), weighing 15–20 g, were kept in two separate groups, one at room temperature 20° C ( $\pm 2^\circ$  C) and the other at 35° C. After 48 h all the mice were inoculated intracerebrally with 3,000 p.r.u. of Sindbis virus. The mice kept at 35° C had access to two feeding-bottles, one with tap water, the other with salt water at 1 part/1,000. The rectal temperature of the mice was measured daily with an electric thermometer ('Ellab Z8' Electrolaboratoriet, Copenhagen). The temperature of the mice kept at 20° C steadily decreased as infection developed, while the temperature of the mice kept at 35° C remained constant at 1.5° C above the initial temperature of the mice of the first group. Most of the mice kept at 20° C developed paralysis and died. In the other group nearly all the mice escaped paralysis and survived (Table 1).

Table 1. RECTAL TEMPERATURE AND MORTALITY AMONG MICE INOCULATED INTRACEREBRALLY WITH 3,000 P.R.U. OF SINDBIS VIRUS

One group of animals is kept at 20° C and the other at 35° C.		
	Rectal temperature* (°C)	Mortality† (per cent)
Environmental temperature (°C)	20 ( $\pm 2$ )	37.3
	35	38.7
		50/55 = 90.9
		4/48 = 8.3

\* Rectal temperature was measured 3 days after infection.

† Percentage of mortality was determined 20 days after infection.

We investigated the development of the virus and the yield of interferon in both groups of mice. Every other day five mice in each group were killed. Their brains were collected and ground with 25 ml. of phosphate buffer saline in a homogenizer at maximum speed for 1 min. Brain suspension (1 ml.) was stored at  $-20^\circ$  C for virus titration. The remaining 24 ml. was dialysed at 4° C for 48 h against hydrochloric acid/potassium chloride buffer (pH 2.0). The acidified samples were neutralized with 1/5 normal sodium hydroxide and were centrifuged at 17,000 r.p.m. for 30 min. Sindbis virus was plaque assayed in chick embryo tissue culture. Mouse interferon was assayed by a plaque inhibition technique in mice embryo fibroblasts or cultures of L cell, using Sindbis or vaccinia virus for challenging. Preparations for assays were diluted in Eagle's medium. Monolayers grown for 24 h in 60 mm plastic Petri dishes received 1.5 ml. of each dilution of interferon. After incubation at 37° C for 6 h the fluids were removed. The cell monolayers were washed with saline and challenged with 60–80 p.r.u. of virus. After adsorption for 1 h at room temperature, 5 ml. of Eagle's medium containing 10 per cent calf serum was used in conjunction with vaccinia virus. The cultures

inoculated with Sindbis virus were overlaid with 0.7 per cent agar in Eagle's medium. The cells were incubated at 37° C in 5 per cent carbon dioxide. After 48 h a solution of neutral red was added to each culture and the plaques were counted. The interferon titre was expressed as the reciprocal of the dilution necessary to give a 50 per cent depression of the control plaque count ( $PDD_{50}$ ). Both techniques were efficient, but the mouse embryo cells, challenged with Sindbis virus, proved the more sensitive.

Throughout three successive experiments, each with sixty mice, we noticed a very significant inhibition of virus development in the mice kept at 35° C. The greatest yield of virus at either temperature was reached on the fourth day after inoculation. At the higher temperature the production of virus never exceeded 16 per cent of the amount observed among the mice kept at the lower temperature.

The same data apply to the production of interferon. The amount of interferon induced in the brains of the animals kept at an ambient temperature of 20° C was 320–1,280  $PDD_{50}$  but it never exceeded 80 in those kept at 35° C. Fig. 1 shows the time course of the production of interferon and virus in one of the three experiments.

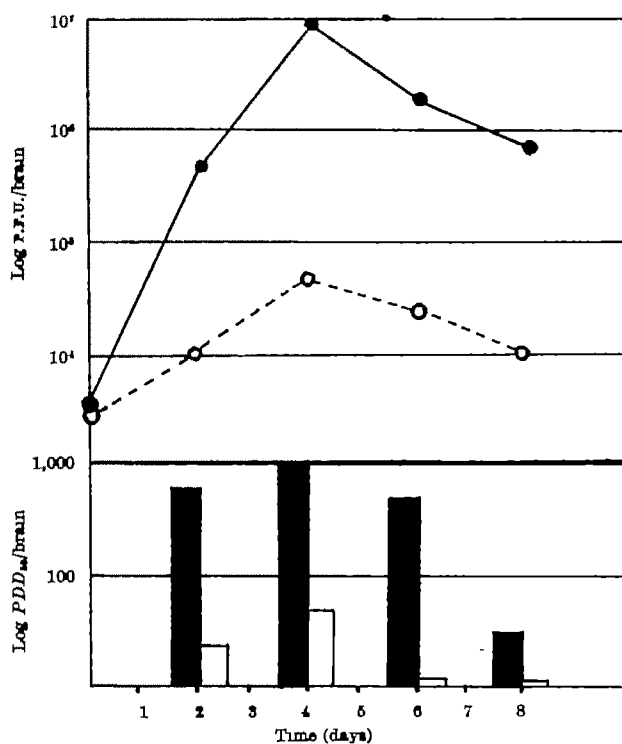


Fig. 1. Time course of interferon and virus production in the brains of mice inoculated intracerebrally with 3,000 p.r.u. of Sindbis virus and exposed to environmental temperatures of 20° and 35° C. (●), Virus (p.r.u./brain) of the mice kept at 20° C; (○), virus (p.r.u./brain) of the mice kept at 35° C. Black columns, interferon ( $PDD_{50}$ /brain) of the mice kept at 20° C; white columns, interferon ( $PDD_{50}$ /brain) of the mice kept at 35° C.

Thus we may assume that there is no relationship between the survival of the mice kept at 35° C and an increase in the production of interferon, particularly because the production of interferon is lower at that temperature. We can therefore conclude that interferon is not active in the defence of the body against infection during experimental hyperthermia in mice infected with Sindbis virus.

These findings lead us to assume that, as we pointed out for mice infected with vaccinia virus, an increase in body temperature has a definite effect on virus multiplication,

probably by checking a thermal-sensitive event in the development cycle.

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### Action of Ethidium Bromide on Growth of Herpes Virus in Cell Cultures

ETHIDIUM bromide (EB) (2,7 di-amino-phenyl phenanthridine 10-ethyl bromhydrate) is an antimicrobial drug which, like proflavine (an acridine), forms reversible complexes with nucleic acids<sup>1,2</sup>. *In vitro*, it inhibits DNA-polymerase and, at a lesser rate, DNA-dependent RNA-polymerase<sup>3</sup>. In the same way, EB and related phenanthridine derivatives inhibit the synthesis of nucleic acids in bacteria<sup>4</sup> and Ehrlich ascites cells<sup>5</sup>; they are also trypanocidal<sup>6</sup>. Moreover, the photosensitization of arboviruses<sup>7</sup>, the inhibition of their development<sup>7</sup> and that of a *Pseudomonas pyocyanea* phage<sup>8</sup> by these compounds have been reported. Although the responses of several virus-infected systems to the action of acridines have been given in detail<sup>9,10</sup>, the comparable interactions with EB have not yet been so clearly described. The present paper details the action of EB on herpes virus and its growth in baboon kidney cells.

We studied first the direct action of EB at 37° C on herpes virus in the absence and presence of light. Rabbit kidney cells, cultured in casein hydrolysate medium with 5 per cent calf serum, were inoculated with herpes virus (strain IP 593055). 42 h after inoculation the infected cells were frozen and thawed in their medium and cell fragments were eliminated by centrifugation at 1,000g for 10 min. 1 ml. of the virus suspension was distributed into each of 13 flat 60 ml. flasks. 0.5 ml. culture medium

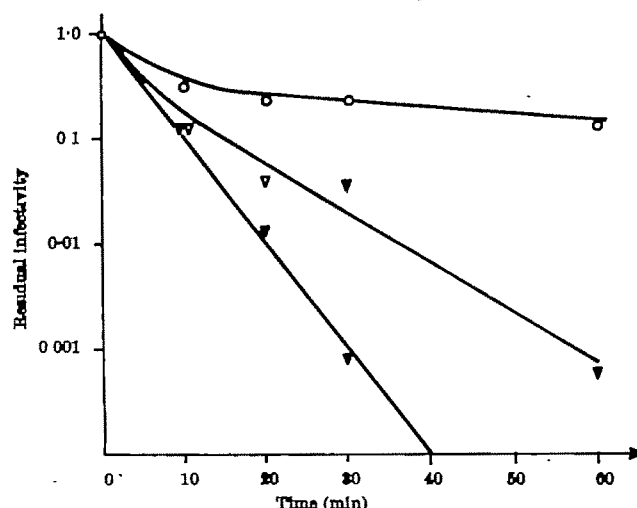


Fig. 1. Action at 37° C of EB on herpes virus in the presence and absence of light. ○—○ 1,000 µg EB/ml.; x—x, 1,000 µg EB/ml.; ▼—▼, 1,000 µg EB/ml. + light.

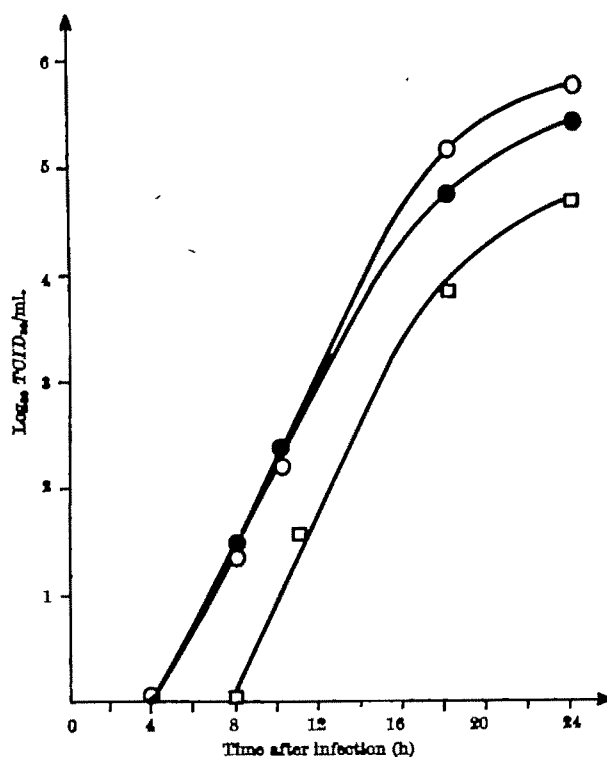


Fig. 2. Action of EB on kinetics of herpes virus growth. ○—○, Untreated controls; ●—●, 1,000 µg EB/ml. 2 h before virus inoculation; □—□, 1,000 µg EB/ml. from the second to the fourth hour after virus inoculation.

containing 3 mg EB/ml. was added to eight of the flasks, and half of these were wrapped in aluminium foil to keep out the light. The five remaining flasks received 0.5 ml. of medium with no EB. All flasks were then kept for various periods at 37° C, 15 cm from three cold white lamps with a total power of 100 W. The residual virulence of each sample was immediately determined on baboon kidney cells by inoculating ten-fold dilutions, each dilution being inoculated into five tubes. The cytopathogenic effect was read on the third day after inoculation, and titres calculated according to the method of Reed and Muench<sup>11</sup> and expressed as log<sub>10</sub> TCID<sub>50</sub>/ml. Fig. 1 shows that EB has both an inactivating and a photosensitizing activity with herpes virus.

Because of this direct effect, the infected cell system should be exposed to EB only during the eclipse phase in order to study the effect of EB on herpes virus growth. 60 ml. flasks containing baboon kidney cell cultures in Eagle's medium<sup>12</sup> with 10 per cent foal serum were distributed into three batches, two of which were inoculated with herpes virus, the multiplicity of infection being approximately 30. After incubation at 37° C for 1 h, the inoculum was removed and the cell layers washed five times with Hanks fluid. Non-absorbed virus was then neutralized for 1 h at 37° C with a hyperimmune rabbit serum, which was afterwards removed. After three washings, each culture was placed for 2 h at 37° C in contact with 2 ml. of a solution of 1 mg/ml. EB in an Eagle medium containing 5 per cent foal serum for the first batch of tubes and 2 ml. of medium for the second batch. The supernatants were removed, the cells washed five times and 5 ml. Eagle medium containing 5 per cent foal serum added to the cell cultures. The cultures were incubated at 37° C. Flasks containing the third batch were incubated with EB for 2 h before inoculation. At different times, two cultures of each batch were frozen and thawed three times, mixed, and titrated.

Fig. 2 shows that EB has little effect on the kinetics of viral growth when added 2 h before inoculation at the maximal concentration, which does not modify the cell

viability for 24 h. On the other hand, when EB is added from the second to the fourth hour after inoculation, there is a delay of 4 h before viral growth begins, although growth from then on is normal.

The photosensitivity of virus grown on cells treated with various stains has been reported for certain arboviruses<sup>8</sup> and for polio virus<sup>14</sup>. In our experiments baboon kidney cell cultures were inoculated with herpes virus, and half of them were then treated with EB from the second to the fourth hour following inoculation. All the cultures were collected 24 h after inoculation, and submitted to three cycles of freezing and thawing. Viral suspensions obtained after centrifugation were illuminated at 37° C for 2 and 4 h under the conditions described. The residual infectivity of the virus from treated cells was 1.6 per cent after 2 h and 0.1 per cent after 4 h of light treatment; it was 7.6 and 1.6 per cent for virus grown in the cells not treated with EB. This finding shows that the virions formed by cells treated with EB also have a certain photosensitivity.

The direct action of EB on normal herpes virus as well as the properties of virus grown in cells treated with EB may be attributed to binding of the stain on the viral genome, which could inhibit its expression or favour its degradation under the action of light. The combination of EB with cellular and viral DNA, causing inhibition of cell synthesis (at high concentrations of dye) and blocking the transcription and duplication of viral DNA, probably also accounts for the effect of EB during the virus eclipse phase. The temporary character of this inhibition is interesting in view of the known *in vitro* reversibility of the DNA-EB complex<sup>1</sup>.

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## Isolation of SV<sub>40</sub> Virus and Its Effect on Renal Function in African Green Monkeys

SV<sub>40</sub> virus is of considerable concern, because it has been shown to be present in a number of samples of inactivated polio virus and adenovirus vaccines produced before 1960, and it is capable of inducing both chromosomal changes and tumours in certain experimental systems. Since the isolation of SV<sub>40</sub> virus from naturally infected monkeys was reported by Sweet and Hilleman<sup>1</sup>, several

investigators have examined experimental infection in monkeys and man with this agent. It has been shown that infection can be produced in African green monkeys by several routes of inoculation<sup>2,3</sup>. Inoculation of human volunteers<sup>4-7</sup> has resulted in low grade infection including some evidence of transient chromosomal changes. Furthermore, administration of this virus to hamsters has resulted in the production of tumours<sup>8,9</sup>.

The experiments reported here were designed to investigate the effects of intravenous infection of the African green monkey with SV<sub>40</sub> virus and to investigate the chief sites of viral growth and the possible effects on renal function in this primate.

African green monkeys (*Cercopithecus aethiops tantalus*) were used. When these animals arrived, they were first quarantined for 3 weeks and then transferred to individual sterile cages. They were tuberculin tested and checked for other infections. The animals were pre-tested for the presence of antibodies to SV<sub>40</sub> virus before infection. Only animals free of antibodies were used. SV<sub>40</sub> virus adapted to African green monkey kidney culture was inoculated intravenously using 1 ml. of infected tissue culture fluid which contained 5.2 TCID<sub>50</sub> (log<sub>10</sub>) of virus (experimental animals) or 1 ml. of control infected tissue culture fluid (control animals). The animals were divided into three groups, and each group consisted of two experimental monkeys and one control. Group 1 animals were killed 2 days after inoculation; group 2 animals were killed 2 weeks after inoculation; and group 3 animals were killed 2 months after inoculation.

Blood and urine specimens were obtained at 2-5 day intervals for 4 weeks, and at weekly intervals for an additional 4 weeks. Kidneys (separated into medulla and cortex), lymph nodes, spleen, liver and lung from dead animals were tested for virus and examined for gross and microscopic pathological changes.

Virus infectivity titres and neutralization tests on all virus isolates from every tissue tested were determined in primary tissue culture cells of African green monkey kidney (AGMK) free of SV<sub>40</sub> virus. Inoculated AGMK tube cultures were incubated in the stationary phase at 37° C and examined microscopically from days 8 to 14 after inoculation for cytopathic effect. Appropriate controls were maintained in each test. Infectivity and neutralization 50 per cent end points were calculated by the method of Reed and Muench<sup>10</sup>.

Laboratory tests<sup>10</sup> to detect possible renal damage and other functional changes were conducted on all surviving animals at 2 or 5 day intervals for 4 weeks, and at weekly intervals for an additional 4 weeks. The tests utilizing the blood specimens included sedimentation rate, total serum protein, albumin/globulin ratio, blood urea nitrogen and cholesterol. Uncentrifuged urine specimens were tested for specific gravity, pH, albumin, sugar, acetone, red blood cells, white blood cells, and casts (also red and white cells and casts after centrifugation).

The concentrations of infectious virus recovered from organs of monkeys killed 2 days, 16 days and 60 days respectively after intravenous inoculation of SV<sub>40</sub> are shown in Table 1. The titres of virus in infected animals 2 days after infection were larger in the cortex of the kidney, the lymph node, spleen and lung. Sixteen days after infection concentrations of virus in these organs had increased by factors of 2-3 log<sub>10</sub> with the greatest increase in the kidney medulla (3.5 log increase). The concentration of virus in the lung, however, decreased ten-fold. These results suggest that virus multiplication is limited to the spleen and kidney in particular and the reticulo-endothelial system in general. Although virus was recovered from all organs tested 2 months after infection, the concentrations were less than ten infectious particles/ml. of 10 per cent suspension of tissue homogenate.

Fig. 1 shows the concentrations of SV<sub>40</sub> virus recovered from the urine and blood at different times from 2-60 days after infection. Viraemia was detectable within 2



Table 1. TITRES\* (IN TISSUE CULTURE) OF SV<sub>40</sub> VIRUS ISOLATED AT DIFFERENT TIMES FROM AFRICAN GREEN MONKEYS AFTER INTRAVENOUS INJECTION

Organs tested	Group: Days:	Days after infection		
		1	2	3
		2	16	60
Kidney medulla		5.4	8.9	+
Kidney cortex		6.4	7.4	+
Lymph node		6.9	8.4	+
Spleen		6.9	9.4	+
Liver		5.4	6.9	+
Lung		7.4	6.4	+

+, Virus recovered but titre less than 1 log<sub>10</sub>/ml.\* Titres expressed as TCID<sub>50</sub> (log<sub>10</sub>)/ml.

days after intravenous inoculation of virus, and within 7 days after infection most of the virus in the blood had disappeared; and at 12, 16, 24, 30 and 60 days after inoculation no virus was detected. The greatest titre of detectable virus in the blood was 2.2 (log<sub>10</sub>)/ml. In contrast, virus was not detected in the urine of inoculated monkeys until 7 days after inoculation; thereafter the virus titre increased until it reached a peak of 4.0 (log<sub>10</sub>) 16 days after infection. This rise was followed by a gradual loss until, 2 months after inoculation, no virus could be detected in the urine.

Microscopic examinations of unspun specimens of urine revealed small numbers of red cells (3-30) and casts in two of four infected animals on the eighth to the thirteenth day after inoculation. Blood from the same animals showed increase of blood urea nitrogen to levels of 52 to 55 (normal control 15 to 30) on the thirteenth day after inoculation, and also elevation of blood cells sedimentation rates of 13 to 33 mm/h (normal control 2 to 10 mm/h) during the 2 weeks following inoculation. All other tests on urine and blood mentioned above were of the same values as in control animals.

None of the animals injected with SV<sub>40</sub> virus showed any clinical sign of virus infection. The investigations carried out on the blood and urine specimens showed that two of four infected animals did have red cells and casts in their urine. Examination of blood revealed moderate elevations of blood urea and nitrogen sedimentation rates primarily during the second week after inoculation; these were transitory effects, however, and did not occur in the three control animals.

There is suggestive evidence that SV<sub>40</sub> virus produced abnormalities of kidney function associated with viruria during the investigation as evidenced by moderate elevations of blood urea nitrogen and red blood cell sedimentation rates. Although silent infections in man

with SV<sub>40</sub> virus inoculated into volunteers<sup>7,8</sup> have been reported, microscopic pathological reports of organs (biopsies or autopsies) have not been available.

The data presented have shown that a rapid multiplication of SV<sub>40</sub> virus occurred in the various organs tested. Only 10<sup>-2</sup> (log<sub>10</sub>) virus was injected, yet within 2 days this ordinarily slow growing virus had multiplied so that more than 100 times the amount injected was present in the lung and over eighty times the amount injected in the spleen and lymph node. All other organs tested contained as much or more virus than was injected. After 16 days the virus in the spleen and kidney medulla had multiplied about ten-thousand-fold. Virus concentration, especially in the kidney medulla, coincided with the detection of virus in the urine at concentrations greater than that found in the blood 2 days after inoculation.

The results reported here confirm some of the observations noted in African primates infected by different routes with SV<sub>40</sub> virus<sup>2,3</sup>. Similar findings in mumps and cytomegalovirus infections of man, in which viruria occurred in the absence of or in the presence of low viraemia, have been reported<sup>11</sup>. In the investigations reported here, virus multiplication occurred at greatest titres in the medulla of the kidney, the spleen and lymph nodes 16 days after inoculation, although viraemia was not apparent at this time.

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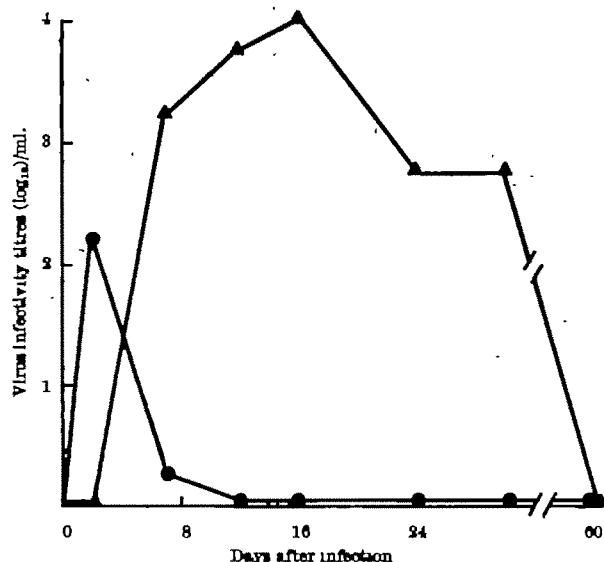


Fig. 1. Concentrations of SV<sub>40</sub> virus recovered from the urine (▲) and blood (●) at different periods from 2 to 60 days after inoculation of African green monkeys.

### Terminology of Bacterial Fimbriae, or Pilli, and their Types

THE nomenclature of the non-flagellar filamentous appendages of bacteria is in a confused state and the need for clarification is increased by a renewal of interest brought about by findings that suggest that some types of these structures may play a part in bacterial conjugation<sup>1-4</sup>. The appendages were first clearly described and distinguished from flagella and extracellular slime by

Houwink and van Iterson<sup>4</sup>, who called them "filaments", an unspecific term which was also used by Brinton *et al.*<sup>5</sup>. The distinctive name, "fimbriae", was proposed by Duguid *et al.*<sup>7</sup> in 1955 and has since been adopted by most authors publishing work on the subject in Britain and elsewhere.

In 1959 Brinton<sup>6</sup> introduced the term "pili" (Latin, hairs) as a synonym, and later<sup>8</sup> urged that it should replace "fimbriae". As chief reason for preferring "pili", he argued that "fimbriae" was "linguistically incorrect" because it is used in English as a collective plural word meaning a bordering fringe, not as an ordinary plural word meaning a number of distinct fibres. This objection is erroneous, and indeed the same kind of objection could be raised to "pilus", which in Latin is sometimes used to mean "the hair" collectively, and in English, as "pile", is generally used in the collective sense.

It is true that some dictionaries, including the *Oxford English Dictionary*<sup>10</sup>, define the English use of "fimbria", or "fimbriae", as a fringe, but others, particularly scientific dictionaries, show also the alternative use of these terms as meaning, respectively, one and several distinct fibres. Thus, the *British Medical Dictionary*<sup>11</sup> gives "fimbriae", "a thread or fringe", and the *Dictionary of Biological Terms*<sup>12</sup> gives: s.v. fimbria, "one of the delicate processes fringing the mouth of tube or duct, as of oviduct, or of siphon of molluscs". In any case, the derivation of a scientific term from a Latin word need not be limited by the current use of the word in English, but may validly be based on any meaning of the word in Latin usage. As stated by Duguid *et al.*<sup>7</sup>, the meanings of "fimbriae" in Latin include those of "threads" and "fibres". The *Oxford English Dictionary*<sup>10</sup> gives: "L. *fimbria* thread, fibre, fringe", and the Berlin *Thesaurus Linguae Latinae*<sup>13</sup> gives quotations from Latin authors that show by context that "fimbria" was sometimes used to mean a single thread or fibre and "fimbriae" to mean several threads or fibres. Thus, "fimbriae" is not incorrectly or inappropriately derived, and since it has temporal priority over, and wider currency than, "pili", it should be used in preference to the latter term. Neglect of priority in such questions of terminology is liable to encourage a burdensome proliferation of synonyms.

A further cause of confusion has now arisen in relation to the classification of the different types of fimbriae. Brinton<sup>6</sup> has published a classification of six types, types I-V and F; type F fimbriae are the scanty filaments evoked by the male fertility factor F and are thought to play a part in bacterial conjugation. Later, Duguid *et al.*<sup>14</sup>, in an article they submitted for publication before they had seen Brinton's article, proposed a classification of seven types (1-6, and F). Unfortunately, only types 1 and F are the same in both classifications. Brinton gives little information about the properties and occurrence of his types II-V. His type II, however, is patently inadmissible, because the only distinguishing information he gives about it is that the filaments are 48 Å in diameter and that they were described by Duguid *et al.*<sup>7</sup>. The latter authors did not describe any fimbriae that were 48 Å in diameter; they described only the fimbriae of *Escherichia coli* that are 75-100 Å in diameter and are designated type 1 in both classifications. It seems that it will be necessary for authors wishing to refer to fimbriae of types other than 1 and F to state which classification they are using. In any case, because of lack of detailed information about some of the types of fimbriae that have been described the present classifications must be regarded as provisional.

A term seems to be required to describe the group of fimbriae associated with bacterial transfer factors. "Type F" is not appropriate for this use because, although F was introduced as an abbreviation for "fertility", it has come to be used exclusively to denote the activities of the F factor, the specific transfer factor of *Escherichia coli* K12. The term "F fimbriae" could therefore perhaps be reserved for the fimbriae dependant on the presence of

F. Only the F and fl+ transfer factors have so far been shown to determine the production of fimbriae, but there is no reason to doubt that fl- transfer factors stimulate the production of functionally similar appendages, which may have different antigenic and phage-adsorption properties from those of the F and related fimbriae. It is suggested that the group of fimbriae associated with transfer factors, that is, F fimbriae and their analogues, should be described as "sex fimbriae". This description will distinguish them from the common types of fimbriae (types 1-6), which appear to be determined by chromosomal genes and not to be essentially associated with bacterial conjugation.

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### Infection by *Hemileia vastatrix*

RAJENDREN<sup>1</sup> has recently published observations on the nuclear life cycle of *Hemileia vastatrix* in which he describes penetration of the leaf as occurring by means of a "bifid infection peg" produced from an appressorium, which he considers does not lie over a stoma. The two branches of the "peg" are shown as growing from the appressorium over the surface of the leaf to penetrate two separate stomata. It is surprising that Rajendren does not comment on the fact that this method of penetration is quite different from that given in the literature and which was first described by Marshall Ward<sup>2</sup>.

During my investigations of the biology of *H. vastatrix*<sup>3</sup>, using a cellulose acetate film stripping technique, I observed several hundred penetrations. The appressorium was almost invariably formed so as to lie over a stoma and an infection peg was produced on its lower surface and grew directly down into the stomatal aperture. This agrees with Ward's observations. I never saw a penetration of the type figured by Rajendren, which would therefore appear, at the least, to be very exceptional.

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## CYTOLOGY

Stomatogenesis and Cell Division in *Euplotes* inhibited by Ultra-violet Microbeam Irradiation

SELECTIVE irradiation of a small part of a living cell with an ultra-violet microbeam has been used to investigate functions of cellular organelles. Functions of the kinetochore, centriole and spindle fibre have been investigated in grasshopper<sup>1</sup> and newt<sup>2-4</sup> cells in culture, as well as functions of the nucleolus in HeLa cells in culture<sup>5</sup>. In addition we have investigated radiation damage and recovery phenomena in a living cell by this technique<sup>6-8</sup>. This work, which involved selectively blocking stomatogenesis, was intended to show the close relationship between stomatogenesis and cell division.

For cell material we used *Euplotes eurystomus* Stock III, supplied by Dr M. Saito of the University of Yokohama. The cells were cultivated in wheat extract at 22° C with *Chilomonas paramecium* as bait. In the culture conditions, the generation time of the cells was 15.5–23 h. The variation is mainly the result of the number and/or the growing condition of *Chilomonas*; when the bait protozoa are growing more rapidly in the culture, the cells have a comparatively shorter generation time and vice versa. The response of the cells to the irradiation is also affected by their growing conditions. The experiments described here were carried out with two types of cells in a logarithmic phase (type A) and in a pre- and post-logarithmic phase (type B). The cells were fed with two types of the bait protozoa in a growing phase (type a) and in a stationary phase (type b) after the irradiation, and so four groups of cells were used in each experiment. For example, when type A cells were irradiated and recultured by feeding with the bait protozoa of type a, the cells were abbreviated as type Aa cells.

The apparatus employed for irradiation was of the Uretz type, constructed by Izutsu<sup>1</sup> in our laboratory, and the microbeam spot was 2  $\mu$  in diameter on the focal plate. Exposure was usually for 2–3 min, using a high-pressure mercury lamp (Toshiba 80 W). One cell was pipetted from the culture and was closed with a small amount of agar-agar paste between a quartz coverslip and a glass slide. After fixing the cell by removing excess medium and paste with filter paper, part of the cell

was irradiated, and the cell was recultured in a glass slide with a hole filled with culture medium containing *Chilomonas* to examine the progress of division. The period of observation after irradiation was 40–60 h, and the cells divided at least once in most cases. Morphological examination was made by haematoxylin-eosin staining, acetic carmine staining, and the Chatton-Lwoff silver impregnation method, as described by Corliss<sup>9</sup>.

From morphological findings of the stomatogenesis on the cell surface and the reorganization bands of the macronucleus, the mitotic cycle of the *Euplotes* cells can be divided into three stages. Stage 1 (about 30 per cent of the generation time) is a period showing no stomatogenesis and no DNA synthesis; stage 2 (about 55 per cent of the generation time) is a period of both phenomena; stage 3 (about 15 per cent of the generation time) is a period of cytokinesis. The stomatogenesis appears synchronously with the DNA synthesis.

In the late stage 1 of the type Ab, Ba or Bb cells, we irradiated for 2.5 min a region adjacent to the oral apparatus, where the oral anlage of the daughter cell appears in the early stage 2. This region was called the stomatogenic region (Figs. 1 and 2, *sr*). In this experiment, none of the twenty-two irradiated cells divided when watched for 41–65 h, and at least three of these cells died on the sixth day. Three of the eight cells irradiated for 2 min divided at least once in this period. On the other hand, when the Aa type cells were irradiated there for 2.5 min all the fourteen cells showed subsequent division in a period of 43–45 h. In the early part of stage 2, when oral formation had begun, the irradiation of the region failed to block the subsequent division in all of the twenty cells irradiated for 2.5 min and even when irradiation lasted 4–5 min.

The irradiated site on the cell surface was observed as a slightly dark portion corresponding to the irradiation spot in form and size with ordinary illumination through a reflecting objective lens. In some cells ciliary movement was weakened shortly after irradiation.

In the control experiment a portion opposite the stomatogenic region across the adoral zone of membranellae (AZM) (Fig. 2 (2)) was irradiated for 2.5 min in late stage 1 of the type Bb cells. All five cells irradiated for 2.5 min divided 40–47 h after irradiation. When one or two portions of the AZM were given this dose, five of the seven cells divided at 39–41 h and each of the remaining two cells divided at 64 h and 92 h. The waving movement of the membranellae was not affected by irradiation. All five cells irradiated at a peripheral part of the cell were able to divide about 40 h after irradiation.

Zeuthen and Williams<sup>10</sup> examined morphogenesis between divisions in heat-synchronized *Tetrahymena*, and indicated that heat shock induced synchronization by interfering with the assembly of oral apparatus from available precursor proteins. Whitson and Padilla<sup>11</sup> have examined effects of actinomycin D on the stomatogenesis and cell division in temperature-synchronized *Tetrahymena*, and concluded that this drug inhibited the former and secondarily the latter. They could not exclude, however, the possibility that actinomycin inhibition in the cell division involves other blocks as well. The results of our selective irradiation strongly suggest that the stomatogenesis is a trigger mechanism in the onset of division in *Euplotes*, and that, when oral formation has begun, its process becomes relatively resistant to ultra-violet irradiation. The resistance of the stomatogenic region in the rapid growing cells may be caused by the recovery phenomena of radiation damage.

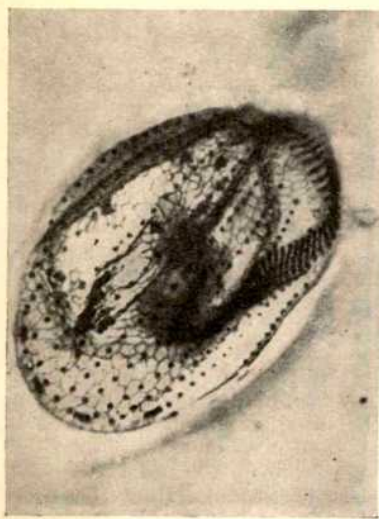


Fig. 1

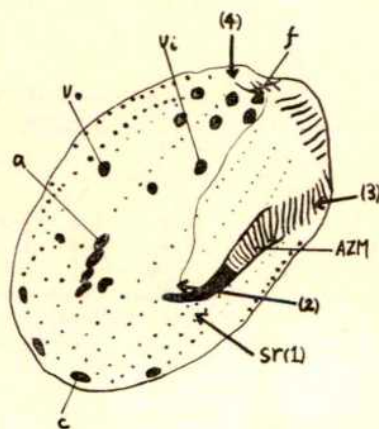


Fig. 2

Fig. 1. A silver-impregnated *Euplotes* cell in early stage 2.

Fig. 2. Diagram of Fig. 1. *a*, Right anal cirrus; AZM, adoral zone of membranellae; *c*, right caudal cirrus; *f*, frontal cirrus; *sr*, stomatogenic region; *vi*, inner ventral cirrus, and *va*, outer ventral cirrus. Arrows marked with numerals show irradiation portion: (1), *sr*; (2), a portion opposite to *sr* across AZM; (3), a portion of AZM; (4), a peripheral portion of the cell.



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### Changes in Surface Charge of HeLa Cells during the Cell Cycle

A METHOD of obtaining large numbers of fixed cells in metaphase arrested by colchicine, from a population of cells growing on a glass surface, by a method dependent on the attenuated adhesion of the cells to the surface has been described<sup>1</sup>. A modification of this method was suggested to obtain a viable population of synchronous cells. This was demonstrated<sup>2</sup> and a method for obtaining large numbers of HeLa cells of which 80 per cent to 95 per cent showed mitotic figures has been described<sup>3</sup>. The decrease in attachment of the mitotic cells was ascribed to a decrease in the area over which the adhesion occurred<sup>1</sup>. It is, however, possible that there is a change in the intrinsic strength of the cell-glass adhesions arising from an alteration in the physical properties of the surface of the cells which round up during mitosis. One of the most easily determined of these properties is the  $\xi$ -potential arising from the cell surface charge, and accordingly we have made determinations of the electrophoretic mobility of parasynchronous populations of HeLa cells at various stages in the cell cycle.

Parasynchronous populations of HeLa cells were prepared by the method of Robbins and Marcus<sup>4</sup>. The mitotic index of such preparations was generally about 60 per cent to 85 per cent and the degree of synchrony as defined by Engleberg<sup>5</sup> was about 50 per cent. Where the cells were maintained in suspension culture throughout the cell cycle, the cell number and the rate of DNA synthesis were also determined as described before<sup>6</sup>.

Determinations of electrophoretic mobility were made in a cylindrical cell apparatus essentially the same as that described by Bangham, Flemens, Heard and Seaman<sup>6</sup>.

Cells to be measured were washed three times in cold 0.146 molar sodium chloride buffered to pH 7.2 with 10 per cent v/v 0.1 molar phosphate buffer. The cells were suspended to a density of about  $2 \times 10^6$  cells/ml. and small quantities of about 0.2 ml. of this suspension were introduced into the field of view of the microscope with a fine polyethylene tube attached to a 1 ml. tuberculin syringe; the electrophoretic cell was already filled with phosphate buffered saline. It was thus possible to obtain reliable values for electrophoretic mobility using about  $4 \times 10^5$  cells. Determinations were usually made at 0°C in order to arrest metabolic changes. Some measurements were made at 25°C, but in any case appropriate corrections for viscosity were made and the results

are presented as equivalent 25°C mobilities. Measurements were made on asynchronous cells from suspension culture, on freshly collected mitotic cells and on asynchronous cells collected from monolayer culture using EDTA. In each determination observations were generally made on between sixteen and thirty cells of the population.

The electrophoretic mobility of HeLa cells cultured in asynchronous suspension was observed on a total of 214 cells to be  $-0.98 \pm 0.06 \mu\text{sec/v/cm}$  at 25°C in the medium defined above. With different preparations values of between  $-0.95$  and  $-1.03 \mu\text{sec/v/cm}$  were obtained. The values obtained for eight separate preparations of synchronous cells at the moment of preparation, that is, at the mitotic peak, are listed in Table 1. These populations clearly display a greater mobility than asynchronous cells.

Table 1. ELECTROPHORETIC MOBILITIES OF SYNCHRONOUS HELa CELLS DURING MITOSIS

Experiment No.	Electrophoretic mobility ( $\mu\text{sec/v/cm}$ ) corrected to 25°C	No. of cells at each determination
1	$-1.21 \pm 0.09$	27
2	$-1.30 \pm 0.15$	40
3	$-1.41 \pm 0.11$	30
4	$-1.14 \pm 0.16$	26
5	$-1.24 \pm 0.10$	30
6	$-1.27 \pm 0.09$	30
7	$-1.25 \pm 0.17$	30
8	$-1.29 \pm 0.10$	24

It is possible that this increase in electrophoretic mobility is a property of cells grown in monolayer as against cells grown in suspension, rather than that it is associated with the period of mitosis. In order to test this suggestion cells were collected from an asynchronous monolayer using EDTA medium<sup>7</sup> and their mobility was compared with that of a synchronous mitotic population treated for a similar period with the EDTA medium. The results of this experiment are contained in Table 2. We thought it desirable to use EDTA rather than trypsin to collect the asynchronous monolayer because it has been reported that trypsinization can alter the electrophoretic mobility of cells<sup>8</sup>. The above experiment demonstrates that treatment of cells with EDTA probably does not affect their electrophoretic mobility and, further, that the enhancement of net negative charge previously seen is associated with the mitotic state and not with the method of cell culture employed.

Table 2. ELECTROPHORETIC MOBILITIES OF SYNCHRONOUS AND ASYNCHRONOUS MONOLAYER GROWN HELa CELLS

Cell population	Mobility ( $\mu\text{sec/v/cm}$ )	No. of cells at each determination	Temperature
Asynchronous monolayer cells collected with EDTA	$-1.06 \pm 0.10$	20	25°C
Synchronous mitotic cells treated with EDTA	$-1.36 \pm 0.10$	16	25°C
Synchronous mitotic cells without EDTA treatment	$-1.39 \pm 0.09$	20	25°C

In a further experiment a synchronous population of cells was collected at 37°C and then allowed to grow on in suspension culture for 26 h. Samples were taken periodically and determinations made of their electrophoretic mobility. It was found that the mobility rapidly fell away, reaching after 60 min a value only just distinguishable from that of asynchronous populations. During the interphase period the value obtained was exactly the same as for asynchronous suspension culture cells, but during the next period of cell division, as indicated by the growth curve, a significant increase in mobility occurred, although the scatter of the observations was increased compared with the freshly prepared mitotic population. These results are contained in Fig. 1 where the standard deviations of the mobility measurements are indicated.

The results reported here confirm the expectation that cells in mitosis in monolayer culture which are readily detached from the substrate display altered surface pro-

perties. Mayhew and O'Grady<sup>9</sup> have described similar changes in tissue culture cells forced into parasynchronous growth by the method of double thymidine blocking. It should be emphasized that this is a transient property and is in no way similar to the generalized increase in surface charge displayed by cells of rapidly proliferating tissues which has been reported by other authors<sup>10,11</sup>. The observed increase in surface charge density is probably only associated with a small time interval during the cell cycle. This would account for the fact that cells with abnormally large mobilities are not generally seen in electrophoretic observations of populations of asynchronous cells when measurements are made on only about twenty individual cells. Furthermore, because the parasynchronous mitotic populations can be expected to contain a comparatively large percentage of cells, dependent on the degree of synchrony, not displaying the transient charge increase, a larger scatter in the individual cell mobilities would be expected. This is reflected in the standard deviations of the mobilities of mitotic populations which are in general about twice those found for asynchronous populations, which difference could easily arise if the mitotic population contained 10 per cent to 20 per cent of cells with the interphase mobility.

The origin of the extra charge remains unknown. Treatment with neuraminidase reduces the mobility of both asynchronous<sup>9</sup> and mitotic cells considerably, indicating that a substantial proportion of the surface charge derives from peripheral sialic residues. It seems unlikely, however, that the increase is simply the result of a surface concentration of charge-bearing macromolecules as the cells round up for mitosis, because all the cells when taken up in suspension for measurement adopted a spherical configuration, thus minimizing their surface area.

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### Natural and Induced Changes in Chromosome Size and Mass in Meristems

PIERCE<sup>1</sup>, in 1937, claimed to have effected large changes in chromosome size in meristematic root tip cells of *Viola conspersa* after treating seedlings with different culture solutions. For example, he reports that in seedlings grown for 2 months in a culture solution with a large content of phosphate the chromosomes were 300 per cent larger by volume than in seedlings grown in a solution without phosphate. We think that his results provide the only direct evidence to show that changes in chromosome size may be induced by external environmental influences. The results pose two questions of particular interest. First, did the induced changes in chromosome volume reflect changes in chromosome mass or merely represent changes in the organization, for example, the coiling, of the chromosome material? Second, were the induced changes associated with quantitative changes in chromosomal DNA and possibly, therefore, in genetic information? This question is especially pertinent because variation in chromosome size between different individuals is, normally, positively correlated with changes in the quantity of nuclear DNA<sup>2,3</sup>. The following is a brief account of an experiment with seedlings of rye (*Secale cereale*,  $2n=14$ ). The experiment completely confirms Pierce's results in showing that changes in chromosome size may readily be induced. The experiment also provides answers to the above questions in relation to chromosome mass and chromosomal DNA. In addition the experiment brings to light a variation in chromosome size, independent of the inducing treatments, that seems to be associated with the "ageing" of the seedlings.

Rye seedlings, 3 days after germination, were transferred to one of three solutions of the kind used by Pierce, that is, a standard culture solution with "normal" phosphate content, a "high" phosphate solution and a culture solution without phosphate. Chromosome size was estimated and expressed as the total volume in each cell. The volume was calculated from estimates at metaphase of the total length of chromatids and of their average width. For the calculation the assumption was made that the chromatids are cylindrical in form. Chromosome volumes at intervals during the treatments appear in Fig. 1.

The results confirm in every essential respect the findings of Pierce. Chromosome size in root tip meristems increases with increasing concentration of phosphate in the culture medium in which the seedlings were grown. The increase is consistent throughout the experiment and at 3 weeks the chromosome volume is 50 per cent greater in the seedlings grown in a medium containing a large concentra-

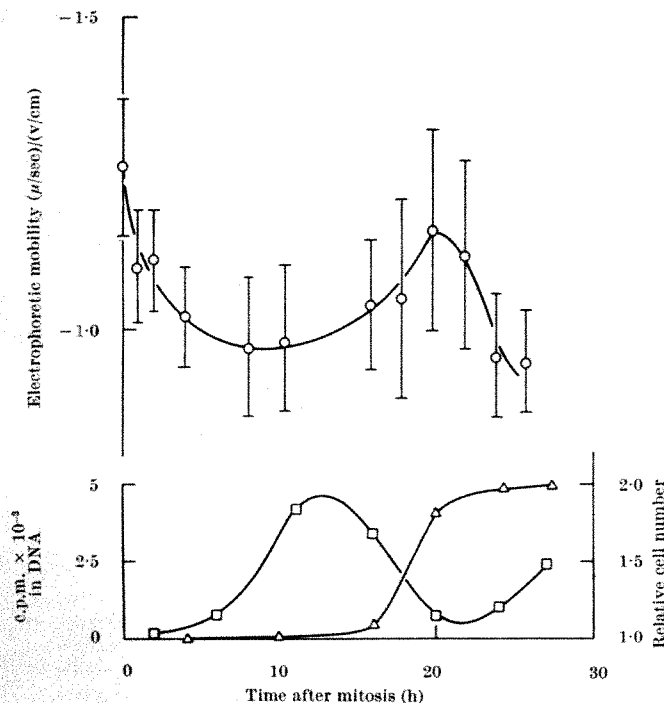


Fig. 1. Electrophoretic mobility of synchronous HeLa cells throughout the cell cycle (—○—). Rate of DNA synthesis (—□—) and cell numbers (—△—) are shown for the same culture.



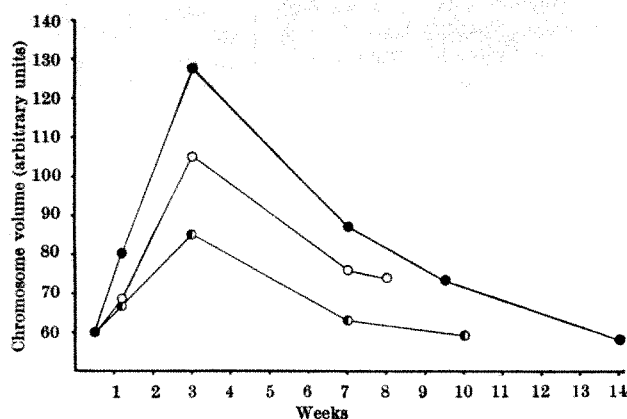


Fig. 1. Volumes of rye chromosomes at metaphase in root tip meristems of seedlings grown in three different culture solutions. Each point represents the mean volume of chromosomes in metaphases from three root tips in each of two plants. ●, High concentration of phosphate in medium; ○, normal concentration of phosphate; and ◐, no phosphate.

tion of phosphate as compared with those in the solution without phosphate.

Apart from the difference in chromosome size associated with the different culture treatments a particularly striking feature of the curves in Fig. 1 is the pattern of chromosome change in relation to the age of the seedlings. In all treatments the size of the chromosomes increases up to about 3 weeks and thereafter decreases to roughly the same level as in the newly germinated seedling. It is worth emphasizing that these changes apply to chromosomes in meristematic cells and thus are not comparable to the more familiar changes in nuclear phenotype which often accompany the differentiation of cells for specialized function. It is worth emphasizing also that "age" in this context applies to the seedlings and not to the roots. The roots examined were in every case young, new roots.

To find out whether the induced changes in chromosome size originate from change in content rather than in reorganization of the chromosome material, estimates were made, by interference microscopy, of the dry mass of isolated nuclei from root tips in each of the three solutions (Table 1). It is appreciated that while the dry mass of chromosomes at metaphase may not correspond exactly with that determined at interphase in the same tissue<sup>4</sup> there is every reason to suppose that the values are correlated<sup>3,5</sup>. Measurements of nuclear DNA, by Feulgen photometry, were also made and are given in the same table. The table shows that the induced variation in chromosome size is associated with variation in mass. There is no detectable change in the content of DNA.

While the precise chemical nature of the chromosome changes needs to be investigated further, the consistency in respect of the content of DNA would indicate that "accessory" non-permanent chromosome material is implicated in the induced chromosome variation. There is evidence that variation in such accessory material is often associated with the degree of synthetic activity of chromosomes<sup>6</sup>. Our observations in rye are consistent with this view. The volume and mass of the chromosomes were greatest in circumstances of rapid growth and, thus, of intense chromosome activity by way of nuclear and cell division

Table 1. MEAN CHROMOSOME VOLUME, NUCLEAR DNA CONTENT AND DRY MASS OF NUCLEI IN ROOT TIP MERISTEMS OF RYE SEEDLINGS GROWN FOR 3-4 WEEKS IN THREE CULTURE SOLUTIONS

Treatment	Chromosome volume (arbitrary units)	DNA content of 4C nuclei (arbitrary units)	Dry mass of nucleus $\times 10^{-11}$ g
40 p.p.m. of phosphate	128	23.65	9.4
18 p.p.m. of phosphate	105	23.92	6.4
No phosphate	85	23.43	5.3

DNA values were taken for ten nuclei in each of three plants, and dry mass values were taken for ten nuclei in each of five plants. The mass of the nucleolus is not included in these averages.

in the meristems; namely, in seedlings at 3 weeks—the period when relative growth rate is fastest—and in the fastest growing seedlings—those in the high phosphate solution.

One of us (M. D. B.) was the recipient of an Agricultural Research Council postgraduate studentship.

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## GENETICS

### Inheritance of Midpiece Length in Mouse Spermatozoa

INBRED strains of mice are known to differ in such aspects of spermatozoan morphology as the size and shape of the head and midpiece<sup>1-3</sup>. When inbred<sup>4</sup> or outbred<sup>5</sup> strains are crossed, clear patterns of inheritance of spermatozoan characteristics emerge. The first heritability estimates for characteristics of mammalian gametes were obtained by Napier<sup>6</sup> from son-sire regressions in the rabbit: for example,  $h^2 = 0.72 \pm 0.18$  for length of the spermatozoan head, and estimates of similar magnitude for its width and projected area. These investigations show in general<sup>7</sup> that genetic effects on the spermatozoan phenotype are paramount and environmental effects seem relatively unimportant.

It should therefore be possible to change spermatozoan characteristics by a genetic selection programme. This has now been tested in mice; the midpiece was chosen because its length can be measured with relative ease and accuracy. Duplicate nigrosin-eosin preparations of spermatozoa were made from each mouse by the method of Beatty and Sharma<sup>8</sup>. One of us (R. A. B.) coded the preparations of each generation and the other (D. M. W.) measured them in randomized order. Ten spermatozoa in each preparation were measured. The spermatozoa were viewed in a projection system consisting of a Gillett and Sibert "Conference" microscope (quartz-iodide light source) fitted with a  $\times 115$  objective, and a  $\times 5$  eyepiece instead of a prism head. On the ceiling an aluminized mirror was positioned to reflect the emergent light beam so that the principal ray fell perpendicular to an inclined drawing platform. Immersion oil was used both above and below the slides. The system gave a linear magnification of  $\times 6,136$ , the element of "empty magnification" being required so as to avoid the use of micro-methods in measuring the images. The length of the midpiece image, defined to include the "neck" (as before<sup>9</sup>), was represented on paper by a single pencil line drawn along the principal axis. The location of the distal end of the midpiece was facilitated by a colour contrast between the stained mainpiece and the unstained midpiece. Drawings were measured with a rotameter read to 1/32 in.

The general pattern of variation and repeatability of results was examined. Thirty-four son-sire pairs were drawn from two unselected generations of five related random-bred groups originating from "Q" strain (D. S. Falconer); nearly every sire was taken from a different litter. Age ranges were 96-114 days (sires) and 68-85 days (sons), but little or no effect of age was anticipated<sup>9</sup>.

Table 1. ANALYSES OF VARIANCE OF MIDPIECE LENGTH ( $\mu$ )

Item	d.f.	Sires		Sons	
		Mean square	Variance component (per cent)	Mean square	Variance component (per cent)
Groups	4	2.43	5	2.59	6
Males in groups	29	1.26*	30	1.35*	34
Preparations in males	34	0.233*	9	0.233*	9
Spermatozoa in preparations	612	0.0901	56	0.0837	51
			100 (0.162)		100 (0.164)

Total computed variance is shown in brackets. d.f., Degrees of freedom. \*  $P < 0.005$ .

From the analyses of variance in Table 1 it was concluded (a) that the principal components of variance are those for differences between spermatozoa within preparations (attributable, at least in part, to the limitations of optical resolution), and for the large and significant differences between males within groups; (b) the minor components are those involved in the differences between groups (non-significant) and in the significant but small differences between duplicate preparations; and (c) the general pattern of variation can be assessed with a large degree of repeatability between different generations, thus indicating reliability of technique and uniformity of material at each sampling level.

The mean midpiece length of each son was plotted against that of his sire (Fig. 1). The best estimate of heritability was considered to be one based on a joint son-sire regression within groups. A test showed that homogeneity of regression slopes between groups could be assumed. Only one sex was being measured, and so the joint regression coefficient was doubled to yield a heritability of  $h^2 = 0.97 \pm 0.36$  on 28 d.f. ( $0.025 > P > 0.01$ ). As in Napier's work with the rabbit, this value is in the higher range of heritabilities in general.

A selection programme was started with the object of realizing the rapid response predictable from the heritability estimate. A detailed account will appear elsewhere. A base population was constructed by crossing the five "Q" groups, and lines were selected for long and short midpiece, with a parallel control line. Spermatozoa were obtained from the vas deferens after killing the male; thus, litters had to be raised before the males were scored, and selection was made retrospectively on the litters. In each generation, between seventy-two and ninety-six males were scored, the litters of twenty-four of them being selected to give the next generation.

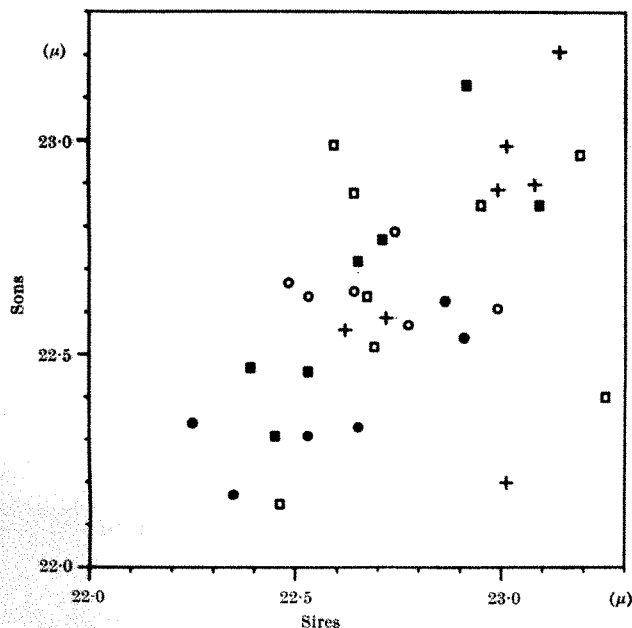


Fig. 1. Relation between mean midpiece length of sons and sires. The mean for each male is based on twenty spermatozoa, ten from each of two slides. The different symbols indicate the five groups.

Selection was practised within families in order to maintain a large effective population, and the matings between sibships followed a cyclical pattern designed to minimize inbreeding. The difference between the means of family means of the long and short lines (long minus short) in the successive selected generations has been  $+0.05$ ,  $+0.17$ ,  $+0.16$  and  $+0.28\mu$ , the trend of the control being intermediate between those of the long and short lines. The regression of these four figures on generation number, arranged to pass through the origin, is highly significant ( $0.005 > P > 0.001$ ). This response, agreeing so far with that predicted from  $h^2$  and the selection differential applied, appears to be the first reported for a purely gametic characteristic. Although small in comparison with the actual length of the midpiece of about  $22.7\mu$  as measured in nigrosin-eosin preparations, the response is large when compared with the variability of spermatozoa between males (for example, the figures of  $0.22$  and  $0.24\mu$  obtained by taking the square roots of the variance components for males within groups, calculable from the two analyses in Table 1). The experiment is being continued with an increased selection differential.

Two points of potential interest are being kept in mind as selection progresses. By increase in gene frequency, selection has been known to bring out in detectable (homozygous) form a recessive gene that was carried only by occasional heterozygotes in the original population, as in the "pygmy" gene of mice selected for small body size<sup>6</sup>. Thus there is the possibility that selection for gametic characteristics might amplify the very small existing list of specific genes known to affect the spermatozoan phenotype. A second interest is that selection for midpiece length is virtually a selection for the quantity or arrangement of mitochondria, and possible effects on the cellular constitution and other properties of the animals themselves are being sought.

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### Heritability of the Plasma Transferrin Protein in Three Species of *Microtus*

ALTHOUGH variation in the electrophoretic mobility of the plasma transferrin protein has been demonstrated in a number of mammals<sup>1-13</sup>, not all investigations include work on the actual heritability of the protein. Cohen<sup>4</sup>, Shreffler<sup>7</sup> and Ashton and Braden<sup>14</sup> have all worked on the genetical control of this protein in the laboratory mouse (*Mus musculus*), but while the first two workers refer to the transferrin proteins, the latter two make reference to  $\beta$ -globulins. This occurs also in other instances in the literature. I therefore suggest that when an iron-binding method is used to identify this protein, as is the case in most of the work already carried out, perhaps the functional name, transferrin, would be more accurate than the general term,  $\beta$ -globulin, for it is possible that not all



the latter is being stained. This communication reports data gathered on the inheritance of the transferrin protein in the meadow vole (*Microtus pennsylvanicus*), the prairie vole (*M. ochrogaster*), and the beach vole (*M. breweri*).

Blood samples were collected from the tail tip in Fisher 'Scientific Red Tip' heparinized capillary tubes. Fisher 'Critocaps', haematocrit tube closures, were used when centrifuging the samples in the capillary tubes. The tubes were then broken just above the erythrocyte-plasma boundary and the plasma was gently blown out into the preformed slots in the starch gel. The starch gel system was run in essentially the same conditions as those described by Smithies<sup>15</sup> as modified by Poulik<sup>16</sup>. Transferrin protein bands were identified, using 'Canalco' series 800 Fe<sup>+++</sup> stain (Canalco, Rockville, Maryland).

The overall starch gel plasma patterns of the three species are very similar, the main difference being the gene frequencies of the transferrin alleles, which will be reported later when the investigation is completed. Because of their identical migration distances and appearances, the transferrins of all three species were named with the same system. This similarity of *Microtus pennsylvanicus* and *M. breweri* is not surprising, for the latter has diverged from the former perhaps within the past 5,000 yr<sup>17</sup>.

Six alleles, the most common being *TrfC* and *TrfE*, have been found in 2,000 *M. pennsylvanicus*; two alleles (*TrfC* and *TrfE*), the most common being *TrfE*, have been found in about 150 *M. breweri*; and two alleles (*TrfE* and *TrfF*), the most common being *TrfE*, have been found in thirty-four *M. ochrogaster*. I am following the suggestion of Cohen<sup>6</sup> in using *Trf* for "the mouse" transferrin locus because *Tf* used for the human transferrin locus might be confused with *tf*, the mouse tufted locus.

In order to see the differences in the gels clearly, the buffer front is allowed to run 15 cm. Fig. 1 shows relative mobilities of the separate alleles, each comprised of three zones. By varying the amount of starch per gel and thus the pore size of the gel, I conclude that the zones are caused by different net charges rather than by different molecular sizes, because less starch or an increase in the pore size only causes the zones to separate farther. This disagrees with the reasoning of Ashton and Braden<sup>14</sup>, who state that paper electrophoresis has no sieve action and thus the reason the zones seen in starch are not resolved in paper. I feel, however, that even with charge differences, the resolution of paper is poor enough that these zones, quite close to each other, could not be seen anyway.

Each main zone is preceded by two zones or bands each of the latter two decreasing in size. The smallest zone, closest to the anode, is sometimes difficult to see clearly. Combinations of two alleles (when they are homozygous the zones are proportionally larger) in each individual suggest the donation of one allele from each parent. Fig. 2 shows three of the transferrin phenotypes as seen

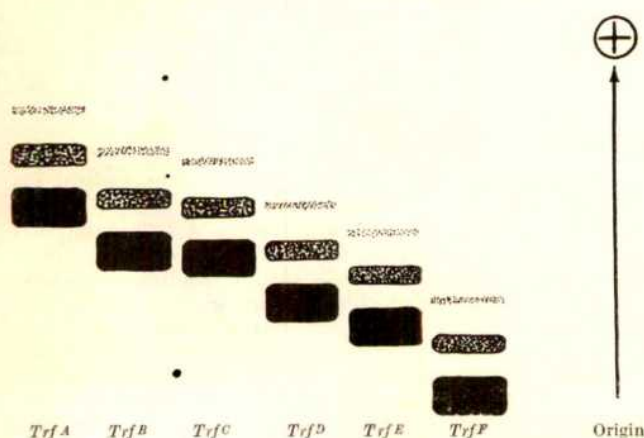


Fig. 1. Relative mobilities of the separate blood plasma transferrin protein alleles in *Microtus*.

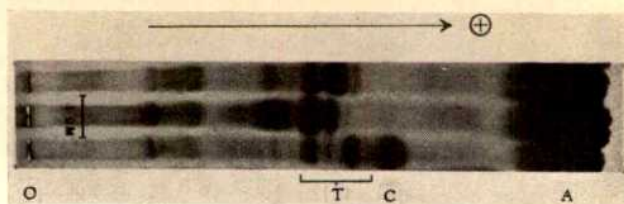


Fig. 2. Three of the blood plasma transferrin phenotypes of *Microtus* as seen with starch gel electrophoresis: (top to bottom) *TrfC/TrfE*, *TrfE/TrfE*; *TrfA/TrfE*. O, Origin; T, transferrin; C, Hb contaminant; A, albumin.

in starch gel. The relative migration of haemoglobin is shown as a contaminant in the right-most sample.

Table 1. RESULTS OF EIGHT LABORATORY CROSSES WITHIN THREE SPECIES OF *Microtus*

Species	Parental genotype Male	Female	Genotypes of offspring
<i>Microtus pennsylvanicus</i>	<i>TrfE/TrfE</i>	<i>TrfC/TrfC</i>	3 <i>TrfC/TrfE</i>
	<i>TrfC/TrfC</i>	<i>TrfE/TrfE</i>	5 <i>TrfC/TrfE</i>
	<i>TrfC/TrfD</i>	<i>TrfA/TrfC</i>	1 <i>TrfA/TrfC</i> , 1 <i>TrfC/TrfC</i> , 1 <i>TrfC/TrfD</i>
	<i>TrfA/TrfC</i>	<i>TrfA/TrfE</i>	1 <i>TrfA/TrfA</i> , 1 <i>TrfA/TrfE</i>
<i>Microtus ochrogaster</i>	<i>TrfF/TrfF</i>	<i>TrfE/TrfE</i>	1 <i>TrfE/TrfF</i> , 1 <i>TrfF/TrfF</i> , 1 <i>TrfF/TrfE</i>
	Male unknown	<i>TrfE/TrfE</i>	4 <i>TrfF/TrfE</i>
			3 <i>TrfF/TrfF</i>
			1 <i>TrfE/TrfE</i>
<i>Microtus breweri</i>	<i>TrfE/TrfE</i>	<i>TrfE/TrfE</i>	2 <i>TrfE/TrfE</i>
			2 <i>TrfE/TrfE</i>
			2 <i>TrfE/TrfE</i>
			2 <i>TrfE/TrfE</i>

\* Backcross, son to mother.

Table 1 gives the results of the laboratory crosses, all of which demonstrate normal non-sex-linked genetic inheritance. More than seventy-five pregnant females or females with nestlings were captured in the field and raised and tested in the laboratory. In all cases each offspring had at least one allele in common with its mother, as would be expected. These results give reasonable assurance that the transferrin protein is the product of codominant alleles in the three species of *Microtus* investigated.

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## HAEMATOLOGY

### Haemocytes and the Measurement of Potassium in Insect Blood

It is normally tacitly assumed that potassium contained in haemocytes contributes insignificantly to whole blood measurements of this ion in insects<sup>1-3</sup>; van Asperen and van Esch<sup>4</sup>, however, explicitly state this to be the case.

Table 1. ESTIMATED CONTRIBUTION OF HAEMOCYTES TO WHOLE BLOOD ION LEVELS IN THE COCKROACH

Ion	No. of samples	Mean haemocyte count in 1,000's/ $\mu$ l.	Mean whole-blood concentration in mmoles/l.	Mean regression coefficient ( $\pm$ S.E.) of ion concentration for 10,000 cells/ $\mu$ l. in mmoles/l.
K <sup>+</sup>	46	31.7	8.8	+0.83 $\pm$ 0.09
Na <sup>+</sup>	44	32.1	148.6	-1.69 $\pm$ 0.52

Tobias<sup>5</sup> seems to be the only worker who has investigated the problem: he measured potassium in both whole blood and in serum, finding a 40 per cent difference. Some scepticism of this result has been expressed<sup>1,6</sup> but the problem has not been re-investigated.

Combined ion measurements and haemocyte counts were made on adult male *Periplaneta americana* of unknown age, but reared under identical environmental conditions. Duplicate haemolymph samples of about 5  $\mu$ l. were collected from each animal. The first sample, suitably diluted with 2 per cent di-sodium ethylenediamine tetra-acetate acid solution, was used for the haemocyte count; specimens were discarded if any clotting occurred. The second sample was mixed with 10 ml. of distilled water and used for the ion determinations (on a 'Unicam SP 900' flame photometer). The haemocyte counts and the ion concentrations are given in Table 1. The mean regression coefficient for potassium is +0.83 mmoles/l. for 10,000 cells/ $\mu$ l. of blood. That this positive correlation is not merely the result of blood dilution factors is indicated by the fact that the equivalent coefficient for sodium is negative, namely, -1.69.

It was not possible to confirm these results by more direct means. Because cockroach haemocytes release cytoplasmic threads within seconds of the animal being wounded, live blood will not separate properly in a centrifuge. It is therefore impossible to produce either uncontaminated serum or a measurable pellet of unbroken cells. A variety of anticoagulants and low temperatures were tried.

Treating the forty-six haemocyte counts on the basis that 10,000 cells/ $\mu$ l. represent 0.83 mmoles potassium/l. of blood indicates that a mean of 29 per cent (S.E.  $\pm$  2 per cent) of the whole blood potassium was contained within the haemocytes. In eleven of the specimens this figure exceeded 40 per cent and in four it exceeded 50 per cent. In the data provided by van Asperen and van Esch<sup>4</sup> for the same species the mean of 67,000 cells/ $\mu$ l. could have represented about 5.6 mmoles potassium/l. or some 70 per cent of their mean whole blood potassium concentration of 7.9 mmoles/l.

In cockroaches, at least, the haemocytes appear to contribute very significantly to the level of whole blood potassium. It seems reasonable to assume that in this intracellular state the ions play no immediate part in any concentration gradients across membranes separating tissues from blood. Several physiological estimations based on the assumption that whole blood potassium is entirely available to such systems may therefore have to be reconsidered. Haemocyte counts in other species<sup>7,8</sup> suggest that this situation may be common.

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## BIOLOGY

### Toxicity of Tobacco Smoke to the Spotted Alfalfa Aphid *Therioaphis maculata* (Buckton)

WE have been attempting to rear the spotted alfalfa aphid, biotype *ENT B* (ref. 1), on artificial diets after the manner of Auclair<sup>2</sup> and Dadd and Mittler<sup>3</sup>. Our initial unsuccessful attempts were conducted in an entomology laboratory. It was then observed that adults placed on potted alfalfa plants (*Medicago sativa* L.) in the laboratory did not produce viable nymphs, and that some of the adults crawled from the plants. Removal of the plants to a small greenhouse enabled normal nymph production after 2 to 3 h. We suspected two factors to explain this: the differences in the illumination or in the atmosphere between the laboratory and the greenhouse. In the laboratory, even with overhead fluorescent light, the aphids were phototropic to daylight. Because of the temperature and humidity fluctuations that occur normally in the field, we ruled out these variables from our consideration.

A group of adult aphids collected from field cages were randomized and placed on plants that had been kept in the laboratory or in the greenhouse for the preceding 18–24 h. A portion of each set was then transferred to the other locale. The adults lived and reproduced normally on the greenhouse plants in the greenhouse, whereas on those plants brought into the laboratory the adults maintained themselves, but the nymphs that were born immediately died and dropped from the plants. The results on the plants acclimatized to the laboratory were comparable. Those that were transferred to the greenhouse maintained the adults, and after a few hours the nymphs that were born were normal. The adults placed on plants kept in the laboratory did not produce nymphs and they usually crawled off the leaves on to the petioles and stems. This behaviour is also exhibited by aphids on resistant alfalfa plants.

Experiments were then conducted in a new, so far unoccupied building nearby. Potted alfalfa plants were placed in several of the rooms and infested with adult aphids. One room had daylight only, another daylight and fluorescent light, and the third daylight, fluorescent and incandescent light. In all cases, after a day, adult behaviour and nymph production were normal. A single cigarette was smoked in one of the rooms. The floor of the room measured 9  $\times$  12 ft. and the ceiling was 10 ft. high. Within 0.5 h the nymphs fell from the leaves and died. The smoke from three additional cigarettes caused the adults to fall from the leaves and die on the floor.

Plants were set up in ten unoccupied rooms and each was infested with ten adult aphids. The next day each plant had the original adults plus 40–60 first stage nymphs. In six of the rooms a single cigarette was smoked (different trade marks from different manufacturers), in the seventh a cigar was smoked 5 min, in the eighth a pipe was smoked the same length of time, in the ninth some paper was burned, and in the tenth fifteen wooden matches were ignited and burned. The nymphs fell from the plants and died in all rooms in which tobacco had been smoked and in no others. Subsequently, dry grass and dry alfalfa were burned in the tenth room, filling it with smoke, but with no effect on the nymphs or adults.

This shows that the spotted alfalfa aphid is extremely sensitive to tobacco smoke and that it is impossible to rear or study this insect in rooms or laboratories where smoking occurs. The nymphs, being killed in a room by the smoke from a single cigarette, are considerably more susceptible than the adults; however, the latter will die if a sufficiently high concentration of smoke is in the air, as during an afternoon coffee break or at tea-time. Also, when heavy smoking has occurred in a room and then is discontinued, nymphs born for several days after the smoking has stopped will die.



The principle in tobacco smoke toxic to the aphid is probably nicotine, an insecticide in use for almost 300 yr<sup>4</sup>. A bell jar experiment corroborated this. Ten adults and 200 nymphs on a plant under a bell jar were unaffected by two puffs of cigarette smoke that had been held in the lungs as long as possible. A single puff taken into the mouth and expelled into a second jar killed all the nymphs and all but one adult also died. Recent estimates place the nicotine content of an average cigarette at 10 mg, of which 6 mg is volatilized with the smoke<sup>5</sup>. The smoke exhaled from the lungs is almost devoid of the alkaloid<sup>6</sup>. This 6 mg would give a nicotine concentration of about 0.2 µg/l. in the air of the rooms used for the experiments. If nicotine is the toxic factor, 0.2 µg/l. is lethal to spotted alfalfa aphid nymphs and about 1 µg/l. to the adults. Previous work with the peach aphid (*Myzus persicae* Sulz.) on plants in a greenhouse showed 99 per cent adult mortality with 50 µg/l. in overnight tests<sup>6</sup>. Other work in fumigation chambers showed 6.5 µg/l. and 12.5 µg/l. to produce 100 per cent mortality with adult peach aphids and pea aphids (*Macrosiphon pisum* Harris), respectively, in 0.5 h tests<sup>7</sup>. These authors also showed that for a given nicotine concentration, mortality was higher at a lower humidity.

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## Lingual Premetamorphic Papillae as Larval Taste Structures in Frogs

RISSER<sup>1</sup> was unable to demonstrate organs of taste in the mouth of the frog tadpole. More recently Helff and Mellicker<sup>2</sup> and Hammerman<sup>3-6</sup> have described lingual premetamorphic papillae on the tongue anlage of the frog. These investigators believe, on anatomical evidence, that the papillae serve as a taste sensor. This communication reports a behavioural study which was aimed at providing further evidence for a gustatory function of the lingual premetamorphic papillae.

First summer *Rana clamitans* and second summer *R. catesbeiana* larvae were tested in a black aquarium having inside dimensions of 16 cm length, 11.6 cm width and a height of 9.2 cm. A black painted partial barrier was placed across the centre of the long axis of the test aquarium. Conditioned tap water at 21.6°C was added to the testing tank to a height 1.7 cm above the top of the barrier. The stimulus substance was boiled leaf spinach, *Spinacia oleracea*, wrapped by a single layer of cheesecloth and lowered into the "test" compartment of the tank before the addition of a tadpole into the "start" compartment. The only source of light was a cool white fluorescent lamp at ceiling level 1.5 m from the test apparatus.

Each animal was tested for a period of 20 min. One minute was allowed to elapse before the timing was begun. A response was scored whenever a tadpole swam over the barrier and entered the "test" section of the aquarium;

a response was not recorded when the animal swam back over the barrier into the "start" compartment.

All tadpoles were anaesthetized in a 1 : 5,000 dilution of Sandoz MS-222 (tricaine methanesulphonate) in conditioned tap water. The olfactory tracts were transected by means of two transverse incisions—one at a level just posterior to the olfactory pits and a second 1 mm posterior to and parallel with the first. The sham operations consisted of two lateral incisions in an anterior-posterior direction, one on either side of the dorsal surface of the head. Premetamorphic papillae were removed by cutting them with irideotomy scissors at their area of attachment on the surface of the tongue anlage. Recovery of the tadpoles in conditioned tap water followed. Operated animals were not tested in the aquarium until at least 24 h after the completion of the operations. Verification of the various operations was made at the completion of the behavioural testing.

Table 1. MEAN CROSSING FREQUENCY OF EACH GROUP

Group	Mean crossing frequency per 20 min behavioural testing period
1 (120)*	8.28 ± 0.392†
2 (120)	2.98 ± 0.139
3 (120)	5.24 ± 0.217
4 (120)	2.57 ± 0.113
5 (50)	3.46 ± 0.203
6 (60)	4.83 ± 0.214
7 (50)	3.08 ± 0.208

The larvae to be tested were divided into seven groups: (1) Intact animals tested with no stimulus; (2) intact animals tested with spinach stimulus; (3) intact animals tested with cheesecloth control stimulus; (4) tadpoles with transected olfactory tracts tested with spinach stimulus; (5) sham operated animals tested with spinach stimulus; (6) tadpoles with both transected olfactory tracts and extirpated premetamorphic papillae tested with spinach stimulus; (7) sham operated animals with their premetamorphic papillae removed tested with spinach stimulus.

\* No. of animals comprising each group is given in parenthesis.

† Standard error of the mean.

The results obtained for each of the seven groups are listed in Table 1. The behaviour of the various groups reveals that when a stimulus, be it cheesecloth or spinach in cheesecloth, is placed in the "test" compartment of the aquarium, the tadpole will remain in this compartment longer than animals tested with no stimulus. When group 1 larvae are compared with all other groups of tadpoles they show a *P* value < 0.001 (Table 2). Thus it can be presumed that some stimulus is being received by the tadpole. No significant difference is noted between the behaviour of intact and olfactory tract sectioned larvae (groups 2 and 4). It can be assumed that the latter group of animals is utilizing a sense receptor other than the olfactory one.

Table 2. STATISTICAL COMPARISON OF BEHAVIOUR BY STUDENT'S *t*-TEST, SHOWING DERIVED VALUES OF *P*

Group compared with Group	<i>P</i>
1	2 < 0.001*
	3 < 0.001*
	4 < 0.001*
	5 < 0.001*
	6 < 0.001*
	7 < 0.001*
2	3 < 0.003*
	4 > 0.236
	5 > 0.268
	6 < 0.013*
	7 > 0.449
4	5 > 0.113
	6 < 0.002*
	7 > 0.259
6	3 > 0.334
	4 < 0.002*
	7 < 0.034*
7	4 > 0.252

\* Significant at the 95 per cent or higher level of confidence.

Comparison of the results of tadpoles in groups 2 and 6 reveals a significant difference. Group 6 larvae, with both the olfactory and postulated gustatory senses removed, are no longer capable of chemoreception. This would account for the increase in the average value shown by group 6 animals. One observes that these tadpoles begin to "wander" away from the spinach, thereby crossing the barrier more frequently. No significant difference is noted when comparing the behaviour of



group 6 with group 3, intact tested with cheesecloth larvae. It appears that tadpoles deprived of both the olfactory and gustatory senses (group 6) show the same behaviour as intact larvae tested with cheesecloth alone (group 3). Group 6 tadpoles differ significantly in their behaviour from larvae lacking only the olfactory sense (group 4). The increase in the average crossing frequency of group 4 tadpoles compared with that for group 6 is apparently due to the fact that the gustatory sensor of the latter group is absent.

The results obtained for group 7 tadpoles are significantly different from those with group 6. If the tadpole has the olfactory sense intact but lacks the premetamorphic papillae, then it probably utilizes whatever chemoreceptor it does have. There is no significant difference in the behaviour of larvae of groups 2 and 7. Because the animals of group 7 lack their premetamorphic papillae, the results indicate that these larvae are utilizing a chemoreceptor other than the gustatory one, namely, the olfactory sense. This may explain the closeness of the averages between the two groups.

No significant difference is noted when groups 4 and 7 are compared. Apparently, if the animal lacks a functional olfactory apparatus, it will utilize the remaining chemoreceptor, namely, the gustatory sensor, and vice versa. An animal with both senses functioning (group 2) probably uses both the olfactory and the taste receptors in combination. If one chemoreceptor of the animal is destroyed or functionally diminished, the tadpole will presumably utilize the remaining chemoreceptor.

A gustatory sensor thus appears to function in frog larvae. The absence of a differentiated taste epithelium indicates that some other structure acts as the gustatory sensor. Lingual premetamorphic papillae are the most likely candidates for this role.

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### Axopodial Filaments of Heliozoa

PROTOZOA of the order Heliozoa are provided with numerous straight radiating processes known as axopods, which in certain genera (*Actinophrys*, *Actinosphaerium*) are known to be supported by axial filaments consisting of microtubules in elaborate array. In transverse section across an axopod these microtubules are seen to form two sheets spirally interwound (Fig. 1)<sup>1-3</sup>.

Further examination of the material prepared by Kitching and Craggs<sup>2</sup> and of additional material has revealed a system of links between the microtubules which form the axopodial filaments of *Actinosphaerium nucleoflum*. The links between tubules (Fig. 1) are of three kinds: tangential, radial and secondary. Tangential links are short and join adjacent tubules within either of the two spirally wound sheets of tubules; the separation between the tubules, centre to centre, is about 26 m $\mu$  in the sectioned material. The radial system of links is built around four primary tubules which form a parallelogram in transverse section. The tubules at two opposite corners each act as centres of a half circle containing six radii, each radius comprising a line of linked tubules. The whole system is divided into twelve sectors by these radii,

and within each sector the tubules of one of the bounding radii are linked with secondary lines of tubules. Thus each tubule of an axopodial filament is located within one of the two sheets and on a radius or secondary line. The spacing of the tubules, centre to centre, is about 47 m $\mu$  along the radii and 45 m $\mu$  along secondary lines, in the sectioned material.

The 30° angle between radial links at the centre suggests that the tubules have a fundamental twelve-fold radial symmetry, although this is never fully realized. Secondary links also conform. Markham rotations of a limited number of transverse sections of microtubules suggest that the wall of a microtubule comprises twelve sub-units—a figure close to that found in other material<sup>4-6</sup>. It is possible that sub-units could act as sites for linkage. Tangential links are thicker and shorter. They do not coincide with the hypothetical twelve-fold radial system, although they may be related to it. No radial or secondary links are found in their neighbourhood. The axopodial filaments are rooted either on the nucleus (*Actinophrys*) or in the perinuclear zone (*Actinosphaerium*) in a region rich in ribosomes, and it is to this region that we must look for an explanation of their origin and organization.

An axopodial filament of the configuration described can be increased in thickness by addition of microtubules to the outer ends of the two spiral sheets without any loss of regularity, but in the fixed and sectional filament of Fig. 1 the slight difference between radial and secondary links leads to increasing concavity within each sector towards the perimeter of the filament.

Although the axopodial filaments appear to act as supports for the axopods, we may speculate on a further possibility. Between six and a dozen independently moving longitudinal streams of protoplasm surround the filament. The sectors of secondary tubules might possibly drive these streams peristaltically. We might thus find justification for the elaborate structure of the filament and the differentiation of linkages. It is interesting that the undulating axostyle of pyrsonymphid flagellates is composed of parallel microtubules in six-fold radial linkage<sup>7</sup>.

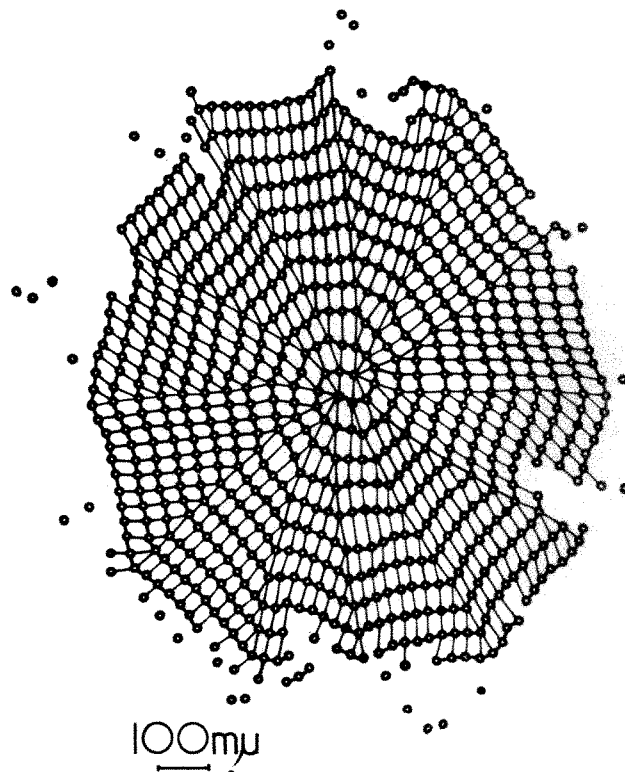


Fig. 1. Schematic tracing of electron micrograph of axopodial filament.

In *Actinosphaerium* the twelve radial lines of microtubules would presumably isolate the active sectors from one another.

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### Swarming of Hyperiid Amphipods

FOLLOWING a period of moderately strong westerly wind, on June 26, 1966, the beaches of Robin Hood's Bay, Filey, and Sandsend, Yorkshire (extending for about 40 km of coastline), were turned white by a covering of vast numbers of hyperiid amphipods.

A sample of these was found to consist of fifty-six large specimens, all but two being sexually mature or nearly mature, and more than a hundred small juvenile specimens. The large specimens were identified as *Parathemisto* (*Euthemisto*) *gaudichaudii* (Guerin), both the *compressa* and *bispinosa* forms<sup>1</sup> being present in about equal proportions. The small specimens could be identified with certainty only as *Parathemisto* sp., but considering that all of them were curled, as within the maternal oostegites, it was obvious that they had just been released.

Specimens of *Parathemisto* had occurred previously in small numbers in April and May 1966. In early June, mature specimens (containing young) were common in the surface plankton off Sandsend. Following the invasion of the shore, large numbers of small specimens of *Parathemisto* were taken in July in plankton hauls off Sandsend and only rarely were specimens taken later in the year. Thus swarming appears to be associated with the reproductive phase.

On June 17, that is, before the invasion, the stomachs of cod (*Gadus morrhua*) of 33–60 cm length trawled in 14 m of water, 1–2 km off Sandsend, were all found to be distended with specimens of *Parathemisto gaudichaudii* (identified by J. E. Kane). The cod were in large numbers and it seems likely that the amphipods had been swarming.

Swarms of *Parathemisto gaudichaudii* have been reported on other occasions; for example, off the Gulf of Maine<sup>2,3</sup>, off the French Atlantic coast<sup>4</sup> and in the Antarctic Ocean<sup>5,6</sup>. Where examined<sup>4,5</sup>, as in the present case, the composition of the swarms showed them to have been in a reproductive phase.

The coastline discussed faces north-east and the residual tidal current is south-east. Westerly winds activated by the Coriolis force might have intensified the south-easterly current and brought the swarming amphipods closer to the beaches. A similar invasion occurred in May 1913, also following a period of westerly wind<sup>7</sup>. Other invasions in February 1892, April 1907 and April 1908 followed periods of more directly onshore winds, north or north-east. Thus it is quite possible that the prevailing wind in each case could have contributed to the invasion of the shore by the swarming amphipods.

A sample collected from the beach at Redcar, Yorkshire, in April 1907 by T. H. Nelson was referred to as *Euthemisto compressa* by Ritchie<sup>7</sup>, and noted as *Euthemisto compressa* (Goës) var. *gracilipes* Norman (Reg. No. 1907–79, Royal Scottish Museum). According to modern usage this species is *Parathemisto* (*Euthemisto*) *gracilipes*<sup>8</sup>. On re-examination, this sample was found to consist of

110 specimens, all but five being sexually mature or nearly mature, many of the females carrying eggs or young. As in the 1966 invasion, the composition therefore suggested a reproduction phase although the species in the two invasions were different.

It appears that the neritic species<sup>8</sup>, *Parathemisto gracilipes*, is generally the most usual one in the southern North Sea<sup>9</sup>, although difficulty in nomenclature tends to confuse this<sup>10,11</sup>. Thus the presence of *P. gaudichaudii* might be considered unusual and, considering its normal oceanic existence<sup>1,8</sup>, indicative of an influx of water from the Atlantic Ocean. Similarly, swarms of the pteropod, *Limnacia lesueurii*, and of the radiolarian, *Phyllostaurus quadifolius*, normally in the Atlantic, occurred off the north-east coast of England in the late summer and early autumn of 1921<sup>12</sup>. These organisms were considered to have been carried around the north of Scotland. More frequently, successive swarms are brought only as far as the north of Scotland<sup>12</sup>. In 1965, for example, the euphausiid *Thysanoessa longicaudata*, which is normally oceanic, was found swarming in Lerwick Harbour in the Shetland Islands, where one would expect *T. inermis* or *T. rashi*<sup>13</sup>. Here, as with the amphipods, the swarm was in a reproductive state and is believed to have been influenced by local wind conditions.

The Sei whale in the Antarctic is reported to feed almost exclusively on *Parathemisto gaudichaudii*<sup>8</sup>, and the tunny (*Thunnus alalunga*) is known to feed on immense surface swarms of this species (as *Euthemisto compressa*) which occur beyond the continental shelf off the French Atlantic coast<sup>4</sup>. The cod off Langesnes, Iceland<sup>14</sup>, and off Bear Island<sup>15</sup>, has been reported to take *Parathemisto obliqua* (prob. = *P. abyssorum*) on some occasions as its predominant food. The feeding to gorging, as shown for the cod off the Yorkshire coast, however, is here reported for the first time.

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### Apparent Photosynthesis and Respiration in Populations of *Lolium perenne* from Contrasting Climatic Regions

MEASUREMENTS of net assimilation rate by growth analysis techniques have shown significant differences between North European and Mediterranean populations of *Dactylis glomerata* in controlled environments at low and high temperatures<sup>1</sup>, and between a similar range of populations of *D. glomerata*, *Festuca arundinacea* and *Lolium perenne* in the winter, spring and autumn at Aberystwyth<sup>2</sup>. Greater net assimilation rates were found

for the North European populations in all these conditions. Variations in the rates of photosynthesis and respiration have also been reported for climatic races of *Solidago virgaurea*<sup>1</sup>, *Oxyria digyna*<sup>1</sup> and *Mimulus cardinalis*<sup>1</sup>.

Our experiments were designed to investigate the effect of temperature on the rates of apparent photosynthesis and respiration in two populations of *Lotium perenne* from contrasting climatic regions: one derived from local Danish material at Pajbjergfonden, Jutland, and the other from Boussarea, Algeria. The seedlings were grown in water culture at 20° C with a light energy of 0.093 cal cm<sup>-2</sup> min<sup>-1</sup> visible radiation, and 16 h duration, from warm white fluorescent tubes. When the seedlings had four leaves the carbon dioxide exchange of the whole plant was measured at 5°, 10°, 20° and 30° C with a null balance infra-red carbon dioxide analyser (Infra Red Developments, Ltd., Model SB/K2). An open circuit was used with clean air pumped at a rate of 2 l./min through a glass assimilation chamber containing the plants in water culture. The net exchange of carbon dioxide was measured by comparing the concentration of the gas in the air before and after passing through the assimilation chamber. The air temperature was regulated in a controlled environment room and the heating effect of the light, a 400 W high pressure mercury fluorescent lamp, was reduced by inserting a water bath below the lamp.

Values for dark respiration were obtained by measuring output of carbon dioxide in the dark at the beginning and the end of each range of light intensities; the results are shown in Fig. 1. There was marked temperature dependence of the rate of respiration, with higher rates in the Algerian than in the Danish population at 5° and 10° C and the reverse relation at 20° and 30° C.

A series of light energies from darkness to 0.233 cal cm<sup>-2</sup> min<sup>-1</sup> were used at each temperature to determine the relationship between apparent photosynthesis and light energy. Measurements were made for an ascending and a descending range of light energies. The results for a particular light energy varied little, therefore the mean of the two values was used. Five groups of plants were treated in this way at each temperature and it appeared that the Danish population reached light saturation at a lower light intensity than the Algerian population.

The following results were for a light energy which was below saturation in both populations.

A comparison of apparent photosynthesis between temperatures was made at 0.17 cal cm<sup>-2</sup> min<sup>-1</sup> visible radiation (Fig. 2); this was the same light energy as that used in growth analysis experiments with *Dactylis*<sup>1</sup>. There were greater values for net uptake of carbon dioxide in the Danish than in the Algerian population at 5° and 10° C but the differences were reversed at 30° C. The fastest rate was found at 10° C for the Danish and at 20° C for the Algerian population. The form of these curves for apparent photosynthesis against temperature at this light energy was very similar to the curves for net assimilation rate in cocksfoot<sup>1</sup>. Values for true photosynthesis derived from the measurements of apparent photosynthesis and dark respiration show the same pattern as the apparent

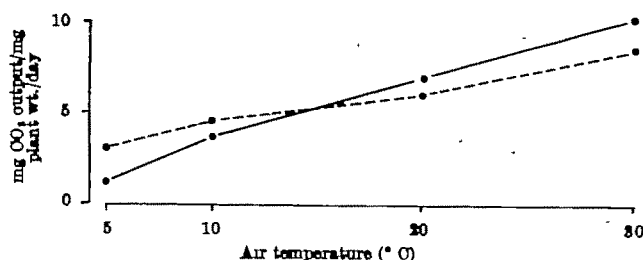


Fig. 1. The relationship between the rate of respiration and temperature for a Danish (solid line) and Algerian (broken line) population of *Lotium perenne*.

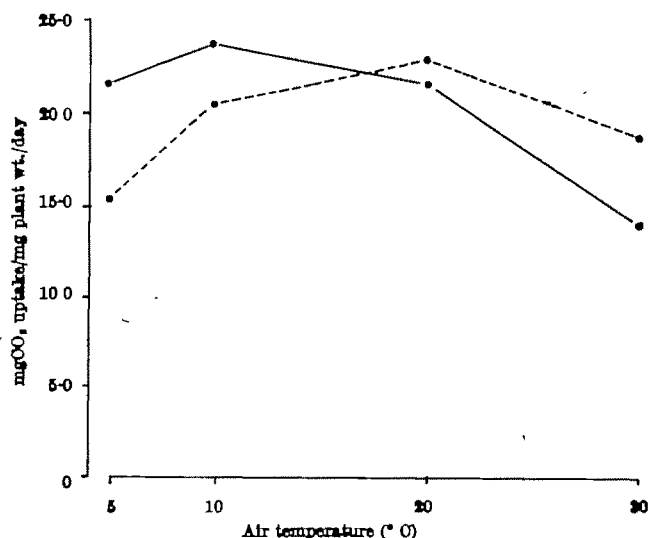


Fig. 2. The relationship between the rate of apparent photosynthesis and temperature for a Danish (solid line) and Algerian (broken line) population of *Lotium perenne* at a light energy of 0.17 cal cm<sup>-2</sup> min<sup>-1</sup> visible radiation.

photosynthesis but with smaller population differences at the temperature extremes.

It can therefore be argued that the lower rate of leaf expansion, and the accumulation of larger concentrations of fructosans in the North European, compared with the Mediterranean populations, at low temperatures in controlled environments<sup>1</sup>, and during the winter in Britain<sup>2,3</sup>, are the result of a different balance between the rates of photosynthesis and respiration. In the North European populations little energy is liberated through respiration and this may limit the rate of energy-requiring processes such as leaf expansion, while the rate of photosynthesis is fast and, because only a small proportion of the assimilates is used to produce new leaf tissue, the remainder are diverted to the base of the plant where they accumulate as fructosans. By comparison, the rate of respiration at 5° C is three times faster in the Mediterranean population, and a large proportion of the products of photosynthesis is used in the production of new leaf tissue; therefore low concentrations of fructosans accumulate in the base of these plants.

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## AGRICULTURE

### *Eperythrozoon ovis* Infection and the Antiglobulin Test

DURING an investigation of *Eperythrozoon ovis* infection of sheep, attempts have been made to find laboratory aids to the normal practice of relying entirely on identification of the organism in blood smears. This method has limitations<sup>1</sup> in that: (a) parasites are detectable for relatively short periods and at a time when clinical symptoms are mild or absent; (b) careful handling of blood samples is necessary and not always possible under field

conditions; and (c) staining methods require exacting techniques and are inconsistent. One promising aid to diagnosis is a modification of the antiglobulin test of Coombs, Mourant and Race<sup>2</sup> described by Dacie<sup>3</sup>. References to the use of the Coombs test in naturally occurring diseases of animals are rare although the test has been used in certain experimentally induced anaemias.

Anti-sheep globulin serum is prepared in rabbits by Slavin's method<sup>4</sup>, inactivated by subjection to 56° C for 40 min and absorbed with washed red cells from the sheep whose serum was used to inject the rabbit. The test is routinely carried out in plastic haemagglutination trays at 37° C. At room or refrigerator temperatures in-saline agglutination may occur at certain stages of the parasitaemic cycle.

This method has shown that washed red cells from uninfected sheep and from sheep that are infected but not in an active phase of the disease show no agglutination at serum dilutions of 1:10. Cells from animals in which infection is or has recently been active, however, agglutinate in dilutions of up to 1:1,280—depending on the potency of the rabbit serum.

Limited observations, which require further investigation, suggest that the test generally remains negative during and after parasitaemic episodes after the first. In the early stages of *E. ovis* infection, therefore, the test should be valuable at least on a flock basis and preliminary observations suggest that this is so. Although the antiglobulin phenomenon in sheep may not be universally diagnostic of *E. ovis* infection, no other condition has been encountered in South Australia in which it occurs; in this state no blood parasites other than *E. ovis* are known to exist and the other known causes of anaemia are mineral deficiencies or excesses or helminths.

The use of sheep infected with *E. ovis* would also seem to offer opportunities for the investigation of the antiglobulin phenomenon itself.

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<sup>1</sup> Sheriff, D., Olapp, K. H., and Reid, M. A., *Austral. Vet. J.*, **42**, 169 (1966).

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## GENERAL

### A New Pseudo-tensor with Vanishing Divergence

MUCH embarrassment has been caused in the general theory of relativity by the fact that the conservation equation for the energy tensor  $T^{ab}$  reads  $T^{ab}_{;b} = 0$  (covariant differentiation) and not  $T^{ab}_{,b} = 0$  (ordinary differentiation). This led Einstein to construct a pseudo-tensor  $\tau^{ab}$  satisfying  $\tau^{ab}_{,b} = 0$  by adding to  $T^{ab}$  a suitably chosen pseudo-tensor formed from the metric tensor  $g_{ab}$  and its first derivatives. The purpose of this note is to offer a new way of reaching the desired equation  $\tau^{ab}_{,b} = 0$ .

As a consequence of the field equations  $G^{ab} = -\kappa T^{ab}$  and the identity  $G^{ab}_{;b} = 0$ , we have the conservation equation

$$T^{ab}_{;b} = T^{ab}_{,b} + K_a = 0 \quad (1)$$

where

$$K_a = \Gamma^c_{ab} T^{ab} + \Gamma^c_{ab} T^{ab} \quad (2)$$

Note that  $K_a$  is not a tensor, and the use of a subscript rather than a superscript is of no significance. It is convenient to use imaginary time ( $x^4 = it$ ), so that for the d'Alembertian operator  $\square$  we have  $\square f = f_{,aa}$ . The inverse d'Alembertian  $\square^{-1}$  is defined by the regarded potential

$$\square^{-1}f(x) = -\frac{1}{4\pi} \int \frac{f(x') d^3x'}{|\mathbf{x} - \mathbf{x}'|}, \quad x'^4 = x^4 - i|\mathbf{x} - \mathbf{x}'| \quad (3)$$

Define the pseudo-vector  $Q_a$  by

$$Q_a = \square^{-1}K_a \quad (4)$$

and the pseudo-tensor  $\varphi_{ab}$  by

$$\varphi_{ab} = Q_{a,b} + Q_{b,a} - \delta_{ab} Q_{c,c} \quad (5)$$

the summation convention for a repeated suffix operating here and throughout. Then

$$\varphi_{ab,b} = \square Q_a = K_a \quad (6)$$

If we now define the pseudo-tensor  $\tau^{ab}$  as

$$\tau^{ab} = T^{ab} + \varphi^{ab} \quad (7)$$

we have the required result

$$\tau^{ab}_{,b} = 0 \quad (8)$$

by virtue of equations (1) and (6). By adding  $\varphi_{ab}$  to  $T^{ab}$ , we have constructed a pseudo-tensor with vanishing divergence.

I refrain from attaching the words momentum and energy to this pseudo-tensor or to integrals formed from it, because I believe that we are barking up the wrong tree if we attach such important physical terms to mathematical constructs which lack the essential invariance property fundamental in general relativity. All that should be asserted is that equation (8) is a logical deduction from the accepted equation (1) and the mathematical definitions involved. The result may be found useful in dealing with solutions of the field equations by methods of successive approximations.

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### Megayear and Gigayear

PROFESSOR RANKAMA<sup>1</sup> has rightly drawn attention to the prevailing disorder in geochronological time-units and the abbreviations used for them, and his advocacy of "megayear" and "gigayear" is worthy of support. But the current international abbreviation for "year", as adopted by the SUN Commission of the IUPAP and the British Standards Institution, is not "yr" but "a" (refs. 2 and 3) and the appropriate abbreviations for megayear and gigayear are thus Ma and Ga. The admittedly incongruous appearance (for English-speaking readers) of the first may perhaps explain why it has not yet been generally adopted.

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Clarendon Press,  
Oxford.

Received May 10, 1967.

<sup>1</sup> Rankama, K., *Nature*, **214**, 634 (1967).

<sup>2</sup> International Union of Pure and Applied Physics (SUN Commission), *Nuclear Physics*, **81**, 701 (1966).

<sup>3</sup> Amendment No. 2 to B.S. 1991: part 1: 1964, p. 3 (1960).

## BOOK REVIEWS

### GUIDANCE FOR APOLLO

Space Navigation, Guidance and Control

Edited by J. E. Miller. (The Advisory Group for Aerospace Research and Development of the N. Atlantic Treaty Organization. AGARDograph No. 105.) Pp. x+373. (Maidenhead: Technivision, Ltd., 1966.) \$15.50.

In June 1965 a series of lectures on space vehicle control and guidance systems was given in Europe by members of the Massachusetts Institute of Technology, under the sponsorship of the Advisory Group for Aerospace Research and Development. This book is based on the material assembled for these lectures; the authors have all been actively engaged on the Apollo programme, and the problems of navigation, guidance and control are discussed with particular reference to this mission.

A manned lunar landing within the next few years may or may not be a laudable objective; but none can doubt the monumental nature of the task, the dimensions of which are vividly brought out in this account of the evolution of the Apollo guidance and control concepts as they now exist. The various phases of the operation—the initial injection into a near-Earth orbit, the Earth-Moon flight, the transfer to lunar orbit, the descent to the Moon's surface of the two-man "excursion module", its ascent and rendezvous with the command module which remains in lunar orbit, the return flight to Earth and re-entry through the atmosphere—present a very wide range of interacting navigational and control problems, all of which are thoroughly explored in a lucid and convincing manner.

The book follows the pattern of the lectures and is divided into seven parts. The fundamental principles of guidance and navigation are elucidated in the first part, which also discusses in some detail the characteristics of inertial instruments such as gyroscopes and accelerometers. The second part sets out the various tasks of the Apollo mission, in which the judgment and adaptability of the astronaut—in particular, his ability to recognize and evaluate patterns, and his decision-making capability in the face of the unexpected—are used to supplement, and sometimes to override, the automatic operation of the machine. Because, it is argued, men have to be carried to fulfil the mission, they should be active crew members rather than passengers.

The third part contains a simplified discussion of the numerous theoretical techniques which have been developed or proposed to ensure that the trajectories are in some sense optimal, while the following two parts of the book give a detailed account of the inertial and optical navigation sensors of the Apollo guidance and control system. On-board digital computers are treated in the sixth part, which includes a chapter on the Apollo guidance computer and a reminder that, in terms of data storage, "in to-day's guidance computer we are realizing an overall density thousands of times greater than in the computers of 15 years ago". The final part deals with the control of spacecraft attitude and of flight path during powered flight, coasting and atmospheric re-entry, again with reference to the Apollo mission.

The subject matter is of necessity drawn from a variety of disciplines in the engineering and scientific fields, and editor and authors are to be congratulated on the way in which they have managed to integrate these many topics into a readable and informative whole. The book

will be widely read, not only by guidance and control specialists, but also by those who wish to gain a deeper appreciation of the formidable yet fascinating problems involved in placing a man on the Moon with a valid return ticket to Earth.

E. G. C. BURR

### LUNAR POINT OF VIEW

Kepler's Somnium

The Dream, or Posthumous Work on Lunar Astronomy. Translated with a Commentary by Edward Rosen. Pp. xxiii+255. (Madison, Milwaukee, and London: The University of Wisconsin Press, 1967.) 88-75; 66s.

KEPLER'S *Dream* on lunar astronomy is a significant document in the development of cosmological thought. It has been overshadowed by Galileo's *Sidereal Messenger* and by Kepler's own work on planetary theory. This English translation, amounting to an impeccable critical edition, should help to redress the balance.

For Kepler himself, the imaginary voyage to the Moon was a life-long, but ill-fated voyage. The details of the journey thither came close to witchcraft, and the popular identification of the mother Fiolxhulde (the name chosen for its Icelandic barbarity) with Kepler's own mother, was an important factor in her subsequent prosecution for witchcraft. The piece itself was composed around 1609, but derived from speculations which had occupied him since 1593. These were an outline of astronomy as it would be developed by an observer on the Moon; their purpose was doubtless to be an indirect support for the Copernican theory. Some ideas on the voyage, and on biological and social aspects of lunar life, were already worked out when Kepler discovered the writing of Plutarch on the same topic. By 1609, he was well convinced of the Moon being an Earth-like planet, possessing mountains and inhabitants as well. The *Dream* itself has the unfortunate account of the voyage, an extended discussion of lunar astronomy, and a sketch of patterns of living there—all before Galileo turned his telescope to the Moon.

The "Notes" and "Geographical Appendix" (also with notes) were composed much later; and in the latter, detailed features of lunar topography are used as evidence for the presence of reason and of social organization on the moon. Galileo prudently abstained from such speculations.

This edition gives translations of the various texts (which were published together in 1634); there are copious notes and appendices on biographical and bibliographical topics. Professor Rosen's massive and meticulous scholarship will make this a standard source for many years to come. Historians of science will have their appetites whetted for closer studies of the influence of this work and of its reputation; and non-specialist readers would welcome an analysis of Kepler's lunar topography in the light of modern knowledge. But as a product of a sort of historical scholarship which is still not sufficiently developed in this field, this edition of the *Dream* deserves praise, study and emulation.

J. R. RAVEN

### GRAPHICALLY DESCRIBED

Connectivity in Graphs

By W. T. Tutte. (Mathematical Expositions, No. 15.) Pp. ix+145. (Toronto: University of Toronto Press; London: Oxford University Press, 1966.) 42s. net.

THE mathematical theory of graphs has become so extensive that an exposition in some depth of a part of the subject is welcome. Professor Tutte's book is such an exposition.

The twelve chapters in this volume (which is intended as the first of a set of three) are on topics which the author regards as related to connectivity. The choice of



such topics is to a large extent a personal one, and is naturally related to the research interests of the author. The treatment throughout is that of the pure mathematician. Definitions are precise, arguments are rigorous and there is no concern with applications. The opening sentences of the book define a graph in terms of an edge-set and a vertex-set which are not necessarily disjoint, and lead one to expect that diagrams of points and lines may not be used to represent graphs. The treatment is not, however, as severe as this and diagrams do illuminate the text in a substantial way.

The book is well written and, like most books on graph theory, demands no previous mathematical knowledge (even the term "group" is defined). The book is not altogether easy reading because the style is that of a research paper with close successions of definitions and precise proofs. There are several places, however, where the intuitive attractiveness of the research problems breaks through the formal rigorous treatment. This is particularly so in the chapter on girth and  $m$ -cages.

Research workers in graph theory as a part of pure mathematics will find much to interest them in Professor Tutte's individual treatment and will look forward to the two further volumes of the set. H. O. FOULKE

## ELECTRICITY AND FLUIDS

### An Introduction to Magnetohydrodynamics

By P. H. Roberts. Pp. vii+264. (London: Longmans, Green and Co., Ltd., 1967.) 60s.

This is a useful addition to the growing library of magnetohydrodynamic textbooks at the advanced undergraduate and postgraduate level. The bias of the book is mathematical, for its origins lie in a postgraduate course for mathematicians. Fortunately, some of the lecture room atmosphere comes across in the book, both in the preliminary comments, which open each chapter, and the way in which the topics are outlined and the central problems posed.

As the author admits, the scope of material he has discussed is strictly limited, and he has preferred to deal with a few problems in detail, rather than to give a general review of those subjects amenable to magneto-hydrodynamic treatment. Within these limits, however, a wide range is possible. The emphasis is geophysical and astrophysical—plasma physics enters only in theoretical exercises on hydromagnetic equilibrium and stability—and a chapter on compressible flow and magneto-gas-dynamics would have been welcome.

The essentials of the subject are given in the first two chapters and the rest of the book is devoted to their application to specific problems. These introductory chapters on the basic equations and electromagnetic induction are particularly good. Although the formulation is mathematical, the text is illuminated by those comments which physical scientists rarely expect from a mathematician. This measures the success of transposing the lecture course into book form.

The electrodynamic effect of the conducting fluid on the magnetic field through which it flows is considered in chapters on induction and dynamo theory. This latter chapter displays Professor Roberts's geophysical research interests.

The other aspect of the fluid magnetic field relationship, that is, the hydrodynamic effect of the magnetic stresses on the conducting fluid motion, follows in the section on hydromagnetic equilibrium, where several magnetic field configurations, familiar in plasma containment experiments, are reviewed.

Both sides of the interaction, the effect of the fluid motion on the field and of the field on the fluid, are then considered simultaneously in a discussion of Alfvén wave phenomena. The book is completed by chapters on

boundary layer theory, experiments on incompressible flow and on stability theory and its applications. Each chapter ends with a comprehensive set of problems.

The price is reasonable for a hard-back edition, but the book will reach many more students if it appears as a paperback. JOHN PAIN

## PLASMA RADIATION

### Radiation Processes in Plasmas

By George Bekefi. Edited by Sanborn C. Brown. (Wiley Series in Plasma Physics.) Pp. xiii+377. (London and New York: John Wiley and Sons, Ltd., 1966.) 125s.

Just over ten years ago the first book on plasma physics as a subject in its own right appeared; in a gradually swelling stream they have been pouring out ever since. Most of these books have been introductory; there have been surprisingly few monographs. One topic which has had scant coverage in any form is plasma radiation (except for spectral-line radiation which has been dealt with very fully in Griem's *Plasma Spectroscopy*). Professor Bekefi's book has now filled this gap.

*Radiation Processes in Plasmas* begins by recalling some results in the transport of radiation and generalizing classical radiation theory to anisotropic, non-thermal media before going on to discuss bremsstrahlung using a binary collision model; quantum modifications to the bremsstrahlung are summarized. The restriction to binary collisions is then relaxed and a more general point of view adopted by establishing the fluctuation-dissipation theorem and applying it to the fluctuations of the electromagnetic field. The dispersion relation for longitudinal waves in a thermal plasma and the damping suffered by these—the Landau damping—are compared with recent experimental results and the treatment of fluctuations ends with an extension of the fluctuation-dissipation theorem to plasmas not in thermal equilibrium—more often than not the case in practice.

The reader is now equipped to tackle the problem of radiation from plasmas more realistically. The considerations of bremsstrahlung introduced earlier may now be extended to bremsstrahlung from collective Coulomb interactions rather than from binary encounters alone. Some recent theoretical results on bremsstrahlung from plasmas which, though stable, contain a flux of supra-thermal electrons are included on the grounds that they provide a possible model (one of many!) for the type II and type III bursts of radiation from the Sun in its active phase. The bremsstrahlung comes, in this case, from the conversion of longitudinal plasma waves to radiation by scattering on the ions in the plasma; this coupling of longitudinal wave energy to the radiation field on a macroscopic scale (for example, through density gradients) is usually more important in laboratory plasmas and is treated in some detail.

The relaxation of the restriction that the plasma be free from magnetic fields adds enormously to the wealth of radiation phenomena. Again it is simpler to ignore collective effects and, to begin with, a single particle model is considered. Cyclotron radiation by non-relativistic electrons, by highly relativistic electrons (with applications to cosmic radio waves) and by "mildly" relativistic electrons (the regime of many fusion experiments) is evaluated before turning to the drastic changes in the emission spectra which appear once collective effects are taken into account.

The development then turns from the emission and absorption of radiation to the scattering of electromagnetic waves by plasma fluctuations. The spectrum of density fluctuations is derived for an isotropic, collisionless plasma and used to treat Thomson scattering and co-operative scattering both from thermal plasmas and those in which,

for one reason or another, the density fluctuations are enhanced.

The book ends with a quick tour through the maze of microinstabilities endemic in plasmas and a chapter on experimental methods.

In his preface the author remarks that the rapidly improving rapport between theory and experiment made this an opportune time to write a book on plasma radiation. On the other hand, one might have adopted the view that because this correlation between theory and experiment is going ahead so quickly the time was not quite ripe. One might back this up by pointing to significant new work not mentioned in the book, for example, results on the co-operative scattering of laser radiation by laboratory plasmas, the formation and heating of plasmas by powerful lasers and recent experiments which appear to establish the coupling between longitudinal plasma waves and the radiation field.

Workers in this branch of plasma physics will be glad that Professor Bekefi chose to write when he did. He has given a clear and balanced account of the subject (although the final chapter has the appearance of a peace-offering to the less theoretically minded). The references to the literature are very complete. The book is attractively produced and most of the mistakes spotted were obvious misprints or trivial omissions. The prospective reader should be familiar with classical electrodynamics and have some knowledge of plasma kinetic theory; he will find the going easier if he also knows something of waves and instabilities in plasmas.

Professor Bekefi has put workers in the field of plasma radiation considerably in his debt. T. J. M. BOXT

## MAKING ISOTOPES VISIBLE

### Techniques of Autoradiography

By Andrew W. Rogers. Pp. xi+335+71 illustrations. (Amsterdam, London and New York: Elsevier Publishing Company, 1967.) 120s.

THIS book is intended for biological research workers who wish to learn or extend the use of photographic emulsion in radioisotope localization and measurement.

The author's aim has been to give accounts of the major techniques with deliberate emphasis on the theoretical and practical aspects of handling nuclear emulsions, rather than the results obtained by autoradiographic methods. The volume comprises eighteen chapters and includes three chapters introducing radioisotopes and the photographic process. Further chapters cover the resolution and efficiency of autoradiographs, background, microscopy and microphotography, relative and absolute isotope measurements, the planning of experiments and histological technique.

Techniques which receive individual treatment include soluble compound methods, stripping film, liquid emulsion grain density and track autoradiography, electron microscope methods, and the treatment of macroscopic specimens.

With a few exceptions these subjects are covered well, sufficient data and references being given to allow the reader a clear understanding of the processes involved. Light microscope techniques for grain density and track methods are described in a comprehensive fashion that would allow the learner to obtain good results with the minimum delay. The data on track technique are especially welcome because these are not readily available elsewhere. The photomicrographic and automated counting techniques are also a valuable source of information, reflecting the author's considerable experience with these methods. Valuable advice is given on the choice of nuclear emulsions and their uses for various purposes, and on the inclusion of appropriate control experiments.

The chapters on soluble compound and macroscopic autoradiography are useful, although much of it has been published elsewhere. The chapter on electron microscope autoradiography is less satisfactory, for only one technique is given. Several other useful well tried techniques exist which might well have merited inclusion. The assessment of electron microscope results is poor compared with the treatment of light microscope preparations. The chief shortcomings of the book stem from the author's insistence on omitting literature concerned largely with applications to specific biological problems. The few examples cited are of a relatively unusual nature. Clearly a vast amount of selection would be required. Many points, however, would have been made clearer. More information would have been useful on preparation of specimens, particularly from the point of view of extraction of labelled compounds during embedding. The book is misleading on this point, especially in repeatedly recommending soluble compound techniques. Such techniques are of great importance. It must be made clear, however, that often useful autoradiographs are only obtained because selective extraction has occurred. The inclusion of some conventional applications together with their biochemical mechanisms would have clarified some of these points.

Dr Rogers has performed a useful service in making available in a single volume a great deal of information on autoradiograph technology. Despite the rather high price the book will find a place in the laboratories of all biologists who use photographic film to localize and measure radioisotopes. M. A. WILLIAMS

## THEORY OF ACCELERATORS

### Particle Accelerators

Edited by Rudolf Kollath. Translated by W. Summer. Pp. xiii+337. (London: Sir Isaac Pitman and Sons, Ltd., 1967.) 75s. net.

DR KOLLATH first published his book *Teilchenbeschleuniger* in 1954, and it was revised and enlarged with the help of other authors for the second edition brought out in 1962. This second edition has been translated by Mr Summer, who has added an appendix to record briefly some of the developments since 1962.

Although there have been no new fundamental ideas for particle accelerators discovered in the past ten years, technical advance in the realization of earlier ideas has been rapid and any book on the subject must be, to some extent, out of date almost before it is published. This book suffers particularly in this respect, for almost all the accelerators described have been running for a long time and some have actually been scrapped. General statements are made which may have been correct when the original book was written, but which progress has made no longer true. Also Dr Kollath falls into the bad habit of reporting (then) projected accelerators in the present tense, so that the reader would be forgiven for thinking that the Canberra ironless synchrotron had accelerated protons to 10 GeV whereas the project was abandoned some years ago. Therefore, one must judge this book on the success with which the principles behind the various types of accelerators are explained, rather than as a progress report.

Despite the fact that Dr Kollath wrote, or assisted in writing, most of the chapters, there is a considerable variation in the standard of previous knowledge expected of the reader. The publisher, in the end papers, states that "Mathematics is involved only where necessary to explain a point and even then is used with restraint" and "the book is highly recommended to degree students and all who have an interest in the study and application of particle accelerators such as medical men and engineers". Such restraint is not very noticeable in the longest

chapter in the book, on the theoretical treatment of accelerators with azimuthally variable magnetic fields, which includes a contribution to the theory of non-linear betatron oscillations. This would be an admirable paper on its own as an example of applied mathematics, but does not seem appropriate to the readership envisaged.

As an introduction to the theory of particle accelerators this book must be compared with Livingood's *Principles of Cyclic Particle Accelerators* and it loses in the comparison. Livingood gives a clearer, more coherent picture, and, despite the title, covers almost as much ground.

The translation is generally good, but some sentences which have been rather literally translated may have to be read twice before the meaning is clear, and some technical terms have suffered from being translated from English into German and then back again. A few errors have crept in, one leading to the statement that "the proton synchrotron supplies a powerful pulse of  $10^{-7}$  sec duration every five to ten seconds". M. C. CROWLEY-MILLING

## SEMICONDUCTOR PHYSICS

Proceedings of the International Conference on the Physics of Semiconductors, Kyoto, 1966

(*Journal of the Physical Society of Japan*. Vol. 21. Supplement, 1966.) Pp. xiii+780. (Minato-Ku, Tokyo: The Physical Society of Japan, 1966.) n.p.

THE eighth International Conference on the Physics of Semiconductors was held in September 1966, at Kyoto Kaikan, Kyoto, Japan, under the auspices of the International Union of Pure and Applied Physics, the Science Council of Japan and the Japanese Ministry of Education. The proceedings are published as a supplement to the *Journal of the Physical Society of Japan*, and contain 146 papers in all, including seven review and seventeen invited papers.

The programme of this conference had been very carefully chosen by the organizers so as to concentrate on new developments in the theoretical and experimental techniques used in the study of semiconductors and on the new phenomena revealed by these methods. There was very little routine reporting of experimental and theoretical work based on well established methods. This was the main reason for the unqualified success of the conference, in that it provided a demonstration of the fact that semiconductor physics is still a rapidly expanding subject with no apparent signs of saturation. The proceedings form an up-to-date picture of the state of the subject and serve to illustrate the increasing sophistication of semiconductor research and the ever-growing overlap with other branches of solid state physics.

The calculation of band structures has become something of a fine art these days, as is reflected in the papers contained in the session on band theory. The experimental determination of band structures is also becoming increasingly more sophisticated and accurate—a number of papers, either dealing specifically with this topic or having an indirect bearing on it, appear in the proceedings. The double session on optical properties contained a fine review of the theory of electromagnetic waves coupled to polarization fields by J. J. Hopfield. This review includes a brief survey of the usefulness of this theory, and it is interesting to note that its application to the problem of exciton spectra leads to very similar conclusions to those arrived at by Y. Toyozawa *et al.*, who treated the problem in terms of the competition between local and band features in the solid. This latter work was reported in the session on excitons.

The sessions on magneto-optics, impurity state, recombination, transport phenomena and quantum transport, while containing excellent reports of experimental and theoretical work, do not break any new ground apart from

showing how sensitive and complex the newer methods are. A typical example of this type of work is described in the paper by J. G. Mavroides on magneto-piezo-optical experiments.

The sessions on hot electrons, electron-phonon interactions and current instability are particularly interesting, not only because of the individual papers, but also because they show how the study of non-equilibrium phenomena has become more popular and fruitful. The past few years have seen great advances in the understanding of these effects—the Gunn effect, for example, has been explained only since the last conference in this series, as was pointed out by H. Y. Fan in his closing address. This part of the conference may well prove to be the most significant in that it contains the beginnings of a new trend in semiconductor research. The general impression given by these papers is that the next phase of development in the field of semiconductor physics will take the form of an investigation of those processes which occur when a solid is not in thermal equilibrium.

Another significant feature of this conference, which illustrates the rapid rate of expansion of the field, is the fact that whole sessions were devoted to both magnetic semiconductors and superconducting semiconductors. These are comparatively new topics which have only been studied for the past three or four years.

A number of new materials seem to have come into fashion. The layer-type semiconductor GaSe was the subject of two papers and tellurium attracted a certain amount of interest. These substances display interesting non-linear optical properties. Superconducting and normal  $\text{SrTiO}_3$  were also discussed. On the subject of material preparation, the general impression gained is that workers on a very wide range of substances are able to "tailor make" their materials to a very much greater degree than they could a few years ago. This state of affairs not only means that devices can be made to very close specifications, but also enables the experimenter to choose the exact conditions he requires for the study of the physical process in which he is interested.

The conference as a whole was a great success and the proceedings give an excellent detailed account of the individual papers including edited versions of the discussions which took place after each paper. The publishers are to be congratulated on making these proceedings available so promptly and on maintaining a very high standard of accuracy throughout. O. H. HUGHES

## RARE ELEMENTS IN ABUNDANCE

The Lovozero Alkali Massif

By K. A. Vlasov, M. Z. Kuz'menko and E. M. Es'kova. Translated by D. G. Fry and K. Syers. Edited by S. I. Tomkeieff and M. H. Battey. Pp. xvi+627. (Edinburgh and London: Oliver and Boyd, Ltd., 1966.) 252s.

LOVOZERO and the neighbouring intrusion of Khibiny, in the Kola peninsula, form one of the most extraordinary igneous complexes on Earth: they are unusually large masses of peralkaline syenite which contain abnormal amounts of rare elements, especially zirconium, niobium and rare earths. Any account of one of them could scarcely be uninteresting, but this large book, full of detailed information, does not match the fascination of its subject. A vast array of mineralogical and chemical data are collected together, and this is perhaps merit enough. But I should have liked a more penetrating description of the field and structural relations, the fabric and the texture, especially of the major rocks and minerals. The discovery at the outset that statements in the text are inconsistent with the illustrated sections (Fig. 5) must strain the credibility of the field descriptions in general. It is impossible to ascertain the form of the

whole massif, although it is implied to be tabular. When the authors come to the pegmatites, and the rare-element geochemistry, they are much more at home, and to this extent the book is mis-titled. Much of the description and the discussion throughout is ponderous and repetitive, although the reader searching for comparative data will find some good summaries and some excellent tables. The plates are of very mixed quality, and a number of figures have unsatisfactory explanations, scales or ornaments. The section on mineralogy is long and of variable consistency—petrologists will be disappointed by the section on rock-forming minerals; the pigeon-holing of some of the others, for example titanosilicates, tends to obscure their affinities. The final section of the book, on geochemistry and genesis, usefully gathers together many loose ends, but some of the averaging of chemical data is open to the criticism that the analyses, and the proportions of each, are not cited. The subsequent account of the mode of formation is not "a stimulating starting point for discussion". Nowhere is the suggested four-stage formation of rocks, as well as pegmatites, clearly substantiated. The remarkable mineral layering at Lovozero is ascribed to crystallization differentiation, but the mechanics of segregation are not postulated, and the phase-conditions governing the suggested sequences of crystallisation are not considered. A serious defect in this discussion is the scant consideration given to the neighbouring Khibiny massif; only the average chemistry is compared with that of Lovozero, and also, strangely, with Vishnevogorsk (only the reader familiar with Soviet geology would appreciate the latter incongruity—Vishnevogorsk is in the Urals province). In short, the factual part of this book is an invaluable, if unwieldy, addition to our knowledge of this uniquely important igneous complex.

D. K. BAILEY

## FEEDING FRUIT CROPS

### Nutrition of Fruit Crops

Tropical, Sub-tropical, Temperate. Edited by Norman Franklin Childers. Pp. xv + 888. (New Brunswick, N.J.: Horticultural Publications, Rutgers University, 1966.) n.p.

Most of the perennial plants that bear fruit crops of economic importance have much in common in their morphology and physiology, and certain general principles underlie their nutrition. Much empirical work has been carried out to determine responses to fertilizers and attempts have been made to provide bases for generalization about the application of results with a given crop to the various conditions in which the crop is grown. Little has been done, however, to integrate the research results to develop a generalized account of the nutrition of fruit plants. Although the present publication does not set out to do this, it performs a useful service in bringing together in one large book reviews on the nutrition of individual fruit crops.

Of the twenty-four chapters, nineteen are reviews, with lists of references, on the nutrition of apple; avocado and mango; banana; blueberry and cranberry; bush fruits; cacao; cherry; citrus; coconut; coffee; grape; olive; edible nuts; peach; pear; pineapple; plum, prune and apricot; strawberry; and tung. These reviews are mostly by American experts concerned with the respective crops. They show great variation in length and treatment; some give a critical assessment of their subject, while others provide a catalogue of experimental results sometimes in extensive tables quite out of place in this book. Many of the chapters retain the same form as in the original edition of 1954, the authors having been content merely to insert additional paragraphs, but some have been re-written. The accounts of the nutrition of banana, cacao, coconut, coffee, olive and pineapple are all new.

The book also includes chapters dealing with the use of leaf analysis as a guide to the nutrition of deciduous fruits and citrus respectively, an account of the use of chelated metals for supplying micronutrients to fruit crops, a chapter on experimentation with orchard trees, a comprehensive table of nutrient concentrations in fruit plant tissues in relation to the appearance of symptoms, and a ninety-eight page insert of monochrome photographs illustrating deficiency symptoms.

The contributions are all virtually independent. With few exceptions, there are not even any cross-references between the texts and the photographic section, so one has to use the subject index, fortunately a good one.

Defects occur, but they do not detract seriously from the value of this collection as a source of reference, on the whole well produced. Those concerned with the nutrition of one type of fruit crop should find it useful to consider what may be learned from the approach of others working with similar crops.

D. W. P. GREENHAM

## ECOLOGY LOOKS AHEAD

### Systems Analysis in Ecology

Edited by Kenneth E. F. Watt. Pp. xiii + 276. (New York: Academic Press, Inc.; London: Academic Press Inc. (London), Ltd., 1966.) \$11.50.

THIS is an excellent compendium of several recent and sophisticated research projects which have utilized electronic sampling devices followed by computer simulation or analysis.

Despite careful selection of the material, however, the reader is left with the impression that this, "the first book to present a detailed exposition of new electronic and computer procedures", is a little premature in its appearance. In several instances it is clear the authors themselves have indulged in some wishful thinking. The realism, for example, of both the direct study of bird navigation by means of orbital satellites, and direct links of computer with experimental situation, are both cases in point.

Many ecologists are not only aware of the complexity of the ecosystem and the desirability of team research but are also aware of the large gaps in our existing knowledge. This in turn leads to highly simplified simulation models. The wide range of the contents of this book does suggest a variety of ways in which the necessary data can be collected and handled by computer and it is in this respect a useful contribution.

It gives a firm jolt to those ecologists who are still prepared to amble along with merely notebook and pencil. Equally it promises those of us with an inadequate mathematical and electronic background some heavy going in the future.

K. A. KERSHAW

## NORTH AFRICAN BIRDS

### The Birds of North Africa

From the Canary Islands to the Red Sea. By R. D. Etchécopar and François Hüs. Translated by P. A. D. Hollom. Pp. xx + 612 + 24 plates. (Edinburgh and London: Oliver and Boyd, Ltd., 1967.) 168s. net.

As I remarked in the notice of the original edition of this book (*Nature*, 205, 9; 1965) it was appropriate that this valuable work of reference should be a product of French ornithology, which has been so notably active in the area. The material in the book is, however, also of great interest to British ornithologists and this English edition is welcome. The translator is one of the authors of the well known *Field Guide to the Birds of Britain and Europe* and himself a frequent visitor to North Africa. The present

book is itself more than a field guide, giving fuller information about behaviour and reproduction than is necessary merely for identification; the authors reasonably excuse its substantial bulk on the ground that travel in the desert anyhow necessitates a vehicle. The book gives a general account of all the bird species, resident and migratory, found in North Africa above about 21° N. latitude, including the Canary Islands. The translation very closely follows the original text, which the authors have not revised except to incorporate information from one recent source. They have, however, redrawn a dozen of the species distribution maps which are a feature of the work. The admirable illustrations by Paul Barruel, mostly in colour, are, of course, repeated.

LANDSBOROUGH THOMSON

## OUTSIDE THE NUCLEUS

### The Plastids

*Their Chemistry, Structure, Growth and Inheritance.* By John T. O. Kirk and Richard A. E. Tilney-Bassett. (A Series of Books in Biology.) Pp. xvi+608. (London and San Francisco: W. H. Freeman and Company, 1967.) 110s.

In recent years the investigations of the fine structure and function of plastids by electron microscopists and molecular biologists have confirmed what has long been apparent to those pursuing the more conventional approaches of cytogenetics and physiology, namely, that plastids have a dual role. They are both the seat of photosynthetic activity and the bearers of extra-chromosomal hereditary information with its associated protein synthetic activity. *The Plastids* is a timely and comprehensive account of both of these activities in which the old and the new are fairly balanced, carefully assessed and put into historical perspective.

The book is divided into four parts: the first is a short general description of the nature of plastids; the second is concerned with the inheritance and genetic autonomy of plastids illustrated by reference to the classical experiments on mutant and normal plastid differences in higher plants. An understanding of the nature of variegations and of the structure of chimeras is essential for a full appreciation of the significance of the breeding experiment and the observations on somatic segregation. The authors endeavour to provide this in the early chapters of the second part, although the combination of exhaustive detail, short sections and extensive cross-referencing makes heavy reading. Nevertheless, these are important chapters because the facts they contain provide the key to understanding how the separation of the chromosomal and extra-chromosomal contributions to the determination of the plastid phenotype has been achieved. But while we are spared none of the details of the origin and structure of variegations and chimeras, the authors' explicit desire "not to burden the reader" leads to a vague account of the mathematical properties of the sorting-out from cells containing a mixture of two kinds of plastids, which is devoid of the simplest statistical expressions, formulae and concepts. The binomial and hypergeometric distributions which are the theoretical basis for all models of sorting-out are not even mentioned.

The third part of the book gives the biochemical basis of plastid autonomy and plastid growth and accounts of the more recent evidence from electron microscopic and biochemical investigations. It contains a particularly good account of the genetical approach to the elucidation of the biosynthetic pathways which are involved in plastid development and in photosynthesis. Although every aspect of plastid structure and function in *Chlamydomonas reinhardtii* is referred to somewhere in the book, the evidence for post-meiotic reassortment and recombination

of extra-chromosomal determinants of the plastid phenotype in this species has been omitted, even though it has an important bearing on some of the speculative discussion in the third part.

The final part of the book is a short summary of the contents of the first three parts followed by speculations about the possible future developments in the field of plastid research. Similar summaries at the end of each chapter or part would have been invaluable in a book of this size and complexity. Unfortunately only three of the fifteen chapters and none of the parts have a concluding summary and many chapters end with a miscellany of material that could not find a place in their principal sub-sections. The book ends with three excellent indices for subjects, taxa and authors, respectively. These supplement the extensive references at the end of each chapter.

Taken as a whole, this book is an impressive work, beautifully illustrated and an invaluable source of reference. Its publication is a landmark in the acceptance of the extra-chromosomal contents of the cell as a partner of the chromosomal system in the heredity, growth and differentiation of green plants.

J. L. JINKS

## OBITUARIES

### Professor H. J. Muller

HERMANN J. MULLER, professor of zoology in the University of Indiana, Nobel laureate in 1946, died in Bloomington, Indiana, on April 5, 1967. He was born in New York City in 1890, and graduated from Columbia College in 1910. Here he had been influenced by the cytologist Edmund B. Wilson and the embryologist Thomas Hunt Morgan, who was beginning his studies of the genetics of the vinegar fly, *Drosophila melanogaster*. Muller began the experimental analysis of heredity at Columbia with the first group of graduate students to devote themselves to work with *Drosophila*, and in the same year, 1915, he obtained his Ph.D. with a dissertation on "The Mechanism of Crossing-over", and became co-author of the book which initiated the new era in genetics, *The Mechanism of Mendelian Heredity*, by T. H. Morgan, A. H. Sturtevant, Muller and C. B. Bridges. While at Columbia he made two discoveries which determined the course of his future work: the recognition of what became known as cross-over suppressors, later shown to be caused by inversion of gene order in part of a chromosome, and this in turn led to the discovery of the first balanced lethal system which provided the clue to the explanation of the recurrent "mutations" which de Vries had discovered in the evening primrose *Oenothera*.

The insights thus provided into the nature of the genetic system led Muller to invent ingenious breeding systems for the quantitative study of the mutation process. Much of this work was carried out at the University of Texas, where he was professor of zoology from 1920 to 1933. His paper on "The Problem of Gene Modification" provided the chief excitement at the Fifth International Congress of Genetics held at Berlin in September 1927. It brought convincing experimental proof that the mutation rate of genes of *Drosophila* could be increased 150-fold and more by treatment with X-rays. What set genetics on a new path was not merely the technical achievement of inducing mutations, both those with lethal and with visible phenotypic effects, in measurable quantitative rates. There was, in particular, the application of these methods to the problems clearly envisaged and pointed out by Muller of the analysis of the chemical and physical structure of the hereditary



material and of the process of evolution. Behind Muller's achievement lay the primary disclosure of the chromosome mechanism of heredity by E. B. Wilson (to whom Muller gave chief credit), T. H. Morgan and his students; after it came the detailed development of cytogenetics and the molecular basis of heredity. Muller participated actively in both the practical experimental and the theoretical development of genetics for more than fifty years, and left on it the indelible stamp of his ideas and vigorous personality.

He left Texas for Moscow in 1933 (he had taken the first cultures of *Drosophila* there in 1922), but abandoned the Soviet Union in 1937 to work first at the University of Edinburgh until 1940, then at Amherst College until taking up his professorship at Indiana in 1945.

Muller made important contributions to human genetics and to evolutionary theory through development of the classical theory of population structure (genetic load of mutations), and devoted increasing attention and enthusiasm to proposals for human betterment through control of human reproduction.

Throughout his work there runs a pattern of persistent use of mechanistic interpretations of biological phenomena. The success of these interpretations in genetics, as in physics and chemistry, led to an attitude of confidence which in Muller's case became overt optimism regarding the prospect of ultimate control by man over his own destiny.

L. C. DUNN

#### Professor F. A. Vening Meinesz

FELIX ANDRÉAS VENING MEINESZ, former professor of cartography and geodesy, and later also of geophysics, at the University of Utrecht from 1927 to 1957, and professor of geodesy at the Technological University, Delft, from 1939 to 1957, died in Amersfoort on August 10, 1966. He had been in hospital for six weeks after a fall at his home which caused a fractured hip; he was 79.

Vening Meinesz obtained his degree in civil engineering from the Technological University at Delft in 1910. Soon afterwards he was appointed engineer of the Netherlands Geodetic Commission and was commissioned to carry out pendulum observations in the Netherlands. This was a turning point which was to influence his whole career. On land he did pioneering work in this field, which he later repeated at sea. He was able to eliminate the disturbances caused by the irregular movements of the weak soil of the Netherlands by the so-called "two-pendulum method", and in 1915 he published the theory of his method in the thesis for his doctorate, entitled "Bijdrage tot de theorie der slingerwaarnemingen", after which he received his doctorate *cum laude*. In 1923 his "Observations de pendule dans les Pays-Bas" were published, describing the application of this method for fifty stations in the Netherlands. At this time Vening Meinesz was beginning to wonder whether this two-pendulum method could be applied with sufficient accuracy at sea as well. Tests made it clear that it would be difficult to fulfil the conditions of this method (equal amplitude and opposite phases of the pendulums) even in a submerged submarine. Vening Meinesz then realized that, independent of amplitude and phase, the difference in the angle of elongation of two isochronous pendulums, swinging in the same plane, was insensitive to horizontal accelerations, and that this angle could be regarded as the angle of elongation of a fictitious pendulum with the same period as the original pendulums. This method was worked out theoretically and instrumentally in his "Theory and Practice of Pendulum Observations at Sea" (1929), and it was used during the many submarine voyages which made him so well known. The results of all these voyages were published in the four volumes of *Gravity Expeditions at Sea*.

In the meantime Vening Meinesz published, in 1928, "A Formula Expressing the Deflection of the Plumbline in the Gravity Anomalies and some Formulae for the Gravity-field and the Gravity-potential Outside the Geoid", which became familiar to geodesists. His extensive gravimetric observations in the Indonesian Archipelago made it possible for this method to be applied for the first time at sea by Dr J. E. Baron de Vos van Steenwijk in his "Plumbline Deflections and Geoid in Eastern Indonesia as derived from Gravity".

Vening Meinesz continually emphasized the relationship between geodesy and geophysics. As a convinced supporter of isostasy, he tried to gather all possible information on the deviations from the state of equilibrium in the Earth. Combining this information with geological and seismological data, he always tried to make the geodesist better acquainted with the background to his science. He paid special attention, however, to trying to explain the reason for the irregular form of the Earth's crust. He considered that convection currents in the Earth's mantle were mainly responsible for the movements and consequent displacement of the continents. He was always able to express very difficult physical problems mathematically, as he had already done with the pendulum theory.

All those who knew Vening Meinesz were impressed not only by his great scientific knowledge, but also by his friendliness and hospitality, and his readiness to exchange ideas. He was awarded many Dutch and foreign decorations, honorary doctorates and the membership of many learned societies. One of his most important Dutch decorations was the "Eremedaille in Goud voor Voortvarendheid en Vernuft van de Huisorde van Oranje".

G. J. BRUINS

#### George Smith

GEORGE SMITH, who died on March 29, 1967, at the age of 71, started his career as a chemist. He graduated from Manchester University in 1916 and obtained his M.Sc. and A.I.C. in 1918. As chemist to a cotton manufacturing firm he met early the problem of mildew on cotton goods, and this led to the study of moulds, which was to become his chief work. In 1930 he joined Professor H. Reistrick at the London School of Hygiene and Tropical Medicine, where he remained till his retirement in 1961. Here he began with studies of the biochemistry of mould products, but became more and more involved in the taxonomy and care of the fungal cultures. During the Second World War he investigated the tropic proofing of military and naval equipment and thus became one of the first specialists in the field of bio-deterioration of materials. On retirement he continued his work on moulds at the Commonwealth Mycological Institute.

In 1939 he produced the first edition of *An Introduction to Industrial Mycology*. This book, with its magnificent photomicrographs, gathered together the information, much of it original, which hitherto scientists working in industry had found difficult to obtain. It is now generally used as an introduction to mycology for all those interested in moulds and moulding. Besides many short papers on the taxonomy, particularly of *Penicillium* and *Aspergillus* species, he produced monographs on the genera *Pasolomyces* and *Scopulariopsis*. All his work was undertaken with painstaking care and a meticulous attention to detail.

He enjoyed field work and was foray secretary of the British Mycological Society for some years, and was president of this society in 1946.

He was a quiet retiring man, kind and considerate, with diverse interests, covering all branches of science and philosophy. His favourite relaxation was music and he was an accomplished performer.

AGNES H. S. OMONS

## University News:

**Edinburgh**  
**DR NEIL CAMPBELL** has been appointed to a personal chair of chemistry.

**Liverpool**

The following appointments have been made: *Senior lecturer*, Dr D. V. Roberts (physiology); *Lecturers*, Dr H. A. Collin (botany); R. A. Latham (child health); Dr K. Lubkiewicz (child health); O. Thomas (civil engineering); P. Mars (electrical engineering and electronics); T. F. Varley (operative dental surgery).

## Announcements

The Medical Research Council has set up a Molecular Pharmacology Research Unit in the Department of Pharmacology, Cambridge, under the honorary directorship of Professor A. S. V. Burgen, to investigate the nature of the interactions between drugs and macromolecules and organized cellular structures. Further information can be obtained from Professor Burgen, Department of Pharmacology, The University, Downing Street, Cambridge.

## Meetings

**PHYSIOS**, Chemistry and Geochemistry of Asbestos Minerals, July 19-21, Somerville College, Oxford (Mr. W. P. Howard, Oxford Conference on Asbestos, 77-79 Fountain Street, Manchester 2).

**THE Mathematics of Solid State Physics**, July 23-26, University of Essex (the Secretary and Registrar, the Institute of Mathematics and its Applications, Maitland House, Warrior Square, Southend-on-Sea, Essex).

**NON-EQUILIBRIUM Processes in Astrophysics**, July 24-28, University of Manchester (Dr J. Hazlehurst, Astronomy Department, The University, Manchester 13).

**SOLUTION Properties of Natural Polymers**, July 25-27, Edinburgh (the General Secretary, the Chemical Society, Burlington House, London, W1).

**INTERNATIONAL Symposium of the International Society for Neurovegetative Research**, July 25-28, Royal Netherlands Academy of Sciences, Amsterdam (Professor J. Ariens Kappers, The Netherlands Central Institute for Brain Research, Ijdijsk 28, Amsterdam-O., The Netherlands).

**SEVENTH International Sedimentological Congress**, August 11-15, University of Reading and University of Edinburgh (Dr P. McL. D. Duff, Grant Institute of Geology, King's Building, West Mains Road, Edinburgh 9).

**FIBROUS Proteins**, August 14-18, Canberra (Symposium Secretary, Division of Protein Chemistry, CSIRO, 343 Royal Parade, Parkville N.2, Victoria).

**SPECTROSCOPY**, August 14-18, Brisbane (Dr D. James, University of Queensland, St. Lucia, Brisbane, Queensland).

**MEDICAL and Biological Engineering**, August 14-19, Stockholm (7th International Conference on Medical and Biological Engineering, Stockholm 60, Sweden).

**RADIOLOGICAL Health Problems**, August 17-20, McGill University (Dr D. Waldron-Edward, Honorary Secretary, Organizing Committee, Donner Building for Medical Research, McGill University, Montreal).

**ERRATUM**. In the communication "Nickel, Iron, and Manganese in the Metabolism of the Oat Plant" (*Nature*, 214, 828; 1967), the fifth sentence of the first paragraph should read: "The analysis of soil and minerals removed from both outcrops and subsoil, however, indicated that nickel alone was responsible for the condition of the crop. The level of cobalt in both soil and affected plants was little different from that in material from unaffected areas."

**ERRATUM**. In the article "Interaction of Basic Magma with Pelitic Materials", by C. D. Gribble and M. J. O'Hara (*Nature*, 214, 1198; 1967), the first sentence of the second column on page 1199 should read: "Residua consisting of an-mu-tr and an-mu, generated at  $t_1$ , and an-mu-coord, generated between  $t_1$  and  $t_2$ , would begin to melt with the appearance of liquid W, leaving residua consisting of an-mu-co, an-co and an-sp-co."

**ERRATUM**. In *Nature*, 214, 1182 (1967) the Fisheries Biochemical Unit at the University of Aberdeen was wrongly described as the Fisheries Biology Unit.

**ERRATUM**. The bibliography of *The Deer and the Tiger* (*Nature*, 214, 1372; 1967) should read: A Study of Wildlife in India. By George B. Schaller. Pp. 370+30 plates. (Chicago and London: The University of Chicago Press, 1967.) \$10; 72s. net.

**CORRECTION**. In Fig. 2 of the article "Three-dimensional Structure of Tosyl- $\alpha$ -chymotrypsin", by B. W. Matthews, P. B. Sigler, R. Henderson and D. M. Blow (*Nature*, 214, 652; 1967), the amino-acid at position 70 should read glu.

## THE NIGHT SKY IN JULY

All times are in Universal Time

PLANETS	MOON		CONJUNCTIONS WITH THE MOON				
	New Moon	Full Moon	Venus	Mars	Jupiter	Saturn	
			11d 00h, 5° S.	15d 01h, 2° S.	24d 10h, 5° S.	27d 14h, 0-9° S.	
Times of rising (R) and setting (S) during the month							
Name	R/S	Beginning	Middle	End	Mag.	$D_p$ (10 <sup>6</sup> miles)	Zodiacal position
Mercury	R	Unfavourable for observation		21h 45m	+2.6	56	Gemini
Venus	S	22h 55m	21h 45m	20h 45m	-4.1	48	Leo
Mars	S	0h 05m	23h 15m	22h 30m	+0.2	93	Boötes
Jupiter	S	22h 00m	21h 10m	Unfavourable	-1.3	563	Cancer
Saturn	R	0h 00m	23h 00m	22h 00m	+0.9	860	Cetus

$D_p$  is the distance of planet from the Earth on the 15th of the month.

## OCULTATIONS OF STARS BRIGHTER THAN MAGNITUDE +6 AT GREENWICH

Star	R/D	Time	Mag.
76 Vir	D	14d 21h 31-0m	+5.4
$\sigma$ Sco	R	17d 22h 14-0m	+3.1
43 Oph	D	18d 22h 06-3m	+5.4

(D, disappearance; R, reappearance)

## OTHER PHENOMENA

- 3d 07h, Mars 1.4° N. of Spica.
- 8d 05h, Venus 0.3° S. of Regulus.
- 24d 10h, Venus at greatest brilliancy.
- 27d 14h, Saturn occulted by the Moon, visible N.E. Asia, N.W. America.

## FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Wednesday, July 5—Thursday, July 6

MUFFIELD COLLEGE OF TECHNOLOGY (at Queensway, Muffield, Middlesbrough)—Symposium on "Mass Spectrometry".

Monday, July 10—Thursday, July 13

INSTITUTE OF ELECTRONICS AND RADIO ENGINEERS; INSTITUTE OF PRODUCTION ENGINEERS; and the INSTITUTE OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at the University, Nottingham)—Joint Conference on "The Integration of Design and Production in the Electronics Industry".

## APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

LECTURER (preferably with a particular interest in mechanical vibration) in the MECHANICAL AND MECHANICAL GROUP of the DEPARTMENT OF MECHANICAL ENGINEERING, to contribute to the teaching and research work of the Department in the field of dynamics—The Secretary to the University Court, The University, Glasgow (July 7).

ASSISTANT LECTURER in the DEPARTMENT OF PHYSICS—The Registrar, The University, Leicester (July 8).

ASSISTANT LECTURER or LECTURER (with an interest in experimental taxonomy, physiological ecology or plant physiology) in the SCHOOL OF BIOLOGICAL SCIENCES—The Registrar, University of East Anglia, Norwich Hall, Norwich, NR8 89C (July 8).

ASSISTANT LIBRARIAN (graduate)—The Secretary, Queen's College, Dundee, Scotland (July 8).

JUNIOR LECTURER in PHYSICS—The Secretary, Trinity College (University of Dublin), Dublin 2, Republic of Ireland (July 8).

LECTURER (highly qualified physical chemist preferably with some experience in polymers and/or ceramics) in MATERIALS SCIENCES to participate in an honours or ordinary degree teaching programme in materials science and physical chemistry—The Registrar, Rugby College of Engineering Technology, Beeston, Rugby (July 8).

LECTURER (insect physiologist with an interest in environmental physiology) in Zoology in the School of Biology—The Registrar, The University, Leicester (July 8).

LECTURER (preferably with an electrical or mechanical engineering background with experience in electronics) in BIOENGINEERING in the DEPARTMENT OF MECHANICAL ENGINEERING—The Registrar, University of Strathclyde, George Street, Glasgow, G1 (July 8).

CHAIR OF STATISTICS AND COMPUTING SCIENCES at Royal Holloway College—The Academic Registrar, University of London, Senate House, London, W.0.1 (July 10).

COMPUTER OFFICER in the CHIEFS COMPUTING OFFICE to assist in the development and maintenance of the software required to help with the University's KDF 9 computer and to help with the systematic documentation of library programmes and other facilities available to users—The Registrar, The University, Nottingham (July 10).

LECTURER or ASSISTANT LECTURER (with broadly based geological training and research experience in one or more of the fields of X-ray crystallography and/or X-ray diffraction, X-ray spectrometry or clay mineral studies) in the DEPARTMENT OF GEOLOGY—The Deputy Secretary, The University, Southampton (July 10).

RESEARCH ASSOCIATE/SENIOR RESEARCH ASSOCIATE in the fields of History of Education or Philosophy of Education—The Assistant Registrar (B), University of Birmingham, 50 Wellington Road, Birmingham, 15 (July 10).

SENIOR LECTURER (with a higher degree and appropriate research, teaching and/or industrial experience) in PHYSICAL CHEMISTRY—The Registrar, Technical College, Sunderland, Co. Durham, quoting Ref. No. 303/8/2 (July 10).

JUNIOR LECTURER (preferably with an interest in physiology) in ZOOLOGY—The Secretary, Trinity College (University of Dublin), Dublin 2, Republic of Ireland (July 14).

LECTURER (with particular research interests in the fragmentation of organic molecules under electron impact) in PHARMACEUTICAL CHEMISTRY AND STRUCTURAL CHEMISTRY—The Registrar, University of Bradford, Bradford, 7, quoting Ref. P80/L/20/M (July 14).

SENIOR SCIENTIFIC OFFICER (with a first- or second-class honours degree or Dip. Tech. or equivalent or higher qualifications in geology or physics and preferably some relevant postgraduate experience) at the Hydrological Research Unit for research in ground-water aspects of hydrology—The Establishment Officer, The Natural Environment Research Council, State House, High Holborn, London, W.C.1 (July 14).

ASSISTANT LECTURER (with special interests in quantitative and mathematical analysis of sociological data) in SOCIOLOGY—The Registrar, The University, Manchester, quoting Ref. 108/67 (July 15).

DEPUTY LIBRARIAN (of suitable academic and professional standing)—The Secretary of the University Court, The University, Glasgow (July 15).

LECTURER (Grade II) (with at least a good honours degree in science or a medical qualification) in HISTOLOGY; a RESEARCH ASSISTANT in EXPERIMENTAL PHYSIOLOGY; and a RESEARCH ASSISTANT in ORAL PHYSIOLOGY in the DEPARTMENT OF PHYSIOLOGY—The Registrar and Secretary, University of Bristol, Senate House, Tyndall Avenue, Bristol, 2 (July 15).

LECTURER or ASSISTANT LECTURER in PHYSIOLOGY—The Registrar, The University, Sheffield (July 15).

LECTURER (S) in the DEPARTMENT OF BACTERIOLOGY—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh (July 15).

ASSISTANT LECTURER/LECTURER in EXPERIMENTAL or THEORETICAL PHYSICS—The Registrar, The University, Keele, Staffordshire (July 17).

SENIOR LECTURER/LECTURER in FLUXUS SCIENCES (Climatology and Geomorphology) in the School of Earth Sciences; and a LECTURER in CHEMISTRY in the School of Chemistry, Macquarie University, Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, July 17).

KINGFISHER POSTGRADUATE STUDENT in TECHNOLOGY—Prof. D. Downon, Department of Mechanical Engineering, The University, Leeds, 2 (July 23).

LECTURER (S) (preferably with special qualifications in mineralogy and structural geology) in the DEPARTMENT OF GEOLOGY, University of Ibadan, Nigeria—The Inter-University Council, 33 Bedford Place, London, W.C.1 (July 28).

SENIOR LECTURER and a LECTURER (honours graduate in mining engineering with either subsequent metalliferous mining experience or research or practical experience in the field of rock mechanics) in MINING ENGINEERING;

and a LECTURER (with an honours degree in metallurgical engineering and a particular interest in extractive metallurgy) in PRODUCTION METALLURGY at the University of Queensland, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia, July 28).

LECTURER (S) in CHEMISTRY at Victoria University of Wellington, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, July 30).

CHAIR OF ORTHOPAEDIC SURGERY at the University of Singapore—The Inter-University Council, 33 Bedford Place, London, W.C.1 (July 31).

HEAD (with an honours degree in physics or a closely relevant science and able to co-ordinate the research programme of a department which is concerned with the application of the physical sciences to problems associated with many aspects of milk production and the utilisation of milk products) of the PHYSICS DEPARTMENT—The Secretary, National Institute of Research in Dairying (University of Reading), Shinfield, Reading, Berkshire, quoting Ref. 67/8 (July 31).

JUNIOR LECTURER/LECTURER in BOTANY at Rhodes University, Grahamstown, South Africa—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, July 31).

LECTURER/ASSISTANT LECTURER (with experience in comparative neurophysiology and/or ultrastructural research) in the DEPARTMENT OF ZOOLOGY and COMPARATIVE PHYSIOLOGY—The Assistant Registrar (S), University of Birmingham, P.O. Box 363, Birmingham, 15 (July 31).

LECTURER/JUNIOR LECTURER in PHILOSOPHY at Rhodes University, Grahamstown, South Africa—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, July 31).

LECTURER (S) in the DEPARTMENT OF PSYCHOLOGY at the University of Melbourne, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (July 31).

RESEARCH STUDENT (honours graduate in oceanography, physics, mathematics or meteorology) to participate in a programme involving the study of air-sea interaction processes—The Registrar, The University, Liverpool 8, quoting Ref. BV/85 (July 31).

SENIOR ASSISTANT in RESEARCH in the DEPARTMENT OF GEOLOGY—The Secretary, Faculty of Geography and Geology, The University, Sedgwick Museum, Downing Street, Cambridge (July 31).

ASSISTANT LECTURER or LECTURER in the DEPARTMENT OF INORGANIC, PHYSICAL AND INDUSTRIAL CHEMISTRY—The Registrar, The University, Liverpool, quoting Ref. BV/82 (August 1).

LECTURER (with a research interest in space physics and preferably research experience in gaseous ion physics) in REMOTE PHYSICS—The Assistant Registrar (S), University of Birmingham, P.O. Box 363, Birmingham, 15 (August 15).

TEACHING FELLOW in INORGANIC or PHYSICAL CHEMISTRY at Rhodes University—The Secretary-General, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1, or The Registrar, Rhodes University, Grahamstown, South Africa (South Africa, August 15).

BOYD BAXTER CHAIR OF BIOLOGY—The Secretary, Queen's College, Dundee, Scotland (September 2).

PATHOLOGIST in the MUFFIELD UNIT FOR LABORATORY ANIMAL PATHOLOGY to work in the field of experimental pathology—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh (September 15).

PROFESSOR OF AGRICULTURAL SYSTEMS AND ORGANIZATION—The Registrar (Room 20, O.R.B.), The University, Reading (September 30).

PROFESSOR OF CROP PRODUCTION—The Registrar (Room 20, O.R.B.), The University, Reading (September 30).

PROFESSOR OF HORTICULTURE—The Registrar (Room 20, O.R.B.), The University, Reading (September 30).

CELL BIOLOGIST (with a good honours degree and postgraduate experience, and preferably experienced in animal cell, tissue or organ culture) in the BIOPHYSICS GROUP, to engage in a programme of mammalian cell culture and experimentation based on existing facilities, and to develop and control a new cell culture unit, planned for the immediate future—The Administrator, Medical Research Council Radiobiological Research Unit, Harwell, Didcot, Berkshire, quoting Ref. GJN/8/3.

EXPERIMENTAL PATHOLOGIST—The Administrator, Radiobiological Research Unit, Medical Research Council, Harwell, Didcot, Berkshire, quoting Ref. BEH 4/21.

HEAD of the DEPARTMENT OF ELECTRONIC AND ELECTRONIC ENGINEERING—Clerk to the Governing Body, Borough Polytechnic, Borough Road, London, S.E.1.

LECTURER in CHEMICAL ENGINEERING—The Registrar, University of Strathclyde, George Street, Glasgow, G1, quoting Ref. 47/87.

LECTURER (graduate in mechanical engineering with an interest in materials) in MECHANICAL ENGINEERING; and a LECTURER (graduate in metallurgy or physics and preferably a knowledge of ceramics) in MATERIALS SCIENCES—The Secretary, Sir John Cass College, Whitechapel High Street, London, E.1.

MASTER to teach PHYSICS to at least "A" level and preferably to University Scholarship standard—The Headmaster, School House, Tonbridge, Kent.

MICROBIOLOGIST (graded S.O./S.S.O.) (with a first- or second-class honours degree, Dip. Tech. or equivalent or higher qualification in an appropriate subject, and for S.S.O. at least three years postgraduate experience) in the BIOMODIFICATION DIVISION which is interested in the detoxification and nutritional improvement of tropical foods by fermentation—The Administration Officer, Tropical Products Institute, 56/58 Gray's Inn Road, London, W.C.1.

POSTDOCTORAL RESEARCH FELLOW (with training in zoology, cell biology, microbiology or biochemistry) to join a team studying the invasive mechanism of cancer cells—The Assistant Registrar, The University of Birmingham, P.O. Box 363, Birmingham, 15.

RESEARCH ASSISTANT (graduate or with a qualification of equivalent standard) to join a small unit working on hypothalamic control of gonadal development in birds—Dr. B. K. Follett, Department of Zoology, The University, Leeds, 2.

RESEARCH ASSISTANT (with a degree in biochemistry or microbiology) for work on the large-scale isolation of enzymes—The Secretary, University College London, Gower Street, London, W.C.1.

RESEARCH ASSISTANT (with a good honours degree in microbiology or a related subject) to investigate the antigens of mycoplasmas—The Secretary, Lister Institute of Preventive Medicine (University of London), Chelsea Bridge Road, London, S.W.1.

RESEARCH FELLOW at the Institute of Sound and Vibration Research for fundamental study of subjective ratings of industrial noise—The Deputy Secretary, The University, Southampton.

RESEARCH SCIENTIST, PALYNOLOGIST (with a degree in geology and/or botany at the doctorate level with specialization in palynology and preferably

experience in the stratigraphic applications of palynology and a knowledge of general botany and plant ecology) at the Institute of Sedimentary and Petroleum Geology in Calgary to carry out investigations on pollen and spores in Mesozoic rocks and to advise on their stratigraphic significance—Biophysical Sciences Program, Public Service Commission of Canada, Ottawa 4, Ontario, Canada, quoting Ref. No. 67-115-19.

## REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

### Great Britain and Ireland

- Northern Ireland: Ministry of Agriculture. Leaflet No. 77: The Installation, Maintenance and Operation of Milking Machines. Pp. 8. (Belfast: Ministry of Agriculture, 1967.) [105]  
 World Directory of Research Workers in Vertebrate Reproduction. Edited by Donn Casey, assisted principally by Isabel Prime. Pp. x+174. (Cambridge: Reproduction Research Information Service, Ltd., 1967.) 70s.; \$18. [105]  
 British Museum (Natural History). Economic Leaflet No. 6: Plantar Beetles. Pp. 8. 4s. Economic Leaflet No. 8: Carpet Beetles. Pp. 3. 3d. (London: British Museum (Natural History), 1967.) [105]  
 The Kent Incorporated Society for Promoting Experiments in Horticulture. Annual Report of the East Malling Research Station, Maidstone, Kent, 1st October 1966 to 30th September 1966 (Fifty-fourth Year). (ASO). Pp. xxiii+250. (Maidstone: East Malling Research Station, 1967.) 21s.; \$8. [105]  
 Admiralty Marine Science Publication No. 12: Hydrographic Observations in North Channel, Irish Sea, H.M.S. Scott, April 1963. By John Harvey and Sinclair Buchan. Pp. 10+12 figures. (London: Hydrographic Department, Ministry of Defence, 1967.) 12s. 6d. [105]  
 British Antarctic Survey. Scientific Reports, No. 58: An Ostracod Fauna from Halley Bay, Coats Land, British Antarctic Territory. By Dr. J. W. Neale. Pp. 50+4 plates. (London: British Antarctic Survey, 1967.) 24s. net. [105]  
 Annual Report of the British Iron and Steel Research Association for the year ending December 31, 1966. Pp. 103. (London: British Iron and Steel Research Association, 1967.) [115]  
 Civil Service Commission. Annual Report 1966 for the period 1st January to 31st December being the Hundredth Report of the Commissioners. Pp. 48. (London: H.M. Stationery Office, 1967.) 6s. net. [125]  
 Office of Health Economics. Alive to Forty-Five. Proceedings of a Symposium held at the College of General Practitioners, London, Sunday, 27 November, 1966. Edited by George Teeling-Smith. Pp. 36. (London: Office of Health Economics, 1967.) 7s. 6d. [125]

### Other Countries

- Rubber Research Institute of Malaya. R.R.I.M. Planting Manual No. 12: Rainfall in Malaysia: a Study of its Occurrence, with Tables of Probability of Rainfall at Selected Stations, and an Introduction to Hydrology in Rubber Plantations. By P. R. Wycheley. Pp. 85. (Kuala Lumpur: Rubber Research Institute of Malaya, 1967.) 2 Malaysian dollars. [95]  
 United States Department of the Interior: Geological Survey. Geophysical Abstracts, No. 243 (March 1967). By James W. Clarke, Dorothy B. Vitallano, Virginia S. Neuhoff, and others. Pp. iii+189-294. \$0.35. Bulletin 1198-G: Copper Mooses as Indicators of Metal Concentrations. By Hansford T. Shackleton. Pp. iii+18. \$0.15. Bulletin 1244-D: Correlation of Upper Triassic and Triassic(?) Formations between Southwestern Utah and Southern Nevada. By Richard F. Wilson and John H. Stewart. Pp. 20. \$0.15. Water-Supply Paper 1819-I: Ground-Water Development in the High Plains of Colorado. By Arnold J. Boettcher. With a section on Chemical Quality of the Ground Water by Robert Brennan. Pp. iii+23+plates 1-3. (Washington, D.C.: Government Printing Office, 1966 and 1967.) [105]

- Australia: Commonwealth Scientific and Industrial Research Organisation. Soil Publication No. 24: Soil Development in Relation to Stranded Beach Ridges of County Lowan, Victoria. By G. Blackburn, R. D. Bond and A. R. P. Clarke. Pp. 44+map. (Melbourne: Commonwealth Scientific and Industrial Research Organisation, 1967.) [105]  
 Annals of the New York Academy of Sciences. Vol. 141, Article 1: Biological Actions of Dimethyl Sulfoxide. By C. D. Leake and 162 other authors. Pp. 1-671. (New York: New York Academy of Sciences, 1967.) [105]  
 American Museum Novitates. No. 2270 (October 27, 1966): A New Subspecies of *Icterus icterus* and other Notes on the Birds of Northern South America. By William H. Phelps, Jr., and Ramón Avelledo H. Pp. 14. No. 2284 (March 10, 1967): New Subspecies and Records of Birds from the Karimn Basin, New Guinea. By J. M. Diamond. Pp. 17. (New York: American Museum of Natural History, 1966 and 1967.) [105]  
 Australia: Commonwealth Scientific and Industrial Research Organisation. Annual Report of the Animal Research Laboratories, 1966-66. Pp. 192. (Melbourne: Commonwealth Scientific and Industrial Research Organisation, 1967.) [105]  
 Bulletin of the American Museum of Natural History. Vol. 185, Article 4: Results of the 1968-1969 Gilliland New Britain Expedition. 4: Annotated List of Birds of the Whiteman Mountains, New Britain. By E. Thomas Gilliland and Mary Leeroy. Pp. 173-216. (New York: American Museum of Natural History, 1967.) \$1.50. [105]  
 United States Department of the Interior: Geological Survey. Water-Supply Paper 1806-B: Determination of Strontium-90 in Water. By J. O. Johnson and K. W. Edwards. Pp. iii+10. (Washington, D.C.: Government Printing Office, 1967.) \$0.15. [115]  
 Proceedings of the United States National Museum, Smithsonian Institution. Vol. 121, No. 3575: Some Bathyal Polynoids from Central and Northeastern Pacific (Polychaeta: Polynoidae). By Marian H. Pettibone. Pp. 16. Vol. 121, No. 3579: Valid Zoological Names of the Portland Catalogue. By Harold A. Rehder. Pp. 51. Vol. 121, No. 3580: The Myodocopid Ostracod Families Philomedidae and Pseudophilomedidae (New Family). By Louis S. Kornicker. Pp. 35+1 plate. Vol. 122, No. 3581: Classification of the Western Hemisphere Belontiids (Homoptera: Cicadellidae). By H. Derrick Blocker. Pp. 56. Vol. 122, No. 3584: The Brindley Sea Mink, with Taxonomic Notes. By Richard H. Manville. Pp. 12. (Washington, D.C.: United States National Museum, Smithsonian Institution, 1966 and 1967.) [115]  
 Museum of Comparative Zoology, Harvard University. Annual Report 1965-1966. Pp. 49. Breviora. No. 261 (31 March, 1967): The *Mesochorus* Group of the Lizard Genus *Asolis* in Hispaniola. By Richard Thomas and Albert Schwartz. Pp. 27. No. 262 (31 March, 1967): A Phylogenetic Survey of Molluscan Shell Matrix Proteins. By Michael T. Ghiselin, Ron T. Degens, Derek W. Spencer and Robert H. Parker. Pp. 25. No. 263 (31 March, 1967): The Hydroid of *Vesicocystis forbesi* (Anthomedusae, Tubulariidae). By Anita Brindmann-Voss. Pp. 10. No. 264 (6 April, 1967): The Chanares (Argentina) Triassic Reptile Fauna. 3: Two New Gomphodonts, *Mesocynodontis paschalis* and *M. teruggii*. By Alfred Sherwood Romer. Pp. 25. No. 265 (6 April, 1967): New Land-Locked Fish Species of the Genus *Galeosoma* from North Auckland, New Zealand. By R. M. McDowall. Pp. 11. No. 266 (6 April, 1967): A New Species of *Varicorhinus* from the Caribbean Sea (Mollusca: Bivalvia: Veneridae). By Kenneth Jay Boss. Pp. 6. (Cambridge, Mass.: Museum of Comparative Zoology, Harvard University, 1967.) [115]  
 State of California. The Resources Agency: Department of Fish and Game Fish Bulletin No. 135: Ecological Studies of the Sacramento-San Joaquin Delta. Part 2: Fishes of the Delta. Compiled by Jerry L. Turner and D. W. Kelley. Pp. 106. (Sacramento: Department of Fish and Game, 1966.) [115]  
 Metropolitan Life Insurance Company. Statistical Bulletin, February 1967 (Vol. 48): Geographic Variations in Longevity After Age 65. Fatal Accidents Increasing Among Men at the Working Ages. The Problem of Arthritis. Death Claim Payments Higher in 1966. Pp. 12. (New York: Metropolitan Life Insurance Company, 1967.) [115]  
 Organisation for Economic Co-operation and Development. Methods and Statistical Needs for Educational Planning. Pp. 363. 35 francs; 50s.; \$8.50. Basic Statistics of Energy, 1961-1966. (Statistical Bulletin). Pp. 290. 20 francs; 20s.; \$5. (Paris: Organisation for Economic Co-operation and Development; London: H.M. Stationery Office, 1967.) [115]

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## COMPUTERS ON THE LINE

PLAINLY it is more than just a coincidence that the British and the American Governments should be bothered at the same time about the problems of arranging that computers should be enabled to communicate with each other by means of the conventional telephone system. By now, there has been talk enough of how computers will, one of these days, enter into the detailed administration of people's lives; how they will cash cheques, make airline bookings and rummage through bibliographies in some distant electronic library. They will so clearly assist the processes of communication that they are bound to be an alternative to many forms of communication by telephone and telegraph. It is only natural that the problems of how the new technology will marry with the old have come to a head in the United States and in Britain at roughly the same time.

This said, there are significant and even characteristic differences. On both sides of the Atlantic, of course, organizations at present established in the handling of telephone and telegraph traffic are anxious to know that they should have a share in the data transmission to and from computers. In Britain, the General Post Office is securely placed, with its effective monopoly on internal telecommunications; although it is theoretically possible for would-be independent operators to apply for licences to set up communications systems of their own, the chances of this happening are small. In the circumstances, it is not surprising that the question now being argued in Britain is whether the Post Office should be allowed to graft on to its communications network enough computer capacity to be able to offer a computing service to the public as a whole. With the approval by the House of Commons of the Data Processing Services Bill on July 4, it is only a matter of months before such a service is launched. In the United States the relationship between the communications carriers and the computing services are necessarily more complicated, and in any case the Federal Communications Commission, which is supposed to regulate the use of telecommunications, is going through a bad spell of indecision. It may, for example, have to allow the Western Union company, recently losing business on telegrams, to provide an integrated data processing system by means of its communications network. As yet, however, nobody knows whether AT & T, which is effectively in charge of the telephone system in the United States, would then follow suit. But the computer manufacturers are almost as concerned as the network operators to see some equitable solution worked out.

Whatever may be said in opposition to the plans the Post Office has been making, there is no denying that it has moved quickly and decisively. Although

there has been talk of a public data processing network for two years or more, it is only in the past few months that the Post Office has made known what it plans to do. To begin with, it plans to sell time on computing machinery already installed within the Post Office system, and it is not surprising that critics of the plan have been paying suspicious attention to the fact that 50 per cent of the capacity of the computers already in service with the Post Office is at present unused. The plan is to install enough computer machinery by the end of the decade to convert the Post Office into what will be the largest computer bureau in Britain. What happens after that is anybody's guess, but it is not hard to see why those who at present operate computer systems are apprehensive.

In principle, however, there is nothing inherently outrageous in what the Post Office plans to do. If it can offer an efficient data processing network, and if it can compete effectively yet fairly with commercial organizations, its intervention could be an asset. The snags, most of which have been clearly recognized in several constructive debates on the subject in the House of Commons, lie principally in the way in which the Post Office would set about the organization of its computer network. The obvious but somewhat abstract danger that a Post Office data processing network would allow the Government to keep an uncomfortably close watch on the way in which commercial organizations run has been acknowledged, and the Data Processing Bill now enjoins the Post Office to see that material in the course of being processed is kept confidential to those who own it.

A more serious problem is that of making sure that the Post Office does not compete unfairly with the commercial operators, possibly by using its great financial resources to undercut competitors. The British Government has been ready to acknowledge that there is a risk of unfair competition, and it has promised that the Data Processing Service will be operated as an independent unit within the Post Office. Unfortunately, however, good intentions are not guarantees, and if the Post Office persists in saying that computing machinery should be amortized over a period of ten years when commercial operators make less cheerful assumptions about the durability of their investments, it may well be able to charge less for data processing than its competitors. The trouble here, of course, is that there is no obvious means by which the charges made by the Post Office will be open to scrutiny and control. When the Post Office becomes an autonomous nationalized industry and not a part of the Civil Service as at present, there will be fewer opportunities than at present for objective criticism of the charges which are levied. And, however keen



civil servants may be to see that the Post Office operates legitimately, their efforts are bound to seem unconvincing to outsiders. In other words, the Government may not yet have recognized all the difficulties of allowing a nationalized industry to compete freely with private companies.

These, however, are immediate problems. Ten years from now, they could easily seem trivial and even pedantic. By then, time sharing computers will be commonplace. Businesses of all kinds will be dependent on computers not merely for huge parcels of data processing but for the solutions of smaller problems as well. This is why the enterprise of the Post Office in moving quickly into data processing should not tempt it to overlook its primary responsibility for efficient communications. Some computer users complain that the Post Office network is overloaded, and that more people would be using computer services already if there were more land lines available. Although the Post Office has been able to multiply the number of computer terminals by three in the past two years, and although complaints about the service now being offered may be exaggerated, it is hard to be confident that planning for the more distant future is being undertaken on a sufficiently ambitious scale.

What, for example, will be the contribution of lasers to communications in the future? What kinds of switching systems will be necessary to handle the gigantic loads on the telecommunications system ten years from now? What use will by then be made of satellites for domestic as well as international communications? Will it make sense to continue operating telephone networks without pulse code modulation? The research programme which the Post Office has in hand seems only inadequately to match the real and urgent needs which are now apparent. It is not reasonable to object that the Post Office is proposing to take a hand in data processing, but there will or should be trouble if it neglects to pursue the improvement of the communications network with the vigour which circumstances require. Even the most cursory examination of the way in which the Federal Communications Commission has been dazzled and even bewildered by the prospect of radically new means of communications should serve to show the Post Office that the problems are not all technical and that few of them are simple.

## COMMITTEE AND AEC

THE relationship between the Joint Congressional Committee on Atomic Energy and the Atomic Energy Commission in the United States has changed a great deal since the time, a decade ago, when the annual examination of the AEC budget seemed more a public spectacle of enmity than an orderly contribution to good government. At the beginning, of course, the AEC was prevented by a combination of arrogance and innocence from learning quickly how to get on

with Congress. The Joint Committee also had to make its reputation in what must then have seemed an exceedingly difficult technical field—and in the event it has succeeded so well that it has not merely become a power in the land on its own account but has also become a model to other committees of Congress which have somehow to make themselves effective critics in technical fields.

The American Constitution is a great help, of course. Congress is as jealous of its independence from the Administration as the British House of Commons is jealous of its independence of the monarchy. In Washington, one result is that the committees of Congress responsible for the detailed scrutiny of legislation are invested with all the prestige and authority that Congress has to muster. Even if the chairman of a committee, who may find that he owes his appointment to nothing but seniority, turns out to be a wayward eccentric, Congress as a whole will not willingly let the Administration make a monkey of him. The committees which make the pace in Washington, however, are those which can somehow establish an authority of their own. The Joint Committee on Atomic Energy has done this, and its success derives almost exclusively from its diligence. Since the early fifties it has been building up an enviable reputation for understanding of and discernment in the operations of the AEC. A decade ago it was largely responsible for persuading the AEC and the other government agencies concerned that there are more problems in regulating the safe use of radioactive materials than could be solved by setting rigid numerical limits for the kind of dose which should not be exceeded. By now, the committee has become expert on a host of technical matters. It can be relied on to know what the AEC is getting at when it says that one type of reactor is less promising than another. It can take a line of its own on the importance of plasma research. It has views on nuclear propulsion for rockets. Its competence has clearly won the respect not merely of other committees of Congress but of the AEC as well.

How has all this come about? Committees elsewhere—the Select Committee of the House of Commons on Science and Technology, for example—should be asking this question. When the prestige of Congress is discounted, the secret of the Joint Committee's success is principally to be found in the way in which it has been able to employ a full-time staff of able people willing to devote themselves to a continuing study of one branch of government administration. One striking proof of how this works is that the man who was for several years the committee's chief of staff, Mr James T. Ramey, became three years ago a member of the AEC. But this, of course, is also a proof of how close has now become the relationship between the committee and the AEC.

But could it be that the relationship is now closer than it should be? There is certainly something in the view that a certain tension between congressional committees and agencies of the Administration is desirable as well as unavoidable. One danger is that

if a committee and the agency which it is supposed to superintend live too closely in each other's pockets, there will be no means of making sure that their combined attitude to the outside world is sound. Another is that a committee which is too knowledgeable and too winsome may find itself persuading a government agency to particular lines of development which become, in retrospect, unwise. In other words, there is a danger that too expert a committee might find itself able to exercise power without responsibility.

On the face of things, the Joint Committee tends towards errors of the second kind. Its report on this year's budget application by the AEC (see page 116) shows how carefully the committee picks over the details of the budget, subtracting a few thousand dollars here and there, and sometimes even adding a few thousand. On one view, this is a splendid illustration of the democratic control of public institutions. On another, it is a sign that Congress and the Administration are too closely entangled.

There is no reason to believe that the committee's work has been unreasonably intrusive in the recent past. On the contrary, the chances are that the prodigal needed to make accurate calculations of the cost of running particle accelerators before constructing them, and the campaign for better regulations to protect the health of uranium miners, have helped to make the policies of the AEC more sensible. But what if the question should arise of whether the AEC in its present form should continue to exist? Would the Joint Committee take kindly even to the much more modest proposal that responsibility for high energy physics might be transferred to the National Science Foundation? There is bound to be a suspicion that the Joint Committee, for all its expertise, would resist too radical a change. The trouble is that the time has probably come for a detailed re-examination of the function of the AEC. The best proof the committee could give of its resolution would be to begin an investigation off its own bat.

## PINK SPOTS GALORE

It is now five years since Friedhoff and Winkle first suggested that the urine of schizophrenic patients may be characterized by something which yields a pink spot in a fairly standard chromatographic procedure (*Nature*, 194, 897; 1962) and the interval has been crowded with ups and downs. At the beginning, of course, there was great excitement. The finding of the pink spot chimed in well with an accumulation of evidence to suggest that the metabolism of schizophrenic patients is biochemically distinctive. Five years ago, however, it must have seemed almost too much to hope for that the appearance of a single chemical substance, identifiable by a comparatively simple technique, might serve to distinguish a substantial proportion—ten per cent or so—of those who suffer from schizophrenia. Obviously a simple pink

spot would have great value in diagnosis, although this was almost the least exciting of many possibilities. Naturally enough there was talk of seeking out people who might be biochemically prone to schizophrenia, but in whom the symptoms had not become overt. But there was also every reason to hope that when the chemical responsible for the pink spot had been properly identified, a means would have been provided for understanding something of the biochemical character of schizophrenia, and possibly of its causation as well. No wonder, then, that hats were thrown in the air when, in 1963, it seemed as if the chemical responsible for the pink spot might be  $\beta$ -3,4-dimethoxyphenylethylamine, or DMPE for short, for it is entirely plausible that such a chemical could have arisen by an error of tyrosine metabolism and that its pharmacological effect might be something like schizophrenia.

Those happy days now seem a long way off. Doubts about the identification of the pink spot with DMPE persisted from the beginning. One difficulty is the obvious problem of working with tiny quantities of material. In circumstances like these, chemical identification is necessarily rather indirect. But there were more serious problems bound up with the difficulties of knowing whether subjects examined for pink spot in the urine were schizophrenic or otherwise, for this is not a field in which diagnosis is easy. Predictably it was not long before apparently normal people were found to yield pink spot. Yet another difficulty was that the substance responsible might be produced by the metabolism of some drug used in the treatment of patients. Finally, a year ago it was demonstrated more or less conclusively that whatever pink spot might be, it was certainly not DMPE. The tale has now taken another turn with the report that the substance responsible is probably *p*-tyramine, and that the material which is responsible for the schizophrenic pink spot also occurs in urine from many patients with Parkinson's disease (see this issue, page 132). Boulton, Pollitt and Majer seem to be quite confident that the pink spots they have been able to find in the urine of patients with schizophrenia and Parkinson's disease are caused by *p*-tyramine. With the benefit of hindsight, it now seems likely that the schizophrenic pink spots reported in recent years may often have been caused by *p*-tyramine and not DMPE. Moreover, while the difficulties of diagnosis must still leave doubt about the association of the pink spot with schizophrenia, it is much more probable that it is causally related to Parkinson's disease. Already speculation has begun about the ways in which errors of metabolism in parkinsonism may account not merely for the *p*-tyramine but for the abnormally high concentrations of dopamine in some brain tissues from patients with Parkinson's disease. The suggestion that there may be a biochemical link between some forms of schizophrenia and Parkinson's disease will not come as a complete surprise. This said, it is bound of course to be a long time before the precise significance of the pink spot is clear, but it is something important gained if its chemical identity is now assured.

## NEWS AND VIEWS

### AEC In Plenty

THE programme of the US Atomic Energy Commission for the new fiscal year has received its customary and predictable blessing from the Joint Committee on Atomic Energy. In the committee's report to Congress, based on a series of hearings which began at the end of January and lasted for seven weeks, the committee has suggested some modest additions to the AEC's own version of its operating budget and has pared some \$7 million from the request for capital funds in the year ahead. If the committee has its way, and there is hardly any chance that it will fail to do so, the AEC will have a total of \$2,634 million to spend, \$2,165 million of it on operating expenses.

Both the Joint Committee and the AEC seem cheerful about the development of civil nuclear power in the United States. The committee is proposing that the AEC should have \$484 million to spend on the development of reactors, more than a fifth of it on systems for naval use. The development of power reactors will cost \$119 million in the fiscal year ahead, and the committee pats the AEC on the back for deciding to stop work on a number of reactor types which have come to seem unpromising now that utility companies in the United States are placing orders for reactors almost as quickly as they can. Among the casualties this year are the beryllium oxide reactor experiment and a design involving heavy water as moderator and an organic liquid as coolant.

The Joint Committee has also accepted the view of the AEC that the time has come for a considerable increase of the amount spent on basic physics of various kinds. Plasma physics will get an extra 15 per cent, or a total of \$28 million, in 1968, which reflects the view now current among plasma physicists that the time has come for a deliberate increase of effort in this field. There is also plenty of money for accelerators, and in particular a cool \$50 million for the meson factory which is to be built at Los Alamos.

### Oxford In Revolt

THROUGHOUT the months of protest since Mr Anthony Crosland increased university fees for overseas students, it has been hard to find a university prepared to support its principles with hard cash. In the end, Oxford remained true to its traditions of eccentricity and down-right dilatoriness. Last week, over six months after Mr Crosland announced the increases, Congregation decided that it did not agree. By 43 votes to 27, the university parliament rejected a motion from the Hebdomadal Council to raise fees from £100 to £150. Even the council's recommendation was something of a rebellion, as Mr Crosland wants to see fees at £250 per year.

What will happen next is still not clear. The decision was apparently taken in an atmosphere approaching open revolt—Mr F. T. B. Millar of Queen's College

suggested that the UGC should have resigned, and Professor Max Beloff regretted that he was unable to impeach Mr Crosland. The cost to Oxford will be £80,000 this year and £120,000 in later years. As a gesture towards payment, a fund has been started by Professor Dorothy Hodgkin of Somerville, Mr George Bennet of Lincoln and Mr R. H. C. Davis of Merton. Each has contributed £5 to the fund, and is busy getting signatures for an appeal and further contributions to the fund. However strongly Oxford dons feel about their decision, though, the fund is hardly likely to soften the blow very much unless wealthy foundations or colleges are prepared to weigh in with substantial contributions.

The Department of Education and Science has nothing to say on the subject. So far as it is concerned, the situation was covered by the original announcement, which assumed that university grants for the next five years would take account of fee income at a rate of £250 per year for each overseas student. Universities charging lower fees than this have a perfect right to do so, but have to find the money themselves. Other universities may feel bitter that once again Oxford's wealth has allowed it more freedom than recent foundations can afford. They may also complain that Oxford will now have first refusal of all the most able candidates from overseas. Oxford itself can claim that its elaborate system of government, often defended on the grounds that it is democratic, has reached a decision more daring than most of its critics would have believed possible. Whether the university can afford to put it into action remains to be seen.

### More about Data Processing

WHEN the Post Office (Data Processing) Bill reached its report stage in the House of Commons on July 4, it was clear that the alarms of the Bill's opponents had not been silenced by the committee stage. The objections to the Bill were much the same as they had been when it was first introduced. First, Mr David Price moved an amendment which would force the Postmaster-General to keep a separate account for the Data Processing Service. He feared that the service would be run at a loss, and subsidized by the other postal services, providing the independent computer bureaux with unfair competition. Undertakings that this would not happen were no equal of a protection in law, he thought. Postmaster-Generals come and go, Mr Price said, and not all of them would feel obliged to conform to Mr Short's undertakings.

Mr Short, the Postmaster-General, denied that financial jugglings of the sort the Opposition were suggesting were even possible. Parliament and the press would see to that—he was always open to challenge. Sir Harry Legge-Bourke wondered if this will still be true when the Post Office is reorganized as a public corporation instead of a government department. The clause, however, was withdrawn.

Sir Harry went on to move an amendment which sought to establish that the first duty of the Post Office was to provide data transmission facilities for others—the service, he said, should not be allowed to diminish in the Postmaster-General's mind the need to press on with telecommunications and the ordinary work of the Post Office. Mr Short gave more assurances, and the amendment was withdrawn.

Mr J. Osborn was afraid that the Bill would open the door to commercial espionage, and moved an amendment to widen the clause covering secrecy of information. Mr Short said the intention of the amendment was simple, but it was unworkable. Once again the amendment was withdrawn, and finally the report stage was completed and the Bill was read for the third time.

## A Louder Voice for Technicians

The largest technicians' union in the world is in prospect with the recommendation by the executives of the ASoW and ASSET that the two associations should amalgamate. The ASoW is the Association of Scientific Workers, and ASSET is the Association of Supervisory Staffs, Executives and Technicians; on July 2 the two executives considered the report of a joint committee, and agreed unanimously that amalgamation would be in the best interests of their membership. Before the merging of the associations can go into effect, there must be a full ballot of the members, which will be preceded by a series of meetings at which the arguments can be put across. If the members agree with their executives, the association could be merged by February 1968.

The reasons for the amalgamation are not hard to find. With a joint membership which the executives claim, perhaps optimistically, at 80,000, the joint union will be both more powerful and more effectively organized than the two could hope to be alone. Not the least of its possessions will be the General Secretary of ASSET, Mr Clive Jenkins, who has been a thorn in the side of the British Government throughout the prices and wages freeze. Employers will have to face some tough battles if the merger goes through, but these will be as nothing compared with the situation if ASoW and ASSET take their arguments to a logical conclusion and start negotiations towards a merger with DATA, the Draughtmen's and Allied Technicians' Association. DATA has a membership of over 71,000, and is recognized as one of the most determined unions in Britain—employers would probably prefer the term intransigent. A full scale merger of this sort could ultimately lead to a membership of 250,000.

## Good Cheer for Neuro-biologists

It seems probable that neuro-biology will profit from the work of a sub-committee of the Science Research Council which has been making a survey of needs in this field in the past few months. One way and another, the sub-committee seems to have convinced itself that there are, in particular, shortages of neuro-anatomists and of biochemists wishing to pursue research in neuro-chemistry, and shortages of funds necessary for their support. One probable result is that the Biology Committee of the Science Research Council may commit itself, and the Science Research Council as well, to a deliberate policy of expansion in neuro-biology, sometimes even going as far as to suggest particular research projects. Another approach will be to provide courses by means of which professional scientists can become skilled in these fields. In this spirit, Professor J. Z. Young at University College, London, is hoping to provide courses in neuro-anatomy at University College, London, lasting from three to

twelve months if there is enough demand—and if the SRC will provide support. In much the same way, the SRC seems now to be at least half committed to a programme for the more generous support for animal behaviour—another of the four fields of research singled out for special study by the Biology and Biochemistry Committee.

These developments are potentially interesting as innovations of principle as well as for the practical benefits which they may bring. For one thing, these activities mark the continuing growth of the interest of the SRC in the biological sciences. For another, it may be an important precedent in the administration of British science that a public grant-giving agency should set out deliberately to stimulate the demand for grants in particular fields of study—this is entirely in line with the way that charitable foundations now seek to work, but public bodies have often been much more content to follow a passive policy. The interest of the SRC in neuro-biology will also raise again the question of how the Science Research Council should relate its work to that of the Medical Research Council, which supports research in neuro-biology. That question would be much easier to answer if the MRC were possessed of a more explicit strategy for the spending of money.

## A Committee for Hovercraft

By the apparently immutable rule that every new task implies a new advisory committee, the National Physical Laboratory is to have a hovercraft committee. The committee will tell the NPL what research is needed to support the development of hovercraft, including hovertrains, and how much money will be needed to carry it through.

The first chairman of the committee will be Mr M. A. L. Banks, who has just retired as the Deputy Chairman of the British Petroleum Company, and the members of the committee will be nominated by all the interested parties, including the Ministry of Defence, the NRDC, the Ministry of Transport, and an organization representing the firms licensed to manufacture hovercraft. Already there is promise of further working parties, to look after marine hovercraft, tracked hovercraft, or, indeed, as the announcement puts it, "any other aspect of the subject where there is need".

## Road Research

Quite suddenly, it seems, the Road Research Laboratory has become a vital part of the planning of the Ministry of Transport. The proposals on road pricing which the Minister, Mrs Barbara Castle, is studying will rely heavily on work done by the laboratory. The recent legislation which compels all new cars to have seat belts is also a result of the laboratory's work. The philosophy—an entirely sensible one—seems to be that it is more profitable to minimize injury when accidents do occur than to hope that by constant exhortation accidents can be banished altogether.

The report of the laboratory for 1965 and 1966 provides some examples of this approach. Collisions with conventional lamp standards produce severe damage, often almost cutting cars in half; but, by using a new type of break-away lamp standard, damage can be considerably reduced. Guard rails mounted along

motorways on weak steel posts can steer cars back on the right carriageway even at impact speeds of 70 m.p.h. or over. Again, damage to the vehicle is reduced, and the danger of head-on impacts with approaching cars is minimized.

Much of the work, of course, is not directly concerned with safety. The traffic section includes predictions of the number of vehicles on British roads up to the year 2010 (27 million by 1980, 40 million in 2010), and gives details of the traffic flows in towns and on the M4 motorway. It also describes the problem a driver faces when approaching a green light at high speed; if it changes to amber too late for him to stop, should he brake and risk skidding or accelerate and hope to be clear of the cross traffic? The dilemma is common enough, but does not seem to have been seriously studied before; the report suggests a solution based on a speed detector which will in some circumstances give fast drivers an extension of the green signal.

In addition, the report describes the problems of road building, both in temperate and tropical climates. One of the responsibilities of the laboratory is to provide technical aid for developing countries, where the development of roads is often an essential forerunner of economic development. Last week, the laboratory opened new buildings at Crowthorne in Berkshire and, for the first time since the war, the work of the laboratory is carried out on one site. The new buildings cost £3.5 million, which is also what the laboratory costs to run each year. The figure given in the Civil Estimates for support for traffic research in 1966 is £3.52 million, showing a sharp increase over the 1965 figure of £2.66 million.

## Detecting Turbulence

WHILE the causes of clear air turbulence remain obscure, it is clear that it is often associated with temperature gradients in the atmosphere. Recent work at Oxford and Reading Universities may offer a way of detecting the gradients, and, by inference, the turbulence. If this can be detected far enough ahead of aircraft, evasive action can be taken.

This, clearly, is the argument which has encouraged the Science Research Council to award a grant of £89,000 to Dr J. T. Houghton of the Clarendon Laboratory at Oxford and to Dr S. D. Smith of the Department of Physics at Reading. Together they have developed a radiometer which detects the infra-red radiation from carbon dioxide in the atmosphere, and the instrument is now being produced in its engineered form by Elliott Bros. (London), Ltd., of Frimley, near Aldershot. In its present form the radiometer will be used in an American satellite, called Nimbus D, to be launched by NASA in late 1969, and is intended to provide a world-wide survey of the temperature of the atmosphere up to about 30 miles. At different heights in the atmosphere, the carbon dioxide band occurs at different wavelengths, so that by choosing a certain wavelength a certain height can be studied by the instrument in the orbiting satellite. In the Nimbus experiment, the temperature of the atmosphere will be studied from cloud top height to about 30 miles, to an accuracy of about  $\pm 1^\circ\text{C}$ .

It is also hoped to develop the instrument for the detection of clear air turbulence. In this role, it would

be mounted in the front of the aircraft, scanning the region of the atmosphere ahead. By detecting temperature gradients, it might be possible to avoid regions of clear air turbulence. Dr Houghton believes that the range of the instrument could be 50 miles, not as great as the operators of supersonic transports might wish—they hope for a range of 100 miles—but better than nothing. The SRC certainly believes the instrument has a commercial future, and has asked the NRDC to consider further development of the design with an industrial firm.

## Rheumatism, Tractable and Otherwise

THE MRC Rheumatism Research Unit housed in the Canadian Red Cross Memorial Hospital at Taplow celebrated its twentieth anniversary last week. Although it had no dramatic announcements to make, it is proud of a steady advance in the treatment of the more tractable forms of rheumatism. The term itself includes any disease of the joints which causes pain and which is aggravated by movement. Professor Bywaters, honorary director of the unit, says that there were 83 different rheumatic conditions at the last count, ranging from sprains to degenerative conditions due to ageing, metabolic disorders such as gout, infective conditions such as rheumatic fever and arthritic diseases the cause of which is unknown.

The unit is chiefly concerned with the arthritic group of disorders, and the most promising line of research centres around the possibility that rheumatoid arthritis involves an immune reaction of the body to its own tissues. Certainly auto-immune reactions like these have been demonstrated in mice and can cause a syndrome similar in appearance to rheumatoid arthritis. But this is only one possibility. Some workers even claim to have found characteristic organisms in the synovia of patients with this disease, suggesting an infective mechanism, but this is in dispute. But there are certainly infective mechanisms in rheumatic fever, one of the more unpleasant possibilities following a sore throat. Over the years the treatment of rheumatoid arthritis has improved considerably, especially with children. Until recently, children with the chronic disease were confined to bed, which often made their condition worse. Nowadays treatment is designed to minimize deformity so that children can lead full lives when they recover—as in time they do. Splinting and physiotherapy play a part, as does the use of drugs to reduce pain and inflammation. Salicylates and corticosteroids are frequently used, but neither is free from side effects; corticosteroids in massive doses, for example, can control pain and inflammation but may cause a child to fail to grow in height. The problem is to weigh the advantages of treatment against the disadvantages of side effects.

## Fossil Fishes

Dr Alan J. Charig writes:

THE Linnean Society of London is marking the retirement of Dr Errol I. White as Keeper of Palaeontology in the British Museum (Natural History) and President of the Society with a special volume of articles mainly on fossil fishes, and five of the twenty papers therein were read at a one-day symposium on June 23. One striking feature of the paper by Professor E. Jarvik of



Stockholm on the lower jaws of dipnoans was that there appears to have been very little change in the dermal bone pattern since the Devonian, at least as far as can be told from a comparison of the fossil genera *Dipterus*, *Scaumenacia* and *Melanognathus* with the recent lungfishes. The dermal bone pattern of all dipnoans is quite distinct from that found in other vertebrates. Jarvik also emphasized the similarities between the Dipnoi and the Holocephali, autostylic jaw suspension and tooth-plates used for chewing and crushing, for example, although he did not claim that these necessarily implied a close phylogenetic relationship.

Dr Bobb Schaeffer of New York then attempted a new interpretation of osteichthyan vertebrae. He had examined both fossil and Recent material, bearing in mind that Gadow's classical "arcualia" theory (that, in all vertebrates, each vertebra originates from four pairs of cartilaginous blocks) had recently been demolished by E. E. Williams as far as the tetrapods were concerned. This, however, had raised more new questions than it had produced answers. In no living holostean or teleost is there any evidence of arcualia; neither is there any division of the sclerotome by the sclerocoel with subsequent resegmentation, as observed by Williams in the tetrapods. Experimental work is urgently needed.

Dr A. Ritchie of Sheffield described in great detail the rare and primitive ostracoderm *Ateleaspis tessellata*, a non-cornuate cephalaspid from the Lower Ludlovian (?) of the Midland Valley of Scotland, and concluded that this resembled the Norwegian *Aceraspis robustus* so closely—differing mainly in the outline and other details of the oral opening—that both species should be placed in the one genus *Ateleaspis*. One member of the audience, however, asserted that the Norwegian form was more advanced. In another fossil from the Middle Devonian of northern Scotland, the pytodont fish *Rhamphodopsis* Watson, Dr R. Miles of Edinburgh was able to demonstrate sexual dimorphism in the pelvic fins—the first time that this has been shown in any placoderm.

Evidence that will bring comfort to the proponents of continental drift has been gathered by Dr D. L. Dineley of Ottawa from the Knoydart Formation of Nova Scotia. The very same species occur in Nova Scotia and the Welsh Borderland: *Traquairaspis symondsii* in the Downtonian and *Peraspis crouchi* in the Middle Dittonian. Between these two in Nova Scotia occurs *Pteraspis whitei*, upon which a true biostratigraphical zone may be recognized; presumably this corresponds to the Lower Dittonian in Britain, containing *P. leathensis*. It is hoped that the establishment of palynological standards for Old Red Sandstone stratigraphy will soon make it possible to measure the dispersal rates of these ostracoderms.

## Active Sites of tRNA

from our Correspondent in Molecular Biology

THE recent determination of the base sequences of several specific transfer RNA molecules, and the discovery of rather extensive common features between them, has provoked strenuous efforts to localize the functional centres. These have for the most part not been overwhelmingly successful, but a number of interesting results have nevertheless emerged.

All the sequences so far determined are consistent with the "cloverleaf" model, based on maximum reasonable base pairing. There are in all cases three large loops, each of about seven unpaired bases. One of the loops apparently contains the anticodon, and it has been generally supposed that all the active sites reside essentially in them. It appears, however, that the common terminal sequence CCA is required for enzyme recognition, and evidence has accrued (most recently in an article by Stulberg and Isham (*Proc. US Nat. Acad. Sci.*, 57, 1310; 1967)) which bears out earlier work that the removal of this tail leads to overall changes of conformation.

A study by Adman and Doty (*Biochem. Biophys. Res. Commun.*, 27, 579; 1967) shows that the alanine acceptor activity of tRNA in the unfractionated state is destroyed on reduction of uracil residues. From the statistics of the process, assuming a random hit mechanism, it seems that no fewer than half the uracil residues must be reduced before the molecule is inactivated. Since the random hit assumption is very reasonable, it seems most likely that the enzyme recognition site in alanine tRNA contains no uracil, and evidence from optical rotatory dispersion indicates that the inactivation results from the breakdown of secondary structure.

A related approach by Lindahl (*J. Biol. Chem.*, 242, 579; 1967) involves irreversible thermal inactivation of tRNA, in respect of three separate functions: amino-acid acceptor activity, ability to incorporate adenylic acid as the chain terminus (a reaction catalysed by an enzyme for which all tRNA species are substrates) and specific attachment of the tRNA to ribosomes. At 90°C, the loss of activity is greatly accelerated by the presence of magnesium ions. Magnesium is known to catalyse the thermal hydrolysis of phosphodiester bonds and in its presence thus appears to be the chief cause of inactivation. Without magnesium, on the other hand, although some chain scission certainly occurs, the number of breaks is not sufficient to account for the loss of activity. Depurination and deamination are similarly ruled out as determining factors. Lindahl therefore concludes that particular heat labile residues must be involved, and the only such known to be present in tRNA are the dihydrouracils (DHU). If this interpretation is correct, then it appears that the DHU must be preserved intact for all three activities. These groups may therefore be thought to function as hinge points for the conformation. Lindahl also notes that valine-specific tRNA, which is the only species known to contain four DHU residues, is very rapidly inactivated in respect of acceptor activity, though less so for adenylic acid incorporation.

It may also be mentioned that a structural change evidently also occurs when tRNA binds its specific amino-acid. The most recent evidence to this effect is the observation (Kaji and Tanaka, *Biochim. Biophys. Acta*, 138, 642; 1967) that the aminoacyl-tRNA sediments more slowly, and therefore probably has a less compact structure, than unloaded tRNA. This recalls the earlier report by Lamborg and Zamecnik, based on optical rotatory dispersion, that some helical structure appears to be lost on amino-acylation. It is curious that polypeptidyl-tRNA is found to sediment more slowly in turn than aminoacyl-tRNA (Kuriki and Kaji, *J. Mol. Biol.*, 25, 407; 1967). When the polypeptide chain is removed from such a species, the resulting tRNA will not accept its amino-acid, and is perhaps in

the "inactive" conformation, described by Fresco and others.

## How Red Cells Lose Nuclei

from our Correspondent in Cell Biology

At long last some convincing electron micrographs have been obtained of nuclear expulsion during the final stages of erythrocyte maturation in mammals (Skutelsky and Danon, *J. Cell Biol.*, **33**, 625; 1967). Mature erythrocytes in circulating mammalian blood have no nuclei. The developing immature erythroblasts are nucleate cells. Two rival theories were proposed to explain the loss of the nucleus at late stages of maturation. One, primarily based on observations of primitive erythrocytic cell lines and some pathological conditions, was that the nucleus breaks down *in situ*, by karyolysis. The other, supported by Bessis and Bricka's microcinematography (1952), was that the nucleus is normally expelled from the cell. The supporters of the karyolytic theory objected that free nuclei are rarely seen in normal erythropoietic tissues.

Several electron microscopic studies failed to yield conclusive evidence for expulsion. Authors have often made vague statements that evidence for nuclear expulsion and/or karyolysis has been found, but have failed to include the vital micrographs in their articles. Part of the difficulty apparently arises from lack of suitable tissue to study. In normal erythropoietic tissue the erythroblasts are scattered among other cell types, which means that the chance of identifying extruded nuclei in thin sections is remote. Moreover, as the work of Bessis and Bricka shows, expulsion is rapid so that it is difficult to catch a nucleus halfway out.

Skutelsky and Danon have overcome these problems by inducing pure clones of erythropoietic cells in mice spleen by injecting syngeneic bone marrow into X-ray irradiated mice. In such clones cells at all the stages of maturation occur close together, so that the final stages involving loss of nuclei can readily be examined. As the micrographs show, nuclear expulsion does occur and the process closely resembles cell cleavage following mitosis. Skutelsky and Danon have found no evidence at all of karyolysis in the 20 clones examined.

Nearly mature erythroblasts develop cytoplasmic processes resembling pseudopods and the nucleus moves into one of these, often being deformed as it passes through the narrow neck. Within a process, the nucleus rounds up again and is surrounded by a very narrow rim of cytoplasm. The plasma membrane begins to constrict about the neck of the process containing the nucleus, leaving it attached to the main body of the cell by a very narrow bridge of cytoplasm. Mitochondria aggregate in the main cell body in the region of the constriction, and presumably provide energy for any contractile process involved in constriction and for the synthesis of new plasma membrane. Vacuoles then appear in the bridge region and effect the complete separation of the nucleus from the young reticulocyte. The nuclei released are soon engulfed by macrophage, so that free nuclei are rarely seen in erythropoietic tissues.

Three questions are raised by these observations. First, what is it that triggers the whole process and brings about nuclear expulsion rather than a cell division? Secondly, what are the properties of the plasma membrane surrounding the expelled nucleus that make it readily recognizable by the macrophage?

Thirdly, can Skutelsky and Danon's observations be generalized to definitive cell line erythroblasts in all mammalian erythropoietic tissue? The answer to the last question is probably affirmative, for it is most unlikely that the method used to induce pure clones of erythroblasts would completely alter the way in which the cells lose their nuclei, and there was no indication of karyolysis.

## Cancer Research

from a Correspondent

THE forty-fourth annual report of the British Empire Cancer Campaign, published this week, includes as in previous years reports of work in a wide variety of disciplines and, even though in 1966 the Campaign collected the record sum of £2 million, the danger that the money will be spread too thinly over a broad front of attack is unlikely to recede.

Some of the more basic projects described in the report for 1966 are concerned with the problems of growth regulation and control. According to P. N. Campbell and E. Lowe (Courtauld Institute), the higher rate of synthesis of protein in regenerating liver is not related to the availability of messenger RNA nor to any factor associated with the polyosomes, but rather to the presence of an inhibitor in the membrane of the endoplasmic reticulum. Professor J. A. V. Butler and his colleagues at the Institute of Cancer Research, on the other hand, are concentrating on the physical and chemical properties of histones because these appear to be involved in the switching on and off of genetic information. G. V. Sherbert, at the same institute, reports that the histone fraction from calf thymus produces apparently specific malformations in early chick embryos. From a study of the control of the renewal of cells in mouse lung, Simnett and Heppleston (Newcastle upon Tyne) conclude that cell division in alveolar tissue is probably regulated by a mitotic inhibitor which persists in tissue culture preparations of lung. The higher mitotic index seen in cultures of newborn lung tissue is associated with the absence of this inhibitor, though systemic stimulatory factors, possibly hormones, may also be involved. Several previously reported experiments have suggested that thymectomy enhances response to chemical carcinogens. Against this background, it is surprising to find the report by A. Flaks (Leeds) that the injection of neonatal thymus tissue into mice increased their response to a carcinogen injected during the neonatal period. But in its time the thymus, like man himself, plays many parts, and it is at present difficult to see the pattern of their interrelationships. According to Koller and his colleagues (Institute of Cancer Research) although thymic cells may restore immune competence after thymectomy, they are not themselves capable of elaborating antibody; but attempts to restore immune competence in thymectomized mice by extracts of calf thymus have so far yielded negative results. A. Glucksmann and C. P. Cherry (Strangeways Laboratories, Cambridge) have made a study of the somewhat neglected epithelial cell components of the thymus. Oestrogens promote the proliferation and secretory activity of these cells and testosterone elicit the opposite response. It is suggested that these effects may be related to the differences in response to chemical carcinogens of the salivary glands and of the genital

tract in castrated female rats treated with the two types of hormone.

According to J. T. Lett and P. Alexander (Institute of Cancer Research), the molecular weight of DNA in mammalian cells may be greater than  $10^{10}$  and possibly be equal to that of a whole chromosome which is of the order of  $10^{11}$ . According to D. Petrovic and A. H. W. Nias (Christie Hospital and Holt Radium Institute), DNA damage induced in HeLa cells by radiation may be repaired more easily in the presence of suitable quantities of deoxyribonucleosides. In *Micrococcus radiodurans* single-strand DNA breaks seem to be repaired in more than one stage, because studies of molecular weights indicate restitution of more than half the breaks within 5 min while the remainder mend more slowly during the next phase of DNA synthesis.

A claim to have transformed rat embryo lung cells *in vitro* by exposing them to urethane (S. Rasheed, Mount Vernon Hospital) will certainly stimulate further investigations because its confirmation would suggest that the agent acts directly as a carcinogen and not by way of metabolite. Organ culture studies which I. Lasnitzki has carried out at the Strangeways Laboratories, Cambridge, have indicated that the human foetal lung is more sensitive to the effects of tobacco smoke condensate than the hamster foetal lung.

The influence of age on susceptibility to carcinogens is a matter of perennial interest. Lasnitzki has found that, under conditions of organ culture, explants from old and young rat prostate glands respond differently to different sex hormones. It is therefore possible that intrinsic changes in the prostate and changes in hormonal status with age are both of importance in the development of cancer in this organ.

The results of a study at St. Mark's Hospital undertaken by Morson *et al.* suggest that one in thirty patients who survive cancer of the large bowel develop a second similar cancer. This higher susceptibility to a second tumour may not apply, however, to all forms of the disease. Certainly the presence of adenomata at the time of removal of the first cancer implies an increased risk. One of the problems peculiar to the less common types of cancer is that few people see enough cases to learn by them. A special study of carcinomata of the nasopharynx (I. W. F. Hanham, Westminster Hospital) has revealed that the commonest presenting physical sign is enlargement of isolated lymphatic glands in the neck. W. T. Smith (Birmingham) has observed a range of associations between cerebral, cerebellar, brain stem, spinal and peripheral nerve lesions, and idiopathic steatorrhoea. He suggests that the syndrome may have aetiological factors in common with carcinomatous neuropathy. It is hoped that by co-operation between thoracic surgeons at the London teaching hospitals light will be thrown on the relation between thymic tumours and certain neurological and immunological syndromes associated with cancer.

Other reports are concerned with environmental chemical carcinogens, the mechanism of chemical carcinogenesis, cancer viruses, cancer epidemiology and prevention, new methods for the early diagnosis and the exact localization of tumours, and with all forms of therapy. All in all, 1966 was a year of numerous tactical gains without any obvious advance of strategic importance.

## Parliament in Britain

### Reactors

DR J. BRAY, Joint Parliamentary Secretary at the Ministry of Technology, said that no current study was being made of the use of nuclear reactors to provide process steam for industrial and district heating purposes, but the UKAEA was keeping the matter under review in consultation with the Ministry of Public Building and Works. Before seeking authorization for constructing the sodium cooled prototype fast reactor, the AEA had assessed the relative merits of fast reactors using other coolants including gas and steam. It concluded that use of sodium coolers offered the best prospect for early commercial exploitation for fast reactors, but evaluation of the possibility of other coolants would continue in the light of developments.

### Breath Testing

LORD STONHAM, joint parliamentary under-secretary of state at the Home Office, defended breath testing devices in the House of Lords. They were not, he said, precise and accurate instruments, and were only to be used for preliminary tests. In tests, eight false positive results had been obtained, all at alcohol contents of less than 80 mg/ml. Despite this, Lord Stonham did not believe that the instruments were significantly less accurate in the lower ranges. (Answer, June 29.)

### Select Committee

AT its final meeting on the study of the British nuclear power programme, the Select Committee on Science and Technology interviewed Mr Duncan Burn, whose criticisms of British developments were published recently by the Institute of Economic Affairs. (*The Political Economy of Nuclear Energy*, 21s. See *Nature*, **214**, 547; 1967). Mr Burn reiterated most of the arguments from his book, and made some suggestions for the improvement of the nuclear industry in Britain.

The CEBG, he suggested, should be re-organized into three separate units, in order to introduce a competitive element into tendering, an idea which prompted some critical questioning from the members of the committee. The UKAEA, Mr Burn claimed, had spent more on construction than on research and development, the exact reverse of the situation in the United States. Britain should try to come nearer to the American pattern, he suggested, and one way in which this could be done would be by converting the AEA to a body more like the AEC. Some parts of the AEA should be transferred to the cons<sup>ortia</sup>, which should themselves be encouraged to seek international links.

Despite his unenthusiastic assessment of the AGR, Mr Burn said that it should continue to be developed, possibly on a wider front than hitherto. He had some more hopeful things to say about another type of reactor, the thorium high temperature reactor (*Nature*, **214**, 1177; 1967). Judging by the committee's reaction, few if any of Mr Burn's recommendations can be expected to find their way into the report when it finally emerges. Whatever the merits of his case, it would undoubtedly have been put across rather better if he had protested rather less. The committee, it is thought, may issue an interim report before its final report is published later in the year.



# Projects for Schools

by

NIGEL HAWKES

Science teachers in British schools are increasingly turning to project work as a substitute for, or as an addition to, conventional practical work. Whatever the educational benefits may be, reports of projects make for good school magazines.

IN a great many British schools it has now become fashionable for experimental work to take the form of project work. Instead of—or as well as—the routine verification in the school laboratory of the supposed laws of science, schoolboys (and schoolgirls) are increasingly being allowed and even encouraged to substitute project work in which the object is to solve quite difficult practical problems. One argument in favour of this is that project work is educationally preferable, partly because it is supposed to bring “creativity” into scientific work and partly because projects present young people with more challenging problems. The fashionable work is “open-ended”.

A number of schools have recently won reputations for themselves by the high standard of their projects, and the movement away from conventional practical work has been lent extra force by a programme designed by the Schools Council for encouraging the use of projects in secondary schools. At least one of the public examinations boards gives credit for project work in some science subjects. But the traditions of school projects go further back than that, and have derived much from the field work of the biologists and geologists, for example. Evidence of how far this type of teaching has spread through the schools is supplied by the project magazines which some schools produce. A number of these have been collected in *Nature* office.

The first impression is that the project magazines tend to be excessively biological, possibly because many of the magazines derive from the school natural history society. Oundle School Natural History Society, for example, produced a report no less than 107 pages long in 1965, which included routine reports of the societies as well as A-level project work. Project work included physiological studies of the melanic forms of the peppered moth, which can be found in the Oundle area. 180 larvae were amassed, and experiments showed some intriguing results—the larvae divide into two types, one green and the other brown. By change of weight measurements, the boys determined that the two types are physiologically as well as morphologically different. The larvae were preserved by members of the school during the summer holidays, and the following year the first adults emerged. Interestingly, the first 17 to emerge were females, an occurrence with a probability of less than 0.01. The boys concluded that there is a distinction between the times of emergence of the sexes of the species under investigation.

Although the Repton school magazine *Biogenesis* is produced by the school Field Club, it does include some physical science. One boy has extracted the tar from cigarettes using a simple smoking machine which drew smoke through a series of condensers. According to his results, French cigarettes produce significantly more tar than English cigarettes. Even when filtered, French cigarettes were worse, but wisely no conclusions were drawn from this. Another boy studied the oxygen consumption of mice kept at different temperatures. Mice were put in a sealed tube to which oxygen was fed under the control of a meter which could also be used for measuring the oxygen consumption in arbitrary units. The air breathed out by the mice was re-circulated, after the carbon dioxide

had been absorbed in soda-lime. Oxygen consumption reached a minimum at 34° C and a maximum at 25° C. Below 25° C, oxygen consumption unexpectedly fell, and future work was intended to show the reason for this. The report hints, though, that experimental technique could have had something to do with it: “It may be that the huddling together of the five mice used for the experiment diminished the need for shivering.”

Eton College produces a glossy magazine called *Atom* which includes general articles as well as some excellent project work. The 1964 issue contains a description of the Eton by-pass, then being built, and this work was followed up in 1965 by a traffic survey in Eton with a Sykes counter, which consists of a rubber tube stretched across the road. Results are plotted as daily flow patterns which show peaks at 8 a.m. and 6 p.m. on weekdays. The weekly graph shows increasing peaks as the week progresses, so that Friday evening, with a flow of 2,000 vehicles per hour, is the very worst time of all to drive through Eton. These results will be particularly interesting if they are followed up by a comparison of the traffic flows before and after the opening of the by-pass.

The 1965 issue also gives details of the building of a land yacht, in order to prove that a wind-driven vehicle can travel, unaided, faster than the wind. Results, the report records, ranged from promising to spectacular, but the aim of the experiment was achieved, and speeds of up to 20 m.p.h. were reached. The best experiment of all, though, is a study of flow patterns around aerofoil sections. The apparatus consists of a trolley arranged to run in a straight line on rails above a water filled tank. The shapes to be studied are suspended from the trolley so that they dip into the water. Mounted on the trolley is a camera fitted with a shutter in the form of a rotating Maltese cross which intermittently interrupts the light falling on the camera lens. The experiment is carried out in the dark. Several small silver paper balls are carefully placed on the surface of the water in a straight line at right angles to the direction of movement of the trolley. The motor is started, the balls illuminated, and the trolley pushed at a steady speed along the rails so

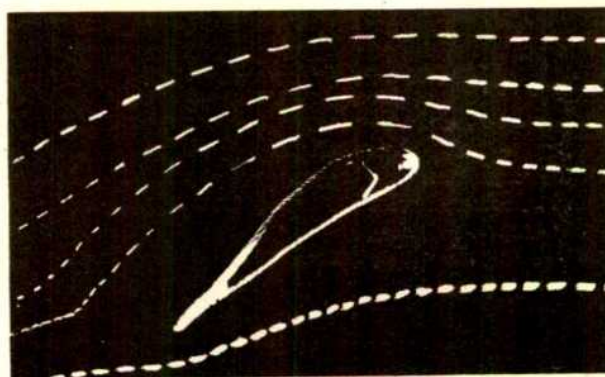


Fig. 1. Smooth flow around an aerofoil section, photographed by H. E. Aubrey-Fletcher at Eton College. The velocity of the flow, given by the lengths of the dashes, is greater above the wing than below it.



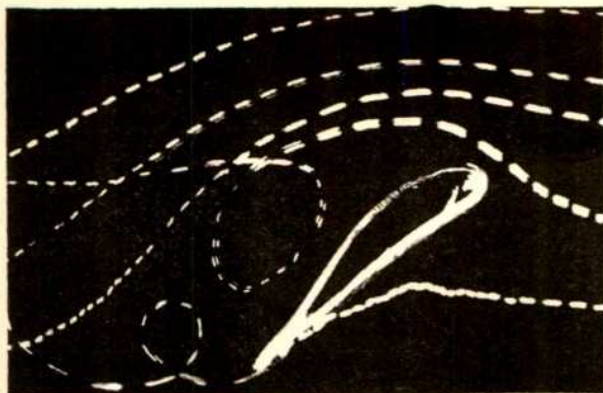


Fig. 2. Turbulent flow around the wing at stalling incidence.

that the aerofoil section passes through the line of balls. The action of the shutter makes each ball appear as a dotted line following the lines of flow around the aerofoil section. Some remarkably good pictures were produced, one in particular showing the disturbed flow occurring at stalling incidence (Figs. 1 and 2).

Not all schools, perhaps, would wish to spend as much on their project magazines as Eton (although the cost does seem to be offset by some advertisements). The ideal lay-out is achieved by *Enquiry*, the magazine of the Harrow County School for Boys. This magazine, printed quarto size by a less expensive printing process, carries several pages of advertisements and a really excellent series of project reports. Three boys studied the common

mole, which is difficult, because moles are rarely seen; the technique was to trap moles alive, ring them, and release them some distance from the trap. The frequency with which the ringed moles were once again trapped showed a strong homing instinct but little capacity for learning. One was caught no less than five times. Dissection of several moles which died—evidently of fright—confirmed that moles feed on earthworms, insect larvae and imagos and a small amount of plant debris.

Three chemists at the same school carried out a survey of the solubility of lead chloride in dilute hydrochloric acid, and the physics section includes a design for a private automatic telephone exchange, an explanation of how a transistorized electronic organ works, and a description of an amateur radio station built by one of the sixth formers. In addition, *Enquiry* includes several village surveys, which seek to relate village size, shape and structure to its geography, and to determine the reasons for its development. These surveys seem to reflect the increasing interest in social science among sixth formers; if the magazine can be criticized, it is for including rather few directly experimental projects.

The Department of Education and Science itself produces a magazine called *Project*, which appears about three times a year. This is intended to stimulate interest in the schools in engineering and the applied sciences, and the latest issue has articles about North Sea gas, automated factories, computer design, medical engineering, tidal power in France, and rotary piston engines, among others. For interest, lay-out and readability, *Project* puts many more senior British technical journals to shame. Ironically, however, it contains no projects by schoolchildren.

## The Flow Behaviour of Blood in the Circulation

by  
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University of Nottingham\*

The effective viscosity of blood in the circulation can vary from infinity down to about 1.3 centipoise. However, the minimum value is not reached in the largest or smallest blood vessels.

As our knowledge of the cardio-vascular system increases it becomes increasingly obvious that the flow properties of blood are sometimes significantly different from those of a simple fluid, such as water or oil, which possess a constant viscosity at all rates of flow and in vessels of all diameters. The problems are to identify the parts of the circulation where divergence from simple fluid behaviour is likely to be appreciable, the reasons for it, the magnitude of any anomaly and the physiological conditions under which it is likely to be most marked. Some of these questions are discussed and analysed here.

### Rheology of Blood

Many measurements of the flow properties of blood in bulk have been made in the past<sup>1</sup>, but it is only in the past few years that it has been possible to codify them with much precision<sup>2,3</sup>. The various parameters are often reduced to a single one—the apparent viscosity—which may be defined as the tangential force which must be applied to unit area of a plane surface situated unit distance from a similar surface immersed in the liquid under test in order to produce a velocity difference of unity (Fig. 1). For a simple liquid it has a constant value; if the force is doubled, the rate of movement is doubled, but in the case of blood a doubling of the force usually more than doubles the resulting velocity. A statistical analysis of viscosity measurements on human

blood (as reported by eight different workers<sup>4-11</sup>) produces a mean apparent viscosity curve and 95 per cent probability limits of the type shown in Fig. 2. Here the shear rate (which is equal to the velocity difference between the surfaces in Fig. 1) is plotted against the apparent viscosity and it will be observed that the velocity effect becomes important at rates of shear which are less than about 50 sec<sup>-1</sup>. Reported results show<sup>1</sup> that blood also exhibits a small yield stress, which represents the tangential force which must be applied to the plane surface of unit area immersed in the liquid in order to initiate flow. This behaviour identifies blood as a Bingham plastic from the rheological point of view; it differs from that of a simple liquid where a tangential force, however small, will always produce continuous movement. The yield stress probably stems from the small attractive force which normally exists between the red cells<sup>1</sup>, and the inherent flexibility of the cells additionally contributes to the inconstant viscosity characteristics shown in Fig. 2 (ref. 12). Illness, disease or shock can seriously alter the rheology of blood, but such abnormal conditions will not be considered here, where attention will be focused on the flow of blood in vessels under normal physiological conditions.

**Large vessels.** When a fluid moves down a tube the rate at which it is sheared depends on its radial position. There is a velocity profile across the tube with a maximum value on the axis and a minimum (usually assumed to

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be zero) at the wall; for a simple fluid flowing smoothly down a tube the profile is parabolic and the resistance to flow is proportional to the mean velocity. The rate of shear is a maximum at the wall and a minimum on the tube axis. In the case of blood in which the apparent viscosity depends on the rate of shear, we must calculate the total resistance to flow by summing the shearing forces across the whole cross-section of the tube, and must expect the result to depend in a fairly complicated way on the mean velocity of flow because this will control the rate of shear at any point.

The rate of shear of blood at the wall of various blood vessels in dogs and humans has been estimated on a few occasions and the data are summarized in Table 1. We can also calculate a mean value, assuming that the velocity profile is parabolic (Table 1) and in both cases the rates of shear are so large that the apparent viscosity of the blood is very near to its minimum, asymptotic value (Fig. 2). We could, in these cases, treat blood as a simple liquid of constant viscosity and introduce very little error by choosing a value which lay within the range where 95 per cent of the measured values were found to be. These limits are 1.7–4.0 centipoise (Fig. 2). If the flow were pulsatile, as in the arterial system, the rise in apparent viscosity at instants when the velocity was small would raise the flow resistance above the anticipated value but, on the whole, the assumption that the blood behaved as a simple liquid with a viscosity of about 3.0 centipoise (that is, about three times that of water) would give a good approximation to the truth.

Table 1. RATES OF SHEAR IN THE CIRCULATION<sup>1,12-18</sup>

Structure	Man		Dog	
	At the wall sec <sup>-1</sup>	Mean* sec <sup>-1</sup>	At the wall sec <sup>-1</sup>	Mean* sec <sup>-1</sup>
Ascending aorta	190†	130		
Descending aorta	120	80	400	270
Large arteries	700	470	600	400
Capillaries	800	530	700	450
Large veins	200	130	50	35
Venae cavae	60	40	200	140

\* Assuming parabolic velocity profile.

† At peak flow velocity.

The quantity flowing down the vessel in unit time, assuming it to be cylindrical, is then given by the well-known Poiseuille equation

$$Q = \frac{P\pi R^4}{8\eta l} \quad (1)$$

where  $P$  is the pressure gradient down the tube,  $R$  is the radius of the tube,  $l$  is the length and  $\eta$  is the viscosity of the blood, which equals 0.028 poise.

For greater precision at low flow velocities an equation proposed and tested by Merrill *et al.*<sup>19</sup> should be used

$$Q = \frac{\pi R^3}{4K_c^2} \left[ \frac{(PR)^{\frac{1}{2}}}{(2l)} - \tau_y^{\frac{1}{2}} \right]^2 \quad (2)$$

where  $\tau_y$  is the yield stress of the blood (assume 0.05 dyne/cm<sup>2</sup>), and  $K_c$  is the flow parameter of blood (assume

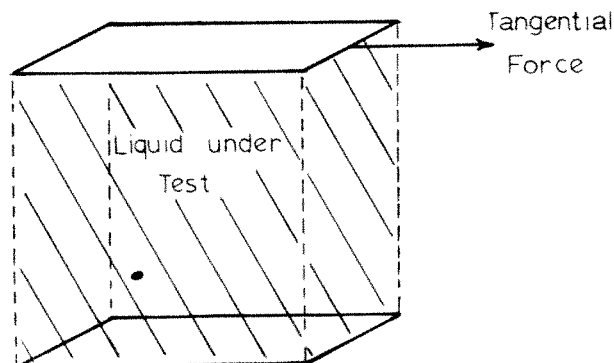


Fig. 1. Tangential force on a unit cube of liquid.

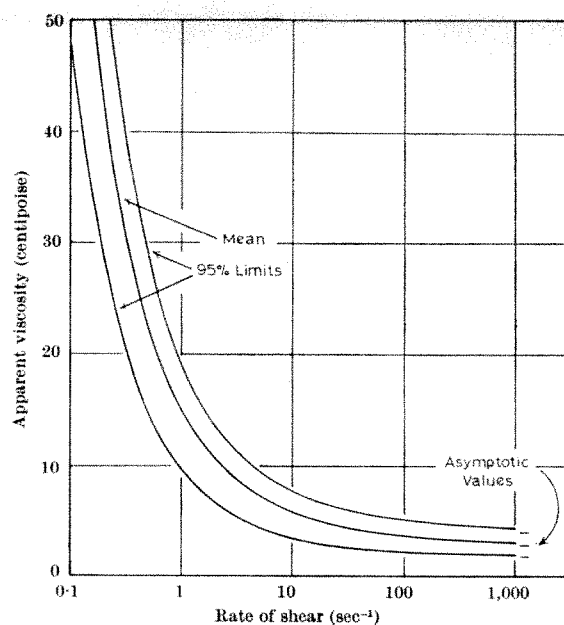


Fig. 2. Variation of apparent viscosity of blood with rate of shear.

0.168 dyne<sup>1/2</sup>sec<sup>1/2</sup>/cm). At high flow velocities equations (1) and (2) give the same result and the importance of the complex flow properties of blood at low flow rates can best be shown by an example. If it were assumed to be a simple liquid with a viscosity of 2.8 centipoise, the mean velocity of flow in a tube 0.2 mm in diameter under a pressure gradient of 0.25 cm of water/cm length of tube would be approximately 1 mm/sec (from equation (1)) and the mean rate of shear across the tube about 30 sec<sup>-1</sup>. If, however, a yield stress of 0.05 dyne/cm<sup>2</sup> and a flow parameter of 0.168 dyne<sup>1/2</sup>sec<sup>1/2</sup>/cm were assumed, the quantity flowing down the tube with the same pressure gradient would be reduced by about a third (from equation (2)).

**Intermediate-sized vessels.** When the diameter of the vessel down which the blood is moving becomes comparable with the diameter of the red cells, it is no longer realistic to represent the blood as a homogeneous fluid. In the case of human blood this condition becomes really noticeable when the vessels are less than 100 or 150  $\mu$  in diameter. The wall of the vessel represents a discontinuity so far as the suspended red cells are concerned because they are unable to penetrate it, and the positions which they can take up in its vicinity are thus restricted. This leads to a change in the nature of the physical interactions between the cells adjacent to the wall. Moreover, the cells which would have flowed down the vessel in positions from which they are now excluded by the wall travel nearer the axis and thus at a higher velocity than anticipated. This leads to a fall in the mean haematocrit in the vessel and a reduction in the resistance to flow<sup>20</sup>. The latter is usually interpreted as a smaller effective viscosity\*. It may at first seem strange that the haematocrit in a narrow vessel can be less than in the larger diameter feeding or collecting vessels, but if it is imagined that some of the red cells, instead of being radially displaced, travel along the narrow vessel on a conveyor belt which moves slightly faster than the plasma with which they entered, then the number present in the vessel at any instant will be less than if the conveyor were absent. The higher the proportion of red cells travelling on the conveyor the lower the mean haematocrit.

As the vessel becomes smaller in diameter the proportion of displaced red cells increases, and the haematocrit (and thus the effective viscosity) falls. This effect was

\* The effective viscosity is defined here as the viscosity of a simple liquid which would give the same volume flow rate as the blood in a vessel of the same dimensions with the same pressure gradient.

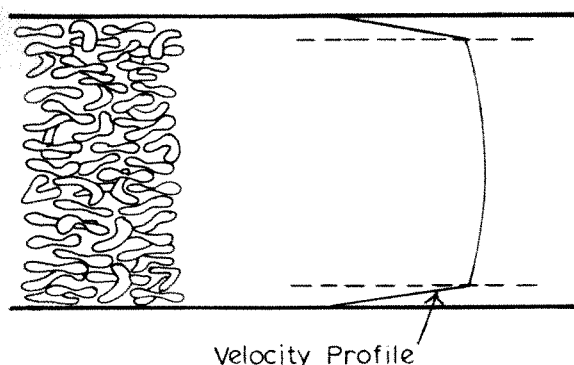


Fig. 3. Shearing core flow.

first studied systematically by Fahraeus and Lindquist<sup>21</sup> after whom it is named. Although there are considerable discrepancies in reported data the results of many experiments made in glass tubes of different sizes indicate that the actual haematocrit in the tube can be estimated fairly well by assuming that the cells behave as though they were concentrated in a core which reaches within about  $4\mu$  of the wall (in the case of human blood), the surrounding annulus consisting solely of plasma (Fig. 3). It should not be assumed that there is, in fact, a continuous annulus of plasma at the wall, and visual evidence that one exists at normal haematocrits is by no means conclusive<sup>4,5</sup>, but the model is convenient for calculation purposes.

The blood in the core is assumed to shear in a similar manner to a simple fluid so that the flow is categorized as "shearing core"<sup>1</sup>, the cells in general tending to orient themselves with their faces parallel to planes passing through the tube axis<sup>22</sup>. This orientation has a profound influence on the light transmission and reflexion characteristics of the blood<sup>1</sup>, and leads to the well known bright streak often seen down the axes of vessels conveying blood<sup>23</sup> and the flashing effect when the direction of flow is reversed. If it is assumed that the plasma behaves as a simple liquid, then the mean haematocrit in the tube is given by<sup>24</sup>

$$\bar{H} = H_f \left\{ \left( \frac{R-4}{R} \right)^2 + \frac{\left[ 1 - \left( \frac{R-4}{R} \right)^2 \right]^2}{2 \left[ 1 - \left( \frac{R-4}{R} \right)^2 \right] + \left( \frac{R-4}{R} \right)^2 \frac{1}{\eta_{rc}}} \right\} \quad (3)$$

where  $H_f$  is the haematocrit in the feed and  $\eta_{rc}$  is the viscosity of the blood in the core relative to that of the plasma.  $\eta_{rc}$  is found by first determining the core haematocrit,  $H_c$ , from

$$H_c = \bar{H} \left( \frac{R}{R-4} \right)^2 \approx H_f \left( \frac{R}{R-4} \right)^2 \quad (4)$$

The relative viscosity of blood at the haematocrit ruling in the core,  $\eta_{rc}$ , can then be obtained experimentally. Alternatively, existing results can be used to obtain a suitable value.

We have already seen, however, that the viscosity of blood depends on the rate of shear, and equation (3) is only true on the assumption that the core, when in movement, behaves as a simple fluid. Thus it can only be used with confidence when the flow rate is large enough for the rate of shear to which the majority of the blood is exposed to be well in excess of  $50 \text{ sec}^{-1}$ . This is probably true in most of the circulation (Table 1). A second complication is that cells may migrate further from the tube walls as the flow rate is raised, leading to an enlarged fall in the haematocrit and effective viscosity. There must at present be some uncertainty regarding the relative importance of these two effects in any particular case<sup>25</sup>, but at normal haematocrits and physiological flow rates

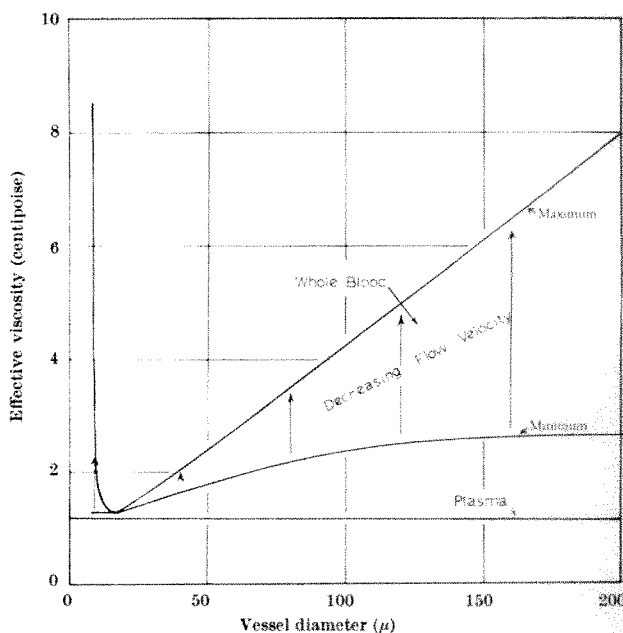


Fig. 4. Variation of effective viscosity of blood with vessel diameter.

the assumptions of the asymptotic value for the viscosity of the blood and an effective annulus width of about  $4\mu$  are not unreasonable. The effective viscosity of the blood, as given by the flow resistance experienced in the tube,  $\eta_{\text{eff}}$ , is then

$$\eta_{\text{eff}} = \frac{\eta_p}{1 - \left( \frac{R-4}{R} \right)^4 \left( 1 - \frac{1}{rc} \right)} \quad (5)$$

where  $\eta_p$  is the viscosity of the plasma.

If the viscosity of the plasma is taken as 1.2 centipoise the relationship between vessel diameter and effective viscosity is then as shown in Fig. 4. When the flow is very slow the yield stress might forbid the blood in the core to shear. This would raise the effective viscosity, and the limiting case—when the core moves as a solid plug—is also shown in Fig. 4.

**Small vessels.** The shearing core model of flow is satisfactory until the diameter of the cells approaches the diameter of the vessel. This corresponds to conditions in the microcirculation, and the concept of the blood shearing in a manner similar to that of a simple liquid becomes unrealistic. The corpuscular nature of blood must be taken into consideration. Observations made *in vivo* suggest that the red cells tend to follow each other axially in single file down narrow vessels with their discoidal surfaces roughly perpendicular to the axis of the vessel but deforming<sup>26</sup> into teardrop<sup>27</sup>, parabolic<sup>28</sup> or bullet<sup>29</sup> shapes and leaving a layer of plasma at the wall. This pattern can be idealized into an axial train containing red cells and some plasma, surrounded by an annulus of plasma in which the shearing predominantly takes place (Fig. 5) (refs. 1 and 22). If the train of cells and plasma is assumed to travel as a single cylindrical unit possessing an infinite viscosity, the mean haematocrit  $\bar{H}$  can be

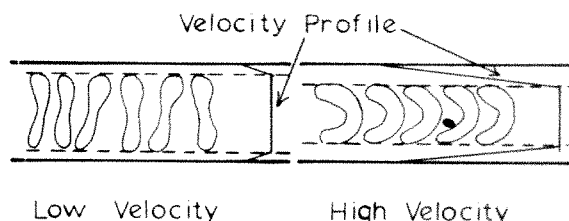


Fig. 5. Axial train flow.

obtained by putting  $\eta_{rc} = \infty$  and  $R - 4 = \text{radius of the train, } R_T$ , in equations (3), (4) and (5) (ref. 22).

Thus

$$H = \frac{H_f}{2} \left[ 1 + \left( \frac{R_T}{R} \right)^2 \right] \quad (6)$$

and the effective viscosity

$$\eta_{\text{eff}} = \frac{\eta_p}{1 - \left( \frac{R_T}{R} \right)^4} \quad (7)$$

This simple axial train model ignores the influence of secondary, or bolus, flow which may develop in the gaps between the cells, but the change in flow resistance stemming from this effect is probably very small. It is also difficult to predict the exact range of vessel diameters over which the model should be applicable. When the vessel is some three or four cell diameters in width, the shearing core model is probably inappropriate, but it is also unlikely that the cells move in single file as required by the axial core model. Taking the diameter of a red cell as  $8\mu$  it can easily be shown from equation (6) that if the cells were compacted into a continuous core of undeformed face-to-face cells, and the vessel diameter were twice the core diameter, then the feed haematocrit would be 40 per cent. In vessels of larger diameter there would be no room in a single, concentric core for all the cells to take up a face-to-face position and some kind of shearing core flow would have to result. As the cells are oriented with the faces predominantly normal to the vessel axis when in axial core flow, but predominantly parallel to the axis in shearing core flow, the cells would probably take up changing, uncertain orientations in this intermediate region giving the "turbulent" flow pattern sometimes reported in the literature<sup>1,9,26</sup>.

In vessels smaller than  $16\mu$  in diameter, axial core flow should predominate and the minimum haematocrit and effective viscosity will be obtained when the cells form a continuous, plasma free, face-to-face axial core. The tendency for the cells to deform to form a single strand on the axis and to displace plasma from the core should increase with the velocity (Fig. 5) until constant minimum values of haematocrit and effective viscosity are reached when the core is made up entirely of red cells. The range of diameters giving these minima should therefore increase as the flow velocity is increased. For a feed haematocrit of 40 per cent the limiting condition corresponds to the core possessing a diameter of one half of that of the vessel, the mean haematocrit in the vessel then being 25 per cent and the effective viscosity approximately 1.3 centipoise (Fig. 4).

When the vessel becomes smaller in diameter than the diameter of the cells, there is a possibility that the cells move as plugs down the vessel. In this case  $R_T = R$  and reference to equations (6) and (7) shows that the tube haematocrit then equals the feed haematocrit and the effective viscosity tends to infinity. This critical condition is probably never reached in normal health, however, a thin layer of lubricating plasma usually being observed at the wall as soon as movement of the red cells commences.

### Minimum Flow Resistance

It has been mentioned that the haematocrit in a vessel down which blood is flowing can only be expected to equal that in the feed reservoir if the vessel is many times greater in diameter than the red cell, or of about the same diameter. Between these extremes there must be a vessel diameter (or range of diameters) which gives a minimum haematocrit and also a minimum resistance to flow. In the case of human blood the diameter is probably less than  $16\mu$  and greater than  $7\mu$  (ref. 22), but the exact value cannot be specified with certainty. Both minima are influenced by the flexibility of the cells and the flow

velocity, but the asymptotic values should be 24 per cent for haematocrit and about 1.3 centipoise for effective viscosity assuming a feed haematocrit of 40 per cent and a plasma viscosity of 1.2 centipoise. The mechanism bringing about this velocity dependent resistance to flow is different from that applying in large vessels where the change occurs at a constant haematocrit which equals that in the feed. Consequently the magnitude of the variation in effective viscosity with rate of flow in small vessels is unlikely to be the same as in large vessels.

If the viscosity of blood is measured by comparing its resistance to flow down a tube with that experienced by simple liquids of known viscosity, values between about 1.3 centipoise and infinity are possible, depending on the diameter of the vessel used and the velocity of flow.

A general tendency is for the apparent viscosity of normal blood to fall with increasing rate of flow. In small vessels this trend is normally accompanied by a fall in the haematocrit but not in large vessels. Where the flow is in large vessels, the viscosity settles down to a reasonably constant value if the rate of shear to which it is exposed is predominantly greater than  $50 \text{ sec}^{-1}$ . Experiments suggest that a value of  $2.8 \pm 1.1$  centipoise is applicable in the case of human blood.

In small vessels a tendency of the haematocrit (and thus of the effective viscosity) to fall with decreasing vessel diameter is superimposed on the normal velocity dependent characteristics of blood. This trend, however, is unlikely to continue down to the smallest vessels and there should be a certain size of vessel (probably between  $7$  and  $16\mu$  in diameter<sup>1,22</sup>) in which a minimum effective viscosity of about 1.3 centipoise is reached. The precise vessel sizes in which the minimum is attained may be affected by the velocity and are crucially dependent on the flexing characteristics of the red cells. Theoretical progress in this region requires better knowledge than is at present available of the elastic properties of the cells.

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# Particles trapped in the Potential Well behind a Mesothermally Moving Satellite

by

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The presence of a negative potential valley in the near wake of a negatively charged satellite suggests the existence of a belt filled with oscillating particles.

THE recent measurements of the ionosphere using satellites have produced some interesting but unexpected results: the plasma probe studies of electron density and temperature of the ionosphere in the wake of the Ariel I satellite showed the possible presence of low frequency plasma oscillations<sup>1</sup>. Plasma resonances found with the Alouette topside sounder satellite occur at multiples of certain characteristic frequencies of high frequency plasma oscillations<sup>2</sup>. With a disturbed tenuous ionospheric plasma, which is characteristically susceptible to numerous aspects of macro and micro-instabilities, it is not difficult to attribute these anomalous results to the general plasma instabilities in the ionosphere. This problem, however, deserves more scrutiny.

It is significant to note the consistency and persistence of the characteristic oscillation frequencies observed in the Alouette ionogram. This apparently suggests that the source and site of these plasma oscillations might be of limited extent and of long duration, possibly associated with the motion of the satellite. This article suggests the possible presence of a specific mode of plasma oscillations by particles trapped in a potential well which is associated with a rapidly moving satellite, thus throwing some light on the anomalous phenomena in the contemporary ionospheric measurements, and proposes a new concept of ionospheric measurement based on the theoretical relation between the ambient plasma parameters and the oscillation properties of the plasma particles.

From the gas dynamic point of view, the motion of a geophysical satellite in the tenuous ionosphere presents a unique plasma interaction between a negatively charged body and a fully ionized medium. The speed of the satellite is usually much larger than the thermal speed of ions [ $v_i = (2kT_i/m_i)^{1/2}$ ] and yet much smaller than that of the electrons [ $v_e = (2kT_e/m_e)^{1/2}$ ] of the ambient plasma. Consequently the gas dynamic disturbances, for example, the particle density distribution in the vicinity of the moving satellite, behave superthermally with respect to the ionic flow; sub-thermally to the electronic flow. The word mesothermal is therefore introduced here to describe the speed of the satellite. The satellite interaction problem is often characterized by the condition:  $d \ll R \ll l$  where  $R$  is the characteristic size of a satellite, for example, the radius of a spherical satellite,  $l$  is the mean free path of the ambient particles and  $d$  the Debye shielding length.

The steady state distribution of particles and potential in the frontal sheath region of a moving satellite is already well known<sup>3,4</sup>. The self-consistent solution of the corresponding problem for the near-wake behind the satellite was obtained only recently<sup>5</sup>. A notable feature of the

potential distribution in the near-wake according to the self-consistent solution is the presence of a negative-potential well in the shape of a kidney extending symmetrically across the wake. The minimum potential with an absolute value in the order  $(kT_e/e)\ln(R/d)^2$  is located at a distance of about  $2R$ , in the case of a spherical satellite with radius  $R$ , from the centre of the satellite<sup>5</sup>. This negative-potential well is populated with an electron-rich mixture of ions and electrons which results from the swifter motion of electrons to fill the wake cavity behind a rapidly moving satellite. The degree of this charge separation which determines the depth of the potential well is governed by the Poisson equation of electrostatics and the prescribed boundary conditions of the wake cavity.

So far our discussion of the problem is restricted to its steady state solution which can, at best, be considered an asymptotic solution if the system is stable. In other words, to find how the behaviour of the system changes with time, we must study the perturbations to the steady state of the system. I do not intend to discuss here the general instabilities of a non-homogeneous plasma wake. Instead, the discussion is restricted to a salient mode of plasma oscillations peculiar to the potential distribution in the wake.

It is well known in plasma physics that a potential well can act as a trap to particles of opposite charge that have energies appropriate to the potential depth of the well. By trapping we mean that the ions with appropriate energies will oscillate between the ridge and the valley of the negative-potential well. To examine the oscillation mechanism of the trapped ions, it is instructive to use a simplified one-dimensional model such that the variation of potential and the oscillation of the ions take place in the  $x$ -direction only. The model may be called a diode analogue, with the free stream, in which charged particles are "emitted" into the wake cavity, identified as its cathode; the satellite surface, where electrons are absorbed and ions neutralized, its anode. A dispersion relation for the standing waves of the ionic oscillations in the potential well can be developed using an approximation similar to the Langmuir-Tonks derivation for a homogeneous plasma. A word of justification for the use of this simplified two fluid approach is in order. It is valid provided that  $T_e \gg T_i$  which is plausible, considering that only ions of low energy are trapped in the well.

Let  $n_i = n_{i0} + n'_i(x, t)$ ,  $n_e = n_{e0} + n'_e(x, t)$  and  $\phi = \phi_0(x) + \phi'(x, t)$  denote the ion density, electron density and potential respectively; the steady state densities  $n_{i0}$  and  $n_{e0}$  may be unequal, but they are taken as their respective average values, therefore independent of space. It is

assumed that the oscillation frequency is so low that the electrons are always able to maintain the Boltzmann distribution with local instantaneous potential.

$$n'_e = n_{e0} [\exp(e\phi'/kT_e) - 1] \approx n_{e0} e\phi'/kT_e$$

assuming  $e\phi' \ll kT_e$ . From the continuity equation we obtain

$$n'_i = n_{i0}(1 - ds/dx)$$

where  $s$  denotes the instantaneous displacement of the ions with respect to their steady state position. These perturbations of the space charges must comply with the Poisson equation

$$\epsilon_0 \nabla^2 \phi' = 4\pi e n_{i0}(ds/dx) + 4\pi e^2 n_{e0} \phi'/kT_e \quad (1)$$

The ionic equation of motion can be written

$$-m_i d^2s/dt^2 = ed\phi_0/dx + ed\phi'/dx \quad (2)$$

Eliminating  $\phi'$  from equations 1 and 2 and noting that  $\epsilon_0 \nabla^2 \phi_0 = 4\pi e(n_{e0} - n_{i0})$ , we obtain an equation for  $s$  similar to that of Langmuir-Tonks.

$$\frac{d^2}{dx^2} \left( \frac{d^2s}{dt^2} + \frac{4\pi e^2 n_{i0} s}{m_i} \right) - \frac{4\pi e^2 n_{e0}}{kT_e} \frac{d^2s}{dt^2} = 0 \quad (3)$$

A dispersion relation between the oscillation frequency and the wavelength  $\lambda$  can be readily obtained from equation 3 by assuming a travelling wave

$$s = A \exp\left(i2\pi ft - i \frac{2\pi x}{\lambda}\right)$$

$$f^2 = \frac{n_{i0} e^2}{\pi m_i + (m_i/8\pi)(\lambda/d)^2} = \frac{f_i^2}{1 + (n_{e0}/n_{i0})(m_i \lambda^2 / \kappa T_e) f_i^2}$$

where the Debye shielding length  $d = (kT_e/8\pi e^2 n_{e0})^{1/2}$ ; the ion plasma frequency  $f_i = (n_{i0} e^2 / \pi m_i)^{1/2}$ . The wavelength ( $\lambda$ ) or its propagation constant ( $2\pi/\lambda$ ), is real for all frequencies less than the ion plasma frequency, the upper cutoff frequency. The fact that propagating waves are obtained from a cold-ion approximation need cause no concern. It is the rapid motion of the electrons, which rush in to negate the fields set up by the ion imbalance, that causes the ion oscillations to propagate. The actual frequencies excited by the ion oscillations must be determined by the boundary condition at the edges of the potential well. The well in question has half-width in the order of  $R$ , the satellite radius, according to the result of the self-consistent solution for a spherical satellite<sup>5</sup>. The wavelength of the oscillation of interest must be such that standing waves appropriate to the geometry of the idealized diode column are obtained; in other words, the lowest harmonic oscillation has a wavelength equal to four times the half-width of the well, namely,  $R$ . It is thus obvious from the assumption,  $d \ll R$ , that we have  $\lambda/d \gg 1$ , consequently the phase velocity  $\lambda f \approx (n_{i0}/n_{e0})^{1/2} (\kappa T_e/m_i)^{1/2}$ . Other than the factor  $(n_{i0}/n_{e0})^{1/2}$ , which is assumed constant in the present analysis, the wave appears like a sound wave. It implies that the ion oscillations propagate through the plasma in a manner quite analogous to the propagation of sound through a neutral gas of temperature  $T_e$  and particle mass  $m_i$ . (A proper account of the adiabatic compression of the gas would lead to an additional factor  $\gamma$ , the ratio of specific heats<sup>6</sup>.) It would be expected, however, if there were thermal equilibrium between the electrons and ions, that is,  $T_e = T_i$ , that the ion sound (lowest harmonic) frequency for the ionosphere at an altitude of 1,000 km for a satellite with  $R = 100$  cm is estimated to be a few kilocycles per second.

The following mechanism can be advanced to account for the excitation of these oscillations. The flux of ions from the free stream enters the region of the potential well where there is an alternating electric field. Under these conditions the ion velocity is modulated. It is anticipated that a modulated ionic flux will cause fluctuations in the depth of the potential well and thus provide the required feedback mechanism.

In view of the non-homogeneity of the plasma and the relative motions between the ions and electrons in the wake, it might be expected that available mechanisms for exciting electron oscillations in the plasma are not lacking. Again using the diode analogy, I would like to draw special attention to the finding of transient modification of plasma non-homogeneity by ion oscillations in a diode discharge in which there is a maximum concentration of plasma electron<sup>8</sup>. It was found that in the interaction between the oscillations of ions and electrons, the ion oscillation may cause the maximum and minimum electron concentrations to shift in space. The coupling found between plasma electrons and ion oscillations is also noteworthy<sup>9</sup>.

When the geomagnetic field is taken into account many more modes of oscillation, particular electromagnetic oscillations, would be possible. Particular attention should be drawn to the Larmor gyration of electrons at electronic cyclotron frequency.<sup>10</sup> Here bunching of the gyrating electrons is caused by the alternating electric field (normal to the magnetic field) which is in turn caused by the bunched electrons. Self-consistent oscillations of this kind might be expected to occur near the harmonics of the electron cyclotron frequency in a very tenuous plasma.

To sum up, I have proposed a theory which throws light on the anomalous mode of ionic oscillations in the near-wake of a satellite on the basis of potential distribution from the self-consistent theory of plasma interaction<sup>5</sup>. The estimated range of frequency appears to agree with the experimental evidence of low frequency oscillations<sup>1</sup> although more specific measurements are needed to bear out the theory. The associated electron plasma oscillation and the electronic cyclotron frequency of the electrons in the potential well should throw light on the source and site of the resonance frequencies observed in the Alouette ionogram. The belt of charged particles with specific oscillation frequencies in the near-wake of a geophysical satellite could make possible a number of ionospheric measurements. Satellite-borne experiments can be devised to detect the ionic oscillations<sup>10</sup>, and electron oscillations<sup>2</sup> as the indirect measurements of the ambient electron density and temperature. A more refined theory, which takes into account the microscopic velocity distributions of the particles and applies to particles trapped in potential wells of either charge, will be presented later. The possible presence of potential wells of alternating charges in the far-wake of a satellite at mesothermal speeds would make it necessary to generalize the present theory to include the mode coupling and damping due to microscopic velocity distributions of the particles.

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# Redox Chemistry of Peroxides: Energy Surface of the Perhydroxyl Ion ( $\text{HO}_2^-$ )

by

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The ground state of the perhydroxyl ion has been studied theoretically by the Pople-Segal self-consistent field molecular orbital method. The results suggest that 2-electron equivalent atom transfer processes may be important in the redox chemistry of peroxides.

THE structure of the perhydroxyl ion ( $\text{HO}_2^-$ ) is not easy to study experimentally, because stable crystalline hydroperoxide salts are not formed and hydrogen peroxide solutions are unstable at high pH. The ion is, however, well suited for theoretical study and this article reports the results of calculations using the Pople-Segal self-consistent molecular orbital method<sup>1</sup>.

Many of the mechanisms proposed for reactions of hydrogen peroxide invoke the perhydroxyl ion as an important intermediate species, although the modes of reaction of this ion and, indeed, of hydrogen peroxide itself have been controversial for many years. This is exemplified by the variety of mechanisms which have been advanced in attempts to describe the catalytic decomposition of hydrogen peroxide by ferric ion and ferric complexes. One aim of our work, therefore, was to examine the changes of energy and electronic charge distribution over the ground state energy surface of  $\text{HO}_2^-$  for indications of likely modes of reaction in peroxidic oxidation-reduction processes.

The system has fourteen electrons (neglecting the oxygen  $1s^2$  core) and the ground-state wave function can therefore be represented by a single determinant. The diagonal elements of the core Hamiltonian matrix ( $H_{\mu\mu}$ ) in the order H  $1s$ ; O  $2s$ ;  $2p$ ; were 13.06, 32.38 and 15.85 eV, respectively; all off-diagonal elements ( $H_{\mu\nu}$ ) were calculated by scaling the standard bond resonance integrals ( $\beta_{\text{O}-\text{O}}^0 = 31$  eV and  $\beta_{\text{O}-\text{H}}^0 = 20$  eV) in proportion to the overlap integrals between the appropriate orbitals for each nuclear configuration. The one- and two-centre electron repulsion integrals were computed theoretically from Roothaan's expressions<sup>2</sup>. Of the fourteen electrons in the  $\text{HO}_2^-$  anion, one originates from the hydrogen atom and twelve from the oxygen atoms. The additional electron cannot be allotted to any particular atom and so

was considered initially to be "smeared out" evenly over all three centres.

A non-linear triatomic system has three degrees of freedom which, in this case, are the O-O and the two OH interatomic distances. In order to reduce the number of calculations the former was first fixed at 1.26 Å, that is, the O-O bond length in the  $[\text{O}_2^-]$  ion<sup>3</sup>. The total self-consistent electronic energies of systems in which the hydrogen atom was considered situated at a series of points in one quadrant of the molecule were calculated from the equation of Pople and Segal<sup>1</sup>.

$$E_{\text{SCF}} = \frac{1}{2} \sum_{\mu, \nu} P_{\mu\nu} (F_{\mu\nu} + H_{\mu\nu})$$

In this treatment both OH distances were allowed to vary simultaneously by expressing them in the spheroidal co-ordinations  $\lambda$  and  $\mu$  (Fig. 1a). These are defined by

$$\lambda = \frac{r_A + r_B}{R} \quad \mu = \frac{r_A - r_B}{R}$$

Thus curves of constant  $\lambda$  are ellipses with the oxygen nuclei as common foci, while curves of constant  $\mu$  are the orthogonal confocal hyperbolae (Fig. 1b). The whole problem is, of course, invariant to rotation about the O-O bond axis.

The limits of  $\lambda$  range from 1 to  $\infty$ , and for  $\mu$  they are 0 to 1 in the quadrant considered. Calculations were performed in which  $\lambda$  assumed the values 1.3, 2.0, 2.5, 3.0, 3.5 and, for each of these,  $\mu$  was varied, in the first instance, over the range 0 (0.2)1. In later work additional calculations became necessary to locate the precise position of the minimum on the energy surface. The energies of the anion at large OH internuclear separations ( $\lambda = 6$ ;  $\mu = 0.2, 0.4, 0.6, 0.8$ ) were also computed, and the case  $\lambda = 6$ ,  $\mu = 0.8$  was taken as the energy zero. In these latter cases and those in which the three atoms were colinear, it was necessary to calculate the energies by hand (*vide infra*).

All the calculations were carried out on the KDF9 computer at the University of Newcastle upon Tyne, using a fully automatic SCF programme.

**Results.** (a) *Energies of the system.* Figs. 2a and 2b are plots of  $E_{\text{SCF}}$  against  $\lambda$  for constant  $\mu$  and  $E_{\text{SCF}}$  against  $\mu$  for constant  $\lambda$ , and so illustrate the energy changes accompanying movement of the hydrogen nucleus along the co-ordinate surface in either direction. The energies of certain geometrical configurations in which the oxygen

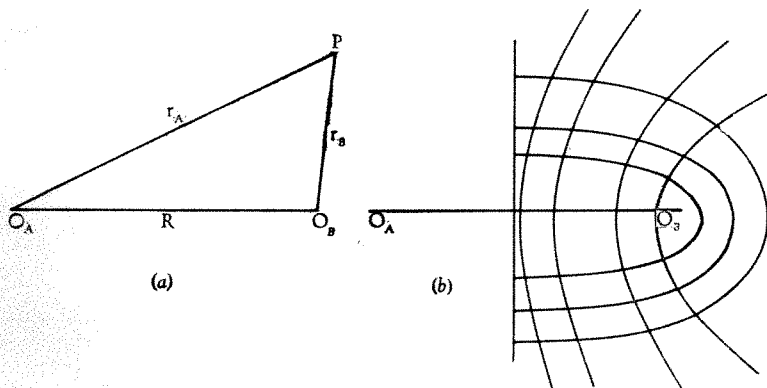


Fig. 1.

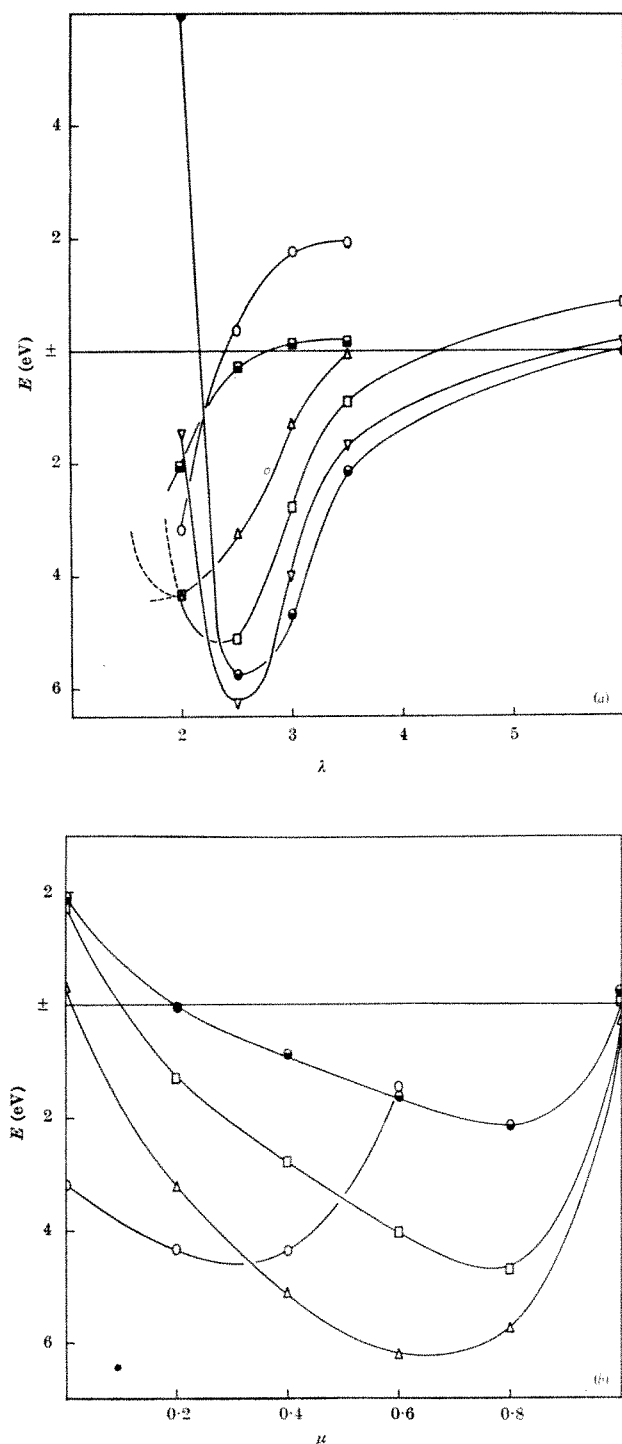


Fig. 2. (a) Variation of  $E_{scf}$  (eV) with  $\lambda$  at constant  $\mu$ .  $\circ$ ,  $\mu=0$ ;  $\Delta$ ,  $\mu=0.2$ ;  $\square$ ,  $\mu=0.4$ ;  $\nabla$ ,  $\mu=0.6$ ;  $\bullet$ ,  $\mu=0.8$ ;  $\blacksquare$ ,  $\mu=1.0$ . (b) Variation of  $E_{scf}$  (eV) with  $\mu$  at constant  $\lambda$ .  $\circ$ ,  $\lambda=2.0$ ;  $\Delta$ ,  $\lambda=2.5$ ;  $\square$ ,  $\lambda=3.0$ ;  $\bullet$ ,  $\lambda=3.5$ .

and hydrogen nuclei lie in close proximity are too high for inclusion on the same scale; this stems from the large nuclear repulsion term in the energy equation. Finally, the lowest energy of the ion, for an O-O bond length of 1.26 Å, results when  $\angle OOH$  is  $105^\circ 18'$ , and the shortest OH bond has a length of 1.197 Å. The ionization potential of this configuration is 9.86 eV.

An interesting feature revealed by the calculations is that, although the minimum energy of the ion lies on the curve for  $\mu = 0.6$  (at  $\lambda = 2.5$ ), the curve for  $\mu = 0.8$  crosses this as  $\lambda$  increases. This means that the lowest

energy route by which a hydrogen atom can leave the ion first entails its crossing from the hyperbola  $\mu = 0.6$  to that of  $\mu = 0.8$ , and then following this hyperbolic path. The energy required to remove the hydrogen completely is  $\sim 6.5$  eV.

(b) *Electronic charge distribution.* The calculations also give the  $\sigma$  and  $\pi$  charge distributions throughout the ion. There is, in fact, no O-O  $\pi$  bonding and a lone pair of electrons resides on each oxygen atom, in an orbital of  $\pi$  symmetry. The distribution of  $\sigma$  electronic charge on the three atoms is extremely informative: (i) in the configuration of lowest energy the hydrogen atom carries an excess charge of 0.127e, the central oxygen 0.269e and the terminal one 0.604e; (ii) from the change in occupancy of the hydrogen 1s orbital, with increasing  $\lambda$  at constant  $\mu$  (Fig. 3), it can readily be seen that, independent of the value of  $\mu$ , the electronic charge on hydrogen builds up to two electrons as  $\lambda$  increases. In this limiting situation there are no off-diagonal elements in the density matrix between the hydrogen and any of the oxygen orbitals. As previously noted, the automatic calculation of the electronic energy of such structures fails, because the lowest virtual and the highest doubly occupied molecular orbitals (which contain no contribution from hydrogen 1s) are near-degenerate. No allowance was made for such a situation in the programme and these energies were therefore obtained from separate hand calculations.

We conclude that for large OH distances, the system approximates to an  $H^-$  ion and singlet molecular oxygen and should yield these initially on decomposition.

(c) *Variation of O-O bond length.* A second series of calculations was carried out to demonstrate the effect of O-O bond lengthening on the electronic charge distribution throughout the "minimum energy" ion. In this the O-O bond length was increased from 1.26 Å to 8.468 Å in steps of 0.945 Å and the self-consistent density matrix calculated for each case. The results illustrate that the excess charge on the terminal oxygen decreases regularly from 0.604e (O-O bond length 1.26 Å) to 0.055e when the two oxygens are separated by 8.468 Å. Hence at very large distances the whole of the "extra" electron resides on the OH moiety, a result which suggests that, when the O-O bond is broken, the ion will generate a neutral oxygen atom and a hydroxyl ion.

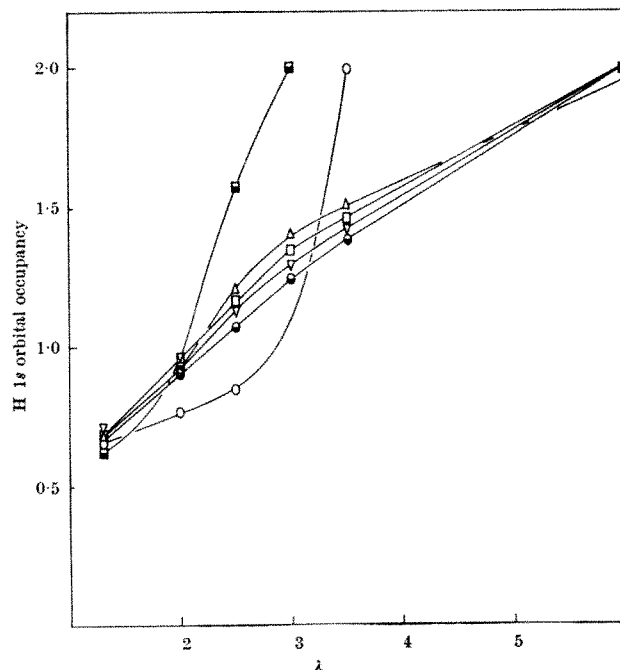
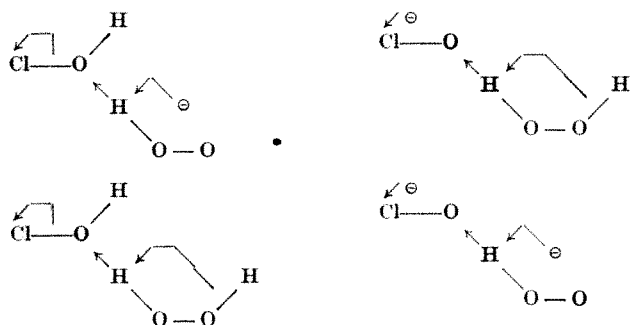


Fig. 3. Variation of H 1s orbital occupancy with  $\lambda$  at constant  $\mu$ .  $\circ$ ,  $\mu=0$ ;  $\Delta$ ,  $\mu=0.2$ ;  $\square$ ,  $\mu=0.4$ ;  $\nabla$ ,  $\mu=0.6$ ;  $\bullet$ ,  $\mu=0.8$ ;  $\blacksquare$ ,  $\mu=1.0$ .

**Reaction mechanisms.** The results of our calculations have possible implications for the mechanisms of a wide variety of peroxide reactions, for example, the types of mechanism indicated are where  $\text{HO}_2^-$  acts (i) as a two electron equivalent reducing agent by hydride ion transfer; the product oxygen molecule thereby released should be a singlet species; and (ii) as a two electron equivalent oxidizing agent by oxygen atom transfer.

(i)  $\text{HO}_2^-$  as a reducing agent. For the oxidation of hydrogen peroxide by alkaline chlorine (that is, hypochlorite) solutions, the evidence for a hydride ion transfer mechanism is compelling. Chemiluminescence accompanies the reaction, and is associated with transitions involving initially produced singlet oxygen molecules<sup>4</sup>. The transition states in these reactions may have different numbers and arrangements of protons, so that a number of contributions to the total reaction can be expected and are indeed indicated by kinetic studies of  $\text{pH}$  effect<sup>5</sup>, although the essential redox process is unchanged. These processes may, for example, be represented by



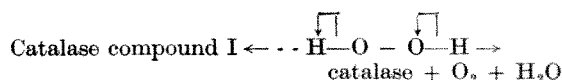
all of which involve a flow of electrons from right to left, resulting in a net two electron equivalent redox process. Reduction by way of the hydrogen peroxide molecule is readily included in these schemes by coupling proton dissociation and hydride ion transfer. Anbar<sup>6</sup> has recently shown that  $\text{NO}_2^-$  and  $\text{NO}_3^-$  are formed when hydrogen peroxide is oxidized *in situ*, by hypochlorite and a number of other oxidizing agents, in the presence of molecular nitrogen. He considers that the oxidation of nitrogen molecules involves attack by ( $^1\Delta_g$ )  $\text{O}_2$  produced by the oxidation of hydrogen peroxide.

(ii)  $\text{HO}_2^-$  as an oxidizing agent. Higginson and Marshall<sup>7</sup> classify hydrogen peroxide as a preferred two-electron equivalent oxidizing agent. The interpretation of the peroxide oxidations of a variety of inorganic and organic species as nucleophilic displacements on peroxidic oxygen by the reductant has been discussed fully by Edwards<sup>8</sup>. Tracer experiments have in several cases shown direct oxygen atom transfer, for example, the oxidation of nitrite ion by peroxyacids<sup>9</sup>. Patterns of substituent effects, both in reductants<sup>10</sup> and peroxidic oxidant<sup>11</sup>, are all consistent with the description of these processes as two electron equivalent oxidations occurring by displacement on peroxidic oxygen. Coupled proton loss, either to the solvent or, in favourable cases such as epoxidation of olefins by peroxyacids, by way of a cyclic transition state to the leaving anion, is also involved in these reactions. Alkyl and, particularly, acyl hydroperoxides are preferred to hydrogen peroxide in these oxidations because the  $\text{RO}^-$  anions are better leaving groups compared with the hydroxide ion. Although there is no direct evidence for the transfer of oxygen atoms in the case of oxidation by hydrogen peroxide and  $\text{HO}_2^-$ , our calculations suggest that these species probably conform to the behaviour pattern, already established for hydroperoxides in general.

(iii) The catalytic decomposition of hydrogen peroxide. For the present, discussion is restricted to ferric complexes as catalysts. Kremer and Stein<sup>12</sup> have suggested that  $\text{FeO}^{3+}$

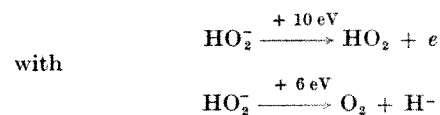
is an important species in ferric ion catalysis and that it is formed by loss of  $\text{OH}^-$  from a ferric ion perhydroxyl ion complex. Wang<sup>13</sup> suggests that the high activity of the triethylenetetramine-ferric complex derives from a situation in which the O-O bond in a co-ordinated perhydroxyl ion is first stretched across two *-cis* co-ordination positions and subsequently generates co-ordinated O and  $\text{OH}^-$ . There is considerable evidence that catalase compound I contains both oxidizing equivalents of its parent hydrogen peroxide molecule (for a review see ref. 14), and Jones and Wynne-Jones<sup>15</sup> have argued that the ferric ion and catalase reactions are probably closely related mechanistically. Yonetani<sup>16</sup> has shown that cytochrome peroxidase compound II contains both oxidizing equivalents of its parent hydrogen peroxide molecule.

If the first step in catalysis is the "activation" of peroxide as a two electron equivalent oxidizing agent, the reaction sequence may then be completed by a two electron equivalent oxidation of a second hydrogen peroxide molecule or a perhydroxyl ion, for example



This has been suggested both for the ferric ion reaction<sup>17</sup> and for the catalase reaction<sup>18</sup>. This model accounts for both kinetic and deuterium isotopic effects and is readily extended to account for peroxidatic oxidations.

Doctrinaire application of exclusive one electron transfer principles is clearly inadequate for complete description of the redox chemistry of hydrogen peroxide, because two electron equivalent atom transfer processes are energetically favourable. Considering the perhydroxyl ion alone we may compare



with

Discussions of, for example, the decomposition of hydrogen peroxide catalysed by ferric ions have largely regarded one electron equivalent and two electron equivalent redox paths as mutually exclusive, whereas it would seem more pertinent to investigate the relative contributions of the two paths to the total reaction in particular cases.

Our results indicate an interesting relationship between the catalytic decomposition of hydrogen peroxide involving two electron equivalent steps and the thermal decomposition of acyl hydroperoxides (peroxyacids). In the former case "activation" of hydrogen peroxide as an oxidant by interaction with the catalyst is rate limiting. This activation, which probably involves O-O bond cleavage, yields mechanisms with rate laws showing a characteristic first-order dependence on hydrogen peroxide. In the latter cases the mutual oxidation-reduction step is rate limiting, O-O bond cleavage and oxygen release are synchronous, yielding a characteristic second-order rate law<sup>19</sup>. In accord with these ideas, very pure hydrogen peroxide is kinetically extremely stable<sup>20</sup>. A subtle feature of peroxyacid decompositions is that the electrophilic centre may not be the carboxyl carbon atom in an acyl hydroperoxide, but may be the outer peroxidic oxygen atom of the peroxyacid molecule. Both centres may contribute to the total reaction, the contributions being distinguishable by isotopic labelling<sup>19</sup>. It would be interesting to compare experiments with calculations of

the structural variation of electronic charge density in these systems.

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## Identity of a Urinary "Pink Spot" in Schizophrenia and Parkinson's Disease

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The substance producing the controversial pink spot on paper chromatograms from urine obtained from patients with Parkinson's disease and some schizophrenics has been submitted to mass spectrographic, ultra-violet and infra-red analysis. The results of these studies would seem to suggest that the pink spot first found by Friedhoff and van Winkle consists of *p*-tyramine and not  $\beta$ -3,4-dimethoxyphenylethylamine as was previously thought.

CONSIDERABLE activity has followed Friedhoff and van Winkle's<sup>1</sup> finding that a substance producing a pink coloration after dipping paper chromatograms through ninhydrin and Ehrlich's reagents occurs only in extracts of the urine of schizophrenic patients. This finding has to some extent been confirmed in other laboratories<sup>2-6</sup>. Perry *et al.*<sup>7,8</sup> and Nishimura and Gjessing<sup>9</sup>, using more sophisticated techniques and patients maintained on carefully controlled diets, failed to locate this pink spot. Von Studnitz and Nymann<sup>10</sup> reported its disappearance from the urine when only glucose, citric acid and water were ingested. Largely from a study of the chromatographic behaviour of the pink spot and some of its derivatives, Friedhoff and van Winkle<sup>11</sup> identified the substance as  $\beta$ -3,4-dimethoxyphenylethylamine (DMPE). Such a finding in schizophrenia would seem to confirm the implications of earlier hypotheses. In 1952 Osmond and Smythies<sup>12</sup> and Harley-Mason<sup>13</sup> postulated an abnormal methylation of catecholamines. More recently<sup>14-16</sup> it has been shown that feeding substances capable of donating a methyl group can cause an exacerbation of schizophrenic symptoms, although normal people also become more excited under the same conditions.

Our own early attempts to locate the pink spot were not very successful. We were misled by various false positive results due to drug metabolites. In an investigation of urine from seventy-three schizophrenic patients we have found seven which show the spot described by Friedhoff. In attempts to increase the sensitivity of detection, and also allow quantitative evaluation, we tried to convert the pink spot zone on paper chromatograms to a fluorophore according to the procedure described by Bell and Somerville<sup>17</sup>. Although the addition of authentic DMPE to urine always produced a blue

fluorescence, this was never observed in any pink spot urine<sup>18,19</sup>. Similarly, Bell and Somerville<sup>20</sup>, using electrophoresis, were unable to observe any fluorescence from urines showing the pink spot. Extensive chromatographic investigations by Faurbye and Pind<sup>5,21</sup> and Williams *et al.*<sup>22</sup> failed to reveal any substance identical with DMPE. Barbeau *et al.*<sup>23,24</sup>, using Friedhoff and van Winkle's method, have recently shown that a pink spot occurs in the urine of 80 per cent of patients suffering from Parkinson's disease. We have confirmed this finding<sup>19</sup> and shown again that the substance is not DMPE, because it also fails to produce the characteristic blue fluorescence. At this stage, therefore, it is confirmed that the pink spot substance occurs in the urine of some schizophrenics and most Parkinsonian patients. It is not DMPE. An eluate from a paper chromatogram of the pink spot material was optimistically subjected to mass spectrometry; the result (Fig. 1d) was very complex and merely confirmed that DMPE was not present. To obtain more pink spot material, phenolic amines from the urine of a Parkinson patient with pink spot urine were concentrated by the procedure of Kakimoto and Armstrong<sup>25</sup>. This technique was used by Nishimura and Gjessing<sup>9</sup> and Takesada *et al.*<sup>4</sup> in their studies on pink spot excretion. According to Perry *et al.*<sup>26</sup> a 96 per cent recovery of DMPE is obtained, whereas with a chloroform extraction from alkaline urine the recovery at best is only about 50 per cent<sup>18</sup>. We were surprised to find that the recovery of pink spot was five times greater than when using chloroform. The extract was applied to the top of a dry cellulose powder (Whatman CC31) column (70 cm long, 2.1 cm diameter) and developed with butanol : acetic acid : water; 60 : 15 : 25 (v/v) (ref. 27). Substances emerging from the column between 75 and 85 ml. had *R<sub>F</sub>* values of roughly 0.55-0.70 on paper

in the same solvent system. This achieved a considerable purification as assessed by subsequent chromatography. The extract was still somewhat contaminated, but the ultra-violet absorption spectrum in 0.001 normal hydrochloric acid ( $\lambda_{\max}$  219, 273 and 280 shoulder  $m\mu$ ) was rather similar to that obtained for DMPE. In 0.001 normal sodium hydroxide, however, there was a marked change ( $\lambda_{\max}$  237 and 293  $m\mu$ ). These spectra were suggestive of a free phenolic group attached to a benzene nucleus. The mass spectrum of this extract was still somewhat confusing, but peaks corresponding to 4-hydroxyphenylethylamine (*p*-tyramine) seemed significant. The extract was finally separated on an 'Amberlite CG-50' column operated at 40° C as described by Perry and Schroeder<sup>28</sup>. The unknown substance was eluted at the *p*-tyramine position as determined by a previous separation of a synthetic mixture of pyrrolidine, DMPE and *p*-tyramine. The infra-red spectrum (potassium chloride disk) of the unknown was identical with that of the synthetic *p*-tyramine obtained from the column.

In order to confirm that *p*-tyramine was the significant pink spot in our studies, extracts were prepared from urines previously classed as positive by the Friedhoff technique. In the first case urine from a schizophrenic, containing 1.1 g of creatinine, was fractionated and concentrated. The second sample, also containing 1.1 g of creatinine, was from pooled urine obtained from four different Parkinson patients. All the fractions from the

cellulose column and the ion-exchange column were analysed by paper chromatography. Pink spots were located with ninhydrin and Ehrlich's reagents<sup>29</sup>. DMPE and *p*-tyramine were run as standards and in relatively uncontaminated extracts it is possible to distinguish between them. In both the schizophrenic and Parkinson samples the only significant (90 per cent or more as assessed visually) pink spot was *p*-tyramine and this was eluted from the ion-exchange column between 110 ml. and 130 ml. with a maximum at 118.5 ml. Many minor spots were observed, some of them pink, but apart from showing in the mass spectrometer that they did not contain DMPE they were not examined further: they may or may not be similar to those described by Perry *et al.*<sup>28</sup>. Nothing was found at the peak elution volume (95 ml.) of DMPE. The pink spot fractions had ultra-violet, infra-red and mass spectra (see Fig. 1*b*, *e* and *f*) identical with those of synthetic *p*-tyramine and in this latter case the identity of the ions producing the significant mass peaks were determined by precise mass measurement and shown to be completely consistent with the structure of *p*-tyramine. The chromatographic (butanol:acetic acid:water; 60:15:25 (v/v), and isopropanol:ammonia:water; 200:10:20 v/v) and electrophoretic (aqueous formic acid 1.56 per cent, acetic acid 2.96 per cent, pH 2.0) properties were identical and none of the fractions, including synthetic *p*-tyramine, fluoresced after treatment with glycine-formaldehyde. *m*-Tyramine, which has somewhat similar ion-exchange and mass spectral properties, was ruled out as a major contributor to the pink spot because of the difference in colour produced with diazotized *p*-nitroaniline<sup>30</sup> on paper chromatograms and because the infra-red spectrum of *m*-tyramine is very different from that of *p*-tyramine. *o*-Tyramine is excluded because it is eluted much later from the CG-50 resin<sup>28</sup>.

Analysis of each fraction from the ion-exchange column, however, by the fluorimetric procedure described by Coulson *et al.*<sup>31</sup> revealed that *m*-tyramine (elution maximum 125 ml.) was present to the extent of 10  $\mu\text{g}/1.1$  g creatinine in the schizophrenic sample and 28  $\mu\text{g}/1.1$  g creatinine in the Parkinson sample. The total amount of *p*-tyramine in the ion-exchange fractions was measured by the fluorimetric procedure described by Udenfriend<sup>32</sup> and was 1,289  $\mu\text{g}/1.1$  g creatinine in the schizophrenic and 2,211  $\mu\text{g}/1.1$  g creatinine in the case of the Parkinson specimen.

It is quite possible that the pink spot identified in these studies is different from those described by other workers, but its identity as *p*-tyramine could account for certain of their observations. Friedhoff and van Winkle, in their original report<sup>1</sup>, stated that the urine extractions must be done quickly because the unknown is readily oxidized in alkaline solution. It is difficult to see why this would be important if the unknown were DMPE. Takesada *et al.*<sup>4</sup> found the pink spot to be present in normal subjects. This is explicable because they used the resin technique which gives a much higher recovery; furthermore, *p*-tyramine is a normal constituent of urine<sup>25</sup>. Because *p*-tyramine runs a little ahead of DMPE during electrophoresis at pH 6.2 in phosphate buffer it might explain the findings of Bell and Somerville<sup>30</sup>. The chromatographic and chromogenic properties of *p*-tyramine and DMPE are sufficiently similar to explain the observations that have been reported so far.

The significance of these abnormally large amounts of urinary *p*-tyramine is not yet understood. In the case of schizophrenia the pink spot has been somewhat elusive, of low incidence and perhaps directly related to exogenous sources<sup>10,12,26</sup>; it may even turn out to be a red herring, as recently suggested by *The Lancet*<sup>33</sup>. In the case of Parkinson's disease, however, the incidence is much more significant. The low concentrations of dopamine in urine<sup>34,35</sup> and certain areas of the brain<sup>36-38</sup> are interesting and may be related to this finding. A longitudinal experiment designed to show whether the excreted

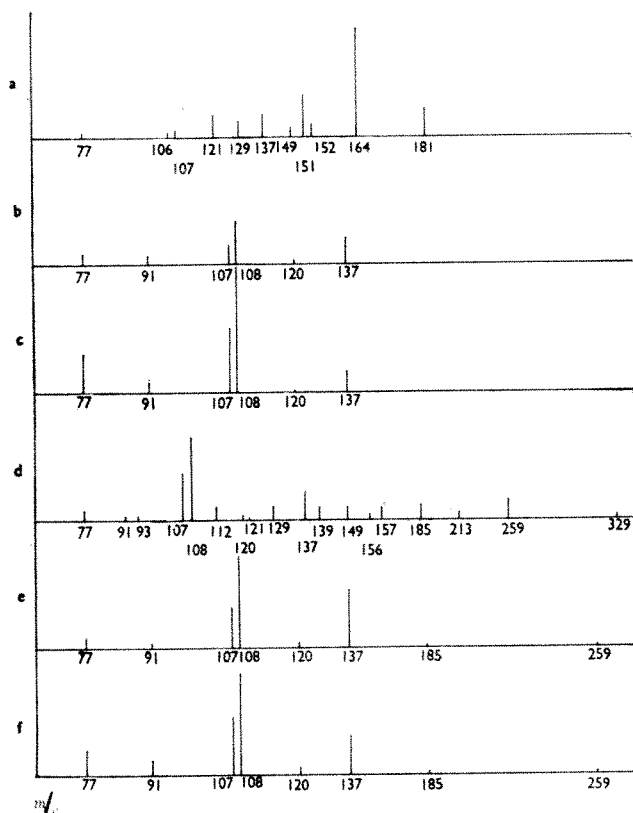


Fig. 1. Simplified monoisotopic mass spectra as obtained using the MS9 A.R.I. mass spectrometer. *a*, *b* and *c* represent DMPE, *m*-tyramine and *p*-tyramine, respectively, in the free base form. *d* is the pink spot zone eluted in 0.01 normal hydrochloric acid from a paper chromatogram. The eluate was adjusted to pH 10.5 with sodium hydroxide and shaken ( $3 \times 15$  ml.) with chloroform. The chloroform extract after drying over sodium sulphate was rotary evaporated to dryness and dissolved in 70 per cent ethanol for analysis. *e* and *f* represent schizophrenic (*e*) and Parkinson (*f*) eluates as obtained from the CG-50 column (see text). The eluates in aqueous pyridine acetic acid buffer, pH 6.32, were rotary evaporated to dryness and dissolved in 70 per cent ethanol for analysis. The small peaks at 185 and 259 in samples *e* and *f* are always seen and may represent contaminants from the resins, buffer or solvent solutions. They were shown to be unimportant by mixing a small amount of samples *e* and *f* with deuterium oxide; on subsequent analysis the *p*-tyramine fragment peaks and the molecular ion peak increased by one in all cases. The 185 and 259 peaks remained the same.



*p*-tyramine is mainly of exogenous or endogenous origin is in progress.

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## Light and Dark Adaptation in the Isolated Rat Retina

by

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A living isolated retina exhibits the initial fast phase, but not the later slow phase of dark adaptation. The irreversible loss of sensitivity with adaptation to bright light in this preparation is directly related to the bleaching of rhodopsin.

A VIABLE isolated retina, because of its thinness and transparency, offers a unique opportunity for combined physiological and biochemical measurements on a piece of living nervous tissue. Sickel<sup>1,2</sup> and Ames<sup>3,4</sup> have developed methods for maintaining the isolated mammalian retina alive while it is mounted as a membrane in a chamber perfused by an enriched medium.

One problem that is particularly suitable for analysis with such a preparation is the relation of visual pigments to visual sensitivity. This problem is difficult to approach because in man, in which it is easy to determine visual sensitivity, it is difficult to measure visual pigments. In animals, conversely, visual pigments are readily measured by extraction, but visual sensitivity is difficult to determine.

Some progress, however, has recently been made with this problem in both man and animals by indirect methods. Rushton has developed an elegant technique for measuring the amount of visual pigment in the intact human eye, and he has been able to compare the content of visual pigment in both rods and cones with visual sensitivity determined in parallel psychophysical experiments<sup>5-8</sup>. One of us (J. E. D.) has carried out similar experiments with rats by comparing sensitivity of the electroretinographic response with the amounts of visual pigment extracted later from the same or similarly treated animals<sup>9</sup>. Both types of experiment indicate that the loss of sensitivity during light adaptation is essentially independent of the amount of visual pigment bleached, but that dark adaptation consists of two components: an initial fast component, independent of concentrations of visual pigment, and a

later slow component, closely related to concentrations of visual pigment. The relation of the slow component of dark adaptation is between the logarithm of visual sensitivity and the concentration of visual pigment.

The isolated retinal preparation permits simultaneous measurement of visual sensitivity and concentrations of visual pigment, and thus provides a more direct test of the foregoing results. It has been known since the time of Kühne<sup>10</sup>, however, that a retina isolated from the pigment epithelium does not regenerate visual pigment. In such a preparation, therefore, one should have the added advantage of being able to separate more clearly the fast, non-photochemical components of adaptation from the photochemical components. Any bleaching of pigment should result in a permanent and stable change in the sensitivity of the preparation—similar perhaps to the stable rise of threshold observed in vitamin-A deficiency of the retina, in which there is not sufficient retinaldehyde (retinene) available to synthesize a full complement of visual pigment in the retina<sup>11,12</sup>.

We have performed the following experiments on retinæ obtained from albino rats anaesthetized with 'Nembutal', dissected out in dim red light and mounted between two pieces of tantalum wire mesh in a 'Lucite' chamber, similar to that described by Sickel<sup>1,2</sup>. The tantalum meshes support the retina and also serve as electrodes to record the electroretinogram. Perfusing fluid, kept at 30° ± 2° C and similar in composition to that used by Ames<sup>3,4</sup> (Table 1), was allowed to flow over the sandwiched retina at a rate of 2-3 ml./min. The sides of the chamber at the level of the retina had previously

Table 1. COMPOSITION OF PERFUSING MEDIUM

Na <sup>+</sup>	146.4 m.equiv.
K <sup>+</sup>	3.1
Ca <sup>++</sup>	0.8
Mg <sup>++</sup>	1.2
Cl <sup>-</sup>	125.4
HCO <sub>3</sub> <sup>-</sup>	24.4
H <sub>2</sub> PO <sub>4</sub> <sup>-</sup>	0.5
SO <sub>4</sub> <sup>--</sup>	1.2
Glucose	28 mmoles/l.
Glutamate	5 mmoles/l.
Plasma	2 per cent
Gas	95 per cent O <sub>2</sub> ; 5 per cent CO <sub>2</sub>
pH	7.4-7.6

Electrolytes expressed in m.equiv., non-electrolytes in mmoles/l. The formula is essentially the same as that used by Ames except for lower calcium and higher glucose concentrations.

been drilled out and replaced by cover glasses to provide a clear optical path through the chamber to the retina. The chamber was then mounted in the sample compartment of a Cary model 14 recording spectrophotometer. Two accessory lamps for stimulating and adapting purposes were mounted above the compartment; and lenses, mirrors and shutters were fitted in to deliver beams of focused light to the retina. The apparatus is shown schematically in Fig. 1. With this technique it is possible to record the absorbance of visual pigment in the retina, and to adapt and to measure retinal sensitivity by the electroretinogram response for a light-intensity range of 7-8 log units.

Most of the retinæ maintained in these conditions remained viable and gave good electroretinogram responses for experiments lasting 4-6 h. In preliminary experiments with another chamber, opened on one side for the insertion of a microelectrode, we recorded the spike activity of single ganglion cells for periods of several hours, and these responses also seem to be entirely normal. The electroretinograms recorded from properly maintained retinæ *in vitro* appear almost identical with those recorded from intact animals. Typical responses from a dark adapted and then partially light adapted preparation are shown in Fig. 2. From the dark adapted retina (Fig. 2, column *a*) only the vitreal-positive *b*-wave is recorded with stimuli of low intensity; stimuli of higher intensity are needed to record the vitreal-negative *a*-wave preceding the *b*-wave. With bright stimuli, large *a*-waves are

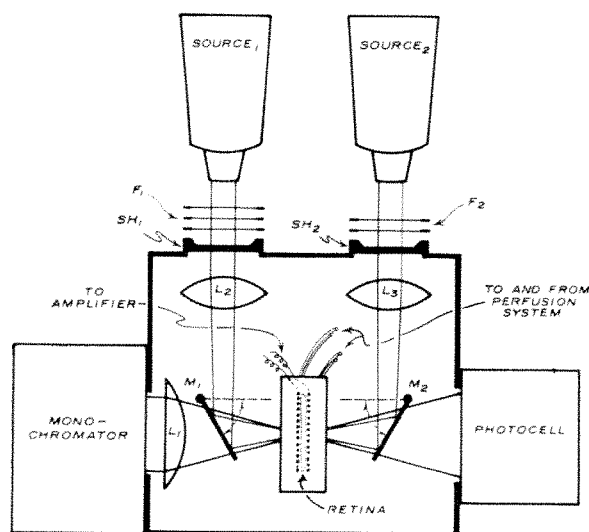


Fig. 1. Schematic arrangement of the perfusion chamber in the spectrophotometer. When mirrors ( $M_1$  and  $M_2$ ) are in down position (solid lines), adapting (left) and stimulating (right) lights are focused on the retina, which is mounted vertically in the chamber. With the mirrors in the up position (dotted lines), the monochromator beam passes through the specimen into the photocell compartment. Light intensity is controlled by neutral density filters ( $F_1$  and  $F_2$ ), and duration by camera shutters ( $SH_1$  and  $SH_2$ ). The spectrophotometer beam is condensed by lens ( $L_1$ ), the adapting and stimulating beams by lenses ( $L_2$  and  $L_3$ ). The specimen is perfused through flexible tubing leading to and from the perfusion system; leads from the tantalum grids, which support the retina, are connected to the recording amplifier.

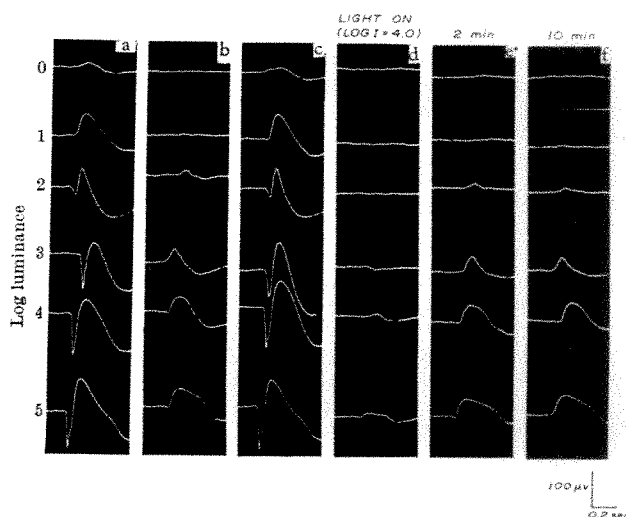


Fig. 2. Electroretinograms from a dark adapted and partially light adapted isolated retinal preparation. (*a*) Electroretinograms from retina in the fully dark adapted state showing increasingly larger responses to brighter light stimuli, the intensity of which is given in log units above the criterion response. Vitreal-positive *b*-wave is shown as an upward deflection, vitreal-negative *a*-wave as a downward deflection. (*b*) Decrease in sensitivity produced by a weak adapting light (log  $I = 2$ ). Threshold response is not achieved until the stimulus light is about 2 log units brighter than that required to produce the criterion response in the dark adapted state. (*c*) Complete recovery of sensitivity 2 min after the weak adapting light is extinguished. (*d*) Greater loss of sensitivity with brighter adapting light on (log  $I = 4$ ). (*e*) Partial recovery of sensitivity 2 min after extinction of the bright adapting light. The criterion response is obtained with a stimulus 2 log units brighter than that required for the dark adapted retina. The responses of column *f* are identical with those of column *e*, showing no further recovery of sensitivity after an additional 10 min of dark adaptation. All records are responses to 1/50 sec flashes.

evoked from the isolated retina, and often a larger *a*-wave (relative to the size of the *b*-wave) can be evoked from the isolated retina than from the eye of an intact animal. No *c*-wave is seen in such preparations, but a *c*-wave can seldom be evoked from the intact eye of an albino rat<sup>12</sup>. With preparations which are not satisfactorily maintained, the *b*-wave selectively disappears and the *a*-wave dominates the electroretinogram response. This is readily detected; when it occurs, the preparation is abandoned.

When background light is shone on a well maintained preparation, the electroretinogram rapidly loses sensitivity and the responses are considerably reduced in amplitude (Fig. 2, columns *b* and *d*). With dim adapting lights, the sensitivity and size of the electroretinogram response quickly recover to the dark adapted condition when the background light is extinguished (Fig. 2, column *c*). With brighter background lights, however, only partial recovery is noted when the adapting light is extinguished. This partial recovery is quickly accomplished; no slow component of dark adaptation is seen in time, such as is observed from an intact eye of a rat<sup>9</sup>. In Fig. 2, columns *e* and *f*, electroretinograms were recorded 2 and 10 min after the extinction of the bright background light. No changes in sensitivity, form or size of the responses were observed during this period.

Fig. 3 shows a record of absorption curves from a viable isolated retina which was dark adapted and then light adapted with background light of various intensities. The broad absorption band of rhodopsin, with a peak at about 500 nm, is clearly seen on the record. Curves 1-6 are recordings made with the retina dark adapted, and after exposure to dim background light that did not significantly reduce the concentration of visual pigment (see legend to Fig. 3 for details). All these curves are essentially identical. Curves 7-12 are runs made after exposures to adapting lights that bleached measurable fractions of the visual pigment. After each 5 min adapting exposure, two runs were made; the first was made 4-8 min after the light adaptation period; the other 20-30 min after the adaptation. In each case the pair of curves is

essentially identical, showing that no regeneration of visual pigment occurs in the living isolated retina. Curves 13 and 14 are runs made after a complete bleach of visual pigment by 10 min of exposure to the brightest light available. Further light adaptation beyond this does not significantly change the absorption curve of the retina. The total change of absorbance from dark adapted retina to light-adapted retina was 0.23. In eight experiments, we obtained an average absorbance of rhodopsin of 0.25.

If we assume that rat rhodopsin has a molar absorbance similar to cattle rhodopsin (40,600) (ref. 14), and that the rhodopsin in the intact rat rod is oriented and has an absorbance 1.5 times that of rhodopsin in solution<sup>15</sup>, then the measured absorbance of rhodopsin in the isolated rat retina corresponds to a concentration of  $4.2 \times 10^7$  molecules of rhodopsin in each rod. Previous estimates from extracts of the rat retina suggested that there are  $3.2 \times 10^7$  molecules of rhodopsin in each rod<sup>16</sup>.

Figs. 4 and 5 present combined physiological and biochemical data from two typical experiments. The only procedural difference between the experiments was that in one experiment (Fig. 4) the adaptation period was 2 min, whereas in the other (Fig. 5) a 5 min adaptation period was used. The open circles and the heavy line indicate the loss of sensitivity or rise of threshold (increment sensitivity) while background light is on. For these experiments, sensitivity (or threshold) was determined by evaluating the intensity of light necessary to evoke a *b*-wave of 20  $\mu$ V. Except with the very dimmest background light, the loss of sensitivity is linearly proportional to the intensity of the background light (log *I*). This is the well known Weber-Fechner relation. A similar relation is seen in the intact rat, but with a slightly lower slope; *in vitro*, the slope of the line is usually about 1;

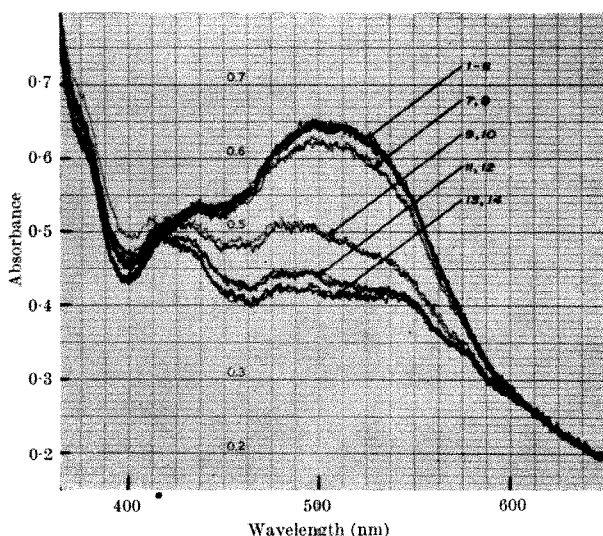


Fig. 3. Absorption curves of viable isolated retina in dark adapted and increasingly light adapted states. Curves 1 and 2 were run with retina fully dark adapted. Curve 3 was made 3 min after 5 min exposure to weak adapting light (log *I* = 1.6). Curves 4 and 5 were made 7 and 10 min respectively after exposure to a somewhat stronger adapting light (log *I* = 3.4). Curve 6 was made 17 min after extinction of that adapting light and 45 min after curve 1. Curves 1 to 6 are virtually identical, indicating that no measurable amount of photopigment has been bleached so far. Curve 7 was run 7 min after a 5 min exposure to a fairly bright adapting light (log *I* = 4.8). Curve 8, superimposed on curve 7, was run 20 min later (27 min after the adapting light was turned off.) Curves 7 and 8 are significantly lower than curves 1-6 at 500 nm, showing that a measurable amount of rhodopsin has been bleached, and that there has been no regeneration of rhodopsin in this time period. Curves 9 and 10 were made 6 and 26 min respectively after exposure to a brighter adapting light (log *I* = 5.9). The two curves are again identical, and show a change of absorbance of 0.145 from the dark adapted curves. Curves 11 and 12 were made 7 and 20 min after a 5 min exposure to the brightest adapting light (log *I* = 7.0) and indicate a further change in absorbance. Curves 13 and 14 were run after a 10 min exposure to both adapting and stimulating lamps, and show the absorption of the tissue in the fully bleached state. The net absorbance change of rhodopsin in this preparation was 0.23.

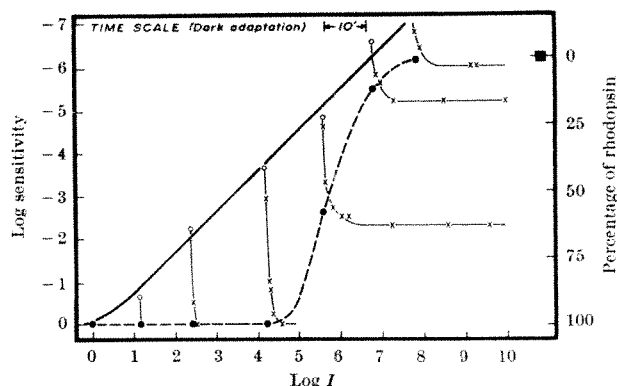


Fig. 4. The effect of 2 min of light adaptation on increment sensitivity (○), rhodopsin content (●) and dark adaptation (×; thin lines). Intensity of adapting and stimulating lights was varied by interposed neutral density filters calibrated at 500 nm. The criterion response was 20  $\mu$ V. As the retina is exposed to brighter adapting lights (abscissa), brighter stimuli (left ordinate) are required to elicit the criterion response (increment sensitivity). These measured points (○) lie on a straight line with a slope of about 1.0 (Weber-Fechner relationship). After the adapting light is extinguished, the sensitivity rapidly increases (×; thin lines). If the adapting lights are dim the recovery is complete; with bright adapting lights, recovery is incomplete and there is an irreversible loss of sensitivity. The level of recovery after a bright light adaptation correlates closely with the content of rhodopsin (●, right ordinate) left in the eye after the 2 min adaptation period. See text for details. The scale for rhodopsin was set by equating the final level of sensitivity (●) with 0 per cent rhodopsin. A 10 min time scale for dark adaptation is indicated at the top of the graph.

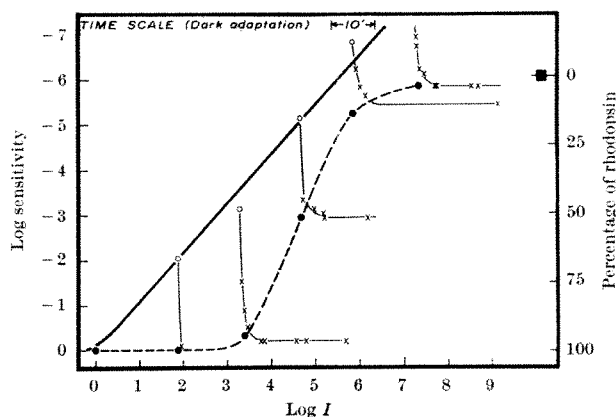


Fig. 5. The effect of 5 min of light adaptation on increment sensitivity, rhodopsin content, and dark adaptation. This experiment is identical to that of Fig. 4, except for the longer period of light adaptation.

in the intact animal it is closer to 0.8. We have no explanation for this slight difference.

When background light is first turned on, the increment sensitivity is established too quickly to be adequately measured by our electroretinogram techniques. This also occurs with an intact animal<sup>9</sup>. When the background light is extinguished, the sensitivity of the preparation rapidly increases (Figs. 4 and 5: crosses, thin lines). If no rhodopsin has been bleached by the adapting light, the recovery of sensitivity is complete. If some rhodopsin has been bleached, the sensitivity returns to an intermediate level and remains there. The rapid increase of sensitivity takes 1-3 min after the adapting light is extinguished. Thereafter, no further recovery is noted, whatever the level which the sensitivity reaches. In Fig. 4 sensitivity levels in some cases were determined for 40 min after extinction of the adapting light, but there was no improvement in absolute sensitivity after completion of the initial fast-recovery phase of dark adaptation. We define absolute sensitivity as the stable sensitivity which the retina reaches in the dark after a light adaptation.

Also shown in Figs. 4 and 5 are the amounts of rhodopsin remaining in the retina after exposure to the various adapting intensities. The scale for the rhodopsin was set

by equating the dark adapted sensitivity with 100 per cent rhodopsin, and equating the sensitivity measured after a complete bleach (filled square) with 0 per cent rhodopsin. The points indicating concentrations of rhodopsin are the averages of runs made immediately after the adaptation period and runs after completion of the physiological measurements (20–30 min later). Usually, no changes in absorption were noted between the two runs. Occasionally, however, the second run showed slightly less absorption, which was perhaps caused by a slight fall of the base line or by the fading of a persisting orange photoproduct. With 2 min of bleaching there was a greater tendency for second runs to be slightly lower (absorbance differences of about 0.01–0.2 at 500 nm), which perhaps favours the latter hypothesis.

In both experiments it can clearly be seen that the absolute sensitivity after the adaptation period is closely related to the amount of rhodopsin bleached. In Fig. 6 another experiment is illustrated in which only one adapting intensity was used ( $\log I = 7$ ) but for varying periods of time. Increasing the duration of the light adaptation period caused bleaching of a larger fraction of the visual pigment, and correspondingly decreased the absolute level of sensitivity. In Fig. 7 is a comparison of the relation of concentration of pigment to the absolute level of sensitivity in the three experiments illustrated. This shows a linear relation between the logarithm of the absolute sensitivity and the concentration of visual pigment. The range of sensitivity in the span from 0 to 100 per cent rhodopsin is about 6 log units. This is a somewhat greater sensitivity range than that found in the intact rat, which is about 5.2 log units<sup>9</sup>. It is, however, likely that a more complete bleach of the pigment was obtained in the present *in vitro* experiments.

These experiments with the isolated retina confirm and extend previous results and interpretations concerning the

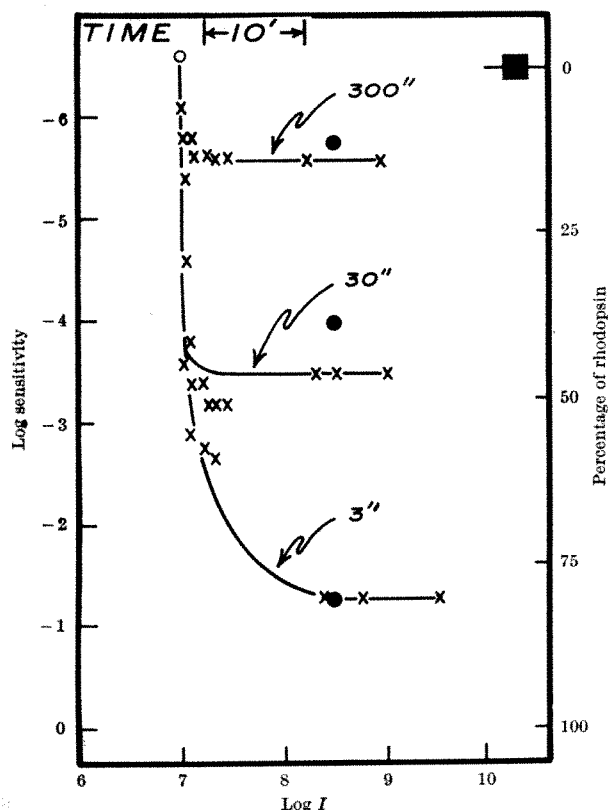


Fig. 6. The effect of varying durations of light adaptation on dark adaptation (x; thin lines) and rhodopsin content (●). A single intensity of light ( $\log I = 7$ ) was used for 3, 30, and 300 sec adapting periods. The increment sensitivity (○) was determined during the latter two periods only, and was the same for each. Sensitivity and rhodopsin content irreversibly decreased during each period.

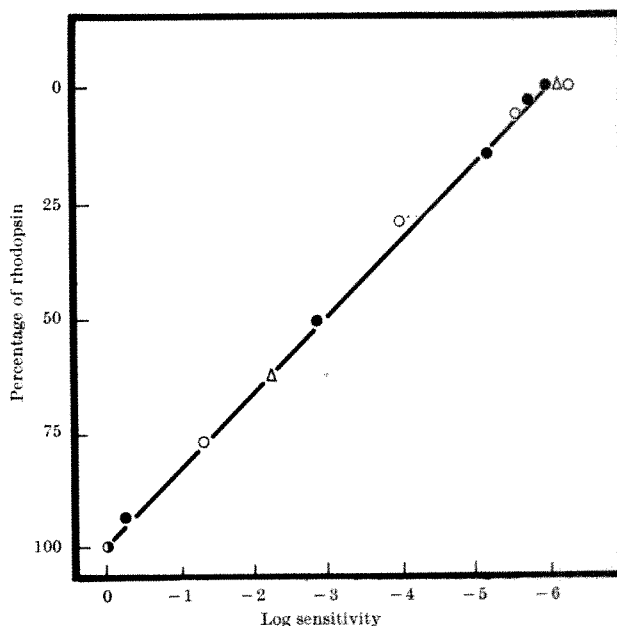


Fig. 7. Relation of pigment concentration to absolute sensitivity. Data from the three experiments above have been combined. This shows a linear relation between concentration of rhodopsin and the logarithm of the sensitivity.

phases of light and dark adaptation. The loss of sensitivity during light adaptation is linearly related to the logarithm of the intensity of the adapting light, for most of the adaptive range of the eye. With dim adapting lights which do not bleach a measurable fraction of the rhodopsin, the eye completely and rapidly recovers its dark adapted sensitivity when the adapting light is extinguished. If bright lights that bleach pigment are used for adaptation the recovery is not complete; instead, the sensitivity rapidly increases to an intermediate level, where it remains.

In summary, light adaptation and the fast component of dark adaptation behave independently of visual pigment levels and thus appear to be mediated by non-photochemical or neural mechanisms. On the other hand, the sensitivity which the isolated retina reaches after the fast phase of dark adaptation (absolute sensitivity) is determined by the concentration of rhodopsin remaining in the rods. Thus, this and later portions of dark adaptation are clearly photochemical. In the intact eye, rhodopsin slowly regenerates, with a corresponding recovery of log sensitivity<sup>9</sup>. *In vitro*, with no regeneration, the sensitivity remains decreased.

Donner and Reuter<sup>17</sup> have suggested from experiments on the frog that the relation during slow dark adaptation is not between the concentration of rhodopsin and log sensitivity, but rather between the logarithm of the rate of rhodopsin regeneration and log sensitivity. These authors postulate that this relation may hold also for the rat and human eye. Our experiments, however, exclude this possibility for at least the (primarily rod) retina of the rat, because in the isolated rat retina there is no regeneration, yet there is a close relation between the amount of visual pigment and absolute sensitivity. By Donner's and Reuter's formulation, we should not observe any loss of sensitivity after a bleaching adaptation in the present experiments, because we have no rate of regeneration to raise the threshold. This is clearly not the case, and our results confirm earlier work that the relation during photochemical adaptation is between the concentration of rhodopsin in the retina and the logarithm of visual sensitivity.

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## Disappearance of Angiotensin from the Circulation of the Dog

by

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Measurements of the rate of disappearance of angiotensin in the blood have previously given contradictory results, with half-lives varying from a few minutes to 37 hours. It is now clear that the blood itself plays little part in the inactivation of angiotensin and that the rapid removal of angiotensin from the bloodstream occurs in the tissues through which it is carried.

RENIN, an enzyme liberated into the blood stream from the kidneys, acts on an alpha-2-globulin in plasma to produce the decapeptide angiotensin I which in turn is converted by another enzyme to the octapeptide angiotensin II (ref. 1). The physiological and pharmacological actions of the renin-angiotensin system such as increase in blood pressure, release of aldosterone<sup>2</sup>, and of catecholamines<sup>3,4</sup> reside in angiotensin II (ref. 5). It is not clear how the activity of angiotensin II in the body is terminated. The inactivation of angiotensin by enzymes in blood has been extensively studied *in vitro*<sup>6,7</sup> as has its inactivation by homogenates of tissues such as intestinal mucosa, kidney, pancreas, spleen and liver<sup>8</sup>. The inactivation of angiotensin II has been studied *in vivo* either by injecting isotopically labelled angiotensins<sup>9,10</sup> or by using the pressor effect of the angiotensin II as a bioassay after the substance has passed through a particular vascular bed<sup>11,12</sup>. One of these methods<sup>9</sup> puts the half-life of angiotensin II *in vivo* between 10 and 37 h, a surprising figure in view of a 70 per cent removal in one circulation through the liver<sup>11,12</sup>.

We have used the blood-bathed organ technique<sup>13</sup> for continuous assay of angiotensin II in the circulation of dogs<sup>14,15</sup> and have estimated the half-life of some angiotensins in the circulating blood. We have also studied the removal of angiotensin II by a number of vascular beds. Some of these results were presented to a meeting of the British Pharmacological Society on April 7, 1967.

The assay organs were a rat stomach strip<sup>16</sup> and two rat colons<sup>14</sup>; these were suspended in polypropylene chambers and superfused<sup>17</sup> in series with Krebs solution while the dog was being prepared. Their movements were recorded on a kymograph by auxotonic levers<sup>18</sup> which produced a magnification of 16:1 with a load of 1-3 g on the tissues. All three organs were contracted by angiotensin, the rat colons much more strongly than the rat stomach strip. The latter was included in the assay system to detect catecholamines which, if released, would relax it<sup>19</sup>. One of the rat colons was perfused intraluminally with propranolol (1 mg/ml. at 0.1 ml/min) to prevent any relaxant effects of catecholamines which would otherwise interfere with the contraction induced by angiotensin.

Dogs of either sex weighing 5-31 kg were anaesthetized with halothane delivered from a Goldman vaporizer; anaesthesia was then maintained with chloralose (100 mg/kg intravenously) and supplemented when necessary with pentobarbitone (5-10 mg/kg, either intramuscularly or intravenously). The trachea was cannulated and the lungs were ventilated mechanically. Polyethylene cannulae were tied into a femoral or carotid artery and a femoral or jugular vein for removal and replacement of blood. Mean arterial blood pressure was also recorded on a kymograph with a mercury manometer attached to a side arm of the arterial cannula.

Heparin (1,000 IU/kg) was injected intravenously and the assay organs were then superfused with arterial blood delivered by a roller pump of constant output at 15 ml/min. The blood then collected in a reservoir and was returned to the dog either by gravity or by a second channel in the roller pump. The assay system could be calibrated by infusing angiotensin II into the stream of blood after it had left the dog; thus an infusion of 15 ng/min into a flow of 15 ml/min would give an absolute change of concentration of 1 ng angiotensin/ml. The assay system could also be calibrated by intravenous or intra-arterial injections into the dog, as described later.

### Inactivation of Angiotensin in Circulating Blood

The inactivation of angiotensin by blood was measured by passing the blood through a length of silicone tubing of 3 mm internal bore and 45 ml. capacity immersed in a water bath maintained at a temperature of 37°C. The arterial blood first circulated through this incubating circuit and then superfused the assay organs (Fig. 1). Thus, by infusing angiotensin at different points in the incubating circuit the drug would be in contact with the blood for known periods of time before reaching the assay tissues. For example, the responses of the rat colons to the same rate of infusion of angiotensin incubated with the blood for 1, 2 or 3 min could be compared with, and bracketed between, infusions of angiotensin given close to the tissues. The inactivation could then be calculated by plotting dose/response curves.

The assay system was sensitive to changes in the concentration of angiotensin of from 0.25 to 5 ng/ml. blood



Table 1. HALF-LIFE OF THREE ANGIOTENSINS

Expt.	Synthetic Asp <sup>1</sup> -NH <sub>2</sub> -Val <sup>5</sup>	Horse and Hog Asp <sup>1</sup> -Ileu <sup>5</sup>	Bovine Asp <sup>1</sup> -Val <sup>5</sup>
1	190	190	—
2	117	174	180
3	97	156	260
4	180	212	405
Mean	146	183	282

Values (in sec) and means refer only to the four dogs in which these multiple estimations were made. Further estimations with the synthetic angiotensin amide alone give an overall value (mean  $\pm$  S.E.) of 113 sec  $\pm$  10.7.

and the half-life of the angiotensins in the blood was measured within this physiological range<sup>20</sup>. Three angiotensins were used: synthetic angiotensin II amide (Asp<sup>1</sup>-NH<sub>2</sub>-Val<sup>5</sup>-angiotensin II; 'Hypertensin', Ciba), subsequently referred to as synthetic angiotensin; the natural horse and hog angiotensin (Asp<sup>1</sup>-Ileu<sup>5</sup>-angiotensin II) and the natural bovine angiotensin (Asp<sup>1</sup>-Val<sup>5</sup>-angiotensin II). A typical assay in which the half-life of synthetic angiotensin was estimated is illustrated in Fig. 2. At first, calibrating infusions of 40, 80 and 40 ng/min were given close to the tissues. An infusion of 80 ng/min incubated with the blood for 180 sec gave the same response as 40 ng/min given close to the tissues. When 80 ng/min was incubated with blood for 120 sec the responses of the tissues were between those given by 40 and 80 ng/min in the close position. Thus, in this experiment, the half-life of synthetic angiotensin was 180 sec.

In four experiments the half-lives of three different angiotensins were estimated (Table 1). Ileu<sup>5</sup>-angiotensin had a longer half-life (mean of 183 sec) than synthetic angiotensin (146 sec) and Val<sup>5</sup>-angiotensin had an even longer half-life (282 sec). Although there was considerable variation from dog to dog in the values of the half-lives, the same general pattern was observed in each individual experiment. Insufficient samples of Ileu<sup>5</sup>- and Val<sup>5</sup>-angiotensin were available to do more comparative experiments. The half-life of synthetic angiotensin in blood was, however, measured in eleven more experiments. The overall mean value was 113 sec (standard error of mean  $\pm$  10.7). In three of these dogs, the half-life was measured three times during the experiment and the same result was obtained each time. Thus the half-lives appear to vary from dog to dog but not in the same dog. When synthetic angiotensin was incubated for 180 sec with Krebs solution in the external circuit instead of blood, there was no loss of activity, showing that the inactivation was due to blood. In one experiment, the incubation circuit was cooled to 5°C and the blood re-heated before superfusing the tissues. The inactivation of synthetic angiotensin was substantially reduced.

When incubated *in vitro* with rat plasma<sup>6</sup>, synthetic angiotensin had a half-life of less than 120 sec, and that of Val<sup>5</sup>-angiotensin was longer. When incubated with

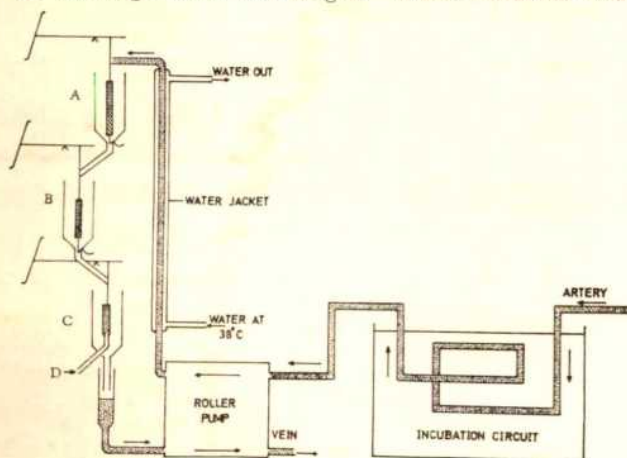


Fig. 1. Blood-bathed organ technique. The diagram shows the assay tissues and the incubation circuit for the estimation of the half-life of angiotensin in blood. The rate of flow of blood through the external circuit is 15 ml/min. A, Rat stomach strip; B, rat colon; C, rat colon ( $\beta$ -blocked); D, propranolol in.

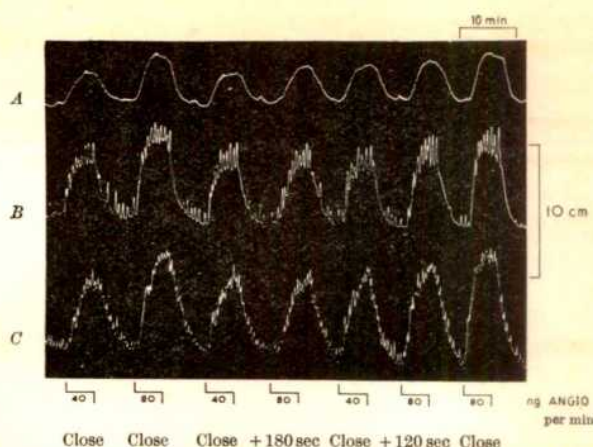


Fig. 2. Inactivation of synthetic angiotensin by circulating blood from a 10 kg female dog anaesthetized with chloralose. Carotid arterial blood was superfused at 15 ml/min over a rat stomach strip (A) and two rat colons (B and C). The angiotensin was infused either close to the tissues or into the incubation circuit to allow contact with blood for two periods of time. Incubation of 80 ng/min for 180 sec reduced the activity to that of 40 ng/min, whereas incubation for 120 sec gave responses intermediate between 40 ng/min and 80 ng/min. In this experiment, the rat stomach strip was unusually responsive to angiotensin.

human plasma, however, synthetic angiotensin had a half-life of 10–20 min<sup>21</sup>.

### Disappearance of Angiotensin in One Circulation through Various Vascular Beds

When angiotensin is infused into the arterial supply to a vascular bed, some of it disappears from the blood and the remainder escapes into the venous effluent, thereby reaching the general circulation. This angiotensin can be assayed by matching the responses of the blood-bathed organs with those produced by infusions made directly into the blood leaving the vascular bed. For example, when an infusion of 1  $\mu$ g/min into the arterial supply to a particular vascular bed gave the same response on the blood-bathed rat colon as an infusion of 0.5  $\mu$ g/min into its venous effluent, 50 per cent of the angiotensin had been removed in one circulation. In all these experiments sufficient time was allowed for equilibrium conditions to be established, as shown by the assay organs reaching a plateau. The disappearance of synthetic angiotensin was studied in the head, the lungs, the liver, the kidneys, a hindquarter and the lower half of the body. For all these experiments, carotid arterial blood was sampled for the assay system except in experiments on the lungs and head, when femoral arterial blood was used.

**Lungs.** For these experiments a catheter was inserted retrogradely through the right carotid artery so that its tip lay in the left ventricle. Infusions of synthetic angiotensin were made either through this catheter, or into the superior vena cava, thus allowing an estimation of the disappearance of synthetic angiotensin in the pulmonary circulation. One of four such experiments is shown in Fig. 3. Angiotensin was infused at two different rates (0.5 and 1  $\mu$ g/min intra-arterially and intravenously); the responses of the assay organs show that no angiotensin was lost in the pulmonary circulation. Indeed, in none of the four experiments could we detect any loss of angiotensin in the pulmonary circulation. This result contrasts strikingly with the 80–90 per cent removal of bradykinin infused into the pulmonary circulation<sup>22</sup>.

**Head.** A carotid artery was divided and both ends cannulated. The circulation was re-established with a loop of silicone tubing through which infusions were made into the carotid arterial circulation; the other carotid artery was tied. These infusions of angiotensin were compared with those made into a jugular vein. In three experiments, one of which is shown in Fig. 4, 40, 50 and 50 per cent of the infused angiotensin disappeared.



Table 2. DISAPPEARANCE OF ANGIOTENSIN II AMIDE IN VARIOUS VASCULAR BEDS

Vascular bed	No. of experiments	Disappearance mean $\pm$ S.E. (%)
Lungs	4	0
Head	3	47
Liver	7	62 $\pm$ 4.3
Hindquarter	5	71 $\pm$ 6.7
Kidneys	10	75 $\pm$ 4.1
Body below diaphragm	10	76 $\pm$ 4.1

**Liver.** A catheter was inserted into a splenic vein and advanced so that its tip lay in the portal vein. Infusions of angiotensin into the portal circulation were then compared with infusions into the superior vena cava (Fig. 4). In seven experiments 62 per cent (standard error of mean  $\pm$  4.3) of the infused angiotensin disappeared in the portal circulation.

**Hindquarter.** A catheter was introduced retrogradely through a ligated femoral artery so that its tip lay just above the bifurcation of the aorta. The venous catheter was introduced into the femoral vein on the same side and advanced the same distance as the arterial catheter. In five experiments 71 per cent (standard error of mean  $\pm$  6.7) of the infused angiotensin disappeared in the hindquarter (Fig. 4).

**Kidneys.** Renal arterial infusions were given in one of two ways. In the first, fine catheters or needles were inserted directly into the exposed renal arteries. In the second, a polyethylene catheter was inserted through a femoral artery and advanced up the aorta so that its tip lay just above the renal arteries; the aorta was then ligated around the catheter just below the renal arteries. In ten experiments there was a 75 per cent (standard error of mean  $\pm$  4.1) disappearance when angiotensin was infused into the kidneys (Fig. 4).

**Body below diaphragm.** To find whether the intestines or other abdominal vascular beds contributed to the removal of angiotensin from the circulation, a catheter was advanced up a femoral artery until its tip lay at the level of the diaphragm. Infusions through this catheter thus reached all vascular beds supplied by the abdominal aorta. In ten experiments the disappearance of angiotensin was 76 per cent (standard error of mean  $\pm$  4.1). The results from all the vascular beds studied are summarized in Table 2.

Our results show that synthetic angiotensin is inactivated relatively slowly in blood and that the natural angiotensins are inactivated even more slowly. Although the nature of dog angiotensin is not known it is unlikely to be inactivated very differently from those already tested. If angiotensin was removed only by enzymes in blood, these half-life values would mean that it would recirculate between ten and twenty times before the activity was reduced to half (assuming a circulation time in dogs

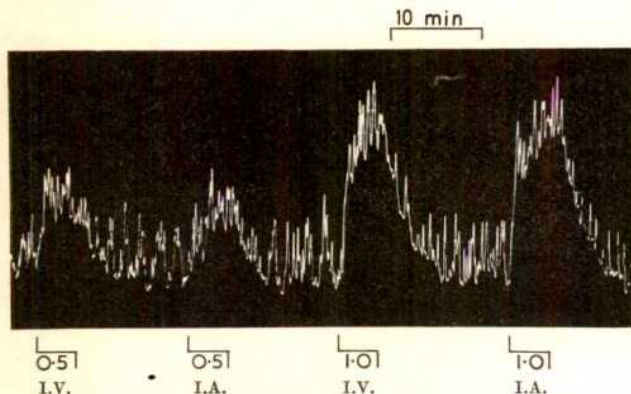


Fig. 3. The failure of synthetic angiotensin to disappear in the pulmonary circulation of a 6 kg dog anaesthetized with chloralose. Femoral arterial blood was superfused over a rat stomach strip and two rat colons. This record is from one of the rat colons. Infusions were made near to the right atrium intravenously (I.V.) or into the left ventricle intra-arterially (I.A.). The responses at each rate of infusion show that there was no loss of angiotensin in the passage through the pulmonary circulation.

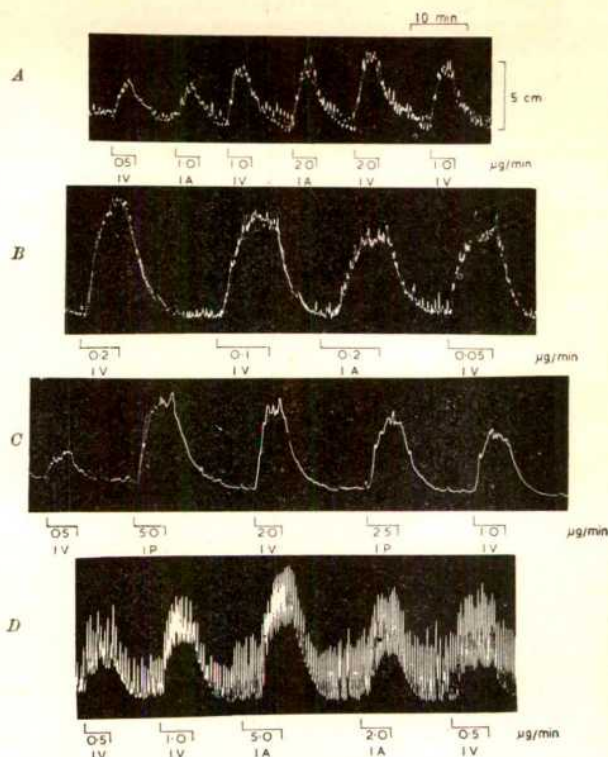


Fig. 4. Disappearance of synthetic angiotensin in some vascular beds. Each panel is from a separate experiment and shows the responses of a rat colon superfused with arterial blood. The estimation of disappearance has been made by comparing responses to infusions on either side of each vascular bed. The disappearance was: 50 per cent in the head (A), because an infusion of 2.0  $\mu$ g/min into the carotid artery (I.A.) gave a response matched by 1.0  $\mu$ g/min into the jugular vein (I.V.); 75 per cent in the hindquarter (B) because an infusion of 0.2  $\mu$ g/min into the femoral artery (I.A.) was matched by an infusion of 0.05  $\mu$ g/min into the inferior vena cava (I.V.); 60 per cent in the liver (C), because an infusion of 2.5  $\mu$ g/min into the portal vein (I.P.) corresponded to between 1.0  $\mu$ g and 2.0  $\mu$ g/min into the superior vena cava (I.V.); 65 per cent in the kidneys (D), because an infusion of 2.0  $\mu$ g/min into the renal arteries (I.A.) corresponded with between 0.5  $\mu$ g/min and 1.0  $\mu$ g/min into the inferior vena cava (I.V.).

of 15 sec (ref. 23)). 47–76 per cent of infused angiotensin, however, disappears in one circulation in all the vascular beds studied. It would therefore appear that the half-life of angiotensin is about one circulation time. Using a different technique Biron, Mayer and Panisset (personal communication) have also found that 50–90 per cent of angiotensin infused into various vascular beds disappears. Similar results have been found in man, where a 70 per cent loss of angiotensin across the vascular beds of the limbs was reported in one study<sup>24</sup> and an almost complete loss between arterial and peripheral venous blood in patients with renal vascular disease in another<sup>25</sup>.

One of the physiological implications of these results is that the inactivation of angiotensin by blood is much less important in the termination of its activity in the body than its removal by vascular beds. It is also interesting that neither the liver nor the kidneys are any more efficient in removing angiotensin than, for example, the hindquarter.

The present results do not show what happens to the angiotensin after it is removed from the circulation by the vascular beds. The angiotensin which disappears in a vascular bed may not be entirely destroyed but only removed temporarily, to re-enter the circulation at a later stage. In this regard, it would be interesting to compare the rate of decline of angiotensin in the circulation with the rate of decline of its effect in a particular vascular bed, such as the hind legs.

Finally, the negligible removal of angiotensin by the lungs indicates that the pulmonary circulation is extremely selective with regard to the substances which are allowed through it into the arterial circulation. Another peptide, bradykinin<sup>22</sup>, is largely removed by the pulmonary circulation, as are 5-hydroxytryptamine and the prostaglandins.



That adrenaline and angiotensin are not removed by the lungs may be related to their physiological role as circulating rather than as local hormones.

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## Causes and Effects of Columnaris-type Diseases in Fish

Diseases affecting salmon, sea-trout and coarse fish and characterized by the occurrence of skin lesions have been recognized for some time in the rivers of England, Scotland and Ireland. During 1966 there were epidemics in parts of Ireland as well as Cumberland, Lancashire and southern counties of England. The British and Irish governments intervened to control the movement of live fish and eggs from infected areas, and a programme of research was begun to identify the causative organisms and the nature of the diseases. The three communications which follow describe some of the results obtained so far in government-sponsored and independent research.

### Columnaris Disease in Roach and Perch from English Waters

DAVIS<sup>1</sup> described an infectious disease caused by a rod-shaped bacterium with an unusual mode of motility, which was responsible for heavy mortalities among warm-water fishes of the Mississippi Valley. He observed that when scrapings from dermal lesions of diseased fish were placed in a drop of water on a slide, the bacteria collected on the edges to form short column-like masses. He called the causal organism *Bacillus columnaris* and the syndrome columnaris disease. Davis failed to culture the organism, but Ordal and Rucker<sup>2</sup> and Garnjobst<sup>3</sup> both claimed to have isolated an organism from diseased fish similar to that originally described by Davis. They agreed to place the organism in the order Myxobacteriales. Ordal and Rucker<sup>2</sup> described the occurrence of fruiting bodies and spherical to oval microcysts in their cultures on tryptone-agar. Thus they assigned the species to the family Myxococcaceae Jahn and the genus *Chondrococcus*, and named it *Chondrococcus columnaris*. Garnjobst<sup>3</sup>, on the other hand, could not demonstrate the production of microcysts; she therefore placed the organism in the family Cytophagaceae Stanier and named it *Cytophagacolumnaris*.

Anacker and Ordal<sup>4,5</sup> investigated the serological and bacteriocin typing of the organism and quoted several reported incidents of columnaris disease in fish in the United States<sup>6-8</sup>. Although striking differences in virulence were noticed between various strains isolated during several years<sup>9</sup>, water temperature was considered to be an important factor in determining the amount of damage caused by the organism in the fish population. Most outbreaks have been reported during summer months<sup>4</sup>.

During the summer of 1966 freshwater fish in the English rivers suffered considerable mortalities. A number of fish caught from different rivers were examined in this

laboratory and we report here observations of columnaris disease in English roach and perch.

Strains of the organism isolated from fish affected in the outbreak described in this paper were examined by Professor E. J. Ordal and Dr T. Gibson. Ordal (personal communication) found them indistinguishable from the organism he had described as *Chondrococcus columnaris*. Four cultures were sent to him and he demonstrated the complete genetic relationship to his own strains by the method of DNA homology. He did not determine whether the cultures produced the structures interpreted by him as fruiting bodies. Gibson (personal communication) was unable to demonstrate the presence of fruiting bodies or microcysts by the methods that he used, and therefore suggests that the organisms should be placed in the genus *Cytophaga*, but he does not discount the possibility that other conditions could give different results or that the culture he examined could be a mutant with impaired functions. For the purpose of this paper we refer to the organism as *Chondrococcus columnaris* (Davis) Ordal and Rucker, or *Cytophaga columnaris* (Davis) Garnjobst. *C. columnaris* will thus be used to cover both names for the same organism.

In the early stage of the disease there were small haemorrhagic patches, usually on the ventral surface at the base of the fins. The soft inter-ray tissues of the fins and tail showed congestion. Haemorrhagic lesions were also seen around the mouth and sometimes the cartilaginous structures in the area of the head were attacked. Some fish, presumably in the later stage of the disease, showed deep ulcerated lesions with disintegration of the muscular and cartilaginous tissue. Gills were usually normal. In some instances there was evidence of pathological changes in the liver and kidney, particularly the former.

Direct smears from clean haemorrhagic lesions showed long flexuous Gram-negative rods. In some instances a few rounded bodies, probably involution forms, could be seen

A modified Ordal's medium consisting of 0.5 per cent 'Bacto-tryptone' (Difco) and 0.8 per cent agar (Davis) was used for the isolation and cultivation of the organism. The pH of the medium was usually about 7.1 after preparation and did not need to be adjusted; over-dried plates seldom proved to be satisfactory. The organism was easily isolated from haemorrhagic lesions free from macroscopic evidence of ulcerative or necrotic changes, that is, from the attachment of the scale or from a cut surface at the base of the affected fin, and not merely from a swab of the surface. The organism was isolated from the liver and kidney of two diseased fish only. The organism was not isolated from any other internal organ.

*C. columnaris* colonies developed after 48 h of incubation at 22° C or at room temperature on the bench; there was no growth at 37° C. The colonies on tryptone-agar medium were golden yellow to orange in colour, varying in size from tiny yellow specks to larger branching colonies (approximately 3 to 4 mm in diameter). The growth stuck tenaciously to the agar and the colonies were so embedded in the medium that usually small chunks of agar had to be removed along with the colony to ensure growth in sub-cultures. The colony could not be emulsified easily and under the plate microscope it showed branching outgrowths with a central focus. The growth of *C. columnaris* was adversely affected in the inoculated area of the plates if there was heavy growth of contaminants. A Gram-stained smear revealed long, slender rods approximately 0.5  $\mu$  wide and 5  $\mu$  or more long, arranged characteristically to give a false branching appearance; short and rounded bodies were also seen. The organism was catalase and oxidase positive, and produced small amounts of hydrogen sulphide in 0.5 per cent tryptone water. No growth occurred in the usual peptone sugar media.

Fish and Rucker<sup>9</sup> described columnaris disease in salmonid fingerlings in which the fin lesions were accompanied by pronounced hyperplasia of the gill tissues. In the present case, the lesions seemed to be confined to the fins and tail only; gills were not affected although the tissues were not examined histopathologically.

The constant pattern of isolation of this organism from the diseased fish that we examined, and its close resemblance to the NCMB 1038 strain called *Chondrococcus columnaris*, form the basis of this report. Pathogenicity experiments were not carried out, but the many reports in the literature support the role of *C. columnaris* as a fish pathogen. Both Ordal and Rucker<sup>2</sup> and Garnjobst<sup>3</sup> described successful pathogenicity experiments with fish.

Aeromonads (*Aeromonas liquefaciens*) and Pseudomonads were isolated in many instances from the badly ulcerated lesions, often in large numbers. The significance of these organisms in diseased fish, however, was regarded as secondary rather than primary. Their presence in early developing lesions was rare; in such lesions *C. columnaris* was the predominant organism.

We thank Professor E. J. Ordal, Dr T. Gibson and Dr S. P. Lapage for their help. We also thank the Fishery Establishments of the Ministry of Agriculture, Fisheries and Food for their collaboration.

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### Species of *Corynebacterium* and *Pasteurella* isolated from Diseased Salmon, Trout and Rudd

THE carcasses of eleven salmon and five trout from many different areas in the United Kingdom were examined between September and December 1966. Of these the carcasses of five salmon and one trout appeared normal, and most of the fish were found to contain large numbers of eggs.

Almost all the diseased fish showed dirty grey areas of discoloured skin, up to 2 in. in diameter, on the body. They were rounded in outline and slightly raised. Pigmentation of the cutaneous lesions varied. The consistency was superficially firm, overlying an area of subcutaneous haemorrhage and a small amount of serosanguineous fluid was present in some. Ulceration of the jaw, the fins and the tail was seen in a few fish. A white fungus covered the lesions in some fish which at places was tinged with blood, thus presenting a velvety appearance. In one fish a tumour-like growth about 0.5–1 cm across was present on each of the two dorsal fins and the tail. Histopathological examination showed these tumours to be pigment-free, undifferentiated melanomas with evidence of malignancy. Visceral lesions included some non-specific degenerative changes in the liver and intense congestion of the spleen. The kidneys, in most instances, were dark, almost chocolate coloured with a soft pulpy consistency. Histologically the glomerular tufts showed congestion and/or an unusual degree of pigmentation, probably melanin, in some cases. Membranous deposits of the type described by Smith<sup>1</sup> on the surface of the kidneys in so-called Dee disease were not seen.

Direct smears from the heart, liver and spleen showed almost complete absence of organisms. Moderate numbers of a Gram-positive coccobacillary organism together with many other Gram-negative and Gram-positive bacilli of varying morphology were seen in smears from the skin lesions. Smears made from the kidneys, however, invariably showed an abundance of Gram-positive coccobacillary organisms with a fairly uniform morphological appearance, some of which seemed to occur intracellularly. The media employed during initial isolation of the causative agent were horse blood agar, Ordal and Earp medium<sup>2</sup>, Dorset egg medium and a modification of Ordal agar. A Gram-positive coccobacillary organism, similar in morphology to that seen in the direct kidney smears, was isolated from the kidneys only of five of the ten diseased fish and from the kidneys, liver and skin lesions of another fish. The description of the organism is given below.

Growth on horse blood agar at 22° C and 30° C was poor, the colonies being less than 0.5 mm in diameter after 48 h of incubation. No growth occurred at 37° C. The organism was a facultative anaerobe. The colonies were non-haemolytic, smooth, entire, transparent and easily emulsifiable. It was a non-motile, Gram-positive rod, measuring 0.5–0.7  $\mu$  × 2–2.5  $\mu$ . Acid but no gas was produced in glucose, maltose, sucrose, lactose, mannitol, sorbitol and salicin. Nitrate was not reduced. It was MR-positive and catalase positive, but indol, hydrogen sulphide and oxidase production were all negative.

Ordal and Earp<sup>2</sup> described the cultural properties of the aetiological agent of "kidney disease" in salmonid fishes and which they regarded as a species of *Corynebacterium*. Smith<sup>1</sup> reported the occurrence of "Dee disease" and concluded that the causal organism was similar to that described by Ordal and Earp<sup>2</sup>. The strain of organism isolated in the present episode, however, appears to be significantly different in cultural characteristics from the organism reported by these workers. Gray and Killinger<sup>3</sup> reported Listeric infection in pond-reared rainbow trout fed with meat from a donkey which had died of an

undetermined cause. They mentioned that the strains of *Listeria monocytogenes* isolated from these fish were somewhat different from the typical strains of this species, but they did not say in what way. Lapage has suggested (personal communication) that our isolate is related to *L. monocytogenes* as the demonstration of motility in cultures of this species was sometimes difficult. Seeliger (personal communication), after intensive investigation of the organism, did not share this opinion and classified it as an unidentified species of *Corynebacterium*.

Experimental infection of goldfish was attempted with the filtrate, as well as with the mashed-up whole kidney, taken from a naturally diseased salmon, but this was not successful. There was no mortality in the test fish in 3 months, after which the experiment was terminated.

An unusual number of fish were found dead in a connecting series of lakes in Sussex during January 1967. Eleven carcasses (9 rudd, 1 chub and 1 perch) were submitted for examination by the Kent River Authority on January 31, 1967. Seven rudd, 1 chub and 1 perch were examined. Another batch of eleven rudd arrived on February 3, 1967, of which eight were examined.

The perch showed no evidence of disease. The chub had lesions similar to those seen in rudd and described below. There were depositions of greyish brown fungus at two, three or more sites on the body of the fish, usually near the base of the fins and the root of the tail, each covering an area of approximately 0.5–1 cm. The skin at these sites was debilitated, discoloured in appearance and soft in consistency, overlying an area of haemorrhages in the musculature underneath; there was no fluid. Microscopic examination of the skin lesions showed the presence of cellular debris, blood cells and bacteria. The gills were congested and had a soft consistency, particularly near the posterior border. The liver, spleen and kidney showed intense congestion.

From twelve of the seventeen fish examined, twenty-one strains of a small Gram-negative bacillus were isolated from the liver, kidney and skin lesions.

In some instances, cultures from the liver and the kidney yielded an almost pure growth of this organism on horse blood agar and on the modified Ordal agar. The organism grew as tiny, non-haemolytic, dew drop-like colonies on horse blood agar at 22° C and 30° C in 48 h; there was no growth at 37° C. The colonies had a margarine-like consistency. The growth was relatively poor on nutrient agar and the colonies were transparent and rough. It was a non-motile, Gram-negative organism with a fairly uniform morphology. Acid but no gas was produced in glucose, sucrose and mannitol; there was no change in maltose, lactose, sorbitol, salicin and dulcitol. The organism did not utilize citrate and failed to grow in potassium cyanide. It showed a negative reaction to malonate and gluconate, and did not produce decarboxylases. It was catalase negative and weakly oxidase positive. The organism seemed closest to the *Pasteurella* group, although it is not typical of any of the recognized species (personal communication from Carpenter). Smith (personal communication), after extensive investigation of the organism, also expressed a similar opinion, although he observed minor differences in certain cultural and biochemical properties.

The present isolate appears to differ slightly from the organism described by Snieszko and his colleagues<sup>4</sup>, who reported the isolation of a bacterium representative of the genus *Pasteurella* and believed it to be the cause of an epizootic in white perch.

The constant pattern of isolation of this organism and its presence in large numbers in the liver and kidney of the diseased fish in almost pure culture have led us to believe that this could be the aetiological agent. The intense congestion of the viscera with an abundance of this organism suggests that the disease was probably of a septicæmic nature. The fungus (identified as belonging to the genus

*Saprolegnia*) would attack the debilitated sites of the skin as a secondary invader.

*Aeromonas liquefaciens* and a species of *Pseudomonas* were also isolated from the skin lesions as well as the viscera of a few fish. Their complete or relative absence from the internal organs of the majority of the diseased fish, however, suggested their role to be of secondary rather than primary significance.

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### Serum Protein Changes in Diseased Atlantic Salmon

SINCE 1964 there have been outbreaks of unusual disease among salmon in some Irish and English rivers. It is not established whether the disease is the same in the two countries. The disease in Irish rivers has been designated ulcerative dermal necrosis and is characterized by, first, the appearance of white patches on the head, trunk and fins; then, the secondary infection by fungal growth; and eventually, the ulceration of the affected areas, particularly the head, fins and eyes. The cause of the disease is as yet unknown.

Total amounts of serum protein and electrophoretic patterns of the sera from diseased and from healthy salmon have been compared during an investigation of the blood of fish affected with ulcerative dermal necrosis. Marked differences have been found between the sera from healthy and diseased fish.

Twenty-seven diseased (SD1–SD27) and five healthy salmon (SN1–SN5) were obtained alive from the River Lee in County Cork between July 1966 and March 1967. The diseased fish included eleven males and sixteen females, weighing from 3 lb. 12 oz. to 22 lb. The healthy fish included two males and three females weighing from 4 lb. 8 oz. to 8 lb. 2 oz.

The concentration of serum protein was smaller in diseased than in healthy salmon. The total concentrations measured in two healthy fish (SN4 and SN5) were 6.84 g per cent and 5.76 g per cent respectively. Total concentrations of serum protein in diseased salmon varied widely, from 2.88 g per cent up to normal amounts (Table 2). The degree of hypoproteinaemia could be correlated roughly with the extent of the disease. Some variation in the concentration of serum protein is to be expected in healthy salmon, because the fish in fresh water metabolize body reserves built up during life in the sea. It seems likely, however, that the decrease in the concentration of protein observed in the diseased fish was largely caused by the salmon disease: (a) one of the healthy fish (SN4), which had been upriver for 4–5 months as shown by scale readings, nevertheless showed a total concentration of serum protein of 6.84 g per cent; and (b) a number of the fresh-run but diseased fish showed small concentrations of serum protein, which could not have been explained as a result of fasting, for example, SD13, SD14 or SD24 (Table 2).

The serum electrophoretic pattern of diseased salmon differed from that of healthy fish. Characteristic change



in some of the fractions could be correlated roughly with the concentration of serum protein.

The pattern of salmon serum protein established from the five healthy fish showed five major fractions, designated B to F in order of increasing mobility (Fig. 1a). The percentage protein in each fraction, obtained by scanning the electrophoresis strips, is shown in Table 1. When a sufficiently large volume of serum was fractionated three fractions of low mobility, A1, A2 and A3, were usually faintly apparent. The following minor fractions were sometimes apparent: *cd* (intermediate in mobility between C and D) found in SN5; *de* found in SN1 and SN2; and *ef* found in SN3.

There was considerable variation in the pattern of serum protein among the diseased salmon. The trend of change from the normal emerged when a large number of sera had been examined.

Among the major fractions B-F in the sera of the twenty-seven diseased fish, fractions B and C remained relatively constant in mobility and quantity; fraction D, a dense diffuse band in healthy fish, tended to disappear. In diseased fish showing a relatively large concentration of serum protein, the E, and more markedly the F bands, showed an increase; in some cases an *ef* band, and in some cases a *cd* band, were evident (Fig. 1b and c). Curiously in diseased fish which had a small total concentration of protein the E and F fractions tended to disappear together with the D fraction, and there was no evidence of fractions *ef* or *cd* (Fig. 1d). Thus what happened to E and F was apparently related to the ability of the diseased fish to produce serum proteins, as indicated by the concentrations of serum protein. The changes in these

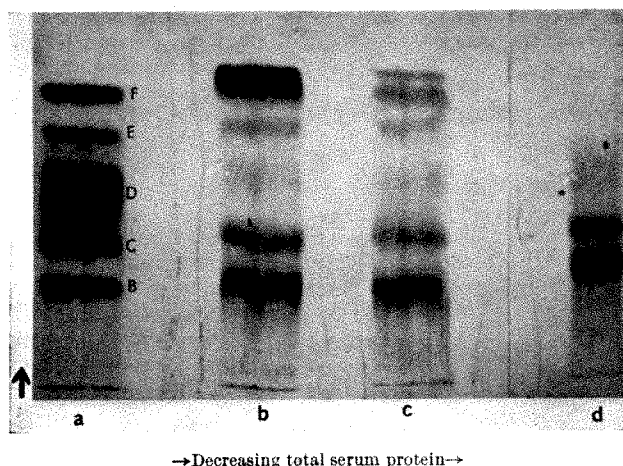


Fig. 1. Change in serum electrophoretic pattern in diseased salmon. Polyacrylamide gel electrophoresis, veronal buffer pH 8.6. (a) Healthy salmon (SN1); (b, c and d) diseased salmon (SD4, SD5, SD15).

Table 1. PERCENTAGE PROTEIN IN MAJOR FRACTIONS OF SERUM FROM HEALTHY SALMON SEPARATED BY ELECTROPHORESIS\*

Fraction	Mean (per cent)	Range (per cent)
B	15	11-17
C	22	17-26
D	46	43-50
E	8	7-10
F	10	3-16

\* Polyacrylamide gel electrophoresis; veronal buffer, pH 8.6.

Table 2. CONCENTRATION AND DISTRIBUTION OF PROTEIN IN SERA FROM HEALTHY AND DISEASED SALMON

Fish No.	Degree of infection with ulcerative dermal necrosis*	Total serum protein g per cent†	Relative conc. of protein fractions‡				
			D	E	F	<i>ef</i>	<i>cd</i>
SN1	—	?	128	27	31	—	—
SN2	—	?	129	39	31	—	—
SN3	—	?	127	11	17	§	—
SN4	—	6.84	129	24	49	—	—
SN5	—	5.76	123	15	7	—	§
SD1	Light	?	142	36	47	—	§
SD2	Medium	?	78	17	18	—	—
SD3	Light	?	120	40	40	—	—
SD4	Heavy	4.32	15	16	4	79	—
SD5	Heavy	3.24	7	15	11	25	—
SD6	Light	4.32	114	29	26	—	21
SD7	Light	6.84	122	29	46	—	22
SD8	Light	6.48	106	23	70	—	—
SD9	Medium	4.32	88	42	30	—	—
SD10	Medium	4.32	166	29	31	—	—
SD11	Heavy	3.24	19	20	43	—	18
SD12	Very heavy	5.76	22	20	28/14	—	—
SD13	Very heavy	2.88	19	9	6	—	—
SD14	Very heavy	2.88	24	0	8	—	—
SD15	Very heavy	2.88	15	2	1	—	—
SD16	Fungus only	3.60	12	0	0	—	—
SD17	Heavy	5.76	19	27	39	9	11
SD18	Medium	?	30	22	13/36/13	—	—
SD19	Heavy	?	14	2	0	—	—
SD20	Medium	?	32	40	40	61	—
SD21	Medium	2.88	18	5	6	§	—
SD22	Heavy	5.40	25	48	99	—	13
SD23	Light	6.48	34	102	70	85	57
SD24	Very heavy	3.24	15	16	13	—	23
SD25	Very heavy	?	35	34	47	§	—
SD26	Very heavy	6.48	18	27	20	§	—
SD27	Light	6.10	34	64	69	§	—

\* For convenience the degree of attack was roughly classified by visual inspection as follows: light, disease evident only while salmon in water; medium, disease patches apparent in or out of water; heavy, disease spread over large areas of body surface; very heavy, widespread disease with obvious ulceration.

† Measured using copper sulphate gravity method<sup>1</sup>.

‡ (B plus C) for each salmon used as base standard; each fraction expressed as percentage of this.

§ Too faint to be recorded on scanner at setting for major fractions.

|| Stripped for hatchery; water in abdominal cavity: total serum protein levels not measured.

fractions in all the diseased fish examined are indicated in Table 2; for purposes of comparison each fraction is expressed as a percentage of the sum of B and C for each fish.

A derangement of serum protein metabolism was suggested by the above results, and so samples of the liver, spleen and kidney of healthy and of diseased fish were compared histologically. I found no generalized histological difference. There are, however, scattered necrotic foci in the livers of at least some salmon with ulcerative dermal necrosis (unpublished results of K. Strickland).

There was no correlation between susceptibility to the disease in salmon, and the length of time which each had spent in the sea or in fresh water, as shown by the scale readings.

The decrease in the concentrations of serum protein in the diseased salmon may be a non-specific response to disease, because (a) one salmon, SD16, which did not have the disease, but which was heavily infected with fungus, showed a total concentration of serum protein of only 3.6 g per cent; (b) a similar decrease in the total concentration of serum protein was observed previously in pike with lymphosarcomatous growths<sup>3</sup>; and (c) Flemming<sup>4</sup> has reported variation in serum protein concentrations in diseased carp.

The changes in the individual protein fractions D, E, F and the development of the *ef* and *cd* fractions in salmon with ulcerative dermal necrosis may be specific effects. This remains to be established. If the changes prove to be specific they may be useful in the diagnosis of ulcerative dermal necrosis, and may provide a clue to the nature of the disease. Further investigation of the concentrations of protein and the electrophoretic patterns of sera from healthy and diseased salmon is in progress to establish (a) the range of variation in healthy salmon; (b) the range of variation in salmon with ulcerative dermal necrosis and its statistical correlation with the extent of the disease; and (c) the range of variation in salmon with other identifiable diseases, for example, furunculosis.

I thank the Fisheries Division, Department of Agriculture and Fisheries, Dublin, for carrying out scale readings on each of the salmon.

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## LETTERS TO THE EDITOR

## ASTRONOMY

## Graphite as Interstellar Matter

GRAPHITE consists of hexagonal crystals. Thus the optical constants depend on the direction of the incident light. For light with an electric vector parallel to the basal planes of the crystals graphite behaves like a metal. In the perpendicular direction, for light propagation through a crystal with an electric vector along optic axis, it behaves like a dielectric particle. Many calculations on graphite as interstellar matter have been made. In these cases the electric vector is in the basal plane. Lenham and Treherne have measured the optical constants for

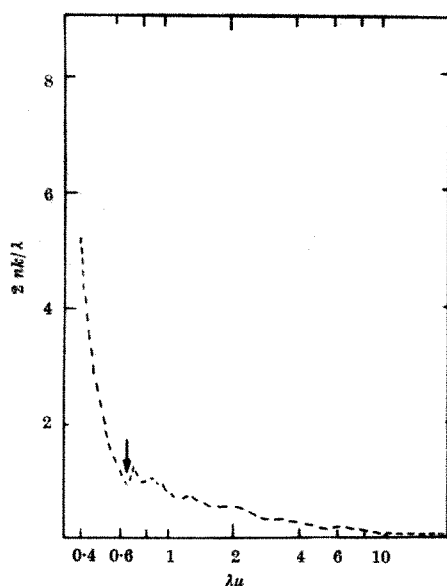


Fig. 1. The optical conductivity against wavelength for graphite with dielectric behaviour.

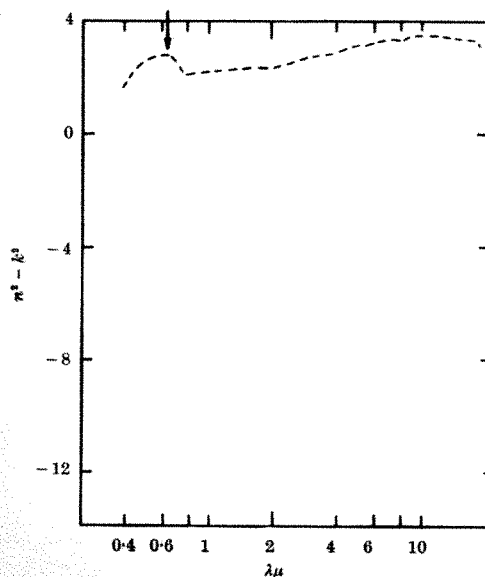


Fig. 2. The real part of the dielectric constant against wavelength for graphite with dielectric behaviour.

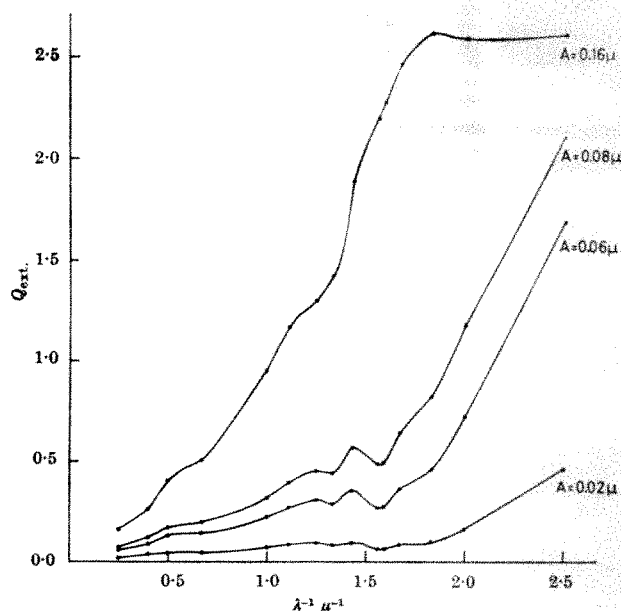


Fig. 3. Computed  $Q$  extinction curves for different radii.

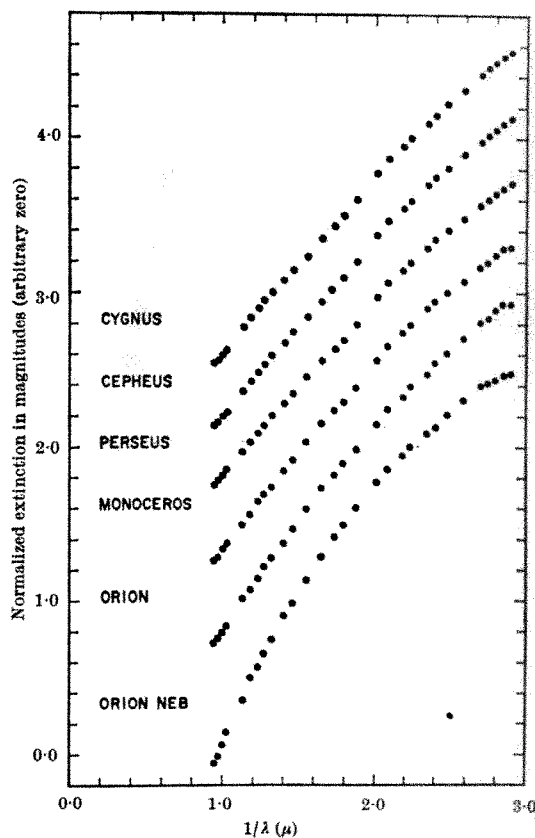


Fig. 4. Observed extinction curves.

graphite with the electric vector along the optic axis (Figs. 1 and 2)<sup>1</sup>. These figures show a sharp minimum in the optical conductivity  $2nk/\lambda$  at a wavelength  $\lambda = 0.64\mu$  and a maximum at the same wavelength in the real part of the dielectric constant  $n^2 - k^2$ . In the computer programme of  $Q$  extinction for spherical particles, " $n$ " and " $k$ " have to be used. They are calculated from  $2nk/\lambda$  and  $n^2 - k^2$  at different wavelengths. The optical constants " $n$ " and " $k$ " have sharp maximum respectively minimum at  $\lambda = 0.64\mu$ , which produces the typical appearance of the  $Q$  extinction curves (Fig. 3). The diagram shows a

broad and deep depression around  $\lambda = 0.64\mu$  between the wave-numbers  $1.5\mu^{-1}$  and  $2.0\mu^{-1}$  for different particle radii. No other material with a similar appearance has been reported which could be interstellar grains. Pure ice is also a dielectric material, but both " $n$ " and " $k$ " are independent of wavelength and give no depression in the  $Q$  curves.

In several observed extinction curves Whiteoak (Fig. 4)<sup>2</sup> has noticed a depression in the range  $1.6\mu^{-1}$ – $2.0\mu^{-1}$ . According to Whiteoak, "it apparently is real, because it is not present in the energy continua of the little-reddened stars".

If interstellar matter consists of graphite grains, all these particles probably have not the same orientation, which means that some of them behave like dielectric particles, some like metals. The depression in the observed extinction curves can be caused by graphite particles with such an orientation that the behaviour is like that of a dielectric particle. Let us assume, for the sake of simplicity, particles orientated at random. Such particles will give a resulting extinction with a shallow depression, because "metallic" graphite has no depression and the resulting extinction is an average value. That would give better agreement with the reported observations. Different combinations of ice and graphite will probably also give a suitable depression.

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Received March 31; revised May 10, 1967.

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## PLANETARY SCIENCE

### Muon Solar Daily Variation at a Depth of 60 Metres Water Equivalent

THE solar daily variation of the underground secondary cosmic ray intensity has shown remarkable changes in phase and amplitude during the recent solar minimum.

Observations at the Holborn Underground Station in London with semi-cubic telescopes show that the solar diurnal wave remained virtually constant in amplitude and phase from November 1960 to July 1964. Table 1 summarizes the results for the relevant periods of operation. The errors quoted are standard deviations calculated from the scatter of monthly groups of data.

In the middle of 1964, the time of maximum intensity,  $T_{\max}$ , changed from an average value of about 14 h, as shown in Table 1, to about 06 h and remained near the latter value until the middle of 1966. This early time of maximum was also obtained with independent cubical telescopes pointing due east and north at  $45^\circ$  to the zenith, which came into operation in October 1964. Table 2 shows the results for the calendar year 1965, roughly corresponding to the period of maximum ground level neutron intensity observed at Deep River during the recent sunspot minimum.

Table 1

Period	Direction	Counts/h	Amplitude of diurnal wave (per cent)	$T_{\max}$ (h local time)
Nov. 1960–Oct. 1961	Vertical	50,000	$0.023 \pm 0.013$	$14.3 \pm 2$
Nov. 1961–Oct. 1962	"	50,000	$0.020 \pm 0.013$	$13.8 \pm 2$
Aug. 1963–July 1964	"	150,000	$0.016 \pm 0.007$	$13.3 \pm 1$

Table 2

Period	Direction	Counts/h	Amplitude of the solar wave (per cent)	$T_{\max}$ (h local time)
Jan. 1965–Dec. 1965	Vertical	50,000	$0.024 \pm 0.013$	$5.3 \pm 2$
" "	East	35,000	$0.043 \pm 0.013$	$2.0 \pm 1$
" "	North	35,000	$0.023 \pm 0.013$	$1.5 \pm 2$

The averaged results for the period November 1960–July 1964 are plotted on a harmonic dial in Fig. 1, together with the results for 1965. The latter data are clearly inconsistent with a diurnal wave having  $T_{\max} = 1400$  h as in the period 1960–1964, but they are consistent with the calculated aberration effect caused by the orbital motion of the Earth<sup>1</sup>. This has a maximum amplitude of 0.055 per cent at 0600 h external to the geomagnetic field. After allowing for geomagnetic deflexion the predicted and observed vectors shown on the harmonic dial for the east, north and vertical directions are seen to be in good agreement, which suggests that there is only a small contribution from atmospheric temperature variations.

Comparison of the observed data with the calculated variation expected on the basis of the usual "corotation" model<sup>2-4</sup> for the origin of the solar anisotropy shows that the upper limiting rigidity ( $R_{\max}$ ) for corotating particles must have fallen to 70 GV or less during 1965. Correction of the October 1960–July 1964 vertical data for the orbital motion effect gives agreement with the corotation model, for this period, if  $R_{\max} = 130$  GV (assuming a maximum free space amplitude of 0.4 per cent).

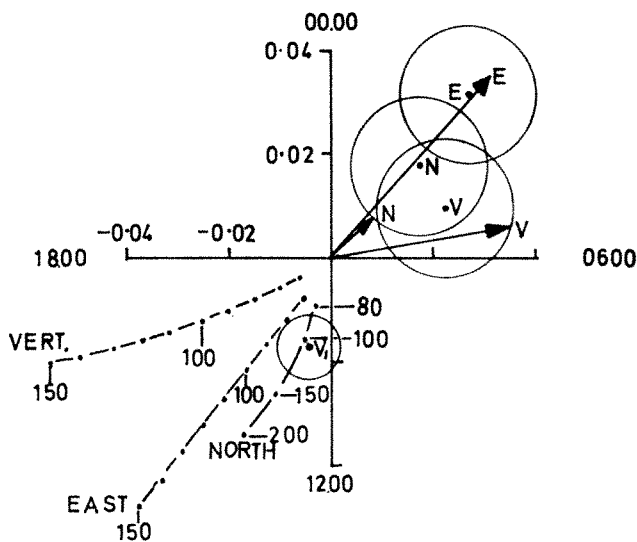


Fig. 1. Harmonic dial in local time, with scale in per cent. Vectors  $E$ ,  $N$ , and  $V$  are the calculated orbital motion effects for east, north and vertical telescopes, respectively. Points marked  $E$ ,  $N$  and  $V$  are the observations for 1965, with their associated standard deviation circles. The point marked  $V$  is the average of vertical observations for the period November 1960–July 1964. The dot-dash curves are the loci of the end-points of the calculated vectors caused by the "corotation" effect; the dots are marked with the corresponding  $R_{\max}$  values (in GV).

Recent data suggest that the time of maximum has returned to 1400 h local time, although this is difficult to establish with certainty from the limited amount of available data. Annual means of the solar diurnal variation have been used to eliminate distortion by possible sidereal effects.

It is interesting to note that during the year 1965 when the underground recorders at Holborn were showing the anomalous early time of maximum, the neutron monitor data from thirteen stations<sup>5</sup> showed a significant reduction in the amplitude of the solar diurnal wave as compared with that of the solar maximum year 1958. There was a similar reduction in the ionization chamber data obtained by the Carnegie Institution of Washington. This reduction in amplitude would be consistent with a corresponding reduction in the amplitude of the free space anisotropy and/or a lowering of the upper limiting rigidity of the variation arising from corotation which have been invoked by us to explain the anomalous behaviour of the underground daily variation. The relatively large changes which occurred underground compared with the rather minor changes in the ground

based data are to be attributed to the vastly different energy responses of the underground and ground level recorders.

So far as is known, the behaviour of the solar diurnal wave as observed by muon detectors has been different during and before the solar minimum of 1964–1965 from that which was observed near the minimum of 1954. During the declining phase of the solar cycle between 1949 and 1953 there were large phase changes in the annual means of the muon diurnal variation at ground level<sup>7</sup>, and near the solar minimum of 1954 there were large phase changes during the course of the year (ref. 6 and others). This pattern of behaviour has not recurred either during the declining phase of the last solar cycle between 1958 and 1964 or around the sunspot minimum of 1964–1965. To this extent the relationship between the cosmic ray solar diurnal variation and solar activity may display a periodicity of 22 yr as well as one of 11 yr, as first pointed out by Thambyahpillai and Elliot<sup>7</sup>.

It would appear that the solar diurnal wave observed underground at Holborn during the period November 1960–July 1964 can be reconciled with the corotation model for the solar anisotropy if the upper limiting rigidity was about 130 GV and the free space amplitude below this limit was 0.4 per cent. The free space amplitude and/or the upper limiting rigidity to which the particles participate in the corotation, however, should have been reduced substantially during 1965 to enable the recorders to register the solar anisotropy caused by the aberration effect which arises from the orbital motion of the Earth around the Sun. There is evidence to suggest that the upper limiting rigidity has again moved to higher values as solar activity increased, but this is not yet conclusive. In contradistinction to the 24 h wave, the semi-diurnal wave has shown no statistically significant variability during the entire period of observation at the underground site.

Drs J. C. Dutt and T. Mathews have contributed to the measurements made with vertical telescopes between 1961 and 1964.

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### Magnetic Properties and Petrology of Rocks near the Crest of the Mid-Atlantic Ridge

MAGNETIC and petrological studies have been made on nine dredge samples taken in the course of detailed geological and geophysical investigations of a small area near the crest of the mid-Atlantic Ridge, 22° 30' N., 45° 30' W. (refs. 1 and 2). Samples were taken in 1964–65 on cruises Chain-44 and Washington 1965–1 of the Woods Hole Oceanographic Institution and the Scripps Institution of Oceanography. The magnetic properties of the samples were examined in order to help in the interpretation of observed magnetic anomalies in the area. The remanent magnetizations of twenty cores (2.5 cm by about 3 cm) taken from the nine samples were measured on an astatic magnetometer at the Scripps Institution. Susceptibilities were measured on a susceptibility bridge. The samples were not demagnetized of any isothermal or viscous remanent magnetization components which may have been present. Because all dredge samples were

unoriented, the cores were also unoriented. Care was taken to avoid coring outer visibly weathered zones in some samples.

The rocks examined in this study<sup>3,4</sup> (Table 1) consist of: fresh basalts (10–85, 4–412 and 5–1); mylonized and brecciated basalts which are partially mineralogically reconstituted (2–1, 2–2 and 2–4); and greenstones (greenschist facies metabasalts) (CH-44; 3–126, 3–3 and 3–2).

Values for the magnetic properties of basalt have been available for some time<sup>5,6</sup>. More recently, direct magnetic measurements of submarine lava rock have also been published<sup>7–12</sup>. One recent approach<sup>13</sup>, however, has been to calculate the direction and magnitude of magnetization of seamounts by considering their volumes and associated anomalous fields. Opdyke and Hekinian<sup>12</sup> have reported the magnetic properties of rocks termed "chloritic". Because the detailed mineralogy was not specified it is not possible, however, to say whether the rocks are similar to the greenstones examined here.

The samples and their respective magnetic properties are given in Table 1. The values tabulated for fresh and brecciated basalts agree well with previous values. The samples with the highest susceptibility and intensity are the unaltered basalts because of their content of fresh iron–titanium oxides (mainly platy crystals of titanomagnetite which are locally marginally oxidized to ilmenohaematite, especially in 10–85). The mylonized basalts, which contain as a whole less fresh iron–titanium oxide, have lower susceptibilities. The greenstones, in which all iron–titanium oxide has been replaced completely by sphene, have still lower susceptibilities.

Additional data on the magnetic properties are obtained by inspecting the Königsberger ratio,  $Q$ , the ratio of the magnitude of natural remanent magnetization to that of induced magnetization evaluated here for 0.5 oersted. All the basalts except 5–1 have the same order of magnitude of  $Q$ . Values of the susceptibility, magnetization and  $Q$  ratio for 5–1 are in accordance with values for fresh basalt<sup>7,9,11,12</sup>. By comparison, the  $Q$  ratios for samples 10–85 and 4–412 are rather low. It is mainly the variability in their remanent magnetizations that causes the variation in  $Q$  (ref. 5).

Sample 5–1 was dredged from near the floor of the rift valley. A glassy crust which surrounds it and its fine grain size indicate that it was removed from the top of a flow and thus cooled rapidly. The grain size of a rock is known to govern its remanent magnetization; rocks of finer grain are more magnetic<sup>5</sup>. This accounts for the higher  $Q$  and remanent magnetization of sample 5–1 than of the coarser grained samples 10–85 and 4–412, although the latter have a greater concentration of magnetic minerals (high susceptibilities). Similarly, coarse grained 4–412 has a lower  $Q$  than medium grained 10–85. Variation in the magnitude of remanent magnetization with cooling rate and grain size has been observed in the Mohole basalt by Cox and Doell<sup>8</sup>. They reported a decrease in the remanent magnetization with depth from the top of the section.

Breccia sample 2–4 has susceptibilities and intensities much greater than those of associated rocks 2–2 and 2–1. Its  $Q$  values are similar, however, suggesting a higher magnetic mineral content for 2–4, but a similar thermal history. All these samples have a similar grain size. As alteration proceeds to produce greenstone, the remanent magnetism is destroyed at a far greater rate than the magnetic mineral content, thus reducing the  $Q$  ratio to less than one (samples 125, 3, 2).

It is clear from Table 1 that hydrothermal alteration can significantly decrease the magnetic properties of basalts. This cannot be assumed for other rocks, however. Low grade hydrothermal alteration produces strong but opposite effects on the magnetic properties of basalts and peridotites. These effects are predictable from the oxide mineralogy of these rocks, and from known relations



Station No.	Sample*		Table 1					Volume (cm <sup>3</sup> )	Dredging depths (corrected metres)	Location of sample	
			Suscep- tibility E.M.U./cm <sup>3</sup> × 10 <sup>4</sup>	Remanent magneti- zation E.M.U./cm <sup>3</sup> × 10 <sup>4</sup>	Error in magni- tude of remanent magneti- zation (%)	Q (T = 0.5 oersted)	Latitude			Longitude	
Fresh basalts											
THV-18	10-85-A	Fresh, medium-grained basalt with about 3 volume per cent equidimensional titanomagnetite crystals. Similar to 4-412 but finer grained	15.74	36.33	0.3	4.617	9.33	3,100-2,590	22° 10.6' N.	45° 15.0'-45° 01.6' W.	
	10-85-B		18.77	36.00	0.3	3.835	8.84	22° 11.2' N.			
THV-8	4-412-A	Fresh, coarse-grained olivine basalt, with approximately 4 volume per cent equidimensional crystals of titanomagnetite. This sample, in view of its unusually coarse grain size, may be an intrusive (dike or sill) rock	26.90	47.38	1.0	3.523	9.33	3,380-2,495	22° 29.0' N.	45° 03.9'-45° 01.6' W.	
	4-412-B		28.64	46.73	0.4	3.264	8.34				
	4-412-C		29.77	50.07	0.4	3.364	8.84				
THV-10	5-1-A	"Quenched", fine-grained olivine basalt with abundant interstitial glass rich in titanomagnetite (?) and ilmeno-haematite in extremely small platy crystals arranged in a rectangular grid-like manner	8.105	112.9	0.3	27.87	9.82	3,525-3,000	22° 22.6' N.	45° 10.8'-45° 132' W.	
	5-1-B		7.528	82.61	0.3	21.95	9.33	22° 21.8' N.			
	5-1-C		6.400	95.51	0.4	29.85	7.85				
Basalt breccias											
THV-4	2-1-A	Brecciated basalt, considerable secondary saponite. Probably a fault breccia. Titanomagnetite altered to sphene and ilmeno-haematite	0.9345	2.484	0.9	5.315	5.4	1,985-1,735	22° 31.5' N.	45° 00.2'-44° 59.8' W.	
	2-1-B		1.122	4.046	5.0	7.315	8.34				
	2-1-C		3.160	6.360	5.0	4.026	12.27				
	2-1-D		2.212	3.575	7.0	3.233	12.27				
THV-4	2-2-A	Fine-grained, saponite-rich, brecciated basalt. Oxides altered to goethite and ilmeno-haematite (?)	0.7588	0.5190	25.0	1.368	12.27	1,985-1,735	22° 31.5' N.	45° 00.2'-44° 59.8' W.	
	2-2-B		0.8802	0.8552	0.9	1.943	12.27				
	2-2-C		1.316	1.329	0.9	2.019	12.27				
THV-4	2-4-A	Basalt fragment from tectonic breccia. Considerable secondary saponite mainly replacing olivine. Interstitial, devitrified glass with abundant platy crystals of titanomagnetite (?)	15.78	22.88	0.3	2.900	12.76	1,985-1,735	22° 31.5' N.	45° 00.2'-44° 59.8' W.	
	2-4-B		18.46	24.50	0.4	2.655	12.76				
Greenstones											
CH-44-3	126	Greenstone. Contains albite, actinolite, epidote, chlorite and sphene in various amounts. Fe-Ti oxides totally replaced by silicates	0.5961	0.1120	6.0	0.3758	9.33	3,400-2,400	22° 38.0' N.	45° 00.7'-44° 58.8' W.	
CH 44-3	3	Same as CH-44-3-126	0.5477	0.05416	1.5	0.19780	8.84	3,400-2,400	22° 38.0' N.	45° 00.7'-44° 58.8' W.	
CH-44-3	2	Same as CH-44-3-126	0.4336	0.01655	1.5	0.07634	11.7	3,400-2,400	22° 38.0' N.	45° 00.7'-44° 58.8' W.	

\* All of the basalts so far examined are olivine basalts. Complete analyses of four fresh basalts show the unusually low K<sub>2</sub>O content typical of "Oceanic Tholeiites" in terms of normative mineralogy; our samples are olivine tholeiites, they are in the normative ternary olivine-diopside-hypersthene.

between continental aeromagnetic anomalies and oxide mineralogy<sup>14</sup>. Basalts of the "oceanic" tholeiite type, the dominant rock in this area, have been found to contain titanomagnetite with ilmeno-haematite, and hence have high susceptibilities. Hydrothermal alteration breaks down these oxides to sphene and other minerals, thus decreasing the susceptibility and remanent magnetization. On the other hand, fresh peridotites, which are mainly free of magnetite, alter to rocks rich in serpentine and magnetite, which have a higher susceptibility than their parents and may also acquire chemical remanent magnetization.

Possible correlations between regional anomalies and hydrothermal alteration zones might thus be expected. Specifically, strong anomalies might be expected over either hydrothermally altered peridotites, or fresh basalt piles. If a terrain were initially composed of alternate zones of fresh basalts and fresh peridotites, strong anomalies might be expected over the former. If this region then underwent low grade hydrothermal metamorphism, high anomalies would be found over the former peridotite zones, and minima over the basalt zones.

We have determined the magnetic properties of the samples, but not the direction of magnetization. By inspecting the  $Q$  factor, we can determine whether the direction of magnetization can be estimated. Table 1 shows  $Q$  varying roughly from 0.1 to 30.0. The  $Q$  factor involves the ratio of only the magnitudes of the remanent and induced magnetizations, not the direction. The direction and magnitude are taken into account in the vector relation for the anomalous magnetization

$$\vec{J} = k\vec{H}_0 + \vec{J}_n$$

where  $\vec{J}$  = anomalous magnetization vector,  $\vec{H}_0$  = Earth's magnetic field vector,  $\vec{J}_n$  = remanent magnetization vector and  $k$  = susceptibility.

Now, by considering this vector relationship we can see that the maximum angular deviation of the anomalous magnetization vector  $\vec{J}$ , from the direction of  $\vec{H}_0$ , will occur when  $\vec{J}_n$  is perpendicular to  $\vec{H}_0$ . Also the maximum uncertainty in estimating the magnitude of the anomalous field  $\vec{J}$  occurs when  $\vec{J}_n$  is parallel or antiparallel to  $\vec{H}_0$ . Obviously these two situations do not occur simultaneously. With  $Q = 30$  the uncertainty in the magnitude of  $\vec{J}$  is only 3 per cent, but the deviation of  $\vec{J}$  from the Earth's field direction can approach 90°. When  $Q = 10$  the uncertainty in magnitude is only 10 per cent but the deviation is still as high as 78.6°. Thus we can see that anomaly interpretation for the basalts in Table 1 (where  $Q$  is large) is essentially indeterminate if the shape of the disturbing body cannot be closely approximated. Clearly, when  $Q = 1$  the maximum uncertainty in estimating the magnitude of  $\vec{J}$  is 100 per cent, while the deviation may be 45°. When  $Q$  approaches 0.1 the uncertainty in magnitude is a maximum of 10 per cent and the direction will deviate as little as 5.6° from the Earth's magnetic field. This is the case with the greenstones; all magnetization may be assumed induced and in the direction of the Earth's field. The susceptibility is now so low, however, that unless the body is of sufficient size, its anomaly will scarcely be recognizable at the surface of the ocean.

Inspection of the map of magnetic intensity<sup>2</sup> shows that the magnetic anomaly pattern in the area studied

may be related mainly to zones of fresh basalts, alternating with hydrothermally altered zones which perhaps surround fractured zones. This offers an alternative hypothesis in contrast to recent models of sea floor spreading of adjacent north-south trending intrusive bodies with alternate normal and reversed magnetization<sup>15</sup>. To sum up, our results show that vertical zones of hydrothermal alteration of basalts or peridotites along fracture zones should strongly influence the magnetic anomaly patterns over the Mid-Atlantic Ridge.

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## Age of Dolerite Dykes in the Vestfold Hills, Antarctica

THE Vestfold Hills, one of the so-called Antarctic oases, make up an area of exposed rock on the coast of Princess Elizabeth Land, Antarctica. The hills, together with the numerous adjacent islands, form a triangular area about 30 km across. The Australian National Antarctic Research Expedition (ANARE) station Davis is situated in the south-west of the hills at lat. 68° 35' S., long. 77° 58' E.

The Vestfold Hills are composed principally of orthopyroxene-quartz-feldspar gneiss and granulite which contain various amounts of garnet, biotite, and hornblende<sup>1,2</sup>. These metamorphic rocks originally crystallized in the granulite facies, but many now show retrogressive mineral changes<sup>3,4</sup>. Orthopyroxene is commonly converted partly to hornblende and biotite or, in a few examples, to garnet, and some plagioclase is partly converted to carbonate and muscovite. Cataclastic deformation of mineral grains is widespread, leading to partial recrystallization in places. Evidence for migmatization, with local potash metasomatism, has been found at several places<sup>5,6</sup>. The ages of five samples of the country rock have been determined by Ravich and Krylov<sup>5</sup>, and range

Table 1. WIDTH AND STRIKE OF DYKES

Sample No.	Width (m)	Strike
GA5429	9	005°
GA5430	12	171°
GA5431	3.5	110°
GA5432	6	005°
GA5433	2.5	115°
GA5434	2.5	115°

from  $1185 \times 10^6$  years (alaskite granite vein) to  $1525 \times 10^6$  years (migmatite).

A swarm of dolerite dykes has intruded the gneisses. The dykes range in width from a few centimetres up to 25 m, although most of them are less than 3 m wide. Individual dykes can be traced on aerial photographs for distances up to 25 km. Most of the dykes strike between 15° and 30° east of north, and most of the remainder between 35° and 45° west of north; a few trend north or east. Ground observations suggest the dip of the dykes is usually greater than 65°, and that at least some of the dykes striking east-west are slightly coarser than those of other directions. There is no other difference apparent between dykes with different trends, and the dykes probably all belong to one episode of intrusion.

At the request of one of us (I. McL.), Ian Black, geophysicist of the Bureau of Mineral Resources seconded to ANARE, collected in 1964 samples of six conveniently accessible dykes for isotopic age determination. Table 1 shows the width and strike of each dyke from which a sample was collected. Sample GA5429 was blasted from a dyke about 400 metres north-north-east of the hut used for filling meteorological balloons at Davis. The others were obtained by sledge hammer from five different dykes along the southern side of Heidemann Bay, ranging from 1.4 km south-south-west to 1.3 km south-south-east of the station.

The samples are typical of the dykes. They are essentially plagioclase-clinopyroxene rocks, with andesine laths up to 0.8 mm long and pyroxene grains up to 0.3 mm in diameter. Rare plagioclase phenocrysts are zoned from sodic labradorite in the core to calcic andesine at the rim. Clinopyroxene, probably augite, is strongly schillerized and is partly replaced by uraltite (some of which is in turn rimmed by minor biotite) and opaque minerals as well as by orthopyroxene in GA5429 and tremolite in GA5431 and GA5432. GA5429 contains a very small amount of secondary interstitial quartz. Thin sections of GA5430, GA5431 and GA5433 are traversed by zones of cataclasis up to 9 mm wide along which carbonate, garnet, and biotite have replaced the plagioclase and pyroxene.

The samples were analysed by one of us (R. R. H.) at the Department of Geophysics and Geochemistry, Australian National University, Canberra, for rubidium and strontium using the isotope dilution method (see Table 2). The indicated age of the six dykes is  $1030 \pm 220 \times 10^6$  yr at the 95 per cent confidence level, calculated according to McIntyre *et al.*<sup>6</sup>. When plotted on a  $^{87}\text{Sr}/^{86}\text{Sr}$  against  $^{87}\text{Rb}/^{86}\text{Sr}$  diagram, the data for six total rock specimens lie on a straight line (within the experimental error). The rather large possible error of  $220 \times 10^6$  yr comes about because of the extremely low rubidium enrichment and low range in rubidium/strontium ratio in these rocks.

The measured age may represent the time of injection of the dykes, or of a later metamorphism which also

Table 2. ANALYTICAL DATA

Sample No.	Rb $\mu\text{g/g}$	Sr $\mu\text{g/g}$	$^{87}\text{Rb}/^{86}\text{Sr}$	$^{87}\text{Sr}/^{86}\text{Sr}$
GA5429	29.3	163.8	0.5163	0.7131
GA5430	18.9	153.0	0.3567	0.7100
GA5431	14.4	269.9	0.1540	0.7073
GA5432	26.5	153.3	0.4997	0.7121
GA5433	14.2	274.3	0.1492	0.7072
GA5434	14.3	273.9	0.1504	0.7077

<sup>1</sup> Rb =  $1.39 \times 10^{-11}$  yr<sup>-1</sup>.

<sup>2</sup>  $^{87}\text{Sr}/^{86}\text{Sr} = 8.3752$ .

Initial  $^{87}\text{Sr}/^{86}\text{Sr} = 0.7052 \pm 0.001$ .

Age  $1030 \pm 220 \times 10^6$  yr.  
(All samples total rock.)

affected the country rocks. The indicated initial strontium-87/rubidium-86 ratio of 0.7052 is within the accepted limits for the composition of "mantle" material, and does not help to decide whether the rocks were intruded or metamorphosed  $1030 \times 10^6$  yr ago.

Evidence for a  $1000 \times 10^6$  yr event, probably metamorphic, has been obtained at places from 1,000 km to 2,200 km east of the Vestfold Hills<sup>5,7-9</sup>. The petrography of the Vestfold Hills dolerites, however, suggests that they have not been metamorphosed to any extent; the primary textures and uraltization of the pyroxene are typically igneous in all specimens. Although GA5430, GA5431, and GA5433 show evidence of recrystallization along narrow zones of cataclasis, the data for all six samples define the  $1030 \times 10^6$  yr isochron to within experimental error, and we therefore regard this age as the time of injection of the dolerite dyke swarm in the Vestfold Hills.

We wish to thank the members of the ANARE expedition who helped collect the samples, and members of the Department of Geophysics and Geochemistry, Australian National University, for discussion and advice about methods of analysis.

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## PHYSICS

### Electron Hole Pairs in the Irradiation of Condensed Systems

RECENT studies on  $\gamma$ -irradiated frozen aqueous solutions at 77° K have led to the elucidation of the kinetics of the reactions of electrons and holes produced by radiation<sup>1</sup>. From this it can be inferred that the nature of the active primary species produced by radiation has to account for the following observations.

The primary species normally undergoes a decay which is kinetically of the first order. Appreciable chemical yields from electrons and/or holes are found only when there are solutes present in the matrix (traps) capable of reacting with both these species in competition with the decay process.

Thus it was concluded that after the initial processes of ionization and excitation subsequent to the passage of radiation, the active intermediate species can be represented as bound electron hole pairs from which the electrons and/or holes can be scavenged by suitable solutes in the matrix.

Recent experimental results on ice and particularly also from electron pulse radiolysis experiments in water in the nanosecond region would suggest that the electron hole pair model proposed for ice is also relevant to aqueous and non-aqueous systems.

The absorption spectrum of pulse irradiated ice<sup>2</sup> (investigated down to temperatures of 200° K) is very similar ( $\lambda_{\text{max}} \sim 720 \text{ m}\mu$ ) to that previously found for water<sup>3</sup>,

and in liquid water, the absorption spectrum of the "hydrated electron" is fully formed after a period of  $\sim 10^{-9}$  sec (ref. 4).

If, as had been previously supposed, one is dealing with an electron trapped in the field of the oriented water dipoles, then on the basis of the theory of the "solvated electron" the absorption maximum in ice should be shifted appreciably towards longer waves, that is, into the infra-red, because the relevant parameter<sup>5</sup>

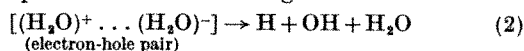
$$\left( \frac{1}{\epsilon_0} - \frac{1}{\epsilon_s} \right)^2 \quad (1)$$

(where  $\epsilon_0$  and  $\epsilon_s$  are the optical and static dielectric constants respectively) which enters into the theory would become very small because of the low value of the  $\epsilon_s$  of ice for frequencies of the order of  $10^6 \text{ sec}^{-1}$  and even lower for frequencies corresponding to the nanosecond region.

Because the absorption spectra of the unstable intermediate in ice and liquid water are practically identical one must conclude that in both instances one is dealing with essentially the same species. The suggested model of a bound electron hole pair, that is, an exciton of small radius, would be fully compatible with these observations.  $\gamma$ -irradiation of a condensed system would, of course, in the first instance lead to free electrons and holes. After a relatively short time, however, the electrons could be captured by the holes into an exciton of small radial extension. The observed chemical reactions of electrons and holes would then be caused by scavenging by suitable solute molecules of either of these species from this exciton state.

An exciton itself would not make a contribution to electrical conductivity. If, however, for example, the hole is made to react with a suitable scavenger the electron would become more or less free and electrical conductivity could manifest itself.

It is known that in aqueous solutions also, the "hydrated electron" decays by a first order process<sup>6</sup> with a rate constant of the order of  $\sim 10^3 \text{ sec}^{-1}$ . On the basis of the present hypothesis this would now become a question of the lifetime of the excitons. These can decay by radiative or non-radiative processes<sup>7</sup>; one of the latter could be a predissociation according to



leading directly to the formation of some hydrogen atoms and hydroxyl radicals in a relatively short time; this could be the type of process first proposed by Allan and Scholes<sup>8</sup>, corresponding to a yield of  $G \sim 0.6$  in water.

The reason for the relatively long lifetime of the majority of the primarily formed electron hole pairs in the absence of scavengers is not immediately obvious. If they possess a certain amount of kinetic energy, then they could only decay by phonon induced transitions. Such processes may be relatively slow and could lead to long lifetimes under certain conditions<sup>7</sup>.

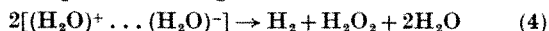
On the other hand, one has to consider also the possibility that one is dealing here with triplet excitons, and in the case of, for example, water or other systems of low atomic number where there is only little spin orbit coupling, one might expect the triplet state to have a relatively long lifetime<sup>9</sup>.

An exact theoretical calculation of the absorption spectra on the basis of a model of bound electron hole pairs (charge transfer excitons) is difficult because hydrogenic wave functions are only applicable for excitons of large radius. As a first approximation, however, one may use hydrogenic wave functions and the optical dielectric constant  $\epsilon_0 = n^2$ . Another parameter which enters into the theory is the reduced effective mass ( $\mu^*$ ) of the electron hole pair. For water or ice, assuming that the effective mass of the electron ( $m_e^*$ ) and of the hole ( $m_h^*$ ) are about equal and do not differ appreciably from the rest mass of the electron ( $m$ ), that is,  $\mu^* = m/2$ , one obtains<sup>10</sup>

$$\Delta E \simeq 0.19 \frac{me^4}{\hbar^2 \epsilon_0^2} \quad (3)$$

which gives a value of  $\sim 1.6$  eV.

The molecular yields of hydrogen and hydrogen peroxide which were previously attributed to the interaction of two electrons and holes respectively should on the present hypothesis be represented by the interaction of two exciton pairs according to



This would correspond to the interaction of two "excited" water molecules which was proposed previously on the basis of some results on hydrogen-deuterium separation factors from irradiated water-deuterium oxide solutions<sup>11</sup>.

Other experimental observations relating to the "solvated electron" can be satisfactorily explained on the basis of the electron hole pair model discussed here. A full account of these considerations will be published elsewhere.

I thank Professor Sir Nevill Mott for helpful discussions.

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### Light Scattering by the Relativistic (Non-linear) Oscillator

A RECENT letter by Hutten<sup>1</sup> contains what in our opinion is an error. The error leads Hutten to the false conclusion that the cross-section of the Rayleigh scattering depends on the amplitude of the relativistic oscillator.

The problem is to find the solution of the non-linear equation

$$\ddot{x} + \omega_0^2 x - \epsilon x \dot{x} = \frac{F}{m_0} \cos \omega_1 t_1$$

where

$$\epsilon = \frac{3}{2} \cdot \frac{\omega_0^2}{c^2}$$

in the harmonic approximation

$$x = A \cos \omega_1 t$$

Assuming the non-linearity to be small, Hutten obtains the following expression for the amplitude of oscillation

$$A = \frac{F_0/m_0}{\omega_0^2 - \omega_1^2 - \frac{\epsilon \omega_1^2}{4} A^2} \quad (1)$$

Hutten concludes that the Rayleigh cross-section may decrease significantly (by several orders of magnitude) when the amplitude of the incident radiation is increased. This is correct if the magnitude of the expression

$$\frac{\epsilon \omega_1^2}{4} A^2$$

in the denominator of equation (1) exceeds the other terms.

It is, however, possible to show that in the case of scattering

$$\frac{\epsilon \omega_1^2 A^2}{4} < |\omega_0^2 - \omega_1^2|$$

always holds, and the cross-section cannot therefore depend significantly on the amplitude of the incident radiation.

Let us evaluate the magnitude of the term

$$\frac{\epsilon \omega_1^2}{4} A^2$$

For the linear oscillator, that is,  $\epsilon=0$ , the amplitude of oscillation and the amplitude of speed are respectively equal to

$$x_{\max} = A; \quad \dot{x}_{\max} = \omega_1 A$$

Consequently, the maximum value (linear oscillator) of the term in which we are interested is

$$\frac{\epsilon \omega_1^2}{4} A^2 = \frac{3}{8} \omega_0^2 \left( \frac{\dot{x}_{\max}}{c} \right)^2 \quad (2)$$

As  $x_m/c \leq 1$ , the effect of magnitude 2 is therefore negligible in the denominator of equation 1, both for Thomson ( $\omega_0 \ll \omega_1$ ) and for Rayleigh ( $\omega_1 \ll \omega_0$ ) scattering.

Even when the incident radiation had a field strength corresponding to the breakdown in a gas ( $10^6$  V/cm) at frequencies higher than  $10^{10}$  c/s

$$x_{\max}/c \ll 1 \text{ and } \dot{x}_{\max}/c \ll 1$$

and a decrease in cross-section cannot therefore be expected as a result of a relativistic effect.

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Dr E. H. Hutten writes (May 30, 1967):

It is quite true that a relativistic effect can be expected only if the speed of the oscillating particle approaches that of light. The published results giving a decrease in cross-section seemed to point to such an effect, but it is likely that they do not bear this interpretation. I mentioned in my letter that the numerical estimate was doubtful and that other types of non-linearity could produce the same effect.

### Quantum Cosmology

IN cosmology a continuous fluid is used to represent the complex contents of the universe. Homogeneous and isotropic models then show that the universe originated from a space-time singularity of infinite density some  $10^{10}$  years ago<sup>1</sup>. Before accepting singularities of infinite density we must ask whether the continuous fluid models are realistic representations of an early dense universe. Given that an infinite density is possible in a continuous fluid, does it also follow that an infinite density is possible in a fluid consisting of particles?

Taking first the simplest view, we suppose that the fluid consists of classical particles which are elementary in the Democritan sense that they are indivisible. If  $m$  is the particle mass and  $\lambda$  the mean interparticle distance, the fluid density is  $\rho \sim m/\lambda^3$ . In its early stages the curvature

and cosmological constants have negligible effect and the age of the universe is  $t \sim (G\rho)^{-1/2}$ , or

$$t \sim (\lambda^3/Gm)^{1/2} \quad (1)$$

The continuous fluid approximation is valid when the particle horizon<sup>2</sup> is large compared with the interparticle distance, or  $ct \gg \lambda$ . From equation (1) it follows that this condition is also equivalent to  $\lambda \gg Gm/c^2$ , and the interparticle distance is therefore large in comparison with the gravitational length of a particle.

If we now go back sufficiently far in time we arrive at the weird situation of  $ct \sim \lambda$ , or

$$\lambda \sim Gm/c^2 \quad (2)$$

$$\rho \sim c^6/G^3m^2 \quad (3)$$

when each particle is isolated by the particle horizon and its neighbouring particles recede at the speed of light. Going back even further, when  $ct < \lambda$ , we are faced with the dilemma that  $\lambda < Gm/c^2$ , and either the particles are smaller than their gravitational length (which is unacceptable), or else they can be subdivided indefinitely into smaller masses (which is contrary to their property of elementarity). The situation in classical cosmology before  $t \sim Gm/c^2$  is, to say the least, paradoxical<sup>3</sup>.

Attempts to quantize the gravitational field encounter formal and conceptual difficulties<sup>4</sup>, and at present in cosmology only superficial and order of magnitude statements can be made. The "size" of a particle is its Compton wavelength  $\lambda = \hbar/mc$ , and the smallest possible value of  $\lambda$  is the gravitational length  $Gm/c^2$ . The mass and wavelength of a limiting particle<sup>5</sup> are found from  $\lambda^* \sim \hbar/m^*c \sim Gm^*/c^2$ , and are the Planck quantities<sup>6</sup>

$$m^* \sim (\hbar c/G)^{1/2} \sim 10^{20}m_n \quad (4)$$

$$\lambda^* \sim (G\hbar/c^3)^{1/2} \sim 10^{-20}\lambda_n \quad (5)$$

where  $m_n$  and  $\lambda_n$  are for a nucleon mass. The coupling constant of such particles is  $Gm^*/\hbar c \sim 1$  and gravitational and strong interactions are of comparable strength. In the early dense universe the mean particle energy is large and there is a multiplicity of particle states<sup>7</sup>. As a crude approximation we assume that ultimately all particles have the same energy  $m^*c^2$ , and by following the previous arguments or using equation 4 in equations 2 and 3, we obtain the Planck density

$$\rho^* \sim c^5/G^2\hbar \sim 10^{95} \text{ g cm}^{-3} \quad (6)$$

when the age of the universe is  $t^* \sim \lambda^*/c$ , or

$$t^* \sim (G\hbar/c^5)^{1/2} \sim 10^{-44} \text{ sec} \quad (7)$$

and the particle horizon is  $\lambda^* \sim 10^{-33} \text{ cm}$ .

The problem of what happens before  $t^*$  can be resolved roughly in the following fashion, by sacrificing the classical picture of the universe. The magnitude of the fluctuations of the metric, "for a quantum of energy  $mc^2$ ", is plausibly<sup>8</sup>

$$\Delta g \sim g(Gm^2/\hbar c)^{1/2} \sim g\lambda^*/\lambda \quad (8)$$

where  $g$  is a typical component of the metric tensor. The fluctuations are maximum at the shortest wavelength  $\lambda^*$ , and Wheeler sees the metric as having a foamlike structure in which the microcurvature has a scale length of  $\lambda^*$ . This microstructure sets a lower limit of  $\lambda^*$  and  $t^*$  on intervals that can be used meaningfully in a space-time manifold. As long as all intervals are large compared with  $\lambda^*$  and  $t^*$ , classical cosmology can be used to construct models of nested hypersurfaces orthogonal to cosmic time. At time  $t^*$ , however, fluctuations smear the spatial hypersurfaces throughout the age of the universe and the notion of an ordered sequence in time breaks down<sup>9</sup>. The study of the universe at time  $t \sim t^*$  now falls within the little known province of "quantum cosmology". The question of what happens before  $t^*$  is therefore apparently

meaningless; an equivalent answer reminiscent of Greek mythology is primordial chaos.

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## CHEMISTRY

### Photosensitized Oxidation of Carbon Monoxide on Semi-conductors supported on Silver

THE recently introduced method of using ultra-violet light to influence reversibly the electronic structure of semiconducting catalysts<sup>1-3</sup> has been extended to a combination of the photovoltaic effect and a catalytic reaction. Illumination with ultra-violet light of thin layers of catalyst, supported on metal, changes the electron distribution in the catalyst reversibly by promoting electrons from the semiconductor into the metal. Other properties, such as porosity and surface area, are unaffected even though they may be changed in doping and alloying. Thus a new method is provided to investigate the relation between electronic structure on the one hand and kinetics and activation energy on the other for catalytic reactions on supported catalysts.

The reaction kinetics and activation energy of the reaction  $2\text{CO} + \text{O}_2 \rightarrow 2\text{CO}_2$  have been investigated in the dark and under irradiation and in the presence of  $\text{Co}_3\text{O}_4$ ,  $\text{NiO}$ ,  $\text{ZnO}$  and  $\text{Ag}$  as well as with the oxides supported on silver. All kinetic measurements have been made over wide ranges of conversion to carbon dioxide in an all 'Pyrex' static system connected with a long necked Erlenmeyer quartz flask as reaction vessel. The reaction was followed by measuring the pressure decrease with time. The catalytic layer covered the bottom of the horizontally mounted vessel. Reaction temperature was measured at the bottom, centre and circumference. An Osram 'HBO 500' high pressure mercury lamp was used for illumination. Due to light absorption by the catalyst, a substantial temperature increase occurred, so that two cooling air tubes, separately controlled by needle valves, were introduced into the furnace, one near the centre of the bottom and one near the periphery. Exactly the same temperatures for corresponding dark and light runs were obtained at all measuring points so that differences of catalytic behaviour between light and dark runs would not be caused by uncontrolled temperature differences but must be the effect of light alone.



Table 1. ACTIVATION ENERGY, PRE-EXPONENTIAL FACTOR AND KINETIC EQUATION

Catalyst	Temperature range (° C)	Plain oxides				Kinetic equation	Supported oxides				Kinetic equation
		(1) Activation energy [kcal/mol]		(2) Log A			(1) Activation energy [kcal/mol]		(2) Log A		
		Dark	Light	Dark	Light		Dark	Light	Dark	Light	
Co <sub>3</sub> O <sub>4</sub>	120-350	9.4	6.0	4.4	1.7	$k_{\text{PCO}}\text{Po}_2^{1/2}$	3.5	1.6	1.6	-0.6	$k_{\text{PCO}}$
NiO	150-250	20.0*	15.2	5.0	3.0	$k_{\text{PCO}}$	40.3	33	7.7	6.0	$k_{\text{PCO}}/p_{\text{CO}_2}$
ZnO	400-600	20.6 <sup>1-3</sup>	10.2	8.3	-0.5	$k_{\text{PCO}}/p_{\text{CO}_2}$ *	9.0	1.8	20.6	9.0	$k_{\text{PCO}}$
Ag	300-550	14.6*	6.0	6.25	1.5	$k_{\text{PCO}}$					

\* In the light  $k_{\text{PCO}}$ .

Kinetic equation was proved by systematic variation of all initial partial pressures. Activation energy was evaluated by plotting  $\log k$  of runs with stoichiometric mixture against  $1/T$  (Arrhenius plot). Log A was extrapolated from the Arrhenius plot for  $1/T = 0$ . Dimensions of A:  $[s^{-1}]$  for first order,  $[s^{-1} \text{ torr}^{-1/2}]$  for 1.5 order,  $[\text{torr/s}]$  for first order with inhibition. A depends on surface area. Because of the preparation of the catalysts in the reaction vessel no BET-areas were measured. All catalysts of the same oxide had approximately the same surface, however, and a change of log A of only  $\pm 0.2$  (at least 7 different layers).

Catalytic layers were prepared by evaporating silver in the presence of 10-20 microns of hydrogen onto the inside bottom of the flask. The oxide in acetone suspension was then painted onto the evaporated silver layer. The reaction mixture was in contact with the oxides only. When the oxide layer did not cover the silver completely, the activation energy of silver was observed. Layer thickness, estimated by weight and geometric coated area, varied from 250 to 2200 Å for both oxide and silver. All layers were transparent when illuminated with the mercury lamp. Illumination was done from the silver side. The transmission of silver layers and spectral distribution of the 'HBO 500' radiation are given in Fig. 1. Even thick silver layers permitted at least 30 per cent of light to pass into the oxide. Catalysts were exposed to 4 runs and then held in the presence of the reaction mixture for one hour to obtain constant activity. Because of dead volume in the neck of the Erlenmeyer flask, a new method of pressure evaluation from the manometer reading was used<sup>4</sup>. Kinetic data and activation energies are given in Table 1.

The principal results are as follows. (1) On plain oxides, illumination of the catalyst significantly lowers the activation energy. Likewise, the pre-exponential factor A is lowered considerably. As a result, the straight lines of the photoactivation energy cross the lines of the dark activation energy in the Arrhenius plot. At temperatures higher than the cross point the absolute rate is higher in the dark; at lower temperatures, the photo-rate is higher. (2) Supporting the oxides on silver completely changes the activation energy, rate constant and reaction order. (3) On supported oxides, illumination of the catalyst considerably lowers activation energy and pre-exponential factor A in two cases (NiO, Co<sub>3</sub>O<sub>4</sub>) and increases it for ZnO. Orders remain unchanged.

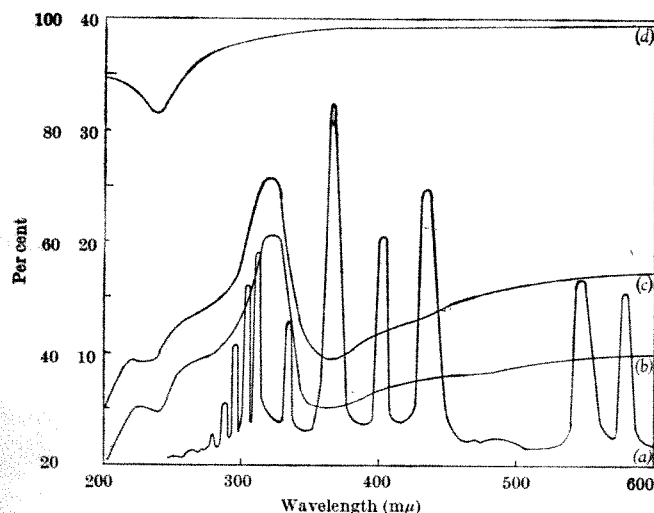


Fig. 1. Right scale, curve a, relative spectral distribution of 'HBO 500' in per cent of total radiation. Left scale, per cent transmission of evaporated silver layers. b, 1500 Å thick; c, 800 Å thick; d, transmission of quartz bottom of the reaction vessel.

It should be noted explicitly that the order of dark and light experiments on a catalyst layer has no influence on the kinetics and activation energy, and that dark and light runs may be alternatively measured; both kinds of measurements are strictly reproducible<sup>2</sup>. Promotion of electrons by light seems to be reversible at least as far as catalytic effects are concerned.

These experimental results may be understood in a manner similar to that for doping experiments<sup>1-3,6-8</sup>. The thickness of oxide layers is comparable with the thickness of space charge layers in semiconductors ( $10^{-7}$ – $10^{-4}$  cm)<sup>9</sup>. In the dark, the work functions of silver and of the oxides will determine whether electrons move from silver to the oxide or in the other direction<sup>7,8,10</sup>. The work function of silver is smaller than that of NiO and ZnO<sup>11,12</sup>. Silver emits electrons into NiO and ZnO, and the former, being a p-type semiconductor, shows a higher activation energy for the oxidation of CO (40.3 kcal/mol instead of 20.0). The activation energy of ZnO, an n-type semiconductor, is smaller (9.0 instead of 20.6). The work function of Co<sub>3</sub>O<sub>4</sub> is smaller than that of silver<sup>11,12</sup>. Even in the dark electrons therefore move to the silver, lowering the activation energy of the p-type Co<sub>3</sub>O<sub>4</sub> (ref. 13) (3.5 instead of 9.4). In the light, electrons are moved into the silver because of the photovoltaic effect<sup>14</sup>. The activation energy of the p-type NiO on silver is decreased to 7.7 kcal/mol, that on the n-type ZnO on silver increased again to the original 20.6 kcal/mol and that on the p-type Co<sub>3</sub>O<sub>4</sub> on silver decreased even further to 1.6 kcal/mol.

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## BIOPHYSICS

Hexagonal Single Crystal Pattern from the Spore Coat of *Bacillus subtilis*

We have already reported by chemical and X-ray diffraction investigations<sup>1,2</sup> that the spore coat of *Bacillus subtilis* is composed of a keratin-like protein with a crystalline structure. This communication reports the results of an electron diffraction investigation designed to give further information on the structure of the spore coat.

The spore coat preparation used in this work was obtained by disrupting the cleaned spores of *Bacillus subtilis* (Marburg strain) in a Braun cell disintegrator and collecting the sedimentable fraction. The detailed procedure used for preparing the spore coat has been described previously<sup>2</sup>. Selected fragments of the spore coat were examined by direct transmission in a JEM-7 type electron microscope at an applied voltage of 80 or 100 kV. Photographs of the micro and dark images were taken on the same specimen.

Fig. 1 shows the direct image of a fragment of the spore coat. The electron diffraction pattern and the micro-image photograph of this specimen are shown in Figs. 2 and 3 respectively. These photographs show that the electron beam was diffracted by the spore coat fragment itself. That this was so is also supported by the fact that a larger fragment of the spore coat which retained the original shape of the intact spore gave the same diffraction pattern as that in Fig. 2.

As will be seen from Fig. 2, the spore coat fragment gave a hexagonal single crystal pattern which has  $C_{6v}$  diffraction symmetry on the basal plane. The unit cell dimension in the direction of the  $a$  (or  $b$ ) axis was calculated to be 5.35 Å from this diffraction pattern. Because the spore coat fragment was a thin plate, as shown in Fig. 1, and it gave the pattern on the basal plane only, we could obtain no information about the direction of  $c$  axis, in spite of our efforts to tilt the specimen up to 20° in the field of an electron microscope. Accordingly, the space group to which the spore coat crystal belongs has not yet been determined.

Comparing the diffraction pattern of the spore coat with that of  $\alpha$ - and  $\beta$ -keratins which were reported by

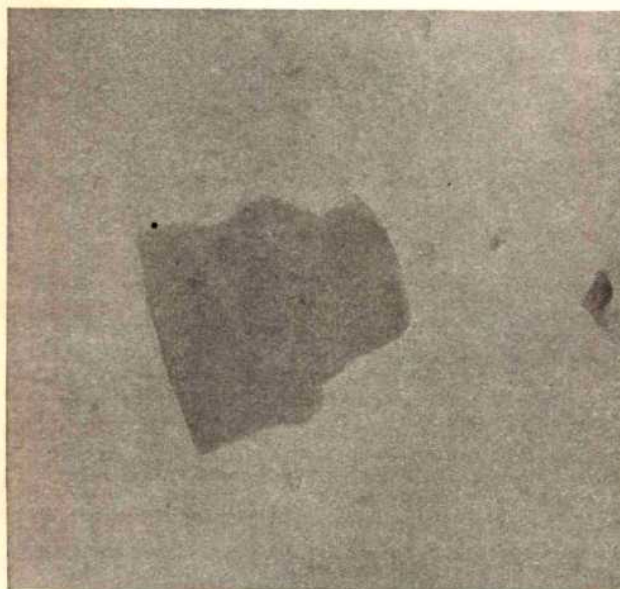


Fig. 1. Electron micrograph (direct image) of a fragment of spore coat. ( $\times 40,000$ .)

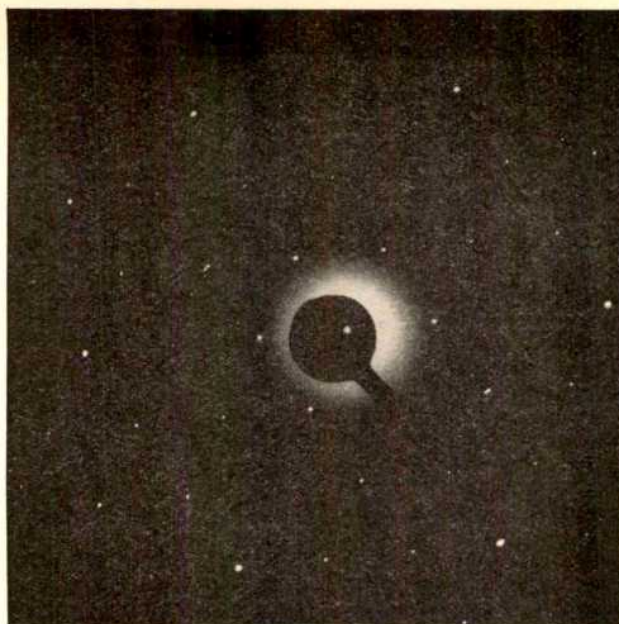


Fig. 2. Electron diffraction pattern of the spore coat specimen shown in Fig. 1. The diffraction pattern shows a hexagonal symmetry.

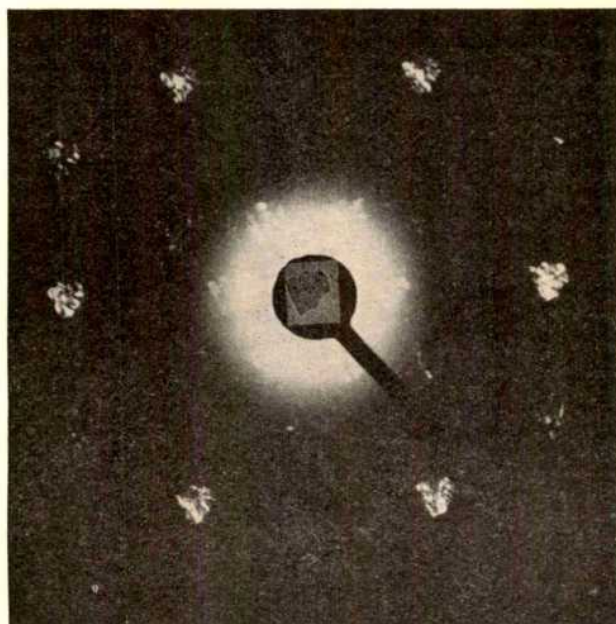


Fig. 3. Micro-image photograph of the specimen shown in Fig. 1. The direct image (centre) shows that the electron beam was diffracted by the spore coat fragment.

Astbury and Street<sup>3</sup>, it was found that the structure of the spore coat crystal was different from that of keratin;  $\alpha$  and  $\beta$ -keratins both showed orthogonal symmetries in the diffraction patterns. These results therefore suggest that the structure of the spore coat protein is similar to that of polyglycine II<sup>4</sup>, rather than to the  $\alpha$ -helix<sup>5</sup> or  $\beta$ -pleated structure of silk fibroin<sup>6</sup>.

Although several workers<sup>7,8</sup> have shown that the spore coat fractions from some bacilli contain a small amount of mucopolysaccharide, this substance was recently found by Warth, Ohye and Murrell<sup>9</sup> to be originally present in the residual cortex or cortical membrane, and not in the spore coat. This observation seems to support our concept that the spore coat is constructed from structural protein only.



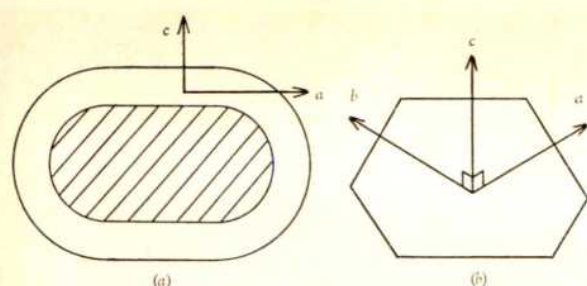


Fig. 4. Diagrammatic representation of the crystallographic structure of the spore coat showing the crystallographic axes on the longitudinal section of the spore (a) and those on the spore coat fragment (b). The spore coat is wrapped in a layer of single crystals of structural protein.

We have already shown<sup>10</sup> that electron micrographs of ultra-thin sections of the spores of *Bacillus subtilis* often indicate that the spore coat consists of about fifteen lamellae, each about 33 Å thick. These lamellae are considered, on the basis of the present results, to be single crystals of the structural protein.

Fig. 4 is a crystallographic diagram showing the proposed structure of the spore coat of *Bacillus subtilis*. The laminated spore coat (Fig. 4a), in which each lamella is thought to be a single crystal, is shown as a distorted O-shaped ring surrounding the spore core (shaded area). The *c* axis (the direction of the polypeptide chain) was perpendicular to the plate of a laminar crystal, whereas the *a* and *b* axes (the lateral directions of the polypeptide chain) were parallel to it (Fig. 4b). A detailed discussion of this problem will be published elsewhere.

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## IMMUNOLOGY

### Allotypic Specificity of Serum Protein in Inbred Strains of Rats

SINCE Oudin's original discovery of allotypes in the rabbit<sup>1</sup> a similar genetically determined intra-species differentiation of plasma proteins has been described in several species<sup>2</sup>. Although most interest was concentrated on immunoglobulins, allotypy is clearly a general biological phenomenon. It provides the biologist in a variety of fields with a useful tool, especially for investigating the molecular structure of proteins and of the genetic

characteristics of the cells producing these proteins. It is therefore important to know the allotypic systems of the experimental animal used.

To our knowledge there has been no report about allotypy in the rat. Perhaps the rat's bad reputation as a producer of precipitating antibodies has delayed the observation which we describe in this communication. This observation has been made during the course of a project in which highly inbred strains of rats, namely, Wistar BB (Department of Anatomy, Birmingham) and black and white hooded (Chester Beatty, London), have been used. Individual rats of the black and white hooded strain were immunized (as control animals for another experiment) in the following way: the animals received weekly intraperitoneal injections consisting of 2 mg of pooled rat serum proteins (Wistar BB) in a volume of 0.25 ml. each, with Freund's complete adjuvant (1 volume of antigen plus 2 volumes of adjuvant containing 2 mg of heat killed *Mycobacterium tuberculosis*/ml.). Four black and white hooded rats were bled a week after the twelfth injection and the sera were tested in 1 per cent agar-gel made up in buffered saline, pH 7.2, against the original material used for immunization (serum of Wistar BB rats). The plates were developed at room temperature for 24 h or longer. Three of the four samples of rat serum showed the presence of an antibody against the material with which they had been injected. The donor of the strongest antiserum (black and white hooded rat No. 24) was then immunized for 3 more weeks and bled out a week later. All the following tests have been performed using this No. 24 rat anti-rat antiserum.

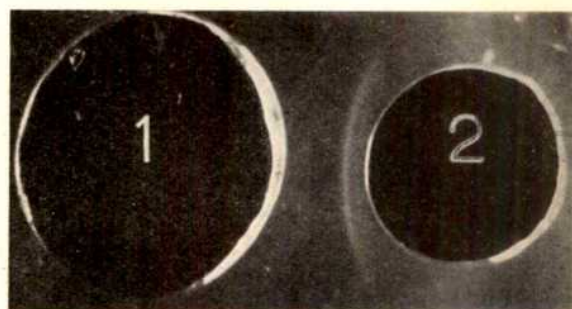


Fig. 1. Precipitation reaction between the rat (black and white hooded) antiserum No. 24 in well 1, and a rat (Wistar BB) pooled serum in well 2.

Fig. 1 illustrates a precipitation reaction between the rat No. 24 antiserum and the pooled rat serum (Wistar BB) used for immunization. Individual serum samples of various inbred strains of rats and mice were examined in the same way; Table 1 summarizes the results. The

Table 1. PRECIPITATION REACTIONS IN AGAR-GEL BETWEEN THE RAT ANTISERUM NO. 24 AND THIRTY-SIX INDIVIDUAL SERUM SAMPLES OF INBRED RATS AND MICE

Serum sample	Reaction	Serum sample	Reaction
Rat Wistar AA No. 1	+	Rat Wistar BB No. 1	+
" " AA " 2	+	" " BB " 2	+
" " AA " 3	+	" " BB " 3	+
" " AA " 4	+	" " BB " 4	+
" " AA " 5	+	" " BB " 5	+
" " AA " 6	+	" " BB " 6	+
" " AB " 1	+	" Albino (Gowans) No. 1	+
" " AB " 2	+	" " " " 2	+
" " AB " 3	+	" " " " 3	+
" " AB " 4	+	" " " " 4	+
" " AB " 5	+	" " " " 5	+
" " AB " 6	+	" " " " 6	+
Black and white hooded No. 1	-	Mouse A	-
" " " " 2	-	" C3H	-
" " " " 3	-	" C57Bl	-
" " " " 4	-	" DBA/BCR	-
" " " " 5	-	" IF	-
" " " " 6	-	" NZY	-

+, Positive reaction; -, no reaction.





Fig. 2. Immunoelectrophoretic pattern of rat (Wistar BB) serum electrophoresed in 1 per cent agar-gel (barbitone buffer, 0.05 molar, pH 8.6) at 15 V/cm for 2 h, and exposed to rat antiserum No. 24.



Fig. 3. Immunoelectrophoretic pattern of the serum from Fig. 2 exposed to rabbit anti-rat serum.

positive reactions were observed with the samples of rat serum from which the immunogen originated—the Wistar BB strain. They also occurred with the individual sera of normal Wistar AA rats, of the cross-breed ( $F_1$ ) Wistar AB and of the albino strain (Gowans). No precipitation, however, was noted between antiserum No. 24 and serum samples of six normal black and white hooded rats, the strain in which the antiserum was produced. This was also true of the single samples from six inbred mouse strains.

The reaction between antiserum No. 24 and a preparation<sup>3</sup> of Wistar BB  $\gamma$ -globulin, as demonstrated by the immunoelectrophoresis patterns (see Figs. 2 and 3), suggests that the allotypic specificity (determinant) being described is located on molecules belonging to the  $\gamma$ -globulin fraction of the serum. 'Sephadex G-200' filtration in 0.5 molar sodium chloride provided further evidence for the active material (antigen) found in the second peak only. We call this rat allotypic specificity (determinant) Ra 1 and the corresponding rat antibody (No. 24) anti-Ra 1.

There is no proof yet for the antibody activity of the molecules carrying the Ra 1 determinants and this should, of course, be investigated further; nor is there any evidence to which sub-class (Fig. 3) of the heterogeneous rat  $\gamma$ -globulin fraction<sup>4,5</sup> these molecules belong. Nevertheless, the reported allotypic specificity may, even at this stage, be of value to research workers looking for biological markers on plasma proteins of the rat.

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### Cytotoxic and Cytolytic Effect of Mouse Anti-embryo Sera on Ehrlich Ascites Cells

FURUSAWA *et al.*<sup>1,2</sup> have demonstrated surface antibodies common to embryonic cells of the mouse and Ehrlich ascites cells. In these conditions we have investigated, *in vivo* and *in vitro*, the action of the anti-embryo sera of the mouse on cancerous Ehrlich ascites cells.

The mouse anti-embryo sera were obtained by injecting guinea-pigs or rabbits with an entire crushed embryo of ML mouse (0.5 ml.) with 0.5 ml. of complete Freund's adjuvant every 15 days for 60 days. *In vitro*, all sera obtained, in the absence of complement, agglutinated

Ehrlich ascites cells even at weak dilutions (as low as 1/512). On the other hand, cytotoxic activity of different sera varied very much, and we have classified our sera into three categories. (a) These sera can only agglutinate Ehrlich ascites cells; they have no cytotoxic activity even when the serum is not heat-treated. (b) These sera have a cytotoxic effect in the presence of complement, as shown by the trypan blue technique; the cancerous cells impaired by the serum are stained with a 3 per cent solution of trypan blue in Hanks solution. This coloration shows the disorders of permeability of the cellular membrane, although by simple microscopic examination the cells do not seem to be destroyed. We have found in controls that 10 per cent to 20 per cent of ascites tumour cells are coloured after 30 min of incubation with trypan blue. When ascites cells were treated with antiserum to mouse embryo cytotoxic for ascitic cells, 40 per cent to 50 per cent of the cells were stained with trypan blue. Cytotoxic serum without complement did not alter the permeability of the cells as detected by staining with trypan blue. (c) These sera have an immediate distinct cytolytic effect in the presence of complement, which can be seen by direct microscopic examination. Under the influence of the sera, the ascites cells are destroyed in large numbers and are observed to burst. This cytolytic activity is usually stronger than that of the control anti-Ehrlich ascites sera.

Sera (c) are obtained more easily if embryos younger than 12 days old are used for the immunization. On the contrary, embryos older than 15 days cannot induce the formation of cytolytic sera. We should, however, emphasize that we cannot constantly reproduce the cytolytic sera (c) because we do not know all the necessary conditions.

Table 1. SURVIVAL OF MICE CHALLENGED SIMULTANEOUSLY WITH ASCITES TUMOUR CELLS ( $10^7$  CELLS) AND 0.15 ML. OF ANTISERUM TO MOUSE EMBRYO

Antisera and controls (0.15 ml.)	Experiment No.	No. of mice	Immunized animals	Mean survival time (days)
Non-cytotoxic antiserum (a)	1	8	G	14.2
	2	10	G	15.3
	3	12	R	14.7
Cytotoxic antiserum (b)	1	8	G	17.1
	2	10	R	16.2
	3	10	R	17.7
Cytolytic antiserum (c)	1	19	G	19.9
	2	4	G	25.2
Saline	1	30		15.2
G normal serum	1	12		12.8
R normal serum	1	15		13.5
Antiserum to mouse adult liver	1	15	G	9.6
Antiserum to ascitic fluid	1	15	G	18.1
	2	10	G	14.8
	3	10	R	13.9

G, guinea-pig; R, rabbit.

We were able to demonstrate the cytolytic effect of the anti-embryo sera on Ehrlich cells *in vivo* in two series of experiments. In the first, simultaneous injection of sera (b) or (c) with  $10^7$  cells of Ehrlich ascites in the peritoneum of ML mice caused a prolongation of the average survival rate (which is normally about 15 days) of 1–3 days (with sera (b)) or 4–5 days (with sera (c)). These results have been supported by several experiments as reported in Table 1. These results indicate that antisera against mouse embryo often inhibit tumour growth more effectively than the control anti-Ehrlich ascites sera.

In the second series of *in vivo* experiments we injected intraperitoneally fourteen ML mice on the fourth day of the development of the Ehrlich ascites with 0.5 ml. of undiluted serum. Five animals died during the first 48 h. Four animals showed prolonged survival (after the twentieth day) and showed either mixed ascitic tumours and solid (two mice) or only solid subcutaneous tumours when the ascites cells were inoculated (two mice). The appearance of these subcutaneous tumours indicates that the ascitic cells are largely destroyed by the anti-embryo serum; but on the contrary, the cells which had diffused into the subcutaneous tissue at the time of inoculation of the ascites cells escaped the action of the serum and were able to develop. Such an explanation



has already been put forward by Colter *et al.*<sup>3</sup>, who also observed the appearance of solid tumours after treatment of Ehrlich ascites. We should, however, point out that we were unable to reproduce this experiment with sera (a) or (b). On the contrary, treatment with these sera shortened the period during which these animals survived.

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### Haemolytic Plaque Formation by Unimmunized Mouse Peritoneal Lymphocytes

BUSSARD<sup>1</sup> has recently reported that when peritoneal cells from unimmunized mice are incubated at 37° C with sheep red blood cells and complement in a film of carboxymethyl-cellulose gum (CMC)<sup>2</sup>, they produce plaques of haemolysis. He also presented some evidence that this lysis results from the production of antibody.

We have studied this phenomenon in the hope that the system might be useful in investigating the mechanism by which some oncogenic viruses depress immunity<sup>3</sup>. We have confirmed Bussard's observation and attempted to determine the type of cells which actually produce plaques, and the effect on them of anti-mouse immunoglobulin serum (AMiGS). Briefly, in the Bussard system, mouse peritoneal cells are suspended in 2.5 per cent CMC in *tris*-buffered Eagle's medium plus antibiotics, with 5 per cent sheep red blood cells and 10 per cent fresh guinea-pig serum absorbed with sheep red blood cells. The mixture is pressed between a slide and a coverslip, sealed and incubated at 37° C. Plaques of haemolysis are counted microscopically and related to the number of peritoneal cells in the film.

In this system, peritoneal cells from unimmunized adult *BALB/c* mice produced a fairly constant number of plaques, and this property was shared by pleural fluid cells, but not by cells from the spleen, lymph node, thymus or bone marrow. The first plaques appeared after incubation for 15 h and reached a maximum number at 72 h. Controls with inactivated complement, heat-killed peritoneal cells, or isologous mouse red blood cells, were all negative, but in the last case observation was limited to 24 h because of their fragility (M. B., unpublished observation).

Between 98 and 99 per cent of the mouse peritoneal cells are either lymphocytes or macrophages. In order to separate these, we used the ability of macrophages to stick more firmly to surfaces *in vitro* than lymphocytes. Because the macrophages could not then be detached, the original Bussard method had to be modified. Three types of peritoneal cell preparation were made in plastic tissue culture Petri dishes 50 mm in diameter:

(1) *Total population*. The cell suspension was allowed to settle for 3 h at 37° C, and the medium gently removed. A microscopic count showed that about 42 per cent of the cells had the appearance of macrophages.

(2) *Macrophages*. After incubation for 1 h at 37° C, cells adhering to the dish were washed three times at hourly intervals, twice gently, the third time vigorously: about 98 per cent of the remaining attached cells were macrophages.

(3) *Lymphocytes*. The first supernatant removed from (2) was incubated for a further hour, and then transferred to fresh dishes. After the cells had been incubated for a further 1.5 h in order to allow the cells to settle the medium

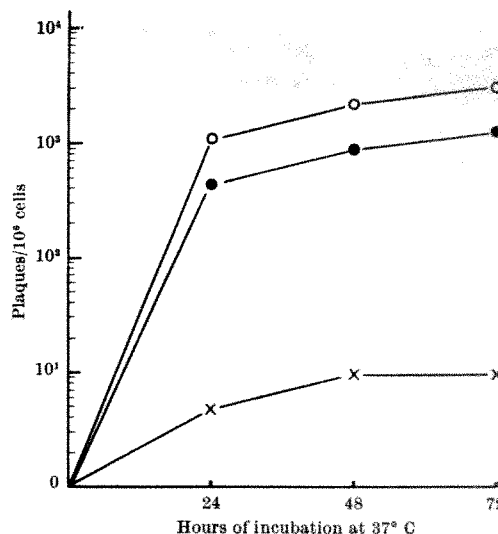


Fig. 1. Production of haemolytic plaques by different peritoneal cell types. ○, Lymphocytes (~92 per cent pure); ×, macrophages (~98 per cent pure); ●, mixed population (~58 per cent lymphocytes).

was gently removed. About 92 per cent of these cells were lymphocytes.

In each case the cells were covered with a drop of the mixture of CMC sheep red blood cells and guinea-pig serum; a coverslip was pressed on to the drop and sealed. The numbers of plaques per 10<sup>6</sup> cells formed by each type of preparation during incubation for 3 days are given in Fig. 1. The total population gave numbers of plaques similar to those observed when the cells were incorporated into the gum. Very few plaques were formed in the macrophage preparation and these could have arisen from residual lymphocytes. More plaques per 10<sup>6</sup> lymphocytes were formed in the 92 per cent pure preparation than in the mixed population with macrophages.

Several workers<sup>4-7</sup> have shown that the presence of AMiGS decreases the number of haemolytic plaques formed in the Jerne test<sup>8</sup> by mouse spleen cells in the first 2-3 days of immunization and greatly increases it in the later stages.

A rabbit AMiGS which reacted immuno-electrophoretically with all five classes of mouse immunoglobulins was kindly provided by Drs Dresser and Wortis. Dilutions of this serum, or of normal rabbit serum, were incorporated in the gum with peritoneal cells, sheep red blood cells and complement. For comparison, the effects of the same concentrations of sera on the production of plaques by spleen cells from mice injected intravenously with  $2.5 \times 10^8$  sheep red blood cells 2 or 9 days before were also examined. The spleen cells were tested both by the Jerne<sup>8,9</sup> and Bussard techniques. The results are given in Table 1.

The action of AMiGS on plaque production by spleen cells from immunized mice was similar in the Jerne and the Bussard systems, namely a slight to almost complete inhibition (depending on concentration of the serum) at 2 days, and a marked enhancement at 9 days, after immunization. Plaque production by peritoneal cells from unimmunized mice in the Bussard system was inhibited in a similar way to that by spleen cells taken 2 days after immunization. Appropriate tests showed that the inhibitory effect of AMiGS on peritoneal cell plaques was not due to anticomplementary activity or to cytotoxicity. It was abolished by absorption with normal *BALB/c* mouse serum. Normal rabbit serum also had some inhibitory effect (not reduced by absorption with mouse serum) on plaque formation by peritoneal cells from unimmunized mice, and by spleen cells taken 2 days after immunization—another similarity between them.

Bussard suggests that peritoneal cells react to a primary antigenic stimulus because they are a mixed population



Table 1. EFFECTS OF VARIOUS SERA ON HAEMOLYTIC PLAQUE FORMATION

Serum†	Fina concentration	No. of haemolytic plaques/10 <sup>6</sup> cells formed by			
		Peritoneal cells from unimmunized mice	Spleen cells from immunized mice 2 days after immunization*	Spleen cells from immunized mice 9 days after immunization*	
		Bussard‡	Bussard§	Jerne	Bussard§
None		1,780	19	20	94
Rabbit anti-mouse Ig	1/2,880	1,570	18	17	496
Rabbit anti-mouse Ig	1/120	< 7	1	3	312
Normal rabbit	1/2,880	1,840	—	20	—
Normal rabbit	1/120	967	—	10	—

\* 2.5 × 10<sup>6</sup> sheep red cells injected intravenously.

† All sera heated at 56° C for 30 min. and absorbed with sheep red cells.

‡ After incubation for 3 days at 37° C.

§ After incubation for 5 h at 37° C.

of macrophages and lymphocytes<sup>1</sup>. Our results show that it is the lymphocytes which actually produce the plaques, and this indirectly supports the view that the plaques result from antibody production because there is no evidence that macrophages produce antibodies. Because our purified lymphocyte suspensions contained 8–9 per cent macrophages, however, an essential role for the macrophage cannot be excluded. The inhibitory effect of AMIgS demonstrates that plaque formation by peritoneal cells is mediated by immunoglobulins, and is a further indication that plaques result from the production of antibody. The close parallelism between this effect on peritoneal cells from unimmunized mice and on spleen cells from mice 2 days after immunization suggests that the former, like the latter, produce 19S antibody.

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### Allograft Survival prolonged by Heterologous Spleen Cells in 'Millipore' Diffusion Chambers

WE have mixed heat-inactivated lymphoid cells from C57Bl mice with rat spleen cells. The mixture was placed in 'Millipore' diffusion chambers which were introduced intraperitoneally into C57Bl mice. Five days later these animals were grafted with CBA skin. We hoped that the rat cells would produce antibody against antigenic moieties present in the mouse lymphoid cells, and that the antibody would diffuse out of the chambers, inactivate host lymphoid cells and thus depress the immune response of the treated animal. Previous workers<sup>1,2</sup>, employing a rather similar experimental model, have shown that lymphoid cells mixed in diffusion chambers with sheep red cells can produce antibody against the latter. The ability of a heterologous anti-lymphocyte serum to prolong allograft survival is now well established<sup>3-6</sup>.

In each of our experiments spleens from two or three syngeneic female Lewis rats (170–200 g body weight) were pooled, finely minced with scissors on a stainless

steel mesh and irrigated with TC 199 (Difco). Lymphoid cells from C57Bl mice were added to this cell suspension in a ratio of a hundred rat cells to one mouse cell. The mixture of cells was gently centrifuged for 5 min; the sediment was resuspended in a small amount of TC 199 and placed in 'Millipore' diffusion chambers. A volume of 0.1–0.15 ml. of cell suspension was placed in each chamber; the number of cells present on each occasion is indicated in Table 1.

The mouse lymphoid cells used were obtained from the spleen, lymph nodes and thymus of male C57Bl mice (20 ± 2 g body weight). The tissue was cut up into small fragments, suspended in TC 199 and gently homogenized by hand in a glass homogenizer. The suspension of cells was filtered through a stainless steel mesh, centrifuged and washed twice with TC 199. Red cells were lysed by adding 5 ml. of distilled water to the pellet of cells with vigorous agitation for 15 sec, after which 10 ml. of TC 199 was added, and the cell suspension was centrifuged and washed twice with TC 199. The suspension of cells was next subjected to 56° C for 20 min in an attempt to prevent the mouse cells in the chambers from reacting against, and inactivating, the rat spleen cells; we had previously found (unpublished observations) that 56° C for 20 min resulted in very significant reductions in the functional viability of lymphoid cells. Finally, the cells were washed twice more with TC 199 and then added to the suspension of rat spleen cells, as previously indicated.

Diffusion chambers, membranes and cement (formulation 1) were obtained from the Millipore Filter Corporation.

Table 1. SURVIVAL TIMES OF CBA SKIN ON C57Bl MICE AFTER INTRAPERITONEAL INTRODUCTION INTO THE C57Bl MICE OF 'MILLIPORE' DIFFUSE CHAMBERS CONTAINING RAT SPLEEN CELLS AND INACTIVATED C57Bl LYMPHOID CELLS

Treatment	No. of cells in chambers	No. of animals with grafts surviving at:					
		<13*	13–15	15–20	20–25	25–30	
Untreated							29
Chambers with rat spleen cells	1.4 × 10 <sup>6</sup>						10
Chambers with rat spleen cells and inactivated rabbit lymphoid cells	1.8 × 10 <sup>6</sup>						10
Chambers with inactivated rat spleen cells and C57Bl lymphoid cells. (L, S, T)	1.3 × 10 <sup>6</sup>						10
Intraperitoneal injection of mixture of rat spleen cells and inactivated C57Bl lymphoid cells. (L, S, T)	1.5 × 10 <sup>6</sup>						9
Chambers containing rat spleen cells and inactivated C57Bl lymphoid cells.							
Experiment (1) (L, S, T)	2 × 10 <sup>6</sup>		1†	2		1	2†, 2
(2) (L, S, T)	1.8 × 10 <sup>6</sup>		4	2			1†, 1
(3) (L, S, T)	0.9 × 10 <sup>6</sup>		4	3			
(4) (L, S, T)	1.5 × 10 <sup>6</sup>		9				
(5) (L, S, T)	1.5 × 10 <sup>6</sup>		3	3	1		
(6) (L, S, T)	1.8 × 10 <sup>6</sup>		2			6	
(7) (L, S, T)	1.5 × 10 <sup>6</sup>		8				
(8) (L, T)	3.2 × 10 <sup>7</sup>		4		2	2	
(9) (T)	10 <sup>6</sup>			2	3		2

\* The figures represent the number of days for which the grafts survived.

† Animal died with intact healthy graft.

L, Lymph nodes; S, spleen; T, thymus. These letters indicate the tissue of origin of the C57Bl lymphoid cells used. Lymphoid cells from male C57Bl mice (20 ± 2 g body weight) were subjected to 56° C for 20 min. These cells were added to a suspension of spleen cells from syngeneic Lewis rats in the proportion of 100 rat cells to 1 mouse cell. Mixed cell suspensions were placed in 'Millipore' diffusion chambers, and each C57Bl mouse (20 ± 2 g body weight) in the experimental groups received one chamber intraperitoneally. Five days later the mice were grafted with skin from male CBA mice (20 ± 2 g body weight).

The chambers had diameters of 14 mm (outer diameter) and 10 mm (inner diameter) and were 2 mm high. Membranes had an average pore diameter of 100 m $\mu$  (Millipore Filter Corporation, No. VCWPO 1400). They were sterilized by dry heat at 70° for 48 h. After introduction of the cell suspension and sealing, one chamber was inserted into the peritoneal cavity of each of the C57Bl mice (male, 20  $\pm$  2 g body weight) in the experimental groups. Cell suspensions and chambers were kept at 4° throughout the course of an experiment.

Five days later the mice were grafted with skin taken from the ventral surface of male CBA mice. Grafts were inspected 9, 11, 13 and 15 days after grafting and daily thereafter. Signs of rejection were considered to be moistness, oedema, loss of epithelium or scabbing and, in the long-standing grafts, scaling or contraction of the graft to less than a quarter of its original size. Grafts were kept covered with a 'Band-Aid' for 15 days, unless rejected earlier.

The results of nine separate experiments of this type, together with appropriate controls, are presented in Table 1. They indicate that the experimental system used was capable of prolonging allograft survival in a significant number of treated animals. In view of the failure to prolong allograft survival in two of the experimental groups, it would not be possible to state categorically that similar results would not have been obtained had the control groups been larger and more numerous. It seems more likely, however, that the prolongation of allograft survival noted here was caused by a diffusible humoral factor, produced as the result of a reaction by viable rat spleen cells against inactivated mouse lymphoid cells in the diffusion chambers.

Allograft survival was unaffected in a number of treated animals. There are many possible reasons for this. First, it may have been caused by deficiencies in the experimental design. The number of cells in the chambers, the ratio of rat to mouse cells used and the timing of skin grafting in relation to the introduction of the chambers, could all have been less than optimum.

Second, failure to prolong allograft survival may have been, and probably was to a large extent, caused by problems inherent in the use of diffusion chambers. The pores of the membrane frequently become blocked with fibrin; this would certainly compromise the viability of cells in the chamber and at the same time prevent the escape of humoral factors. Very occasionally, cells have leaked from chambers which were defectively sealed. When, however, chambers from animals on which allograft survival had not been prolonged were examined, the two most common findings were infection around and clot formation in the chambers. Infection, which is usually of a low grade, results in encapsulation of the chamber with fibrin, serous fluid, omentum and gut; the contained cells die. Clot formation, which has been previously described by others<sup>1,2</sup>, occurs with unpredictable frequency and results in the formation of a gelatinous clot in which most of the cells are incorporated. Infection and clot formation were rarely found when chambers were examined from animals on which graft survival had been prolonged.

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## HAEMATOLOGY

### Nature of Luteinizing Hormone Releasing Factor in Hypophysial Portal Blood

In 1966 Worthington<sup>1</sup> described a method for the collection of hypophysial portal blood from the cut pituitary stalk of rats. This method was used by Fink, Nallar and Worthington<sup>2,3</sup> to demonstrate the presence of luteinizing hormone releasing factor (LRF) in portal blood obtained from rats in proestrus and from rats hypophysectomized at least two weeks before collection. The chief disadvantage of this method of collection of portal blood was that contamination of samples by blood flowing back through pituitary sinusoids and bearing significant quantities of

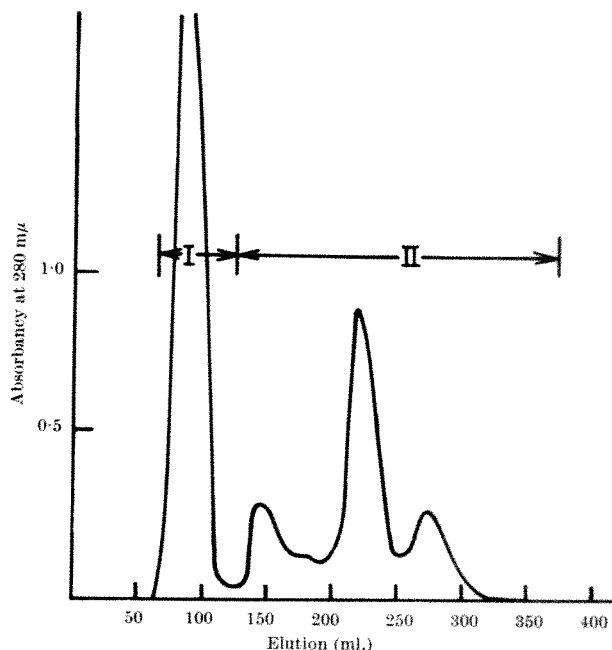


Fig. 1. Elution profile on 'Sephadex G-25'. 9 ml. portal plasma from nine ovariectomized rats extracted with acid alcohol. Supernatant evaporated, redissolved in 4.5 ml. of distilled water and loaded on a column of 'Sephadex G-25' (fine), 35.5  $\times$  2.5 cm. Eluted with 0.3 molar acetic acid; 5.0 ml. fractions; rate 0.5 ml./min. Fraction I contains substances of molecular weight > 5,000; Fraction II contains substances of molecular weight < 5,000.

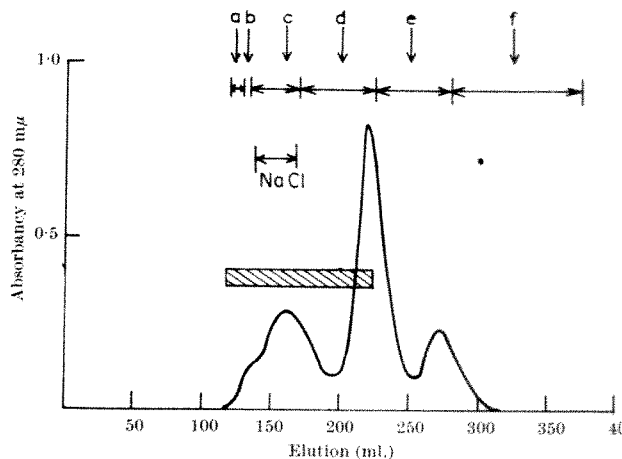


Fig. 2. Elution profile on 'Sephadex G-25'. 9 ml. portal plasma from nine animals extracted with acid alcohol. Supernatant evaporated and redissolved in 4.5 ml. distilled water. Loaded on to a column of 'Sephadex G-25' (fine) 35.5  $\times$  2.5 cm; eluted with 0.3 molar acetic acid; 5.0 ml. fractions; rate 0.5 ml./min. Fraction II lyophilized, redissolved in 4.5 ml. distilled water and refractionated on same column. Eluate divided into six fractions and assayed by OAAD method. LRF activity found in shaded region. Note activity in d suggesting adsorption ( $K_d > 1$ ).

anterior pituitary hormones could not be prevented. Thus, because the ovarian ascorbic acid depletion method<sup>4,5</sup> was used to assay the plasma, some of the activity exhibited by portal plasma from animals in proestrus probably resulted from luteinizing hormone (LH).

The exclusion of LH from samples of hypophysial portal plasma has been achieved by use of an unpublished method devised by Dr C. P. Fawcett in which the plasma is extracted with acid alcohol and fractionated on 'Sephadex G-25'. Substances of molecular weight greater than 5,000 are excluded from the gel particles and so, therefore, any trace of LH which remains after extraction appears in the void volume (fraction I, Fig. 1), whereas substances such as LRF, with molecular weights lower than 5,000, are included in the gel particles and appear in fraction II (Fig. 1). This method has been used to demonstrate the presence of LRF in hypophysial portal blood from hypophysectomized and ovariectomized rats, and from rats at various stages of the oestrous cycle<sup>6</sup>.

The aim of the experiments reported here was to use gel filtration to obtain an estimate of the molecular weight of LRF extracted from hypophysial portal blood of rats which had been ovariectomized 3-8 weeks before collection. The ovarian ascorbic acid depletion assay<sup>4,5</sup> was used to determine LRF activity.

The behaviour of LRF when fractionated on 'Sephadex G-25' was investigated first. As is shown in Fig. 2, LRF activity was present in the first four subfractions of fraction II. Because activity persisted beyond the electrolyte region, adsorption played a part in the retention of LRF by the gel. This precluded the use of calibrated columns of 'Sephadex G-25' to estimate the molecular weight of LRF.

It was decided, therefore, to use 'Sephadex G-10', which has an exclusion limit of 700-900, to determine whether the molecular weight of LRF in portal blood was above or below this range. Evidence from studies on LRF in which extracts of hypothalamus and stalk median eminence were used<sup>7</sup> has suggested that this substance is a polypeptide, with a molecular weight of 1,500-2,000. Consequently, it was thought that LRF would be excluded from 'Sephadex G-10'; the results, however, were quite unexpected.

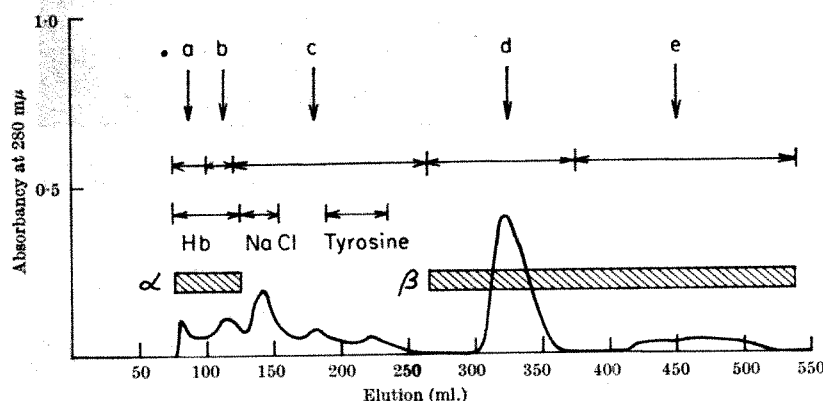


Fig. 3. Elution profile on 'Sephadex G-10'. 4.0 ml. of Fraction II from 'Sephadex G-25' (2 × concentrated) loaded on column of 'Sephadex G-10' 43.0 × 2.5 cm. Eluted with 0.3 molar acetic acid; 5.0 ml. fractions; rate 0.35 ml./min. Eluate divided into five fractions and assayed by OAAD method. LRF activity in shaded region. Note retention of β beyond tyrosine.

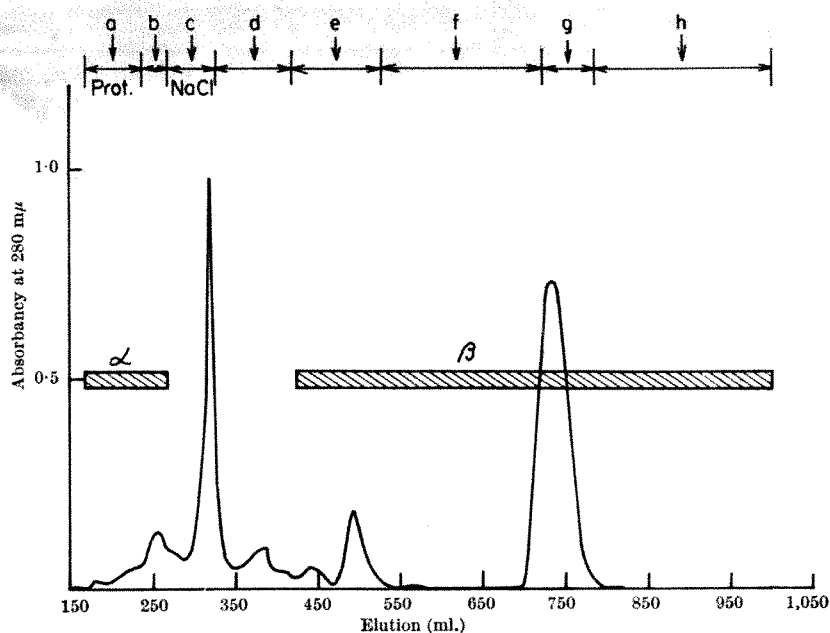


Fig. 4. Elution profile on 'Sephadex G-10'. 4.0 ml. of Fraction II (concentrated from 24 ml. of portal plasma) from 'Sephadex G-25' loaded on column of 'Sephadex G-10' 94.0 × 2.5 cm. Eluted with 0.3 molar acetic acid; 5.0 ml. fraction; rate 0.5 ml./min. Eluate divided into eight fractions, aliquots of which were assayed by the OAAD method and by determining release of LH from the anterior pituitary glands of ovariectomized, oestrogen, progesterone blocked rats. LRF activity found in shaded region.

Hypophysial portal plasma was extracted and fractionated on 'Sephadex G-25'. The small molecular weight fraction (II) was lyophilized to dryness, redissolved in distilled water and fractionated on 'Sephadex G-10' (Fig. 3). Activity was found in two regions, α and β, separated by an interval which included the electrolytes. Whereas α was largely excluded from the gel particles, β was markedly adsorbed. This experiment was repeated twice using longer columns of 'Sephadex G-10' (Fig. 4). Similar results were obtained.

In order to exclude the possibility that α and β had caused depletion of ovarian ascorbic acid by direct action on the ovary rather than by the release of LH from the anterior pituitary gland of the test animals, aliquots of fractions from the experiment shown in Fig. 4 were injected intravenously into ovariectomized, oestrogen, progesterone treated rats<sup>8</sup>. Fractions from regions α (a and b) and β (e, f, g and h) caused a significant rise in the level of LH in the plasma of the intermediate animals (assayed by the ovarian ascorbic acid depletion method), whereas fractions c and d from the intervening region had no effect.

These results indicate that the LRF activity of hypophysial portal blood from ovariectomized rats may be caused by two specific substances. It is possible, however, that β is the active molecule and that α consists of a carrier molecule to which β is attached. Dissociation of a proportion of α during extraction and fractionation would then explain the finding of two active substances. The alternative explanation, that α is a polymer of β, must also be considered. The possibility that the activity of these substances was caused by vasopressin or by a non-specific factor introduced during the collection of portal blood has been excluded in previous experiments<sup>9</sup>.

The molecular weight of α probably lies in the range of 900-5,000; however, because of the strong adsorption



of  $\beta$  by 'Sephadex G-10', a valid estimate of the molecular weight of this substance may not be made. The adsorption of  $\beta$  suggests that it probably contains a heterocyclic or aromatic component. Because of the low pH of the eluent and the presence of considerable quantities of electrolytes, it is unlikely that electrostatic forces played a principal part in the adsorption of  $\beta$  by the gel<sup>9</sup>.

It is of interest that recently Fawcett, Reed, Charlton and Harris (personal communication), using anion exchange chromatography and gel filtration in a partially organic medium, found two regions of LRF activity in extracts of hypothalamus.

I thank Professor G. W. Harris for suggesting this project and for advice. I also thank Dr D. B. Hope for criticism. This work was supported in part by the Medical Research Council and the US Air Force.

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### Localization of Transferrin in Human and Rat Liver by Fluorescent Antibody Technique

FLUORESCENT antibodies have been used to demonstrate intracellular albumin and fibrinogen in human liver<sup>1</sup>. We have shown the presence of transferrin in normal liver cells of man and rat using anti-transferrin and fluorescein-labelled anti-globulin.

Human transferrin was supplied by Behringwerke AG., and rat transferrin was prepared by the method of Gordon and Louis<sup>2</sup> combining electrophoresis of rat serum on 'Pevikon C870' and chromatography with DEAE 'Sephadex A-50'. Both transferrin samples were found on immunoelectrophoretic analysis to contain small amounts of  $\gamma$ -globulin and were therefore further purified by passage through 'Sephadex G-100'.

Rabbits were injected intramuscularly with 10 mg of transferrin and Freund's complete adjuvant, equally divided between five sites. Booster injections were given with pure transferrin 4 and 8 weeks after the primary injection. Antibody titre was assessed by the tanned sheep cell haemagglutination technique after Stavitsky<sup>3</sup>, by gel diffusion and by immunoelectrophoresis. Potent antisera were collected after the second booster injection; neither antiserum showed absolute specificity to human or rat transferrin, showing the difficulty of preparing wholly pure antigens from serum proteins. For use the antisera were rendered specific by absorption of the anti- $\gamma$ -globulin component.

Rabbit IgG was prepared by separation of 40 per cent ammonium sulphate precipitated rabbit serum on DEAE 'Sephadex A-50', the fraction eluted with 17.5 mmolar phosphate buffer, pH 6.8, and kept for immunization. Sheep anti-rabbit IgG was prepared using the schedule already described. Titre was estimated by the haemagglutination of group A rabbit red cells sensitized with anti-A serum. Sheep anti-rabbit IgG was conjugated after the method of Goldstein, Slizys and Chase<sup>4</sup>. Before conjugation with fluorescein isothiocyanate, the sheep immunoglobulin was prepared in a pure form and after conjugation the fluorochromed protein was fractionated on DEAE 'Sephadex A-50' as described by Goldstein

*et al.*<sup>4</sup>. Conjugate fractions with a fluorescein/protein ratio between 0.4 and  $2.5 \times 10^{-3}$  with a protein concentration of 10 mg/ml. were saved for use. The conjugate was diluted 1:3 for staining.

Human liver was obtained fresh from an operation and was immediately frozen at  $-50^\circ\text{C}$ . Rat tissues were taken fresh when required and directly frozen at  $-35^\circ\text{C}$  on a cryostat chuck. Sections, 2 $\mu$  thick, were cut in a cryostat and the fluorescent staining was performed as an indirect technique on unfixed tissues. The following blanks were set up with all preparations: (1) unstained tissue (autofluorescence); (2) tissue plus sheep anti-rabbit IgG conjugate (non-specific fluorescence); (3) tissue plus rabbit anti-rat serum absorbed with pure rat transferrin followed by conjugate. Slight autofluorescence was seen in the liver capsule, but this was not obtrusive. Non-specific fluorescence was absent. Absorption of the anti-transferrin serum resulted in the loss of the specific staining pattern.

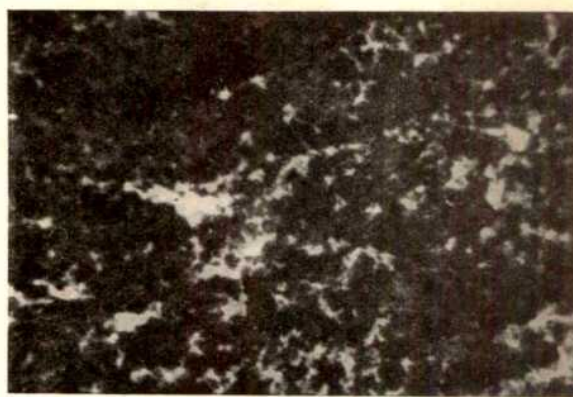


Fig. 1. Fluorescent transferrin cells in human liver. Low power view showing the scattered distribution.

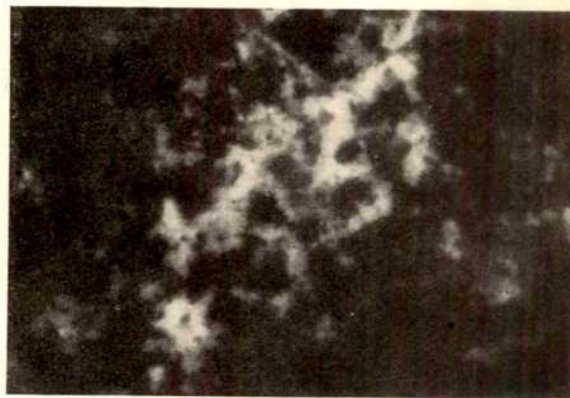


Fig. 2. Fluorescent transferrin cells in human liver. Oil immersion field showing cytoplasmic granularity and absence of nuclear staining.

**Human liver.** Cells exhibiting bright fluorescence and staining specifically for transferrin were seen in all preparations (Figs. 1 and 2). There was a scattered distribution of such cells throughout the liver tissue within the lobule; there seemed to be no special pattern associated with the centrilobular vein or round the periphery of the lobule. A much greater percentage of staining was seen in the parenchymal than in Kupffer cells, and in both fluorescence was cytoplasmic with no nuclear staining. High power examination revealed that the fluorescent parenchymal cytoplasm was granular





Fig. 3. Fluorescent transferrin cells in rat liver. Low power view showing the general distribution comparable with the human liver, but with a diminution in the number of staining cells.



Fig. 4. Fluorescent transferrin cells in rat liver. Oil immersion field showing pan-cytoplasmic fluorescence and absence of nuclear staining.

and that some cells showed only cytoplasmic granules rather than pan-cytoplasmic fluorescence.

**Rat liver.** The pattern of staining was the same in rat liver as in human liver (Figs. 3 and 4), but fewer cells showed fluorescence. The stained cells were evenly distributed throughout the tissues. High power examination (Fig. 4) revealed a close similarity with human liver, that is variability in intensity of fluorescence, no nuclear staining, but obvious cytoplasmic granularity.

These results indicate that the distribution of transferrin in the liver is similar in many ways to that of albumin and fibrinogen. The human and rat livers selected for the investigation of transferrin were considered to be normal, and so the results may be representative for the liver in the steady state. We hope that further experiments aimed at stimulating increased synthesis of transferrin will result in an altered pattern of distribution and this is now being studied.

This work was begun during the tenure of a research fellowship from Fisons Pharmaceuticals, Ltd. The work was directed by Dr A. D. T. Govan in the Research Department of the Royal Maternity Hospital, Glasgow. I thank Professor P. L. Mollison for his advice and encouragement.

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## Serum Transferrin Polymorphism in the Desert Sheep of the Sudan

SINCE the introduction of starch-gel electrophoresis by Smithies<sup>1</sup>, the intraspecific polymorphisms of several types of iron-binding *B*-globulins (transferrins) in the sera of different species of farm animals have been examined by a number of workers. In sheep the number and frequency of the transferrins have been found to vary from one breed to another: Ashton<sup>2</sup> and Ashton and Ferguson<sup>3</sup> have reported as many as five to seven zones in any one breed. This communication deals with the results of a preliminary investigation of serum transferrin polymorphism in the desert sheep of the Sudan.

In all, 393 samples of blood serum were available for this study. The blood samples were obtained from the sheep as they were being killed at Omdurman abattoir, which receives animals from most of the arid parts of Northern Sudan.

Table 1. COMPARISONS OF THE TRANSFERRIN ZONES IN DESERT SHEEP WITH SHEEP REFERENCE SERA OBTAINED FROM WAGENINGEN

No. of zones	Decreasing mobility											
	1	2	3	4	5	6	7	8	9	10	11	12
Wageningen samples, nomenclature as accepted at Paris conference	I	A	G		B	C	M	D	—	—	E	—
Desert sheep, local nomenclature	—	A	—	B	C	D	E	—	—	—	F	—
											G	

Starch-gel electrophoresis using a *tris*-discontinuous buffer system was used to identify the serum transferrin types. Seven transferrin zones, each represented by one densely stained and another faintly stained, were found to occur in serum from desert sheep of the Sudan. This number of observed zones is similar to that reported by Ashton and Ferguson<sup>2</sup> in Australian Merino sheep. Fig. 1 shows the five out of the observed seven transferrin zones. Starting from the fastest zone the following nomenclature was assigned to the seven observed zones: TfA, TfB, TfC, TfD, TfE, TfF and TfG. To overcome the confusion already existing in the nomenclature of sheep transferrins, reference sera coded according to the recent nomenclature approved at the tenth (Paris) conference of the European Society for Animal Blood Group Research were obtained with the kind co-operation of Dr C. C. Oosterlee, of Wageningen, The Netherlands. Table 1 shows a comparison between the transferrin types identified in the desert sheep and those obtained from Wageningen. Figs. 2a and b show some of these comparisons.

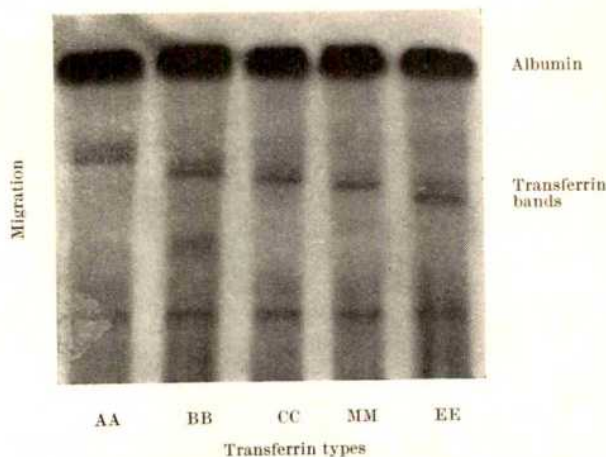


Fig. 1. Desert sheep serum transferrin types after starch-gel electrophoresis.



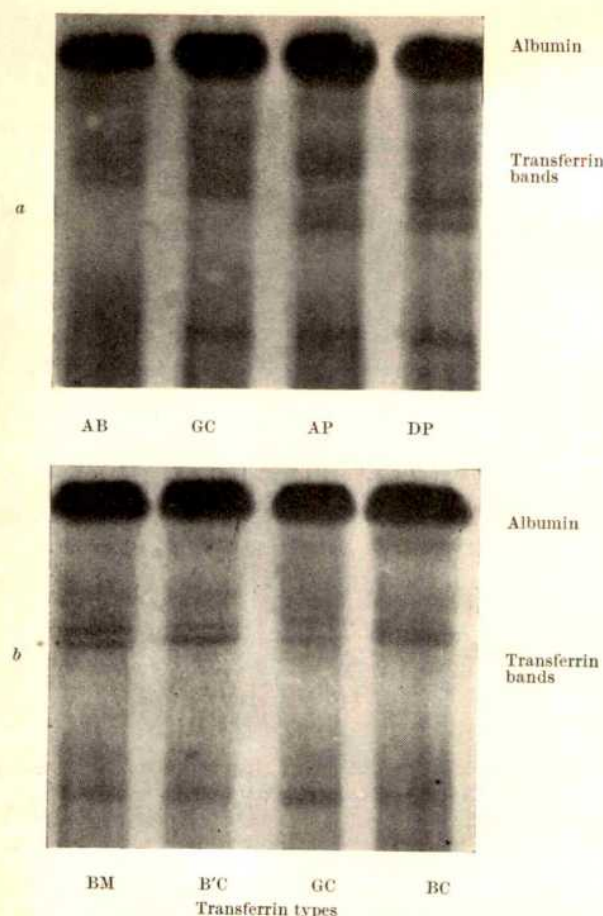


Fig. 2. Sheep transferrin types after starch-gel electrophoresis. Transferrin types GC, BM and DP are the Wageningen standards.

The results in Table 1 show that the fastest zone in the desert sheep corresponds with the second fastest in the Wageningen standards. Furthermore, the results show that there is an extra zone in the desert sheep which lies between the Wageningen TFG and TFB transferrin zone. If this new zone is called TFB' and the others are re-coded according to the Paris nomenclature the transferrin zones in the desert sheep of the Sudan would then correspond to TFA, TFB', TFB, TFC, TFM, TFE and TFP. Assuming that each zone is produced by the action of one gene, a maximum of twenty-eight phenotypic combinations would be expected from an allelic series of seven genes. Table 2 gives the calculated frequency of the seven co-dominant transferrin alleles. It can be seen from Table 2 that the allele TFB was the most frequent in

Table 2. FREQUENCY OF THE SERUM TRANSFERRIN ALLELES IN DESERT SHEEP

Transferrin allele	Frequency
TFA	0.19
TFB'	0.03
TFB	0.32
TFC	0.21
TFM	0.17
TFE	0.08
TFP	0.01

Table 3. OBSERVED AND EXPECTED FREQUENCIES OF SERUM TRANSFERRIN PHENOTYPES IN 393 DESERT SHEEP

Pheno-type	Observed	Expected	O-E	Pheno-type	Observed	Expected	O-E
AA	18.00	13.71	4.29	B'C	20.00	4.44	15.56
B'B'	0.00	0.27	-0.27	B'M	0.00	3.65	-3.65
BB	41.00	39.77	1.23	B'E	1.00	1.57	-0.57
CC	20.00	17.17	2.83	B'P	0.00	0.24	-0.24
MM	17.00	11.75	5.25	BC	45.00	52.23	-7.23
EE	3.00	2.20	0.80	BM	59.00	43.23	15.77
PP	0.00	0.04	-0.04	BE	20.00	18.75	1.25
AB'	0.00	3.97	-3.97	BP	4.00	2.75	1.25
AC	40.00	46.73	-6.73	CM	12.00	28.41	-16.41
AM	34.00	30.73	3.27	CE	12.00	12.32	-0.32
AE	23.00	25.43	-2.43	CP	1.00	1.81	-0.81
AP	11.00	11.02	-0.02	ME	8.00	10.17	-2.17
B'B	3.00	1.61	1.39	MP	0.00	1.49	-1.49
	0.00	6.76	-6.76	EP	1.00	0.63	0.37

Adjusted  $\chi^2$  (14 d.f.) = 26.02  $P$  = 0.05-0.02.

desert sheep, followed by TFC. TFP was the least frequent. Table 3 gives the actual and expected frequencies of the twenty-eight possible transferrin phenotypes. As chi-square tests cannot be carried out in those classes represented by less than five individuals, adjusted chi-square value was calculated after pooling or merging the phenotypic classes containing less than five individuals with larger ones. The adjusted chi-square value is given at the foot of Table 3 and is significant, suggesting that random mating was not necessarily operating. This may suggest that some phenotypes may have certain selective advantage in certain environments.

A general survey of the serum transferrin types, sodium and potassium levels in the blood of desert sheep from different geographical regions and from other Sudanese types of sheep is under way.

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### Post-heparin Lipolytic Activity and Blood Platelets

THERE have been several reports indicating a possible interrelationship between plasma lipoprotein lipase activity and blood platelets. Mitchell<sup>1</sup> and Gardikas *et al.*<sup>2</sup>, using changes in the optical density of plasma as indicators of lipolytic activity, have reported an inhibition of lipoprotein lipase activity by platelets. The presence of platelets contributes materially to plasma turbidity, however, and the changes in optical density, particularly proportionate changes, may not be an accurate measure of lipase activity. Hollett and Nestel<sup>3</sup> have shown that the clearing activity after administration of heparin, as measured by changes in optical density, and lipolytic activity, as determined by glycerol production, do not necessarily parallel each other. More recent results indicate that the lipoprotein lipase activity and "stickiness" of blood platelets are both altered in human cardiovascular disease<sup>4</sup>. We therefore decided to reinvestigate the relationship between lipoprotein lipase and blood platelets using heparin as an inducer and the release of free fatty acids (FFA) as a measure of the lipolytic activity.

Non-fasting adult Sprague-Dawley rats of either sex were injected intravenously either with 10 mg heparin/kg body weight or with an equivalent volume of saline. Blood samples were drawn by heart puncture into a heparinized syringe 10 min after the injection and divided into two aliquots. One aliquot was centrifuged at 4°C and 160g for 10 min to obtain platelet-rich plasma, the other part at 4°C and 4,000g for a similar length of time to produce platelet-poor plasma. The plasma samples were then incubated at 37°C for 1 h and the FFA concentrations determined by the method of Dole<sup>5</sup>. In other studies, 1 ml. portions of platelet-rich plasma taken after administration of heparin were centrifuged at 4°C and 4,000g

Table 1. EFFECT OF POST-HEPARIN BLOOD PLATELETS ON RELEASE OF PLASMA FREE FATTY ACIDS

Treatment	No. of rats	Plasma		Difference (μequiv./l.)
		Platelet-poor (μequiv./l.)	Platelet-rich (μequiv./l.)	
Heparin	34	944	1,085	141 ± 30*
Control	12	458	478	16 ± 38
Heparin	15	604	1,903†	1,299 ± 34
Heparin	7	589	798‡	209 ± 34
Control	3	463	525§	62

The heparin blood samples were obtained 10 min after intravenous injection of 10 mg heparin/kg body weight. All plasma samples were incubated at 37°C for 1 h.

\* Mean ± standard error.

† Post-heparin platelets resuspended in control plasma.

‡ Post-heparin platelets washed with saline and resuspended in control plasma.

§ Control platelets resuspended in control plasma.

for 10 min; the resulting platelet buttons were then resuspended in 1 ml. platelet-poor control plasma and incubated at 37°C for 1 h. In an additional series, platelet buttons from 1 ml. portions of post-heparin platelet-rich plasma were washed in 2 ml. of physiological saline, recentrifuged at 4,000*g*, resuspended in 1 ml. of control plasma and again incubated at 37°C for 1 h. In the control series, platelet buttons from control plasma were resuspended in control plasma and processed as described.

On incubation, the post-heparin platelet-rich plasma released significantly more FFA than the corresponding platelet-poor samples ( $P < 0.01$ , paired comparison, Table 1). No such differences were observed with platelet-rich and platelet-poor control plasma. Post-heparin platelets incubated in control plasma released 1,299  $\mu$ equiv. FFA per litre of plasma; washing the platelets with saline reduced the FFA release to 209  $\mu$ equiv./l. Incubation of control platelets in control plasma (Table 1) or in post-heparin plasma produced a negligible release of FFA. Neither control nor post-heparin erythrocytes or leucocytes showed any consistent enhancement of lipolysis<sup>6</sup>.

It would seem, therefore, that the presence of post-heparin platelets in plasma can enhance the post-heparin lipolytic activity. The mechanism of this effect is not clear at present; however, the observation that the lipolytic activity can be reduced by washing the platelets suggests that the enzyme (or enzymes) involved is absorbed at the cell surface. A possible correlation between the amount of lipolytic activity on the platelet surface and platelet "stickiness" remains to be investigated.

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### Effect of Heparin on Mixed Lymphocyte Cultures

Mixed lymphocyte culture may well offer a valuable assay of histocompatibility and it has been suggested that it can be used in donor selection for allotransplantation<sup>1,2</sup>. Goldsmith<sup>3</sup>, however, finds that this technique produces variable results when it is repeated with cells from the same individuals. Bach and Voynow<sup>4</sup> have also reported contradictory results on consecutive days. One reason for the lack of reproducibility may be the method of collecting samples of blood.

Taylor and Culling<sup>5</sup> have recently demonstrated that heparin inhibits the cytopathic effect of immune spleen cells in fibroblasts. Previously Johnson and Bencze<sup>6</sup> had shown that the lupus erythematosus cell phenomenon

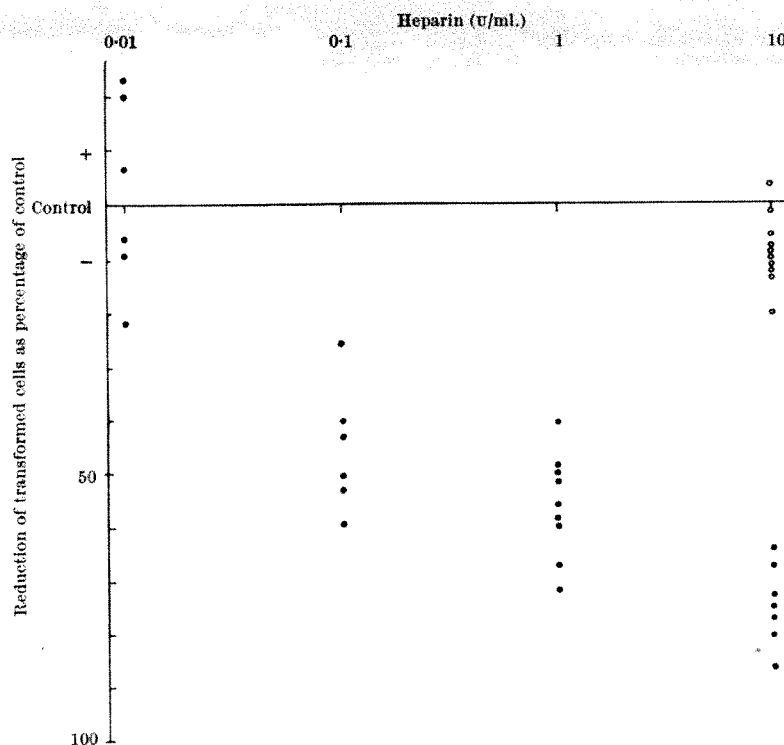


Fig. 1. The effect of serial dilutions of heparin on mixed lymphocyte cultures (●) and phytohaemagglutinin-stimulated lymphocytes (○). Depression of transformation is expressed as a percentage reduction of each untreated control result.

*in vitro* is suppressed by heparin, which is a commonly used laboratory anticoagulant, often used in the preparation of lymphocyte suspensions<sup>1,4,7,8</sup>. An investigation of the effect of heparin on mixed lymphocyte cultures was therefore performed.

Lymphocytes were isolated from defibrinated peripheral venous blood of healthy unrelated volunteers using the technique described by Coulson and Chalmers<sup>9</sup>. Mixed lymphocyte cultures were set up in medium 199 containing 25 per cent normal human serum. The cell concentrations were adjusted to 10<sup>6</sup> lymphocytes/ml. Control cultures of single donor cells were also established. Crystalline heparin (Boots, 167 U/mg) was made up in the culture medium in serial ten-fold dilutions. Single donor cultures were treated with 2.5 per cent phytohaemagglutinin and incubated with and without heparin. All cultures were left in inclined glass test-tubes for 72 h at 37°C. Smears of each culture were made, air dried, fixed in methanol and stained by the Jenner-Giemsa method. The morphologically blastic and mitotic cells in each culture were counted. The percentage of transformed cells in each culture without heparin was regarded as the control and depression of lymphocyte transformation was expressed as a percentage reduction of this figure for each experiment. Lymphocytes from eighteen donors were used and the results obtained are expressed in Fig. 1.

The results demonstrate that at a concentration of 100 U/ml. and more heparin was profoundly toxic to lymphocytes. There was gross distortion and fragmentation of all nucleated cells in the cultures. At lower concentrations lymphocyte transformation was depressed in the absence of morphological signs of toxicity. This depression occurred at concentrations as low as 0.1 U/ml. Heparin, however, produced very little reduction in transformation induced by phytohaemagglutinin, even at 10 U/ml., a concentration at which there was considerable depression in the mixed cultures. Control cultures of single donor lymphocytes all revealed less than 5 per cent blastic transformation.

The fact that heparin suppressed lymphocyte transformation caused by allogeneic cells but not that



caused by phytohaemagglutinin indicates that the "transformability" of the lymphocytes was not affected. The inhibitory effect of heparin must therefore occur on the afferent arm of the transformation process, possibly suppressing antigen detection. Taylor and Culling<sup>6</sup> have explained the inhibitory effect of heparin on immune spleen cells as possibly involving modification of the cell surface. Heparin is a powerfully electro-negative substance and has been shown to coat cells *in vitro*<sup>10</sup>. By increasing the already high negative surface charge of the normal lymphocyte<sup>11</sup> it would increase intercellular electrostatic repulsion and could therefore act by inhibiting cell contact. Weiss has speculated<sup>12</sup> that surface charge may regulate the "magnitude of potential energy barriers to contact" between cells. Failure of lymphocytes to make contact with cells containing alloantigens could therefore account for the reduction in the formation of blast cells caused by heparin.

I suggest, therefore, that the variable results from mixed lymphocyte cultures can be explained by variations in the concentration of heparin. Heparin should thus be avoided in the preparation of lymphocyte cultures.

This work was carried out while I was a recipient of a Saltwell scholarship from the Royal College of Physicians, London.

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## BIOCHEMISTRY

### Action of Ultra-violet Light on Soluble Collagens

It has been shown<sup>1</sup> that rat tail tendon becomes less soluble after exposure to short wave ultra-violet light and that the amount of soluble collagen in mouse skin was decreased after irradiation<sup>2</sup>. Fujimori<sup>3</sup>, working with acetic acid solutions of calf skin collagen, recently showed that irradiation resulted in the acquirement of ability to fluoresce in long wave ultra-violet light. These changes in properties have been ascribed to the formation of cross-links from the aromatic side-chains of phenylalanine and tyrosine<sup>3,4</sup>. Working with suspensions of acid-soluble and neutral salt-soluble collagens from rat skin we have also found that short wave ultra-violet light does produce fluorescence, and we have sought to measure the fluorescence quantitatively and correlate this with changes of solubility and shrinkage temperature after different periods of irradiation.

Purified collagens soluble in acid and neutral salts were prepared from the skin of 6 week old rats by the method of Jackson<sup>5</sup> as modified by Gross and Kirk<sup>6</sup>. Solutions of these collagens were stored at 4° C, the former in 0.1 molar acetic acid and the latter in 0.14 molar sodium chloride, containing 0.02 molar phosphate, pH 7.2. The skin remaining after extraction was treated by incubation

with trypsin in *tris* buffer, pH 8.2, to remove non-collagen protein, followed by treatment with chloroform to remove fat, followed by two further trypsin treatments. The material ("extracted rat skin collagen") was then washed with water and autoclaved in water for 2 h at 120° C and the solution separated from the insoluble residue of hair and other non-collagen. This solution suitably diluted was used as reference in fluorescence measurements. Hydroxyproline<sup>7</sup> and nitrogen<sup>8</sup> analyses were carried out on this solution and on autoclaved aqueous solutions of the soluble collagens. The hydroxyproline nitrogen was 7.5, 8.3 and 7.9 per cent of the total nitrogen for the extracted rat skin collagen, acid-soluble and neutral salt-soluble collagens, respectively.

Acid-soluble collagen was precipitated from 0.1 molar acetic acid solution by neutralization to pH 7-7.5 and incubation at 37° C. The fibrous precipitate was washed with water. Freshly prepared aqueous suspensions fluoresced relatively weakly compared with the extracted rat skin but acquired increased fluorescence after standing for some days or weeks. This increase with time, measured against a suitable standard, varied from batch to batch and appeared to require daylight. We then turned our attention to the production of fluorescence by irradiation with ultra-violet light. About 5 ml. of aqueous collagen suspension containing about 10 mg/ml. was placed in a crucible, stirred magnetically and exposed to light from a 'Chromatolite' lamp (Englehard Hanovia, Ltd.) with a maximum emission of 254 mμ. The base of the crucible was 4 cm from the lamp. Samples (about 0.5 ml.) were withdrawn at intervals. From these samples a small amount of fibres was removed, suspended in 0.14 molar sodium chloride for *T<sub>s</sub>* measurement in capillary tubes<sup>9</sup>. Fibres teased out from the skin of rats 6 weeks old (native collagen) served as reference material for *T<sub>s</sub>* measurements. The remaining suspension was made to 2-3 ml. with water, sealed and autoclaved for 2 h at 120° C. The resulting solutions (containing the equivalent of 1-2 mg collagen/ml.) were used to measure fluorescence and hydroxyproline content. Fluorescence at maximum emission was measured in the Locarte fluorimeter, Mark 4. The exciting light in this apparatus came from a mercury vapour lamp through a Wood glass filter, the fluorescent light passing through a monochromator. The reference material was a solution of extracted rat skin collagen of known hydroxyproline content as already mentioned. The results are shown in Table 1. Fluorescence figures are adjusted for equal hydroxyproline concentrations.

A second irradiation experiment was carried out to compare neutral salt-soluble with acid-soluble collagen. In this case the same conditions as previously could not be used, because the neutral salt-soluble collagen became soluble on washing with water. The neutral salt-soluble collagen was precipitated from its salt solution by incubation at 37° C, part retained for autoclaving in water and part (about 5 ml. containing about 1 mg collagen/ml.) was irradiated. After irradiation for 1.5 h the collagen was no longer soluble in water and the irradiation was thereafter continued on the collagen suspended in water. There was not sufficient neutral salt-soluble collagen available to allow periodic sampling for fluorescence and hydroxyproline as in the first experiment, so these

Table 1. EFFECT OF ULTRA-VIOLET IRRADIATION ON ACID-SOLUBLE COLLAGEN (WATER SUSPENSION)

Time of irradiation (h)	<i>T<sub>s</sub></i>	Solubility in acetic acid (0.1 molar)	Relative fluorescence of aqueous autoclaved solution
0	45-52°*	Completely	19
0.5	45-52°†	Partially	29
1	46-56°	Insoluble	36
4	50-63°	"	38
8	54-60°	"	47
Extracted rat skin collagen	45-49°	"	100
Native collagen	59-62°		

\* Dissolved completely at shrink temperature.  
† Dissolved partially at shrink temperature.



Table 2. COMPARISON OF EFFECTS OF ULTRA-VIOLET LIGHT ON ACID-SOLUBLE AND NEUTRAL SALT-SOLUBLE COLLAGENS

Irradiation carried out on suspensions in 0.14 molar sodium chloride solutions for the first 1.5 h and thereafter on suspensions in water

Acid-soluble collagen			Neutral salt-soluble collagen		
Time of irradiation (h)	$T_s$	Relative fluorescence* (aqueous autoclaved solution)	$T_s$	Solubility (in 0.14 molar NaCl at 0° C)	Relative fluorescence* (aqueous autoclaved solution)
0	45-52°†	14	45-52°†	Completely	26
0.5	50-54°‡	—	49-53°‡	Partially	—
1.5	52-59°	—	53-58°	Insoluble	—
3.5	54-60°	—	53-57°	"	—
5.5	54-59°	—	58-62°	"	—
9.5	54-59°	57	59-63°	"	32

\* Extracted rat skin collagen = 100.

† Dissolved completely.

‡ Dissolved partially.

Table 3. SPECTRAL DATA (UNCORRECTED) FOR FLUORESCENCE OF AQUEOUS AUTOCLAVED SOLUTIONS OF COLLAGENS

	Acid-soluble collagen		Neutral salt-soluble collagen		Extracted rat skin collagen
	Not irradiated	Irradiated	Not irradiated	Irradiated	
Excitation maxima (m $\mu$ )	330	335	320	345	350
Fluorescence maxima (m $\mu$ )	405	410	400	425	415

estimations were carried out before and at the end of the irradiation period. A similar amount of acid-soluble collagen, freshly prepared from acetic acid solution, was irradiated under the same conditions. In fluorescence measurements of samples before irradiation, the salt solution was replaced by water. The results of this experiment are given in Table 2.

The spectral characteristics of autoclaved solutions of irradiated and non-irradiated soluble collagens and of the extracted rat skin collagen were measured in the Aminco-Bowman spectrofluorimeter (we thank Dr J. R. Daley, West London Hospital, for the use of this apparatus). The results (uncorrected) are given in Table 3.

The results show that short wave ultra-violet light has a marked effect on the properties of the soluble collagens. Irradiation causes a decrease in solubility and an increase in  $T_s$  and fluorescence. These effects are noticeable after only a short exposure, and are consistent with the formation of new cross-links and increased hydrogen bonding. The fluorescence of acid-soluble collagen in suspension increases with time of irradiation (Table 1), as Fujimori<sup>3</sup> also found for collagen in solution, but the  $T_s$  tends to level off with time, approaching that of the native collagen. We have also found in other experiments, however, that prolonged irradiation of acid-soluble collagen suspension resulted in a slight fall in both fluorescence and  $T_s$ , which would be consistent with the breakage of bonds by ultra-violet light of sufficient energy<sup>4,10</sup>. Comparison of the results for acid-soluble collagen in Tables 1 and 2 indicates that initial conditions may have some influence on the final effects. In the former the  $T_s$  ranges are somewhat greater than those of the latter. The results in Table 2 show some differences between the behaviour of the two types of collagen towards ultra-violet light. Neutral salt-soluble collagen attains its maximum  $T_s$  after 1.5 h of irradiation, whereas the acid-soluble collagen requires a longer exposure. The final  $T_s$  of neutral salt-soluble collagen is the same as that of the native collagen, whereas that of the acid-soluble collagen is somewhat lower. On the other hand, the relative increase of fluorescence is much greater for acid-soluble collagen than for neutral salt-soluble collagen. The relatively high initial fluorescence of neutral salt-soluble collagen could be due to traces of contamination, but the relatively small increase of fluorescence with irradiation is almost certainly a property of the collagen itself.

If fluorescence is due to a certain type of covalent cross-link (and the spectral results in Table 3 suggest a similarity between such cross-links in irradiated and non-irradiated collagens) then it appears that this type of linkage is not the only factor in determining high  $T_s$  and low solubility. This conclusion is supported by the relatively high fluor-

escence of extracted rat skin collagen, which has a low shrinkage temperature. Prolonged exposure to acetic acid in the extraction process has evidently brought about extensive disruption of hydrogen bonds but not of those bonds responsible for fluorescence. Fluorescence cross-linking may, however, play a part in the final stabilizing of the structure of native collagen as previous observations<sup>9,11</sup> suggest.

One of us (J. A. K.) is a Beit Memorial Fellow.

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### Effect of Rotenone on the Alcohol Dehydrogenase of Yeast Mitochondria

CHANCE<sup>1</sup> applied the crossover theorem to a study of intact yeast cells, and reported an apparent crossover point (or phosphorylation site) between pyridine nucleotide and flavoprotein (site I), between cytochromes *b* and *c* (site II) and between cytochrome *c* and cytochrome oxidase (site III). These observations led to the expectation that intact isolated yeast mitochondria would exhibit no anomalies with respect to their phosphorylation capacity. More recently Mattoon and Sherman<sup>2</sup> used loosely coupled yeast mitochondria and obtained indirect results supporting the observations of Chance.

Ohnishi *et al.*<sup>3</sup>, using a preparation of tightly coupled yeast mitochondria, and Schatz *et al.*<sup>4</sup>, using a yeast respiratory particle preparation, however, were unable to

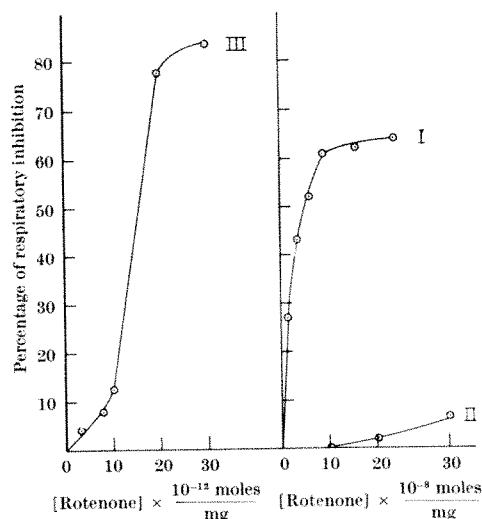


Fig. 1. Effect of rotenone on the respiration of rat liver and yeast mitochondria. In addition to rotenone, the 3 ml. polarographic reaction chamber contained: (I) 1.5 mg yeast mitochondrial protein, 133 mmolar ethanol, 25 mmolar semicarbazide, 42  $\mu$ molar dinitrophenol, 0.6 molar mannitol, 10 mmolar sodium phosphate buffer, pH 6.5; (II) 0.75 mg yeast mitochondrial protein, 16 mmolar succinate, 42  $\mu$ molar dinitrophenol, mannitol and buffer as in (I); (III) 10 mg rat liver mitochondrial protein, 33  $\mu$ molar  $\beta$ -OH-butyrate, 8  $\mu$ molar dinitrophenol, 0.25 molar mannitol, 10 mmolar potassium chloride, 10 mmolar *tris* buffer, 5 mmolar potassium phosphate and 0.2 mmolar EDTA, pH 7.4. The temperature was 25° C.

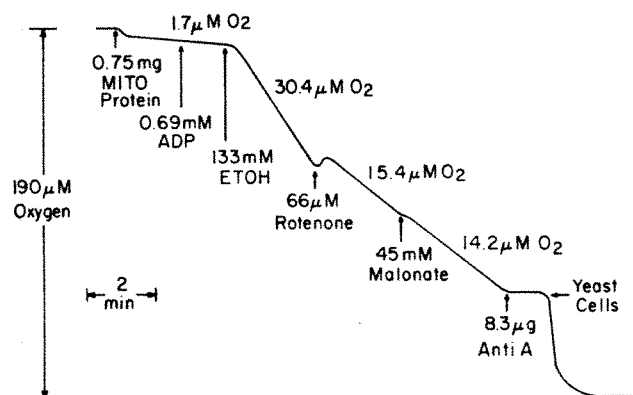


Fig. 2. Representative polarographic trace illustrating the effect of rotenone, malonate and antimycin A on rapidly respiring (state 3) yeast mitochondria. The conditions were similar to those described in Fig. 1. The final concentrations are given below the polarographic trace while the rates in  $\mu$ molar oxygen/min are indicated above the trace. Excess rapidly respiring yeast cells are added to obtain a zero oxygen calibration point.

obtain ratios of phosphorus : oxygen greater than 2 with pyridine nucleotide-linked substrates. These observations, together with the fact that isolated mitochondria are not sensitive to rotenone, seem to lead to the conclusion that yeast mitochondria exhibit a fundamental difference in their respiratory processes when compared with other types of mitochondria.

While testing the effect of rotenone on tightly coupled yeast mitochondria in our laboratory, we increased the rotenone concentrations to values exceeding those generally observed to be effective with mammalian mitochondria. In these conditions rotenone exhibited a pronounced inhibition of the uptake of oxygen with ethanol-supported respiration, although the same concentrations of rotenone had little or no effect on the rate of succinate or pyruvate plus malate oxidation. Further investigation of this phenomenon has revealed that rotenone appears to exert its effect through a direct action on the yeast mitochondrial alcohol dehydrogenase.

Yeast mitochondria were prepared either from baker's yeast purchased from the Federal Yeast Co., Baltimore, Maryland, or from a laboratory strain of *S. cerevisiae* (strain D-261) according to a modification of the method of Mattoon and Balcaavage<sup>5</sup>. This method consists of a gentle grinding of the yeast in a 0.6 molar mannitol medium containing 0.2 per cent bovine serum albumin (Sigma fraction V), and  $10^{-4}$  molar EDTA, using an Eppenbach 'Micro-mill', followed by careful separation of the intact heavy mitochondria from other cellular material by differential centrifugation. The resulting preparation exhibits respiratory control ratios of 1.5–3.5 with 16 mmolar succinate as substrate and 1.5–2.5 with 133 mmolar ethanol as substrate. The ratios of phosphorus : oxygen with either of these substrates have not been observed to exceed 2.0.

Crystalline yeast alcohol dehydrogenase (ADH) (Calbiochem, Los Angeles) was assayed in conditions similar to those described by Kägi and Vallee<sup>6</sup>. The rate of ADH reaction was followed as the increase in absorbancy at 340 m $\mu$  (NAD $\rightarrow$ NADH). Mitochondrial respiration was followed using a 'Teflon' covered Clark oxygen electrode polarized at  $-0.8$  V, immersed in a 3.0 ml. semi-closed reaction vessel. Rotenone (K and K Laboratories, Inc., Plainview, N.Y.) was prepared as a stock solution of  $1 \times 10^{-3}$  moles/l. in methanol. Two 80  $\mu$ l. portions of this solution were added to the reaction mixtures to achieve the final concentrations indicated. All other chemicals used were of reagent grade. Rat liver mitochondria were prepared according to the method of Schneider<sup>7</sup> and assayed in the medium described by Chance and Hollunger<sup>8</sup>.

In Fig. 1 the effect of rotenone on the respiration of yeast mitochondria during the catalysis of ethanol oxidation (I) is compared with its effect on rat liver mitochondria during  $\beta$ -hydroxybutyrate oxidation (III). It is apparent that the observed inhibitions differ both qualitatively and quantitatively. With rat liver mitochondria inhibition by rotenone occurs at the concentrations described by Ernster *et al.*<sup>9</sup> and approaches 100 per cent inhibition. The inhibition of yeast mitochondria during ethanol respiration requires much higher concentrations of rotenone, and an inhibition exceeding 75 per cent has not been observed. Curve II of the same figure illustrates a small effect of high rotenone concentrations on yeast mitochondria catalysing succinate oxidation.

The residual ethanol respiration is insensitive to malonate (even at concentrations as great as 45 mmoles/l.) but is completely blocked by antimycin A as demonstrated by the polarographic trace reproduced in Fig. 2. Because the residual respiration (15.4  $\mu$ molar oxygen/min) exceeds the endogenous respiration (1.7  $\mu$ molar oxygen/min) and is insensitive to malonate, it would seem that the residual respiration must be supported by ethanol oxidation, thus indicating an incomplete inhibition of this process.

Because pyruvate plus malate or isocitrate oxidation was not inhibited by similar concentrations of rotenone, it can be concluded that the inhibition of ethanol oxidation probably does not occur at the level of the respiratory chain. Control experiments showed that methanol alone had essentially no effect on mitochondrial respiration.

Crystalline yeast ADH was then examined for its sensitivity to inhibition by rotenone, and as shown in Fig. 3 the crystalline enzyme exhibits behaviour qualitatively similar to that demonstrated by the enzyme *in situ*; that is, maximum inhibition of only 60 per cent. Kinetic analysis of the rotenone inhibition, as presented in Fig. 4, is consistent with the hypothesis that it is of the mixed type with respect to both NAD and ethanol.

These results thus extend the known physiological effects of rotenone to include a site of attack other than site I within the electron transport chain. Because yeast ADH is at least in part associated with the isolated mitochondria<sup>3,5,10</sup>, it is conceivable that the effect described here may be an example of a more general effect of rotenone on other mitochondrial dehydrogenases. Alternatively, the effect of rotenone on yeast may be specifically associated with ADH and as such may be a useful tool in future studies on the *in vivo* function of this enzyme.

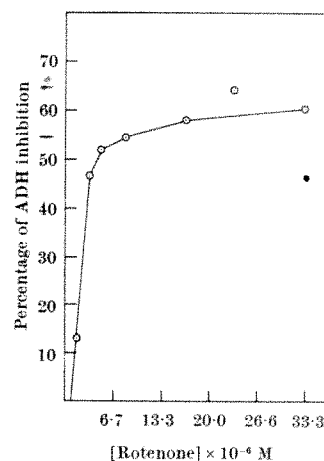


Fig. 3. Effect of rotenone on commercial crystalline yeast alcohol dehydrogenase. The 3 ml. cuvettes contained: 2.6 ml. pyrophosphate buffer (0.115 moles/l.), pH 8.5; 0.15 ml. 50 per cent ethanol; 0.1 ml. NAD (0.03 moles/l.) and 0.1 ml. methanol or rotenone in methanol to achieve the desired final rotenone concentration. The reactions were started by adding 50  $\mu$ l. of an enzyme solution ( $A_{340} = 0.090$ ) to the cuvette and followed, using a Zeiss spectrophotometer attached to a Sargent strip chart recorder, as the increase in absorbancy at 340 m $\mu$ .



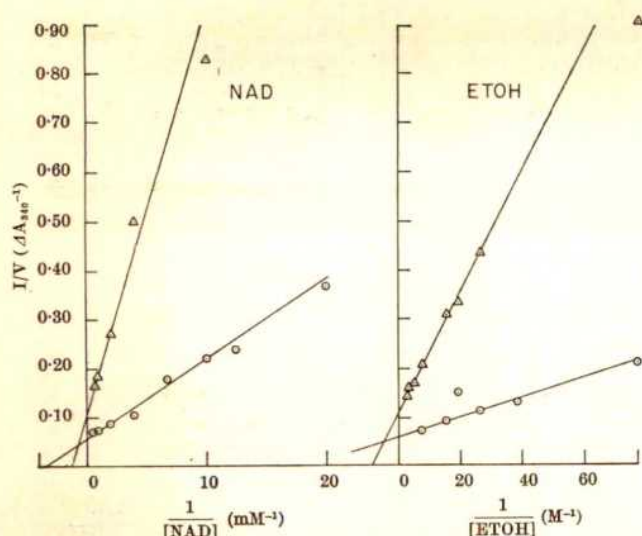


Fig. 4. Lineweaver-Burk analysis of the effect of rotenone on the affinity of yeast alcohol dehydrogenase for NAD and ethanol. The conditions are similar to those described for Fig. 3. O, Substrate dependency curve obtained in the presence of methanol alone.  $\Delta$ , Substrate dependency curve in the presence of  $3 \times 10^{-4}$  molar rotenone.

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### Caesium Chloride in the Preparation of Membrane Fractions from Human Cerebral Tissue

THE standard procedure for the isolation of myelin and other membrane fractions involves differential and density gradient centrifugation in sucrose solutions. Two bands of myelin, light and heavy<sup>1</sup> or small and large<sup>2</sup>, and many bands of microsomes<sup>3</sup> have been obtained by these techniques when applied to cerebral tissues, and a similar multiplicity of bands has been noted with the use of 'Ficol' gradients<sup>4</sup>. One explanation may be that the membranes exist as vesicles, which are impermeable to sucrose, and thus are separated by buoyant density rather than matrix density<sup>5</sup>. The two bands of myelin obtained show an almost identical lipid composition<sup>6</sup>.

Meselson *et al.*<sup>7</sup> used caesium chloride for the separation and the molecular weight determination of nucleic acids. Caesium chloride should be permeable to membranes existing in vesicular form, and so the technique has been applied to cerebral tissues to prepare purified myelin and microsomes, and the preliminary results of this work are reported here.

Cortex and white matter from three apparently normal human brains were each homogenized (10 per cent w/v) in 0.32 molar sucrose; myelin was prepared from the white matter and microsomes from the cortex. The white matter homogenate after an initial centrifugation at 1,000g was adjusted to 0.25 molar sucrose and layered on to 15 per cent caesium chloride (1 : 10), centrifuged in an MSE refrigerated centrifuge at 4°C in a swing-out rotor for 18 h at 100,000g. The single myelin band at the top was removed and extracted in chloroform-methanol (1 : 1).

The cortex homogenate was centrifuged at 17,500g for 1 h and the supernatant centrifuged at 100,000g in a small angle head for 1 h. The crude microsomal pellet was taken up in 15 per cent caesium chloride and centrifuged at 100,000g in the swing out head for 18 h, when the microsomal pellet was removed and extracted with chloroform-methanol (1 : 1). Dr W. G. P. Mair examined both preparations by electron microscopy and these were either pure myelin of characteristic appearance or microsomes respectively (Fig. 1).

Thin layer chromatography was performed as previously<sup>8</sup>, using as solvent either chloroform, methanol, formic acid and water (70 : 30 : 7 : 3) for phospholipids or chloroform, methanol and water (14 : 6 : 1) for sphingolipids. The phosphorus content of each band, after visualization by spraying with 50 per cent sulphuric acid and subsequent charring, was estimated with recovery of total phosphorus from the plate of between 95 per cent and 98 per cent. Sphingosine was estimated from each sphingolipid band as seen after spraying with 0.2 per cent (w/v) di-chlorofluorescence in 95 per cent ethanol containing 0.02 per cent (w/v) butylated hydroxy toluene with a recovery of 90 per cent to 95 per cent.

Table 1. PHOSPHOLIPIDS IN HUMAN MYELIN AND MICROSOSES (AS PERCENTAGE OF TOTAL PHOSPHORUS)

	Myelin		Microsomes	
	Caesium	Sucrose	Caesium	Sucrose
			"Standard"	"Heavy"
Cardiolipin and phosphatidic acid	0.5	3.0	0	1.0
Phosphatidyl ethanolamine	38.0	38.0	28.8	30.0
Phosphatidyl serine	17.7	16.5	11.2	9.0
Phosphatidyl choline	22.8	19.0	44.6	38.0
Monophosphoinositide	0.5	1.5	0	4.0
Sphingomyelin	20.5	20.0	15.2	13.0

Results from duplicates from each of three brains.

The results of the phospholipid analyses for both myelin and microsomes are shown in Table 1 and the results compared with similar estimations using standard procedures involving sucrose gradients.

The distributions of phospholipids in myelin are comparable by both techniques. It can be mentioned that

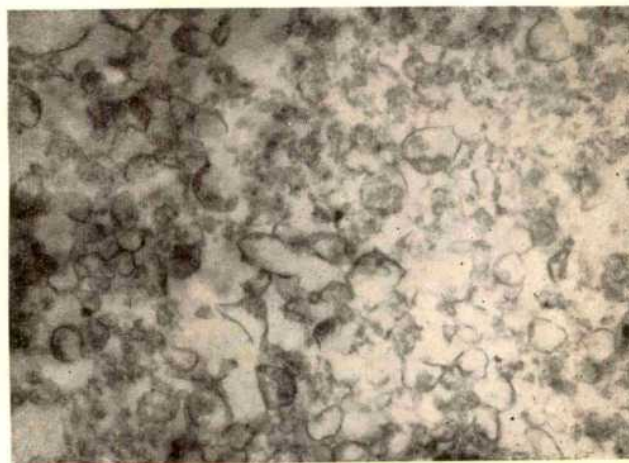


Fig. 1. Electron microscopy of microsomal pellet prepared with caesium chloride. ( $\times 30,000$ .)



Fig. 2. Thin layer chromatogram to show gangliosides of lipid extract from microsomes. The solvent was chloroform, methanol and 10 per cent aqueous ammonia (60:35:5); the spray was resorcinol reagent<sup>8</sup>.

from the determinations already carried out the ratios of the sphingosine of cerebroside, sulphatide, sphingomyelin and ganglioside in the microsomal preparations are as 1:0.3:1.5:0.6 and that gangliosides are present in microsomes but not in myelin (Fig. 2). The myelin results agree well with the figures given by Eichberg *et al.*<sup>2</sup> and by Cuzner *et al.*<sup>9</sup>. A single pure microsomal preparation results from the use of caesium chloride as compared with a number that can be obtained when using sucrose, and the phosphorus containing compounds in this one preparation show a pattern very similar to that of "standard" microsomes as prepared from sucrose.

The simplicity of the procedure is such that it can be readily applied to a wide range of pathological material and both this and the distribution of the sphingolipids in myelin and microsomes is at present being carried out.

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### Changes of Gamma-aminobutyric Acid, Glutamic Acid and Aspartic Acid in Various Brain Structures of Cats deprived of Paradoxical Sleep

KNOWLEDGE of the role of free amino-acids in the activity of the central nervous system (CNS) and in the basic neural processes of excitation and inhibition has accumu-

lated over a number of years. The pool of free amino-acids in the brain is the source from which amino-acids liberated by the breakdown of proteins are returned<sup>1</sup>. The brain has the highest concentration of glutamic acid, gamma-aminobutyric acid (GABA) and aspartic acid, and these compounds do not occur to any significant extent in the other tissues of mammals. GABA especially occurs only in the CNS of mammalian organisms, and it has been shown that this substance has characteristic physiological effects on the CNS (ref. 2). In nervous tissue, as in other organs, the carbon skeleton of non-essential amino-acids is derived ultimately from glucose by way of the citric acid cycle. Active brain transaminases catalyse the reversible amination and deamination of essential and non-essential amino-acids<sup>3</sup>.

Studies on sleep have revealed clearly the metabolic and enzyme nature of the mechanism of sleep, and especially its paradoxical phase (PS)<sup>4</sup>. Our previous investigations showed that the exogenous and endogenous increase in the amount of GABA in the CNS vigorously suppresses the paradoxical sleep of cats deprived of paradoxical sleep but has no influence on slow sleep<sup>5</sup>. The amino-acid content of the CNS reflects its state and deprivation of paradoxical sleep causes a selective accumulation of paradoxical sleep in the recuperative period, and it seemed interesting to investigate the changes in the amounts of GABA, glutamic acid and aspartic acid in different brain structures, with the object of finding how the general and regional metabolism of these amino-acids in the CNS is related to the mechanisms of paradoxical sleep.

The experiments were carried out on adult cats, which had been deprived of paradoxical sleep according to the technique devised by Jouvet<sup>6</sup>. The animals were decapitated and their heads quickly placed in liquid air. Certain brain structures were extracted from the frozen tissue in a cool chamber and then measured and homogenized in 80 per cent ethyl alcohol. This homogenate was centrifuged at 6,000 r.p.m. After this, supernatants were separated and chloroform added to precipitate proteins. The upper layer of water and alcohol evaporated to dryness. Later, the dry residues were dissolved in 0.1 normal hydrochloric acid (1 ml./g of fresh tissue). All amino-acids were separated by two-dimensional ascending paper chromatography and quantitative analyses were carried out by ultra-violet spectrophotometry at 505 mμ (ref. 7). For each series of experiments standards were prepared from pure amino-acids in hydrochloric acid.

The results of the experiments (Fig. 1) show that in control animals the highest concentrations of GABA occur in the superior and inferior colliculi and in the caudate nucleus and that significantly lower concentrations of GABA occur in the cortex, midbrain reticular formation and thalamus. The concentration of glutamic acid was highest in the caudate nucleus and the thalamus, lower in the frontal cortex, reticular formation and occipital cortex and lowest in the colliculi. The concentrations of aspartic acid in the various cortical and sub-cortical regions of control animals were very similar, but in the thalamus the concentration was slightly higher. The quantities of these amino-acids in animals deprived of paradoxical sleep undergo changes which are primarily regional in character. The largest changes in animals deprived of paradoxical sleep occur in the concentrations of GABA, which show highly significant increases in the reticular formation (39.4 per cent;  $P < 0.01$ ), the thalamus (26.2 per cent;  $P < 0.01$ ), the frontal cortex (15 per cent;  $P < 0.01$ ), and decreases in the colliculi (29.1 per cent;  $P < 0.01$ ) and the caudate nucleus (21.9 per cent;  $P < 0.01$ ). The concentration of GABA decreased in the occipital cortex, but the decrease was not significant (10.9 per cent;  $P > 0.05$ ). The concentration of aspartic acid increased significantly in animals deprived of paradoxical sleep in the thalamus (29.2 per cent;



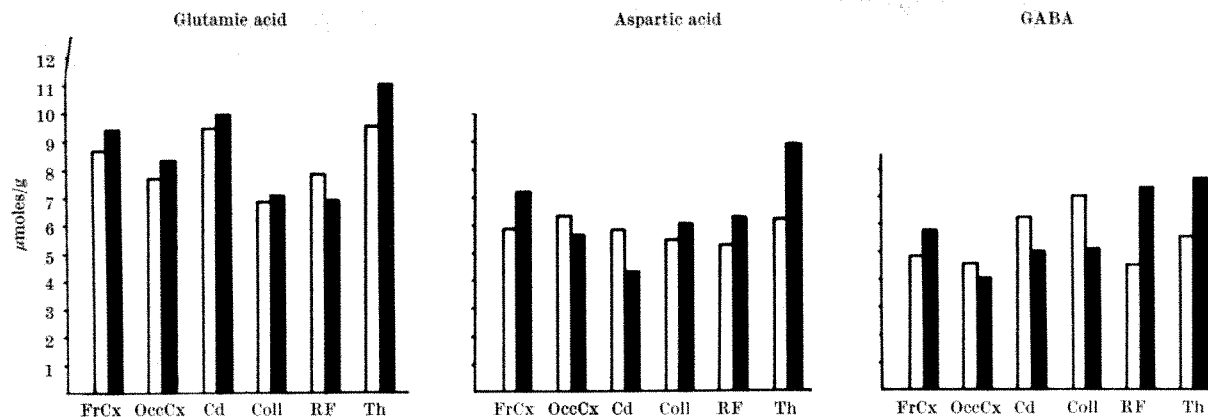


Fig. 1. Changes of amino-acids. □, Normal; ■, deprivation.

$P < 0.01$ ), the frontal cortex (18.3 per cent;  $P < 0.01$ ), the reticular formation (16.1 per cent;  $P < 0.01$ ), and decreased in the caudate nucleus (26.0 per cent;  $P < 0.01$ ). The changes in the occipital cortex and the colliculi were not statistically significant. Glutamic acid undergoes less change than the other two amino-acids in the experimental conditions used here and the changes in its concentration are not significant, apart from the slight increase in the thalamus (15.3 per cent;  $P < 0.01$ ) in animals deprived of paradoxical sleep.

The results obtained show that the changes in the concentrations of the amino-acids in animals deprived of paradoxical sleep have a high regional specificity which points not only to a specificity of metabolism but also to the functional importance of different regions in paradoxical sleep. We can speculate that the decrease in the concentrations of GABA and aspartic acid, especially in the caudate nucleus—a structure known to exert inactivating influences (electrographic and behavioural) on higher nervous functions<sup>8</sup>—might implicate this structure in the mechanism of paradoxical sleep, depending on the metabolism of these two amino-acids. It is necessary, however, to point out that glutamic acid decarboxylase is very susceptible to the action of its inhibitors in the caudate nucleus<sup>9</sup>. The absence of reciprocal parallelism in the quantitative changes in the concentrations of GABA, glutamic acid and aspartic acid indicates that these amino-acids, which are specially required in the metabolism of various brain structures, do not always follow known metabolic pathways<sup>10</sup>. It is possible that under special conditions GABA, glutamic acid and aspartic acid are transformed into new compounds or are utilized more effectively in the citric acid cycle<sup>11</sup>.

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### A 3-Amino-3,6-dideoxyhexose from the Lipopolysaccharide of *Escherichia coli* 071

For a long time fungi were the only known source of naturally occurring 3-amino-3,6-dideoxyhexoses<sup>1</sup>. It was only in 1965 that a representative of this group of amino sugars was reported to be present also in bacteria by Ashwell *et al.*<sup>2</sup>, who isolated 3-amino-3,6-dideoxy-D-galactose from a phenol soluble lipopolysaccharide of *Xanthomonas campestris*. Later 3-amino-3,6-dideoxy-D-galactose was also isolated from the lipopolysaccharides of several *Escherichia coli* strains (unpublished work of Jann, Jann and Müller-Seitz). Recently, a 3-amino-3,6-dideoxyhexose was found in a glycoprotein complex of *Citrobacter freundii*<sup>3</sup>. In the lipopolysaccharides of *Salmonella* group M a 3-amino-3,6-dideoxyhexose was detected (Lüderitz, O., private communication). This communication concerns the isolation of a 3-amino-3,6-dideoxyhexose from the lipopolysaccharide of *E. coli* 071 and its tentative identification as 3-amino-3,6-dideoxyglucose.

Acetone dried cells of *E. coli* strain P10a (071:K? : H12) were obtained from I. and F. Ørskov of the International Escherichia Center, Copenhagen. Culture conditions are described elsewhere<sup>4</sup>. The lipopolysaccharide was isolated by extraction with 45 per cent phenol at 65°C for 10 min and purified by repeated centrifugation at 105,000g (ref. 5). The sugar components of the lipopolysaccharide are heptose, 2-keto-3-deoxyoctonate (KDO), galactose, glucose, rhamnose, glucosamine, galactosamine, and the new amino sugar (amino sugar X). It should be mentioned that glucosamine is present in lipid A only and is no constituent of the polysaccharide. From the hydrolysate (2 normal hydrochloric acid, 100°C, 3 h) of the lipopolysaccharide neutral sugars were removed on small (1.5 × 20 cm) columns of 'Dowex 50-H+' by percolation with water. Amino sugars were then eluted with 0.5 normal hydrochloric acid. The amino sugar fraction (glucosamine, galactosamine, and amino sugar X) was resolved by fractional elution with 0.33 normal hydrochloric acid from long (1.2 × 80 cm) columns of 'Dowex 50-H+' × 10 at a flow rate of 2 ml/h. After evaporation of the hydrochloric acid, the amino sugar remained as an oil which could not be crystallized.

Amino sugar X was N-acetylated with acetic anhydride/sodium bicarbonate according to Roseman and Ludowieg<sup>6</sup>.

When sprayed with periodate followed by nitroprussid/piperazine both amino sugar X and its N-acetyl derivative formed blue spots on paper chromatograms. This reaction is specific for deoxysugars<sup>7</sup>. During periodate oxidation (pH 4.5) of amino sugar X as well as of the N-acetylated amino sugar acetaldehyde was liberated, which was determined by the colour reaction with *p*-hydroxydiphenyl<sup>8,9</sup>. In the conditions used—microdistillation of acetaldehyde into the reagent—the determination

was not impaired by formaldehyde. This was verified by analysing the absorption spectrum of the colour reaction. Maximum absorption was at 570 m $\mu$  (for acetaldehyde  $\lambda_{\max}$  = 570 m $\mu$ ; for formaldehyde  $\lambda_{\max}$  = 646 m $\mu$ ). Formation of acetaldehyde during periodate oxidation is indicative of a 6-deoxy-group in amino sugar X.

Amino sugar X did not react in the Morgan-Elson assay modified according to Strominger *et al.*<sup>10</sup>. After controlled periodate oxidation, however, the *N*-acetylated amino sugar X was reactive in the Morgan-Elson assay. This modification was introduced by Ashwell *et al.*<sup>11</sup> as a specific test for 3-amino sugars.

From the results described so far we conclude that amino sugar X is a 3-amino-3,6-dideoxyhexose.

For further identification of amino sugar X its elution pattern from 'Dowex 50-H<sup>+</sup>' was compared<sup>12</sup> with those of 3-amino-3,6-dideoxy-D-galactose, 3-amino-3,6-dideoxy-L-glucose, 3-amino-3,6-dideoxy-D-mannose and 3-amino-3,6-dideoxy-L-talose. Peak positions relative to glucosamine are given in Table 1.

Table 1. RESULTS OF CHROMATOGRAPHY ON 'DOWEX 50-H<sup>+</sup>'  $\times 10$  OF AMINO SUGAR X AND REFERENCE SUBSTANCES

Amino sugar	Peak position, relative to glucosamine
Amino sugar X	1.37
3-amino-3,6-dideoxy-D-galactose	1.54
3-amino-3,6-dideoxy-L-glucose	1.38
3-amino-3,6-dideoxy-D-mannose	1.28
3-amino-3,6-dideoxy-L-talose	2.35

Column dimensions: 0.8  $\times$  55 cm; eluted with 0.33 normal hydrochloric acid at a flow rate of 2 ml./hr; 1 ml. fraction; tested with ninhydrin.

Table 2. PAPER CHROMATOGRAPHIC MOBILITIES OF *N*-ACETYLATED AMINO SUGAR X AND REFERENCE SUBSTANCES

<i>N</i> -acetyl-amino sugar	Mobility, relative to fucose, in		
	A	B	C
<i>N</i> -acetyl-amino sugar X	1.32	1.52	1.38
3-acetamido-3,6-dideoxy-D-galactose	1.23	1.42	1.18
3-acetamido-3,6-dideoxy-L-glucose	1.32	1.52	1.38
3-acetamido-3,6-dideoxy-D-mannose	1.38	1.60	1.60
3-acetamido-3,6-dideoxy-L-talose	1.50	1.92	2.33

Whatman No. 1 paper; descending chromatography in solvents, A: pyridine/*n*-butanol/water (4 : 6 : 3); B: *n*-butanol/acetic acid/water (5 : 1 : 2); C: pyridine/ethyl acetate/water (10 : 36 : 11.5); stained with alkaline silver nitrate<sup>13</sup>.

As can be seen from Table 1, amino sugar X has the same elution value as 3-amino-3,6-dideoxy-L-glucose. Paper chromatography of the *N*-acetylated amino sugar in three different solvents (Table 2) also indicates identity of amino sugar X and 3-amino-3,6-dideoxyglucose.

On paper chromatograms in solvent systems A, B, and C, free amino sugar X and 3-amino-3,6-dideoxy-L-glucose had identical mobilities and could be differentiated from the other reference substances. *R<sub>F</sub>* values vary on individual chromatograms, however, and are not easily reproducible.

Quantitation of amino sugar X was hampered by its instability, a property which it shares with 3-amino-3,6-dideoxy-L-glucose<sup>14</sup>. Following hydrolysis for 3 h (4 normal hydrochloric acid) 7 per cent of amino sugar X was found in the lipopolysaccharide using the procedure of Ashwell<sup>11</sup> with 3-acetamido-3,6-dideoxy-D-galactose as reference. On prolonged hydrolysis this analytical value decreases (4 per cent of amino sugar X after 12 h).

To the best of our knowledge 3-amino-3,6-dideoxyglucose has not previously been found in bacteria. The *N*-dimethyl derivative of the D-enantiomer was shown to be a constituent of carbomyein<sup>1</sup>, which was proved by synthesis<sup>14</sup>. Meanwhile we have detected amino sugar X in several other strains of *E. coli*.

We thank Drs I. and F. Ørskov for the *E. coli* strain and the culture of the bacteria, Dr G. Ashwell for a gift of 3-acetamido-3,6-dideoxy-D-galactose, Dr J. D. Dutcher for a sample of 3-amino-3,6-dideoxy-D-mannose, and Dr R. C. Richardson for methyl-3-amino-3,6-dideoxy-L-glucoside and methyl-3-acetamido-3,6-dideoxy-L-taloside. We also thank Miss Ch. Heidkämper for technical assistance.

Note added in proof (June 26, 1967). Meanwhile, 3-amino-3,6-dideoxyglucose has also been found in lipopolysaccharides of *Citrobacter* and *Salmonella* (Lüderitz, O., *et al.*, *J. Bact.*, **93**, 1681; 1967).

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## MICROBIOLOGY

### Density Dependent Inhibition of Cell Growth in Culture

Abercrombie and Heaysman<sup>1</sup> coined the term 'contact inhibition' to describe the arrest of locomotion of animal cells which are in contact with one another. It was based on various parameters such as the behaviour of fibroblasts migrating from confronting explants, speed of movement in relation to numbers of contacts between cells, and nuclear overlap counts. Phase contrast cinematography of living cells by Abercrombie and Ambrose<sup>2</sup> later confirmed that the inhibition of movement depended on contact between cells. During these experiments it was found that certain sarcoma cells were less sensitive to inhibition than normal fibroblasts, and that they moved freely over one another and over normal cells<sup>3</sup>.

The concept of contact inhibition has attracted particular interest since the advent of tissue culture investigations on neoplastic transformation. Cells transformed by tumour viruses, *in vitro* or *in vivo*, show a marked tendency to pile up in culture in disorderly array, and to form layers several cells thick, in contrast to their untransformed precursors which tend to form monolayers of cells arranged in parallel. This change from the normal pattern has been attributed to loss of contact inhibition of movement, which in at least one instance has been confirmed by time-lapse cinematography (Ambrose, E. J., and Sheppley, K., personal communication, 1964).

Normal cells also differ from transformed cells in other characteristics, however, and the use of the term contact inhibition has been gradually extended to explain other phenomena, such as restriction of growth in dense cultures. We wish to suggest that the extension in use of this term is misleading, because it carries the implication of a common mechanism for which no evidence exists at present.

The notion that immediate contact between cells inhibits growth as well as movement has arisen from the tendency of cells to form monolayers and for the cell sheets formed to grow slowly, if at all, despite the continuing abundance of nutrients in the medium. Abercrombie originally suggested that this tendency to form monolayers may result from arrest of movement in contiguous cells, but he has not claimed that the contiguity between

cells is also responsible for inhibition of growth<sup>4</sup>. Unfortunately, movement of cells is not usually investigated by those investigating their growth and no precise work has been reported to show that the two are correlated. The available evidence in fact suggests that inhibition of movement and of growth are unrelated.

Cells of the BHK21 line, for example, form well oriented cell sheets and have been shown by time-lapse cinematography to be subject to contact inhibition of movement; yet they will grow to form multilayers with the same densities as their transformed derivatives which have lost movement inhibition<sup>5,6</sup>. We have also found independently that freshly isolated fibroblasts will continue to grow, without decrease in rate, for some time after they have formed monolayers in culture, provided that the medium is frequently changed<sup>7</sup>, or that the volume is large in relation to cell covered surfaces<sup>8</sup>. With continuous perfusion of fresh medium, Kruse and Miedema<sup>9</sup> produced multilayers of normal cells 5-17 cells thick, with no diminution of growth rate at the monolayer stage. Despite the absence of direct investigation of movement, these observations are scarcely compatible with the notion that growth and movement are simultaneously inhibited through a common mechanism which depends on surface contact between cells.

This is not to deny that in conventional culture conditions there are restrictions on cell growth which are apparently related to the population density of the cells<sup>10,11</sup> and which may be particularly important in connexion with the change following neoplastic transformation. Even with rapid exchange of medium, the growth of multilayered dense cultures of normal cells eventually slows or stops. Certain cell types such as 3T3 cells are particularly sensitive to this density restriction, in contrast to their transformed derivatives<sup>12</sup>.

It is even possible that contact might still play a part in inhibiting growth, but on a different basis from that involved in movement inhibition. For example, a small diffusible inhibitor might achieve an effective intracellular concentration in dense cultures with highly contiguous cell membranes through direct transfer from cell to cell and not through the medium where dilution or inactivation could occur<sup>13-15</sup>.

There is at present no direct evidence that contact is the primary requirement for the inhibition of growth, however, and other attributes of dense cultures should therefore be considered. One explanation which has not been subjected to rigorous analysis is that dense cultures may alter the extracellular environment in their immediate vicinity without making significant changes which can be detected in the bulk medium. Cells add and remove a variety of molecules in their immediate environment and there must be a series of concentration gradients which extend outwards from the cell membranes.

On first consideration this may seem unlikely to cause strong local effects close to the cell surface because in most cultures there is continuous agitation by vibration and thermal convection and a deliberate increase in mechanical agitation does not noticeably affect cell growth. Experimental observations and theoretical consideration of flow theory, however, show that fluid in the immediate vicinity of a solid-liquid interphase moves at a rate approaching zero, whatever the turbulence in the bulk fluid. As a result, movement in medium close to the cell surface will be restricted to molecular diffusion<sup>16</sup>. Concentration gradients will be maintained and not be greatly disturbed by movement in the overlying medium.

Thus it is quite possible that inhibitory conditions obtain in the immediate vicinity of cells in dense cultures and yet the bulk of the medium may retain the capacity for support of cell growth in another culture. For example, the local inhibitory conditions could result from accumulation of inhibitory molecules, depletion of nutrients, or local changes in pH or oxygen tension. In fact, it can be shown that a large number of cells attached to one surface

can inhibit the growth of a small number of cells on another surface if the distance between the two surfaces is small<sup>16</sup>.

It is not the purpose of this communication to examine these various hypotheses in depth, and the exercise would be of little value without further experimental data. We simply wish to draw attention to the inappropriateness of the term contact inhibition for use in relation to growth. It is misleading in that it implies a common mechanism for inhibition of cell growth and movement and it may misdirect the attention of investigators by tacitly implying a direct and immediate contiguity between cell surfaces as the basis for growth inhibition.

We therefore propose that the term "density dependent inhibition" be used to describe the restriction of growth in crowded cultures of animal cells, and that contact inhibition be restricted to arrest of movement as defined by Abercrombie and Heaysman<sup>1</sup>.

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## Haemagglutinin of Rubella Virus

STEWART and his associates have developed a method for detecting a haemagglutinating antigen for rubella virus<sup>1</sup> which depends on two discoveries: first, that the calf sera ordinarily incorporated into media for the maintenance of cells infected with rubella virus contain an inhibitor for haemagglutination and, second, that the best agglutination was seen with blood cells from newly hatched chicks. In their original method BHK21 cells, which have been shown to produce the virus in high titre<sup>2</sup>, were infected with rubella virus and maintained in 2 per cent kaolin-treated foetal calf serum. Although this method provided a signal advance in the serology of rubella, in our laboratory production of haemagglutinin has been variable and, when found, the peak titres observed were only 1/32 per ml.

During an investigation of the nature of the rubella haemagglutinin, we have found a simpler method for its preparation. When tissue culture media containing normal foetal calf serum from infected cell cultures were placed on a 5 per cent to 60 per cent preformed sucrose density gradient, the haemagglutinin and infectivity appeared in the fractions around the density 1.18 g/ml. while the serum inhibitor remained at the top of the gradient (Table 1). Haemagglutinin could be demonstrated only when EDTA was incorporated in the gradient. It was then found that addition of 0.01 molar EDTA (final concentration) to crude tissue culture fluid and incubation for 2 h at 4° C, followed by centrifugation at 32,000 × g (16,000 r.p.m.) in a Servall RC2 for 2 h, resulted in sedimentation of haemag-

Table 1. SUCROSE DENSITY GRADIENT CENTRIFUGATION OF RUBELLA-INFECTED BHK21 TISSUE CULTURE FLUIDS

Fraction No.	Infectivity (P.F.U./ml.)*	HA ( $\mu$ /ml.)	Inhibitor†	Density (g/ml.)
1	<10 <sup>3.4</sup>	<1:2	N.T.	1.297
2	<10 <sup>3.4</sup>	<1:2	N.T.	1.283
3	<10 <sup>3.4</sup>	<1:2	N.T.	1.257
4	<10 <sup>7.1</sup>	32	N.T.	1.220
5	10 <sup>7.1</sup>	128	N.T.	1.188
6	10 <sup>3.4</sup>	64	N.T.	1.153
7	<10 <sup>3.4</sup>	<1:2	N.T.	1.122
8	<10 <sup>3.4</sup>	<1:2	<1:4	1.098
9	<10 <sup>3.4</sup>	<1:2	<1:4	1.076
10	<10 <sup>3.4</sup>	<1:2	<1:4	1.066
11	<10 <sup>3.4</sup>	<1:2	1:80	1.050
12	<10 <sup>3.4</sup>	<1:2	1:80	1.036

Tissue culture fluid (0.5 ml.) was layered on 4.5 ml. of 5 per cent to 60 per cent sucrose, containing 0.001 molar EDTA. The tube was centrifuged in the SW39 rotor of a Spinco centrifuge for 9 h at 35,000 r.p.m. at 4° C. Fractions (0.4 ml.) were collected from the bottom. Tube No. 12 is the top of the gradient. HA, Haemagglutinating antigen; N.T., not tested.

\* Titrated by plaque formation in RK13 (ref. 3).

† Tested against 4 u of haemagglutinin.

Table 2. CHARACTERISTICS OF RUBELLA HAEMAGGLUTININATING ANTIGEN

Treatment	Titre		
	Experiment 1	Experiment 2	Experiment 3
None*	1/128	1/320	1/64
Filtration	<1/4	-	-
'Tween'-ether	-	1/640	-
'Tween'-ether, filtration†	-	1/160	-
Sedimentation‡ (supernatant)	-	-	<1/4
(precipitate)	-	-	>1/4

\* The initial material used in each experiment was antigen obtained by concentration from EDTA-treated tissue culture fluids.

† 100 m $\mu$  Millipore filter.

‡ 32,000g for 2 h.

glutinin. After suspending this sediment in one-fifth the volume of phosphate buffered saline, we have obtained haemagglutinin titres of 1/256 and 10<sup>7.9</sup> P.F.U./ml. of virus. A titre of 1/640 was obtained when a sediment was re-suspended in one-tenth the original volume. Thus with this technique it is possible to prepare concentrates of haemagglutinin from large volumes of ordinary tissue culture fluid.

The mechanism for the effect of EDTA appears to be the removal of cations which may attach the inhibitor to the virus, because addition of calcium or magnesium chloride to the tissue culture fluids prevented the release of haemagglutinin from EDTA-treated fluids.

The fact that rubella haemagglutinin and infectious virus were associated with each other in the density gradients agrees with our observation that haemagglutinin produced by cells maintained with kaolin-treated serum<sup>1</sup> can be sedimented and does not pass a 100 m $\mu$  filter except after treatment with 'Tween'-ether (Table 2). Rubella haemagglutinin prepared from tissue culture fluid is therefore not a soluble antigen.

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### Application of Particle-counting to a Leafhopper-borne Virus

THE electron microscope particle-counting technique of Backus and Williams<sup>1,2</sup>, which involves the use of shadow-cast specimens, has been generally favoured in investigations of viruses involving particle counting. Watson<sup>3</sup> modified the method by using phosphotungstate negative

staining<sup>4,5</sup> to reveal the virus particles in more structural detail and to make identification easier. The modification facilitates counting of virus particles in relatively impure preparations.

We have used the electron microscope particle-counting technique of Backus and Williams<sup>1</sup>, as amended by Watson<sup>3</sup>, to estimate concentrations of wound-tumour virus (WTV) in extracts from root tumours of sweet clover plants and from the insect vector infected with the virus. The method also permitted the determination of several other parameters for WTV.

Root tumours from infected sweet clover plants, *Melilotus officinalis* (L.) Lam, Clone C<sub>10</sub> (refs. 6 and 7), were generally used as a source of WTV. The clarified tumour extract used for electron microscopy was prepared by triturating the tumours with pestle and mortar in three times their weight of a solvent, containing 0.05 molar potassium phosphate and 0.01 molar sodium sulphite, until the pulp attained the consistency of a finely ground meal. The crude extract at approximately pH 7.0 was squeezed through one layer of cheese cloth and then clarified in a Servall SS-1 centrifuge for 5 min at 7,200 r.p.m. In order to estimate the concentration of wound-tumour virus in infected leafhoppers, *Agallia constricta* Van Duzee, healthy nymphs in about the second instar were exposed to crimson clover (*Trifolium incarnatum* L.) infected with WTV until the infective virus attained a maximum concentration<sup>8</sup>. Then samples of forty to sixty infected leafhoppers were collected at random, counted and triturated in 0.1–0.2 ml. of a solution containing 0.1 molar glycine and 0.01 molar magnesium chloride adjusted to pH 7.0 with 1 normal sodium hydroxide. The crude extract was transferred to a cellulose nitrate tube (size 3/16 × 5/8 in.) by means of a Pasteur pipette. The preparation was centrifuged as described here by supporting the tubes in special 'Lucite' adapters made to fit the tubes of a Servall SS-1 centrifuge. The supernatant containing the virus was carefully removed with a 22 gauge needle fitted to a 1 ml. syringe, avoiding the fat layer floating on the surface of the extracts. These extracts were used for electron microscopy. The percentage of infected insects in the colony was assessed by haemagglutination tests<sup>9</sup> on twenty-five individual insects taken at random from the population, and on this basis the concentration was corrected to represent the number of extractable particles from infected insects only. The weight of an insect was always taken to be 1 mg.

The polystyrene latex suspension used consisted of particles 0.126 ± 0.0043 $\mu$  in diameter and was supplied by the Dow Chemical Company from Run No. LS-052-A. The concentration of particles was calculated from the dry weight of a given volume<sup>2</sup>. For electron microscopy, 0.1 ml. of the clarified tumour or insect extract prepared at or near 0° C, as described here, was mixed with 0.1 ml. of the latex suspension (diluted to contain 9.34 × 10<sup>11</sup> particles/ml.). To this suspension 0.2 ml. of 2 per cent potassium phosphotungstate and 0.05 ml. of a 1.0 per cent solution of bovine serum albumin were added. The mixture was sprayed from a commercial low pressure atomizer onto electron microscope specimen grids coated with a parlodion film stabilized with a second film of evaporated carbon. The grids were scanned in a Hitachi model HU-11A electron microscope at a constant magnification of × 7,000. Two grids were usually examined for each sample, and nine or ten droplets were photographed in each sample. Virus and latex counts were made on the original negative. The consistency of the ratio of virus particles to latex particles in a number of droplets was checked statistically by means of the  $\chi^2$  test, and the error in each count was estimated from the 95 per cent confidence limit calculated as previously described<sup>2</sup>.

The virus concentration of WTV isolate VI<sub>64</sub>, expressed as number of particles extracted/g of tumours, was 14.31 ± 1.76 × 10<sup>11</sup> in one experiment and 11.07 ± 1.20 × 10<sup>11</sup> in another.



Assuming the weight of a single leafhopper to be 1 mg, the average number of extractable virus particles/g of infected leafhoppers was  $9.6 \pm 1.0 \times 10^{11}$  in one experiment and  $14.0 \pm 3.5 \times 10^{11}$  in another. The maximum concentration of virus in the leafhopper is therefore almost exactly the same as that in the sweet clover tumour.

It was possible to determine, by direct particle count, the concentration of virus for the peak region of the curve of virus increase and decline in the insect vector after acquisition of virus by feeding on infected plants. Reddy and Black<sup>8</sup> had earlier determined this same curve for a greater range of concentration by measurements of relative infectivity. An average curve for relative infectivity constructed from their Fig. 7 corresponded closely with our count of virus particles in the region of the peak. We concluded that the two peaks could be equated and that, at the peaks, their relative infective virus concentration of  $10^{7.72}$  equals our concentration of  $10^{9.26}$  virus particles per infected leafhopper. Any virus concentration value on their relative scale can be converted to the number of virus particles per ml. of solution injected into assay leafhoppers by multiplying their value by  $10^{2.54}$ . Information from the curves permits the determination of the minimum infective dose for injection of a leafhopper as  $10^{2.6}$  virus particles. This value compares with a minimum infective dose of between  $10^{4.4}$  and  $10^{4.6}$  for the inoculation of cultures of the vector cells (Gámez, R., and Chiu, Ren-jong, personal communication).

In duplicate experiments comparisons of the concentration of extractable virus/g of tumour incited by WTV isolates VI<sub>66</sub> and VL<sub>49</sub> yielded values of  $14.2 \times 10^{11}$  and  $11.2 \times 10^{11}$  for the former and  $1.6 \times 10^{11}$  and  $0.4 \times 10^{11}$  for the latter. Other isolates had intermediate concentrations roughly related to the length of time they had been maintained in sweet clover without passage through the vector.

It was also established that the current purification procedure isolates only 2.5 per cent of the virus extracted from the tumours, and that 1 mg of protein in this purified virus corresponded in duplicate experiments to  $7.1 \times 10^{12}$  and  $7.7 \times 10^{12}$  virus particles.

At serological end-points determined by the precipitin ring test and indirect haemagglutination the number of virus particles/ml. in duplicate experiments was  $4.0$  and  $9.2 \times 10^9$  and  $8.1$  and  $40.0 \times 10^7$ , respectively (unpublished results of Gámez and MacLeod).

On the basis of serological data and Merrill's curve<sup>10</sup>, Black<sup>7,11</sup> had estimated values for the concentration of WTV in tumours, leafhoppers, and the minimal infective dose for the vector, which were close to the same order of magnitude as the more direct and precisely measured values presented here.

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### Interferon and Murine Leukaemia. III: Efficacy of Interferon Preparations administered after Inoculation of Friend Virus

INTERFERON has been most effective *in vitro* and *in vivo* when administered before viral inoculation, considerably less effective when inoculated at the same time as the challenge virus, and often ineffective after viral inoculation<sup>1-3</sup>. The necessity of pretreatment of cells or animals to obtain an anti-viral effect has raised doubts as to the value of interferon in the treatment of viral diseases<sup>4-5</sup>.

We have shown that concentrated preparations of interferon inhibited the development of splenomegaly in Friend disease as long as mice were treated with interferon before viral challenge and twice daily thereafter<sup>6,7</sup>. Administration of interferon for only 3 days was ineffective although treatment preceded inoculation of virus. These results contrasted with those obtained in mice treated with interferon and inoculated with encephalomyocarditis and Semliki forest viruses. In these acute lethal diseases, interferon had a prophylactic effect and afforded only negligible protection after viral inoculation<sup>8,9</sup>.

This communication reports the results of experiments in which treatment with interferon initiated 48 h after inoculation of Friend virus inhibited the development of splenomegaly in Swiss mice. We think that these are the first results which show interferon preparations to possess significant anti-viral activity after infection has been well established in the animal host.

The methods of preparing and titrating Friend virus have been described before<sup>6,7</sup>. Swiss mice, 4-6 weeks old, were inoculated intraperitoneally with 0.2 ml. of a 15 per cent extract of leukaemic spleen. Spleen weight at the end of the experiment served as a criterion of disease<sup>10</sup>. Mouse interferon was prepared by the method of Finter<sup>11,12</sup>. The brains of diseased weanling mice infected with West Nile virus were ground in a mortar and the interferon was extracted as described before<sup>7</sup>. To increase the interferon titre, preparations were concentrated ten-fold by pressure dialysis. Control normal mouse brain was extracted by techniques identical to those employed in the preparation of interferon. The interferon preparations were assayed by standard techniques with mouse embryo fibroblasts in monolayer cultures challenged with 50-100 P.F.U. of vesicular stomatitis virus. Interferon titres were expressed as the dilution (2 ml.) of the original preparation responsible for a 50 per cent reduction in the number of plaques as compared with virus control cultures<sup>13</sup>.

Table 1 summarizes the results of two experiments designed to evaluate the effect of mouse brain interferon on the development of splenomegaly in Swiss mice infected with Friend virus. In one of the two treatment groups, interferon was administered intraperitoneally

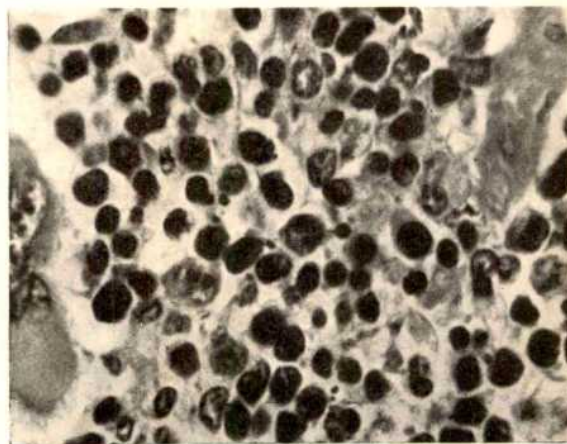


Fig. 1. Spleen of uninoculated Swiss mouse stained with haematoxylin and eosin ( $\times c. 660$ ). Note absence of immature cells.



Table 1. EFFECT OF MOUSE BRAIN INTERFERON ON DEVELOPMENT OF SPLENOMEGALY IN FRIEND DISEASE OF SWISS MICE

Experiment	Normal		Viral controls		Leukaemic <sup>1</sup>		Interferon treatment initiated		No. Spleen wt mean $\pm$ S.E. mice	
	Uninoculated	No. Spleen wt mean $\pm$ S.E. mice	No. Spleen wt mean $\pm$ S.E. mice	Treatment normal mouse brain	No. Spleen wt mean $\pm$ S.E. mice	Before viral <sup>2</sup> inoculation	No. Spleen wt mean $\pm$ S.E. mice	After viral <sup>3</sup> inoculation		
1	10	215 mg	15	975 mg	25	871 mg	25	322 mg <sup>4</sup>	25	391 mg <sup>4</sup>
		2-30		2-98		2-94		2-50		2-59
		$\pm 0.03$		$\pm 0.07$		$\pm 0.07$		$\pm 0.05$		$\pm 0.06$
2	5	185 mg	22	579 mg	22	675 mg	22	395 mg <sup>4</sup>	22	395 mg <sup>4</sup>
		2-26		2-76		2-82		2-59		2-59
		$\pm 0.03$		$\pm 0.04$		$\pm 0.05$		$\pm 0.06$		$\pm 0.05$

In experiment 1 Swiss mice, male and female, were killed 22 days after viral inoculation. In experiment 2 Swiss mice, female, were killed 16 days after viral inoculation. (1) The virus was a 15 per cent extract of leukaemic spleen and 0.2 ml. was injected intraperitoneally. (2) In this group interferon treatment initiated 24 h and 3 h before inoculation of Friend virus was continued twice daily until the end of the experiment. Treatment consisted of twice daily injections of 0.2 ml. of interferon intraperitoneally. (3) In this group interferon treatment initiated 48 h after inoculation of Friend virus was continued twice daily until the end of the experiment. Treatment consisted of twice daily injections of 0.2 ml. of interferon intraperitoneally. (4) Titre of interferon employed was 56,000/2 ml. (5) Titre of interferon employed was 44,000/2 ml. Spleen weight was the geometric mean spleen weight in mg. Mean  $\pm$  S.E. was the geometric mean in logarithms  $\pm$  standard error.

24 h and 3 h before inoculation of Friend virus and twice daily thereafter. In the other group, interferon treatment was initiated 48 h after viral inoculation and twice daily thereafter.

There was no significant difference between the mean spleen weights of the two viral control groups (that is, those mice receiving virus alone and those mice receiving virus and concentrated normal mouse brain). A significant inhibition of splenomegaly, however, was observed in both groups of mice treated with interferon compared with the two viral control groups. (Experiment 1:  $P < 0.0001$ ; experiment 2:  $P = 0.001$ ). There was no significant difference between the mean spleen weights of the two groups of mice treated with interferon.

At the termination of these experiments most of the untreated leukaemic mice had large spleens in which the normal parenchyma was extensively replaced by "Friend cells" (Fig. 2)<sup>14</sup>. Relatively small spleens with only scattered foci of Friend cells were noted in a few of the untreated mice. Of the interferon treated mice, a considerable number had small spleens, and these could not be distinguished histologically from the small spleens of the untreated leukaemic mice. In the larger spleens of mice treated with interferon, however, not only was the extent of Friend cell involvement less marked than that observed in control leukaemic mice, but there appeared to be numerous foci of erythroid maturation involving the primitive Friend cells (proerythroblast), and their

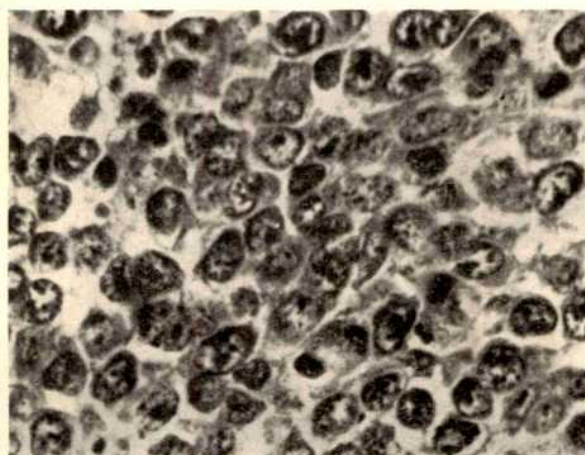


Fig. 2. Spleen of untreated leukaemic Swiss mouse stained with haematoxylin and eosin ( $\times c. 660$ ). Note sheet of large immature "Friend" cells.

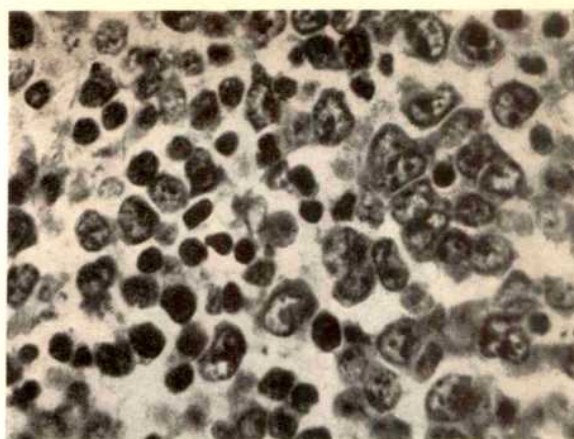


Fig. 3. Spleen of leukaemic Swiss mouse treated with interferon, stained with haematoxylin and eosin ( $\times c. 660$ ). Note large immature "Friend" cells at right and focus of erythroid maturation on the left.

conversion to normoblastic elements (Fig. 3). This cellular maturation was seldom observed in the spleens of untreated leukaemic mice. A similar phenomenon has been observed before in Swiss mice infected with Friend virus and treated with erythropoietin (unpublished work, F. Zajdela); namely, inhibition of splenomegaly accompanied by histological evidence of erythroid maturation.

The therapeutic effect of administration of these interferon preparations after inoculation of Friend virus clearly demonstrated that in this system interferon pretreatment was not essential. Other viral diseases characterized by a long latent period or a sub-acute or chronic course may also be attenuated by adequate interferon treatment after viral infection.

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### Failure of Bacteriophage $\phi$ X 174 to multiply in Serum Spheroplasts

SPHEROPLASTS are bacteria that are deficient in cell wall and can be prepared by a variety of methods from Gram-negative bacteria. One way to examine the degree of cell wall removal is to study the infection of spheroplasts with bacteriophage, which attaches to receptors on the cell wall. Bacteriophage adsorption and multiplication in spheroplasts have been reported by a number of authors<sup>1-7</sup>. The ability of spheroplasts prepared by treatment with serum to multiply phage has not yet been investigated.

We compared the capacity of spheroplasts, made with serum and by three other methods, to adsorb and multiply  $\phi$ X 174 bacteriophage. Spheroplasts were prepared from *Escherichia coli* C in the log phase of growth. Lysozyme spheroplasts were prepared by treatment with EDTA, tris, and lysozyme (ETL) as described<sup>8</sup>. Glycine spheroplasts<sup>9</sup> were made from bacteria suspended in brain-heart infusion broth containing 3 per cent glycine and 0.02 molar magnesium chloride. The suspension was aerated by bubbling air through the broth. The transformation of the bacteria to spheroplasts was virtually complete after 80 min. Penicillin spheroplasts were prepared by the method of Lederberg<sup>10</sup>, and serum spheroplasts were prepared as previously described<sup>11</sup>. Briefly, serum from a healthy adult donor was added to suspension of bacteria in brain-heart infusion broth with 0.01 molar magnesium chloride. After 30 min the transformation of rods to spheroplasts was complete.

Spheroplasts were collected by centrifugation, resuspended in brain-heart infusion broth with 0.05 molar magnesium chloride, and adjusted to an optical density (OD) of 0.15 at 650 m $\mu$ . The addition of magnesium and the use of broth both seemed to be essential to the prolonged stability of spheroplasts. About 10–20 per cent of the spheroplasts lysed during centrifugation and resuspension. The final suspensions therefore contained about 80–90 per cent spheroplasts and the remainder of the bacterial forms were ghosts. Less than 1 per cent of the bacterial forms in the final suspensions were rods.

Bacteriophage  $\phi$ X 174 was grown and purified as described by Adams<sup>12</sup>. Phage was added to the spheroplast suspensions in a ratio of 10 plaque-forming units

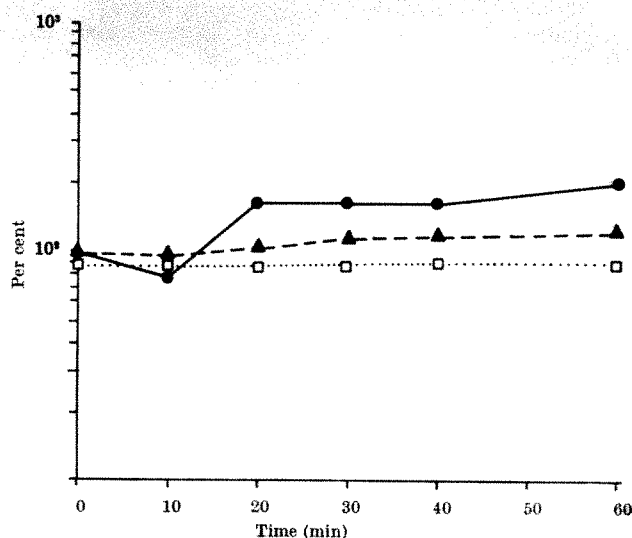


Fig. 3. Phage multiplication in serum spheroplasts. Symbols as in Fig. 2.

(P.F.U.) to bacterial form. Two control tubes were run with each experiment. One control consisted of bacteriophage alone; all phage suspensions remained stable during the hour of incubation. The second control tube contained suspensions of spheroplasts without bacteriophage. The spheroplast suspensions remained stable for more than 1 h without significant change in OD or in the differential count of spheroplasts and ghosts.

After bacteriophage was added, samples were taken at 10 min intervals for 1 h of incubation at 37°C. For differential counts, samples were fixed in 2 per cent formalin and were examined using a phase contrast microscope. The optical density was determined, and samples were taken for determination of phage titre by the agar-layer method. Samples for phage titre were diluted in saline with 0.05 molar magnesium chloride.

For comparison with phage multiplication in spheroplasts, *E. coli* C rods were infected with bacteriophage, and changes in bacterial morphology and phage titres were measured in a similar manner (Fig. 1). The phage titre decreased at 10 min and then rapidly increased. The OD increased slightly in the first 20 min, but then decreased to 40 per cent at 60 min. At 30 min the rods began to lyse by changing into rod-shaped ghosts. The change in rod morphology ran parallel with the change in optical density and the increase in phage titre. At 60 min only 2.5 per cent normal rod forms remained.

The results of experiments with ETL, glycine and penicillin spheroplasts were similar. As an example, phage multiplication in glycine spheroplasts is shown in Fig. 2. Phage adsorption proceeded as with the normal rods, but the phage titre began to increase earlier. Remarkably, neither the OD nor the differential count changed during the experiment. Spheroplasts did not lyse but became larger and somewhat paler, as did the uninfected spheroplasts in control tubes.

In sharp contrast, little phage adsorption and multiplication occurred with the serum spheroplasts (Fig. 3). Furthermore, there was no evidence of phage multiplication when the spheroplasts were lysed by freezing and thawing.

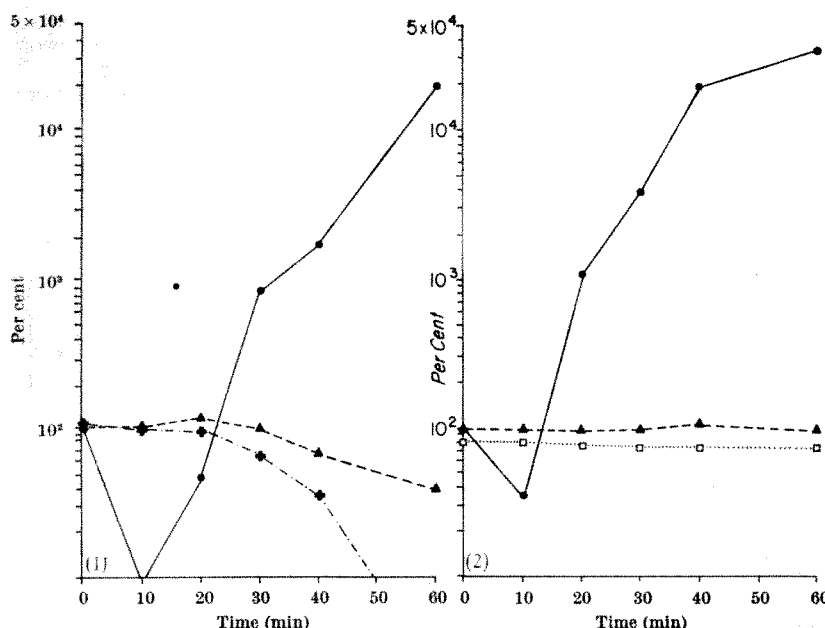


Fig. 1. Phage multiplication in *E. coli* C. ●—●, Phage; ▲—▲, optical density; +—+, rods; □—□, spheroplasts.

Fig. 2. Phage multiplication in glycine spheroplasts. ●—●, Phage; ▲—▲, optical density; □—□, spheroplasts.



The effect of human serum on glycine spheroplasts was also studied. Glycine spheroplasts were treated with human serum for 20 min, resuspended in broth and infected with phage. Glycine spheroplasts treated with human serum did not support phage multiplication. Treatment of glycine spheroplasts with serum inactivated by heat had no effect on phage multiplication.

When phage multiplication in normal rods is compared with that in penicillin, glycine, and ETL spheroplasts, there are two striking differences. The first is that the phage titre increases more rapidly in the spheroplast suspensions. The loss of part of the cell wall may allow more rapid penetration of phage into spheroplasts. This view is supported by reports that some phage DNA particles infect spheroplasts but not intact bacteria<sup>12</sup>. The second notable difference is that the rods lysed after infection and the spheroplasts did not. Infection of rods by  $\phi$ X 174 may activate an autolytic enzyme in the bacteria<sup>14</sup>. Either the autolytic enzyme is not induced or it is not effective in spheroplasts. The presence of magnesium as a stabilizing agent in the spheroplast suspensions does not seem to be the cause of the lack of lysis because normal rods lysed in the presence of magnesium. One strain of bacteria is known to produce phage without lysis of the host organism<sup>15</sup>. The mechanism of phage multiplication and release in that strain may be similar to that in the spheroplasts.

Spheroplasts have been the subject of considerable interest, because they retain many of the metabolic capacities of the parent organisms<sup>16</sup>. As shown here, spheroplasts prepared by different methods may have different properties. Serum treatment may have damaged the phage receptor sites while the other methods of spheroplast preparation did not.

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### Comparative Growth of *Candida lipolytica* on Glucose and *n*-Hexadecane

MUCH attention has been paid to yeasts grown on hydrocarbons since Champagnat<sup>1</sup> suggested their potential use as fodder-yeast. Few differences have as yet been reported to be induced in these yeasts by the hydro-

Table 1. YIELD OF *Candida lipolytica* GROWN ON GLUCOSE AND ON *n*-HEXADECANE

Phase	Substrate	
	Glucose	<i>n</i> -Hexadecane
Exponential	2.00* ( $\sigma = 0.31$ )	1.99 ( $\sigma = 0.59$ )
Stationary	2.22 ( $\sigma = 0.13$ )	3.96 ( $\sigma = 0.51$ )

\* g/10<sup>11</sup> dry cells.

Cells were collected in the exponential phase when the broth contained 10<sup>8</sup> cells/ml. and in the stationary phase 1 day after the growth curve levelled off. Dry weights were obtained by drying equal portions of the washed cell crop at 105° C for 3 h. For comparison, the cell yield was calculated for 1 l. broth and at a cell concentration of 10<sup>8</sup> cells/ml. in all cases. Each value is the mean of at least ten separate experiments.

carbons, other than an increase in their lipid content<sup>2,3</sup>. We wish to report a striking difference in the growth of *Candida lipolytica* when *n*-hexadecane replaces glucose as the sole source of carbon and energy.

*C. lipolytica* was obtained from the C.B.S. (Delft) as a pure strain, not as a mixture of two<sup>4</sup>. The original slant was twice sub-cultured on malt agar. One slant served to inoculate a 250 ml. flask containing 50 ml. of a yeast nitrogen base medium (Difco), to which 1 per cent (weight/volume) glucose (A.R.) or *n*-hexadecane (B.D.H.) was added. Incubation lasted for 3 days at 27° C on a rotatory shaker (E. Bühler, Tübingen, type 150100) at 140 r.p.m. The pH (about 6.5) was adjusted twice a day with 5 normal ammonia. Enough of this starter culture to make 10<sup>7</sup> cells/ml. was transferred to a 1 l. flask containing 200 ml. of the same medium. Incubation was resumed in the same way. The growth curve was then determined by plotting the number of cells present against time (Fig. 1). The break point *a* in the curve was always observed when *C. lipolytica* was grown on *n*-hexadecane.

An explanation for this phenomenon was found when cell yields in the exponential phase of growth were compared with those in the stationary phase (Table 1). The cells were collected by centrifugation at 16,300g for 30 min, in a refrigerated Sorvall 'RC II-B' centrifuge. The excess *n*-hexadecane solidified as a pellet at the surface and the yeast cells collected at the bottom of the cups when the broth was at room temperature at the beginning of the centrifugation and allowed to cool to +4° C during the run. Most of the cells were in the true yeast form when they were collected and little if any pseudomycelium was present.

From the break in the growth curve and the fact that individual cell weights increased by a factor of two, it appears that the culture on hydrocarbon, on reaching a certain age, shows a lesser tendency for cell division which is compensated by an increase in individual cell size. Thus *C. lipolytica* may well show an exponential

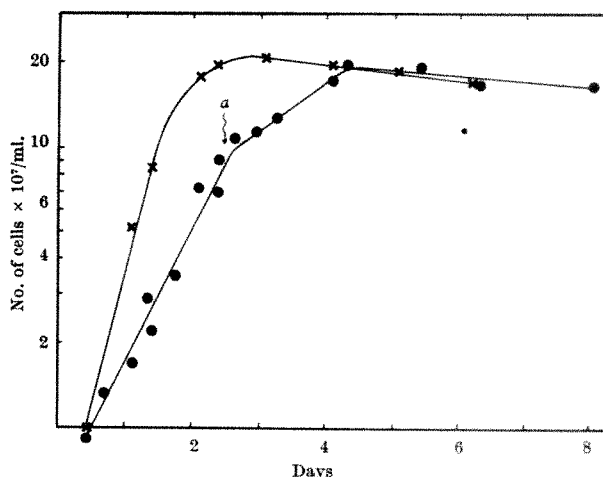


Fig. 1. Growth of *Candida lipolytica* on glucose (x) and on *n*-hexadecane (●). The cells were counted using a Thomas haemocytometer. Cell clusters were broken up by adding three drops of a 10 per cent ethanolic solution of 'Tween 80' per ml., and mixing for 1 min on a 'Whirlmix' (Cenco).



overall mass increase on both glucose and *n*-hexadecane, but in quite a different way. Further work is being carried out to correlate this varying growth behaviour with biochemical differences.

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### Electron Microscopy of Monkey Kidney Cells infected with both SV40 and Measles Virus

THE ultrastructure of cells infected with SV40 alone has been described previously by several investigators<sup>1-4</sup>. The morphology of the internal component of measles virus in infected cells has also been described<sup>5-7</sup>. Single cells infected with these two viruses have not been reported, however. In a previous study, a mixed infection with both SV40 and measles virus was obtained in a lot of cell cultures prepared from the kidney of a sick green monkey<sup>8</sup>. Mixed infection with these two viruses was later experimentally induced in tissue culture, and doubly infected cells were noted on examination by light microscopy<sup>9</sup>. The present communication describes the electron microscopy of cells in cultures doubly infected with SV40 and measles virus, and observation of the fine structure of both viruses in the same nucleus.

Primary kidney cells prepared from both African green and rhesus monkeys were used. Freshly trypsinized cells were seeded into 3 oz. prescription bottles or Leighton tubes containing 11 × 22 mm coverslips. SV5 antiserum to a final concentration of 0.2 per cent was added to a medium

consisting of Hanks balanced salt solution, 0.5 per cent lactalbumin hydrolysate, and 2 per cent calf serum. When confluent monolayers were obtained, the cultures were inoculated with measles virus and SV40, either simultaneously or in alternating sequence with an interval of 1-5 days between the first and second virus inoculations. All viruses were used in a multiplicity of 1 or greater. Infected cells were fixed for electron microscopy 3-7 days after the final inoculation, depending on the condition of the cell as observed by light microscopy. The infected cells in the prescription bottles were removed from the glass, centrifuged, and fixed in cold 3 per cent phosphate-buffered glutaraldehyde. They were then post-fixed in 1 per cent phosphate-buffered osmium tetroxide, dehydrated in graded alcohol, and embedded in 'Maraglas'. The infected cells on coverslips were left *in situ*, fixed as described and finally embedded in 'Epon'. All sections were examined with a Siemens-Elmiskop I electron microscope.

As shown in Fig. 1, the nucleus of a rhesus monkey kidney cell infected with both SV40 and measles showed many slightly electron-dense SV40 particles and, in addition, aggregates of fibrils, 150 Å in width, either linearly arranged or sectioned across their width. Morphologically, these fibrils were similar in appearance to the fibrils in the nuclei of measles-infected dog kidney cells described by Tawara<sup>6</sup>, and to those observed by us in the nucleus of a green monkey kidney cell infected with measles virus alone (Fig. 2). It is possible that these fibrils were the early development of the internal component of measles virus. In the same photograph (Fig. 2), coiled strands of filaments were observed in the cytoplasm of the cells infected with measles, which are identical in appearance to the internal component, described in recent reports, found in the cytoplasm of cells infected with other myxoviruses<sup>10,11</sup>.

In a study of SV40 in green monkey cells, Granboulan *et al.*<sup>12</sup> noted the presence of a similar fibrous structure in the nucleus of the SV40 infected cells during the process of cellular alteration, but considered these fibrils to be of unknown significance. Because "normal" monkey kidney cell cultures have often been found to harbour a variety of viruses, it appears that the structures observed earlier may be due to an unrecognized latent infection with measles virus or some other agent.

Mixed infections with two DNA viruses have been demonstrated by O'Connor *et al.*<sup>13,14</sup>. Both SV40 and an adenovirus, and SV40 and herpes simplex virus, have been

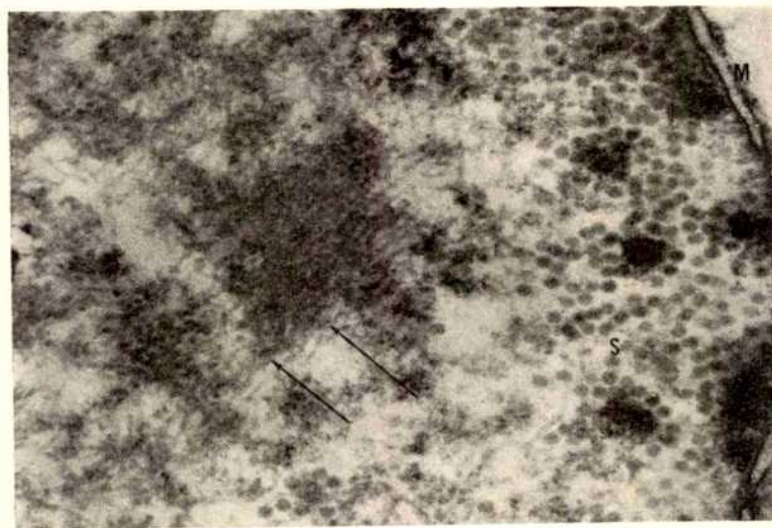


Fig. 1. Electron micrograph of a portion of a nucleus in a rhesus monkey cell showing low electron-dense SV40 particles (S) near the periphery of the nuclear membrane (M) and the linearly arranged fibrils (→) in the centre portion of the nucleus. (× c. 50,665.)



observed in the same nucleus. Other investigators have noted the occurrence of adeno-SV40 hybrids in monkey cells infected with these two viruses<sup>15,16</sup>. Evidence is presented here that one nucleus can be infected with both a DNA virus (SV40) and an RNA virus (measles). The phenotypic mixing of SV40 and measles would appear to be a possibility and is being studied.



Fig. 2. Electron micrograph of a portion of an African green monkey kidney cell infected by measles virus showing closely packed, linearly arranged fibrils (→) in the nucleus (N), and coiled strands of filaments (↪) in the cytoplasmic matrix (C). ( $\times 35,000$ .)

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### Segregation during Transfer of Infectious Drug Resistance in Enterobacteriaceae

MANY reports of infectious drug resistance suggest that all the resistance determinants of a multiple resistant donor strain are invariably transferred to the sensitive recipient strain.

A new technique which we have developed has shown that, by using different recipients and different methods of selection, various patterns of resistance are obtained in colonies selected at random from the converted recipient population.

Equal volumes of overnight broth cultures of both donor and recipient strain were mixed in nutrient broth to produce a final dilution of 10 for each culture. After overnight incubation at 37° C, the mixed culture was plated on to a selective medium. This selective medium was tryptone soya agar (Oxoid CM 131) containing 15 µg nalidixic acid/ml. and one other antibiotic. Nalidixic acid was added because all the recipient strains used were mutants resistant to nalidixic acid<sup>1</sup>; this facilitates the isolation of the converted recipients from the donor strains which were all sensitive to nalidixic acid. The other antibiotic which was added to the selective medium depended on the resistance pattern of the donor strain. For example, if the donor strain was resistant to tetracycline, streptomycin and sulphonamide, three plates were used each containing one of these antibiotics in addition to nalidixic acid. The colonies which grew on these plates after incubation were formed by bacteria to which drug resistance had been transferred from the resistant donor strain. The converted recipients were purified on nutrient agar to free them from contamination by the donor strain.

A hundred colonies were selected at random, and individual bacterial suspensions were prepared from each purified colony. The pattern of resistance of the individual colonies was determined by growing the bacteria in drops of antibiotic solutions on the surface of flat 'Perspex' plates. These plates are modifications of those originally described for a complement-fixation test<sup>2</sup>. In the original method, the 'Perspex' plates were 16 in. square and 1/8 in. thick. These proved to be rather clumsy, and so smaller plates of the same thickness but only 12 in. square were substituted. The top surface of the plate was engraved with a centrally placed grid of a hundred 1 in. squares arranged in ten rows of ten. A rack to hold ten of these plates was constructed<sup>3</sup>.

To investigate the converted recipients, we sterilized the plates by immersion in 10 per cent hydrochloric acid, rinsed them in sterile distilled water, allowed them to dry and finally wiped them with petroleum ether.

Eight antibiotic solutions were prepared in tryptone soya broth. They were 25 µg/ml. of oxytetracycline; 12.5 µg/ml. of nalidixic acid; 12.5 µg/ml. of streptomycin; 100 µg/ml. of sulphonamide; 20 µg/ml. of furazolidone; 25 µg/ml. of neomycin; 30 µg/ml. of ampicillin; and 25 µg/ml. of chloramphenicol.

Each square in the tenth column of a 'Perspex' plate received two drops of tryptone soya broth, and each square in the ninth column received one drop of the broth from a dropping pipette calibrated to deliver 0.04 ml. With individually calibrated pipettes, 0.04 ml. of each of the eight selected antibiotic solutions was delivered on to the squares of the first eight columns of the plate so that each column contained a different antibiotic; the squares in the ninth and tenth columns received no antibiotic.

To each square in the first nine columns of the first row was added a drop of 0.02 ml. of a bacterial suspension. The square in the ninth column containing no antibiotic checked the viability of the culture and the square in the tenth column receiving no bacteria was a control of sterility. The next row on the plate received a drop of another bacterial suspension, and in this way ten individual bacterial suspensions could be accommodated on each plate and one hundred on the complete set of ten plates.

As an indicator, a sterile 0.1 per cent solution in distilled water of 2,3,5-triphenyltetrazolium chloride was prepared. With a calibrated dropping pipette, 0.02 ml. of this indicator solution was added to every square on the plates. As they were completed, the plates were placed in the rack, which was then put into a box and sealed to prevent evaporation of the drops. The box was incubated overnight at 37° C.

On the next day, the drug resistance pattern of the converted recipients was determined by inspection of the plates. If, in a particular pool on a 'Perspex' plate, the bacteria had multiplied they would have absorbed the tetrazolium salt and reduced it to a red formazan. That pool would therefore contain a red deposit and would be scored positive.

All the squares in the ninth column should be scored positive, because these squares contained no antibiotic, and the squares in the tenth column testing sterility should be negative. The pattern of red deposits in the first eight columns determined the resistance pattern of the individual suspensions.

With this technique we have shown that a converted recipient does not always receive the complete resistance pattern of the donor, but that the particular resistance markers which appear in the converted recipient depend on which antibiotic has been added to the selective medium and also on the type of sensitive recipient used. An example is shown in Table 1: three different resistance patterns can be recognized with the six antibiotics used.

The advantage of this new technique in comparison with the use of solid media or replica plating is that very

Table 1. THREE TYPES OF RESISTANCE PATTERNS RECOGNIZED IN CONVERTED RECIPIENTS SELECTED ON A NEOMYCIN CONTAINING PLATE FROM A MIXED CULTURE CONTAINING *E. coli* R (Te, S, Su, C, N, Amp) AS RESISTANT DONOR AND A FULLY SENSITIVE STRAIN OF *E. coli* 026 : B6 AS RECIPIENT

Colony	Te	S	Su	N	Ampi	C	Control*	Control†
1	+	+	+	+	+	+	+	-
2	+	+	+	+	-	+	+	-
3	+	+	+	+	-	+	+	-
4	+	+	+	+	-	+	+	-
5	+	+	+	+	-	+	+	-
6	+	+	+	+	+	+	+	-
7	-	+	+	+	+	+	+	-
8	+	+	+	+	-	+	+	-
9	+	+	+	+	+	+	+	-
10	+	+	+	+	-	+	+	-

Te, Oxytetracycline; S, streptomycin; Su, sulphonamide; N, neomycin; Amp, ampicillin; C, chloramphenicol.

\* Viability control.

† Sterility control.

small amounts of reagents are required and a full set of ten plates containing one thousand mixtures can be set up in less than 2 h.

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## RADIOBIOLOGY

### Radiation Dose due to the Cosmic Rays

THE present interest in the effects to populations of low levels of radiation exposure emphasizes the importance of accurate estimates of the dose due to the cosmic radiation. This dose has usually been estimated from measurements of the ionization produced in high pressure ionization chambers and the estimates have varied over a wide range of values. Lowder and Beck<sup>1</sup> have recently made a critical assessment of these measurements.

By far the principal components of the cosmic ray dose at sea-level are due to  $\mu$  mesons and cascade showers of electron-photons. The  $\mu$  meson dose is the larger of these two contributors. To calculate the  $\mu$  meson dose the energy lost by the  $\mu$  meson in the absorbing material is required together with the integrated intensity of the particles. Barnaby<sup>2</sup> and Crispin and Hayman<sup>3</sup> have discussed, with experimental verification, the energy loss theory for  $\mu$  mesons in an organic material. They showed that above about 300 MeV the average loss of energy by collision processes in a plastic phosphor (NE 102) is virtually constant at about 1.7 MeV/g cm<sup>2</sup>. The cosmic ray integrated intensity for  $\mu$  mesons at sea-level (lat. 50° N.) is 1.01 particles/cm<sup>2</sup>/min (ref. 4), of which at least 95 per cent have energies above 300 MeV, and the dose due to  $\mu$  mesons in plastic therefore becomes 15 mrad/year. The error in this figure would be less than  $\pm 1$  mrad/year. The average energy loss for a  $\mu$  meson of a given energy in an absorber is a function of both the ratio  $Z/A$  and density. The composition of the human body<sup>5</sup> is such that the mean  $Z/A$  is 0.550 compared with 0.545 for the plastic. The density of the plastic is 1.03 g/cm<sup>3</sup>, and therefore the tissue dose due to  $\mu$  mesons will not be significantly different from the dose in plastic at sea-level.

The contribution of the electron-photon showers is harder to estimate. If, however, we assume that the rate of energy loss is the same, then, using Rossi's figure for the



flux of the shower component, the total sea-level dose becomes  $21 \pm 2$  mrads/year. This figure is in reasonable agreement with the value of  $26 \pm 1.5$  mrads/year in water obtained by Lillierap<sup>6</sup> using Čerenkov radiation detection techniques.

The cosmic ray  $\mu$  meson flux in the basement of a five-storey London building has been measured using the large liquid scintillation counter described elsewhere<sup>7</sup>. The flux was found to be 0.63 particles/cm<sup>2</sup>/min. Because of the size of the counter (138 cm  $\times$  74 cm in area and 10 cm thickness) the measured figure is a value between the flux of particles traversing the counter in a downwards direction and the total flux extended over all directions. It is the latter that is needed for calculating the  $\mu$  meson dose. The ratio of these fluxes is 3:4. An accurate calculation of the total flux is difficult, but from the measured flux it cannot be greater than 0.84 particles/cm<sup>2</sup>/min. The contribution of the electron-photon shower in these conditions is very small and therefore the total tissue dose due to cosmic radiation is about 12 mrads/year.

The figures at present accepted for the cosmic-ray dose<sup>8</sup> are higher than the values given here. The probable reason for this lies in the different measuring techniques used and it is clear that further measurements using solid detectors are desirable, particularly because of the considerable variations among the various ionization chamber data.

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### Depression of Reticulocytes by $\gamma$ -Globulin after Splenic X-irradiation

It has been suggested that local splenic X-irradiation leads to the release of a substance which inhibits mitosis<sup>1-3</sup>. It has also been found that when blood serum withdrawn from rabbits 3 h after local splenic X-irradiation is injected intramuscularly into normal healthy rabbits, it induces a similar drop in the number of reticulocytes as does an injection of blood plasma withdrawn from isolated spleens which had been irradiated<sup>3</sup>.

To isolate the substance responsible for this drop we fractionated the serum proteins of rabbits 3 h after their spleens had been irradiated with 400 r. (THX-250 X-ray apparatus, 180 kV, 15 m.amp, 0.5 mm copper, 30 cm focus skin distance, 111 r./min).

The serum was fractionated step by step by chromatography on DEAE-cellulose columns<sup>4</sup>. Five peaks were determined in diluted samples (see Fig. 1). The fractions were dialysed, concentrated by pervaporation, their homogeneity checked by paper electrophoresis and, when necessary, rechromatographed. By means of paper, starch-gel and free boundary electrophoresis, fraction I was shown to be homogeneous gamma-globulin.

Three mg of fraction I/kg body weight was injected intramuscularly into rabbits. A similar effect was observed

3-17 h after injection as on the first or second day after serum which contained 100 mg protein/kg was injected (ref. 4, see Table 1). Serum fraction I withdrawn from untreated rabbits had no such effect (see Table 2). Thus it would seem that the factor in the serum responsible for reducing the reticulocyte number after local splenic X-irradiation is associated with gamma-globulin obtained by DEAE-cellulose chromatography of the serum.

Table 1

Serum fraction I	No. of Treated rabbit	Intramuscular injection of fraction I (mg/total)	(mg/kg)	Minimum reticulocyte number as a percentage of pre-injection reticulocyte number
25	161	7.7	3	71*
	162	7.7	3	43*
31	194	8.45	3	31*
	197	6.5	2.9	39
	198	6.5	3.2	40
34	200	8.4	3	60
	208	6.9	3	46
	212	7.3	3	32
	216	7.9	3	30
36	232	8.2	3	38
	234	7.2	3	23
	236	7.2	3	58
41	253	8.1	3	40
	254	7.05	3	42

\* First determination 17 h after irradiation only.

Table 2

Serum fraction I	No. of Treated rabbit	Intramuscular injection of fraction I (mg/total)	(mg/kg)	Minimum reticulocyte number as a percentage of pre-injection reticulocyte number
22	156	10-12	4	93
35	223	6.9	3	71
	229	5.4	3	88
	230	9.0	3	71
38	243	7.9	3	165
	245	6.6	3	73
	246	7.1	3	110

It is possible that the same serum factor is responsible for inhibiting the growth of Jensen sarcoma<sup>5</sup> or fibroblast cultures<sup>7</sup>. It could well be the same factor, as is suggested by refs. 1 and 2, that is found to be released from the spleen following X-irradiation<sup>3,4</sup>.

Investigations are in progress to prove these results by experiments *in vitro*.

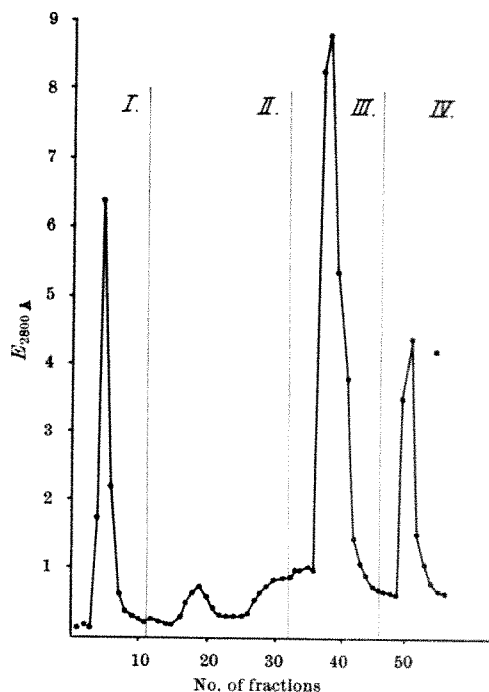


Fig. 1. Step by step chromatography of rabbit serum proteins (No. 36) on DEAE-cellulose. Fractions of 15 ml/6 min were collected at room temperature. Sodium phosphate buffer: I, 0.0175 moles/l., pH 6.3; II, 0.04 moles/l., pH 5.9; III, 0.10 moles/l., pH 5.8; IV, 0.40 moles/l., pH 5.2. Buffer changes as indicated by vertical lines.



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## Reduction of the Absorption and Retention of Strontium in Rats

THE absorption and retention of dietary strontium can be reduced by supplementing the diet with an equimolar mixture of barium and sodium sulphates<sup>1,2</sup>, calcium phosphates<sup>3</sup>, or sodium alginate<sup>4,5</sup>. The possibility of remedial measures to protect a population exposed to a high concentration of strontium-90 in the diet makes these results particularly interesting. Promising results have been obtained from each of the dietary supplementations, and the purpose of the present investigation was to compare the effectiveness of the different treatments in rats of the same strain and age in similar conditions.

Sixty-three female albino rats, 8-9 weeks old and weighing about 130 g, were used. All rats had been fed the standard diet (1.0 g calcium, 0.5 g phosphorus/100 g diet) with water *ad lib*. since weaning. They were divided into seven groups of nine animals each and fed for 8 days on the standard diet or diets supplemented with the additives shown in Table 1. On the sixth day, the rats were placed in separate metabolism cages and given tracer amounts of almost carrier-free strontium-85 and calcium-47 in the drinking water for 2 days before they were killed on the eighth day. Urine was collected from each rat during the last 3 days of the experiment and the gut was removed after death, before drying and ashing the separate carcasses. All assays of radioactivity were made on a single channel gamma scintillation counter<sup>6</sup>.

The results are shown in Table 1. The amounts of strontium-85 in the carcass and in the urine plus carcass are measures of the body retention and the absorption of the radioactive marker, respectively. For calcium-47, however, when dietary calcium is increased by supplementation with calcium and phosphorus, the body retention and absorption of the marker decreases, and the fall in the percentage of calcium-47 in the carcass and in the urine plus carcass which we observed is to be expected.

Table 2 shows the comparative absorption and retention of strontium relative to that in the control animals (no supplementation). It should be noted that the

Table 2. ABSORPTION AND RETENTION OF STRONTIUM-85

Supplement	<sup>85</sup> Sr per cent control	
	Absorbed	Retained
Sulphates	46	46
Phosphate	27	29
Alginate 1	33	37
Alginate 2	25	26
Alginate	19	15
Alginate + phosphate	15	13

sulphate and phosphate supplementations were equimolar (60 mmoles/l.).

The decreased retention and absorption of strontium-85 shown in Table 2 were achieved with only about 30 per cent change in the absorption and retention of calcium. The combined therapy with alginate and calcium phosphate supplementation gave the greatest reduction in the strontium absorbed or retained that we have obtained so far from any dietary supplementation.

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## PATHOLOGY

### Adverse Effects on Offspring of Tranquillizing Drugs during Pregnancy

It has recently been shown that the injection of certain tranquillizing drugs during pregnancy produces loss of weight and defects of maze learning depending on the stage of pregnancy in which the injections are given<sup>1</sup>. The same workers obtained inconclusive evidence of the effect of tranquillizers on conditioned avoidance training. The present study investigates further the possible effect on conditioning of such treatment and attempts to verify the effects on weight.

Twelve groups of female rats were used in a 4 × 3 factorial design. One independent variable, the drug administered, involved four groups—experimental subjects the mothers of which were given reserpine (Res), meprobamate (Mep), chlorpromazine (Chl), or distilled

Table 1. EFFECT OF DIETARY SUPPLEMENTS ON THE MEAN CONTENT OF STRONTIUM-85 AND CALCIUM-47 IN THE CARCASS AND URINE PLUS CARCASS OF 8 WEEK OLD RATS

Group	Total dietary		Additive/ 100 g diet	Percentage of oral dose			
	Ca (g/100 g diet)	P (g/100 g diet)		<sup>85</sup> Sr Carcass	<sup>47</sup> Ca	<sup>85</sup> Sr Urine + carcass	<sup>47</sup> Ca
Control	1.0	0.5	—	11.5 (0.7)†	35.4 (2.5)	15.0 (0.8)	40.5 (2.4)
BaSO <sub>4</sub> + Na <sub>2</sub> SO <sub>4</sub>	1.0	0.5	5.7 g SO <sub>4</sub>	5.3 (0.3)	32.7 (0.7)	6.9 (0.3)	36.0 (0.4)
KH <sub>2</sub> PO <sub>4</sub> + CaCl <sub>2</sub>	2.5	2.4	5.7 g PO <sub>4</sub>	3.3 (0.3)	9.9 (0.8)	4.1 (0.4)	10.3 (0.8)
Na alginate 1 (B.D.H.)*	1.0	0.5	10 g	4.2 (0.2)	27.0 (1.1)	5.0 (0.2)	28.6 (1.0)
Na alginate 2 ('Manucol' SS/LD/2)†	1.0	0.5	10 g	3.0 (0.2)	27.3 (0.9)	3.7 (0.2)	28.6 (1.0)
Na alginate 2	1.0	0.5	20 g	1.7 (0.1)	22.3 (0.9)	2.9 (0.2)	24.1 (0.7)
Na alginate 2 and KH <sub>2</sub> PO <sub>4</sub> + CaCl <sub>2</sub>	2.5	2.4	10 g + 5.7 g PO <sub>4</sub>	1.5 (0.1)	9.6 (0.5)	2.3 (0.2)	10.4 (0.5)

\* British Drug Houses (batch No. 2402290).

† Alginate Industries, Ltd., London.

‡ Figures in parentheses are standard errors of the mean.

water (control) during pregnancy. The three tranquillizing drugs are representative of three different chemical groups, respectively *Rauwolfia* derivatives, substituted propane-diols, and phenothiazine derivatives. The second major independent variable was whether the drug was administered in early, mid- or late pregnancy. The mothers were obtained from the Charles River Breeding Co., Boston, and were of a special Sprague-Dawley stock delivered by Caesarian section. The offspring were delivered normally and a total of 144 female rats selected randomly for the experiment, with twelve in each cell of the factorial design.

The experiment comprised two phases. In the first phase, during four successive days in early, mid- or late pregnancy, each gravid female was given subcutaneous dorsal injections of one of the three drugs or distilled water. Injections were given to each animal three times a day, at 8 a.m., 4 p.m. and 12 p.m. Drugs were freshly prepared every 48 h and administered in the following daily doses: reserpine, 0.1 mg/kg; chlorpromazine, 6.0 mg/kg; and meprobamate, 60.0 mg/kg. The solutions used were so adjusted that animals of equal weight would receive an equal volume of fluid (including animals receiving water only) and were as follows: reserpine, 0.0017 per cent; meprobamate, 1.0 per cent; and chlorpromazine, 0.1 per cent. Animals in "early" pregnancy (I) were injected on days 5-8, those in "mid-" pregnancy (II) on days 11-14, and those in "late" pregnancy (III) on days 17-20. The offspring were weaned at 21 days of age and placed in large community cages until they were 84 days old. In the second phase of the experiment, animals were weighed at age 84 days and conditioned avoidance training was begun. Animals were given twenty trials per day of conditioned avoidance training until a criterion of seven out of eight successive correct avoidance responses was achieved. The rat was placed in one side of a shuttle box (5 in.  $\times$  5 in.  $\times$  10 in.) which was completely dark. The experimenter opened the door, which automatically turned on a light and sounded a buzzer. If the rat did not go into the goal box within 5 sec, a 0.6 m.amp shock was administered for 0.75 sec. The trials for each rat were run consecutively, with an interval between trials of about 10 sec. On the day after the acquisition criterion was reached, extinction training was begun. Each animal was given twenty trials per day of extinction training, which was identical to the acquisition training except that the shock was disconnected. The criterion for extinction was seven out of eight successive failures to give a conditioned avoidance response.

The mean weight at 84 days is shown for each group in Table 1. The analysis of the weight results showed significant effects ( $P < 0.001$ ) of drug administration depending on the period of administration. In group I, the control animals weighed significantly more than Res animals and Chl and Mep animals weighed significantly more than both control and Res animals. No differences were found in group II. In group III, control animals weighed significantly more than Mep animals and Chl animals weighed significantly more than all other subgroups. The analysis of variance also showed a significant overall effect of the drug, the Chl animals weighing significantly more ( $P < 0.001$ ) than all the other groups. All post-analysis of variance tests were done using the Duncan range test<sup>2</sup>.

The score for conditioned avoidance acquisition was the number of trials taken to reach the criterion. These means are shown in Table 2. Analysis of variance indicated a significant effect only in the drug/period interaction ( $P < 0.05$ ), caused by the fact that the Mep II group was significantly slower in acquisition than control II and Chl II groups and that Chl III animals were significantly slower than control III animals. No other differences within periods were significant.

The score for conditioned avoidance extinction was the number of trials taken to reach the criterion. These means are shown in Table 3. Analysis of variance indicates

Table 1. MEAN WEIGHT IN GRAMS AT 84 DAYS

	Stage of pregnancy when drug administered			Mean
	I	II	III	
Control	224	232	229	229
Meprobamate	232	231	215	226
Chlorpromazine	235	231	238	235
Reserpine	214	233	230	226

Table 2. MEAN TRIALS TO ACQUISITION CRITERION

	Stage of pregnancy when drug administered			Mean
	I	II	III	
Control	14.7	12.2	11.0	12.6
Meprobamate	12.3	18.3	11.8	14.2
Chlorpromazine	14.6	11.4	15.7	13.9
Reserpine	11.1	14.5	13.9	13.2

Table 3. MEAN TRIALS TO EXTINCTION CRITERION

	Stage of pregnancy when drug administered			Mean
	I	II	III	
Control	43.5	89.5	113.3	82.0
Meprobamate	55.3	54.4	53.3	54.4
Chlorpromazine	63.1	64.5	58.1	61.9
Reserpine	111.8	75.9	50.5	79.4

a significant effect ( $P < 0.05$ ) of the drug administered, with both the Chl and Mep animals extinguishing more rapidly than the control animals. The drug/period interaction was also significant ( $P < 0.001$ ), comparisons within periods of administration showing that in period I all groups extinguished faster than the Res animals, in period II the Mep animals extinguished faster than control animals, and in period III all drug groups extinguished more rapidly than the control group. The speed of extinction is usually considered to be an inverse function of the strength of the conditioning.

These results show conclusively that the administration of tranquillizing drugs to pregnant rats affects the weight and the acquisition and extinction of a conditioned avoidance response. As would be expected from previous research<sup>1,3</sup>, the effect depends on the drug used and the period of pregnancy in which it is administered. Weight scores, presumably reflecting general health, are adversely affected by administration of reserpine in period I or meprobamate in period III. The rate of acquisition of the conditioned avoidance response is adversely affected by administration of meprobamate in period II or chlorpromazine in period III. The extinction rate is adversely affected by the administration of reserpine in period I, meprobamate in period II, and chlorpromazine, meprobamate or reserpine in period III.

The exact findings of this experiment differ somewhat from previous research<sup>1,4</sup>, as would be expected from the fact that an aversive response was used instead of a mild appetitive response. In all likelihood, performance in avoidance conditioning involves considerable emotional components in addition to learning ability. Also, the strain of rats used for this research was somewhat larger and healthier than in previous work. The general conclusion from all these studies is inescapable, however: the administration of tranquillizing drugs to gravid rats produces adverse health and learning effects in the offspring. All the drugs used had some adverse effect, depending on the period of administration, and none was uniformly deleterious. The implications of the use of such drugs by pregnant women clearly need to be examined.

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### Animal Model of Viral Oncogenesis

ELECTRON microscopy showed a virus in the tissues of a strain of guinea-pigs in which leukaemia arose spontaneously. This strain of guinea-pigs dates back to 1906 when inbreeding began at the Bureau of Animal Industry of the US Department of Agriculture<sup>1</sup>. We are at present using one of these families, now known as strain No. 2, in our research. In 1940, these animals were transferred to the National Cancer Institute where inbreeding of this strain has continued until now. A high degree of genetic homogeneity has resulted from this method of breeding. In 1954, Congdon and Lorenz reported several forms of acute lymphatic leukaemia<sup>2</sup>. Ten transplantable tumours were found, and of these four were carried and three were subsequently lost.

Electron microscopy of animals carrying this transmissible leukaemia has revealed a new virus. The leukaemia is at present being transmitted with cell-free leukaemic material, such as plasma or tissue extracts, prepared by repeated ultracentrifugation at speeds up to 40,000 r.p.m. The infective agent crosses the placental barrier in the leukaemic mother and has been recovered from the gastrointestinal and urinary tracts. Feeding leukaemic spleen cells has also produced the disease. Lines of guinea-pigs other than strain No. 2 are almost entirely resistant; but the disease can be serially propagated, with a 100 per cent mortality rate, in  $F_1$  hybrids originating from cross-mating susceptible strain No. 2 animals with resistant Hartley guinea-pigs.  $F_1$  hybrids, irrespective of age, are now used exclusively. After injection by various routes, the disease has an average incubation period of 18 days and ends in death with blast cell counts in the peripheral blood up to 350,000 cells/mm<sup>3</sup>. Autopsy shows widespread leukaemic involvement of the entire haematopoietic apparatus<sup>3</sup>. The leukaemia is of special interest because the experimental disease resembles the human disease very closely in its haematological and pathological responses. Tissues from animals in the terminal stages of acute leukaemia were examined with light and electron microscopes. Viral particles could readily be demonstrated in plasma pellets, lymphoid tissue and megakaryocytes in all animals examined<sup>4</sup>. Control animals had no particles.

The guinea-pig leukaemia virus is about 85 m $\mu$  in diameter. It resembles the type C particles associated with murine leukaemia but differs in detail. The virus is smaller and the intermediate layer is not as electron dense (Fig. 1). The virus particles can be seen to bud into the cisterna of the endoplasmic reticulum (Fig. 2). The particles were present in high-speed plasma pellets, bone marrow, spleen

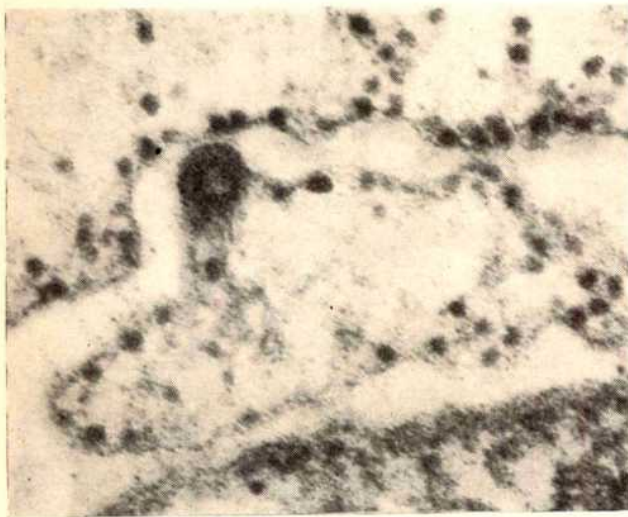


Fig. 1. Section of part of a cell from the bone marrow of a leukaemic guinea-pig. The particle can be seen budding from membranes of the endoplasmic reticulum. ( $\times c. 142,500$ .)

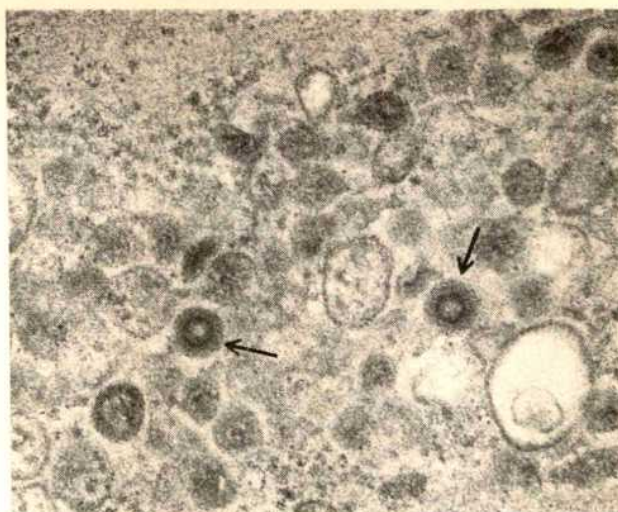


Fig. 2. Particles present in a plasma pellet from a leukaemic guinea-pig. ( $\times c. 86,700$ .)

and lymph node biopsies when the guinea-pigs had peripheral white counts of more than 100,000 cells/mm<sup>3</sup> (ref. 5). Light microscope examination showed that almost all organs had been infiltrated by neoplastic lymphoblasts. This virus is destroyed by exposure to ultra-violet light or X-rays, by heating for 30 min at 56° C, by shaking with ether or acetone and by formalinization. The virus is resistant to trypsin. The infectivity of leukaemic spleen cell suspensions treated with glycerine and calf serum can be preserved by storing them at -90° C for up to 5 months.

Leukaemia in guinea-pigs appears to be limited to this strain and associated with the presence of viral particles. Virus recovered from the blood of guinea-pigs with induced leukaemia, using resuspended pellets obtained by ultracentrifugation, has induced leukaemia when injected into susceptible guinea-pigs. We have found that the virus acts as a potent antigen in homologous host species. It seems that a new animal model system for work in viral oncogenesis has been opened up by the demonstration of a new viral leukaemia in a strain of inbred guinea-pigs.

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### Recognition of the Individuality of Tumour Strain by Sensitized Peritoneal Lymphoid Cells

Odashima<sup>1</sup> and Yoshida<sup>2</sup> have demonstrated the biological individuality of more than fifty different strains of rat ascites hepatomata. Even in strains originating from the same animal, there are marked differences in the pattern of growth as well as differences in such cytological characters as chromosome constitution or sensitivity to anti-tumour agents<sup>3</sup>. Antigenic differences among these strains have not, however, been investigated.

Over the past 2 years, four different strains of ascites hepatoma, AH-64A, AH-64B, AH-64C and AH-64D, and six sub-strains have been established, all of which originated from primary tumour ascites or separate hepa-



Table 1

Hepatoma strain	Transfer generations	Modal No. of chromosomes	Islands in tumour ascites (per cent)	Tumour take (per cent)	Mean survival (days)
AH-64A	90	66	1.0	100	14.0
AH-64B	90	71	83.0	100	14.5
AH-64C	100	81	5.0	100	10.5
AH-64D	90	72	7.0	100	11.5

toma nodules in an individual female Donryu rat fed 0.05 per cent 3'-methyl-4-dimethylaminoazobenzene (3'-Me-DAB) for 5 months. They have been maintained by intraperitoneal passage in isologous Donryu rats that have had their genetical homogeneity checked by intrastrain skin grafting<sup>4</sup>. The biological characteristics of the first four strains, shown in Table 1, are individually distinctive. The Donryu rats are quite susceptible to all these tumour strains and 100 per cent tumour takes were recorded in the twenty most recent transfer generations. AH-64B is the only strain which has many "hepatic islands", that is, small or large clusters of tumour cells.

When the Donryu rats were immunized with tumour cells previously attenuated with nitrogen mustard N-oxide ('Nitromin'), they acquired a marked resistance to further transplantation of the tumour strain used for immunization<sup>5</sup>. The percentage of animals that developed a resistance for AH-64A, AH-64B, AH-64C and AH-64D tumours was 38.7, 82.4, 36.5 and 27.3 respectively. This may have been due to differences in the immunizing capacity of the individual tumour strains. Sensitized peritoneal lymphoid cells from each group of resistant animals were pooled and separated by centrifugation into supernatant and cell fractions. The cell fraction was washed with glucosol solution and adjusted to a suspension of  $10^7$  cells/ml. Lymphoid cells from normal intact rats or from rats sensitized with normal rat liver cells were also prepared to serve as controls. An equal volume of a viable tumour cell suspension containing  $10^5$  cells/ml. of each tumour was then added to the separate fraction, and incubated at 37° C for 15 min. The ratio of the number of lymphoid cells and the number of tumour cells was made constant in every case at 100:1. After incubation, 1 ml. of the mixed cell suspension was inoculated intraperitoneally into five to sixteen intact female rats of 80-100 g body weight. Table 2 shows that the mean survival time, when half the host animals were killed by the progressive accumulation of tumour ascites, was markedly prolonged only when the tumour cells were mixed with lymphoid cells. Moreover, as indicated by the percentage increase in the survival time of the hosts, the differences between the effects of the cell-free and cell fractions were most marked when the tumour cells were exposed to sensitized lymphoid cells obtained from animals resistant to the corresponding tumour strain. The tumour strain-specific reaction of the sensitized lymphoid cells seemed to be relatively mild when AH-64B cells were used. In addition the growth of this strain was inhibited only slightly when the cells were exposed to lymphoid cells sensitized with other tumour strains or normal liver tissue.

Differences in antigenic specificity and immunogenicity between individual tumours produced in a single animal have been reported by a few workers. Globerson and Feldman<sup>6</sup> have shown that each of two sarcomata produced in a mouse by simultaneous applications of benzo-pyrene had distinct antigenic specificity. Rosenau and Morton<sup>7</sup> have pointed out that spleen cells sensitized with each of a pair of sarcomata induced by methylcholanthrene in the same mouse showed an inhibitory effect only on the corresponding tumour, both *in vivo* and *in vitro*. Similar results were obtained by Baldwin<sup>8</sup> on rat liver carcinomata induced by 4-dimethylaminoazobenzene. He found that the sensitized peritoneal lymphoid cells only prevented the growth of the tumour used for immunization when they were inoculated together with the tumour cells into intact animals.

From the present experiment it must be emphasized that four different strains of ascites hepatoma induced by 3'-Me-DAB in the same animal showed marked differences in their antigenic as well as morphological characteristics, and that the peritoneal lymphoid cells sensitized with each tumour strain could react specifically with the corresponding tumour strain used for immunization.

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### Increased Tumour Induction by Adenovirus Type 12 in Thymectomized Mice and Mice treated with Anti-lymphocyte Serum

TUMOURS<sup>1</sup> have been induced in three out of thirteen C3Hf/Gs mice inoculated neonatally with adenovirus type 12; no tumours were seen in inoculated DBA/J or A/J mice. Tumours<sup>2</sup> have been seen in C3H/HeN and BALB mice only after surgical thymectomy, and there is evidence<sup>3,4</sup> that tumours induced by adenovirus type 12 in mice have a specific antigen which elicits in the host a cell-mediated, homograft type immune response. The marked depression of cell-mediated immunity after thymectomy presumably accounts for the increased tumour formation after this procedure<sup>5</sup>; this is observed after neonatal exposure to those viruses that do not induce tolerance (that is, with polyoma, SV40 and adenovirus)<sup>6</sup>.

Experiments with immunosuppressants have provided important information about the role of immune responses in controlling tumour induction and growth<sup>6,7</sup>, and it is

Table 2

Tumour strain used for immunization	Peritoneal fluid	No. of rats	50 per cent survival (days) of the animals transplanted with $10^5$ cells of			
			AH-64A	AH-64B	AH-64C	AH-64D
AH-64A	Cell-free	25	15	21	19	10
	Cells	41	(87)	(43)	(5)	(10)
AH-64B	Cell-free	33	14	13	17	10
	Cells	45	(0)	(>100)	(6)	(0)
AH-64C	Cell-free	26	22	16	15	11
	Cells	40	(23)	(88)	(74)	(18)
AH-64D	Cell-free	20	27	30	26	13
	Cells	35	(6)	(67)	(40)	(23)
Normal liver	Cell-free	20	30	23	17	11
	Cells	25	(0)	(74)	(12)	(0)

Percentage increase in survival time shown in parenthesis.



useful to have at our disposal effective immunosuppressants that work in different ways. The use of antiserum against lymphoid cells (ALS), which has been shown to prolong greatly the survival of skin grafts in adult mice across an H-2 barrier<sup>8-10</sup>, has aroused some interest. We therefore decided to examine the effects of this antiserum on the induction of tumours by viruses. Antisera were prepared in rabbits of the New Zealand strain by two intravenous injections of  $10^9$  thymus cells from *CBA* mice at an interval of 14 days and bleeding 7 days after the second injection. Before use sera were decomplexed by subjecting them to a temperature of  $56^\circ\text{C}$  for 20 min, and they were absorbed with washed *CBA* mouse erythrocytes until there was no further agglutination.

Fifteen newborn *CBA* mice were injected subcutaneously with 0.05 ml. of adenovirus type 12 (strain 1131, isolated and propagated in human embryonic kidney cells, with a titre of  $10^{7.2}$  TCD<sub>50</sub>/ml.). The mice were given 0.05 ml. of ALS on the day after birth and 0.1 ml. of this antiserum on the eighth and fifteenth days. A second group of twelve mice was given the same inoculum of adenovirus on the day of birth and surgically thymectomized 2 days later. A third group of twenty-four controls received virus alone subcutaneously on the day of birth. The cumulative incidence of tumours is plotted in Fig. 1. Most control mice developed tumours between 3 and 8 months after inoculation of virus. Thus the *CBA* strain is more susceptible to adenovirus oncogenesis than other strains so far tested. Some acceleration of tumour formation was observed in mice treated with ALS or thymectomized, but the effect was not marked and does not attain formal statistical significance. This result suggests that the relatively long latent period for tumour induction by adenovirus in mice is caused chiefly by factors other than the immune response of the host.

Another experiment was carried out with a much smaller virus inoculum (approximately 100 times less than the first). Only one of the twenty-four controls developed a tumour, whereas nearly half the twenty-four mice receiving ALS or thirteen thymectomized mice developed tumours (Fig. 2). The differences between these two groups and the controls were very significant ( $P \approx 0.01$ ). Although the tumours began to appear sooner after treatment with ALS than after thymectomy, the differences

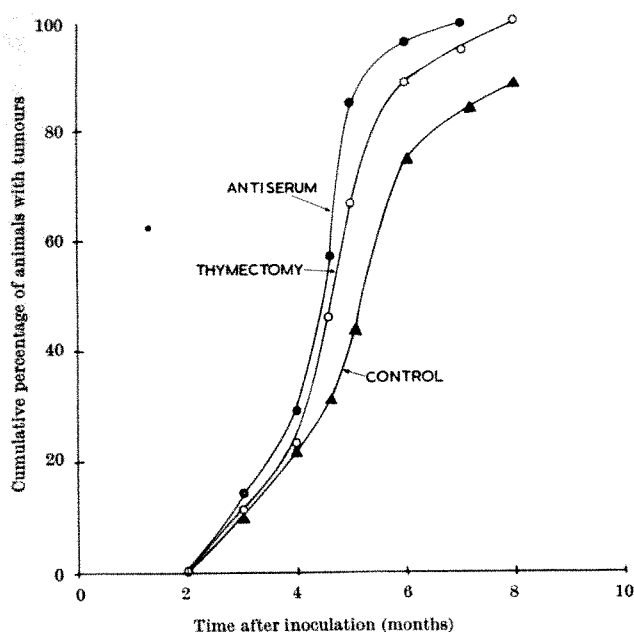


Fig. 1. Appearance of tumours after inoculation of newborn *CBA* mice with a large dose of adenovirus type 12.

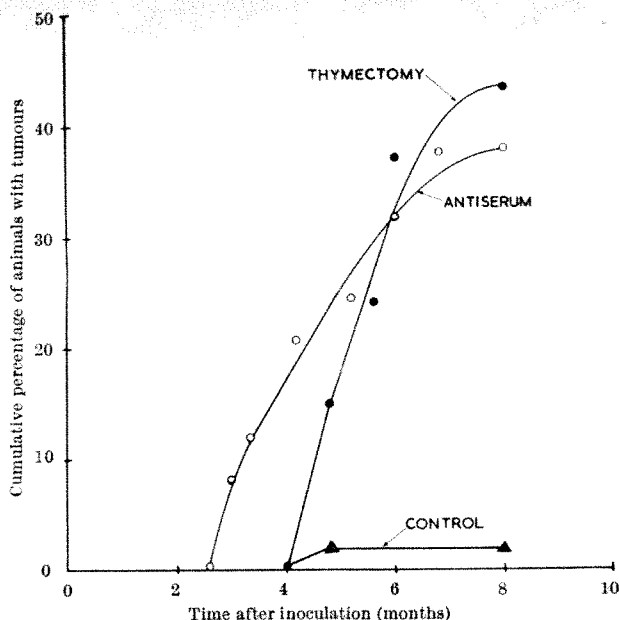


Fig. 2. Appearance of tumours after inoculation of newborn *CBA* mice with a small dose of adenovirus type 12.

between the two treated groups are not statistically significant.

A third experiment was carried out using 0.05 ml. of undiluted adenovirus type 12 in *C3H/He* mice. At the time of writing, 6 months later, nine out of twelve mice treated with ALS have developed tumours whereas no tumours have appeared in controls (Fig. 3). This potentiation is very significant ( $P < 0.001$ ) and even more marked than that seen by Kirschstein *et al.*<sup>2</sup> after thymectomy.

In all experiments tumours appeared subcutaneously, at the sites of inoculation, and had the histological appearances characteristic of tumours induced by adenovirus<sup>1,4</sup>. Some of the *CBA* tumours contained antigen reacting specifically in complement-fixation tests with sera from hamsters bearing tumours induced by adenovirus 12. Sera of tumour-bearing mice contained no haemagglutination-inhibiting antibodies against polyoma virus. In experiments with adenoviruses in mice polyoma virus must be excluded, for the two have a synergistic effect on oncogenesis<sup>11</sup>.

The results show that ALS administered in relatively small doses to newborn mice increases tumour induction by small doses of adenovirus 12 in a susceptible strain of mice (*CBA*) or by a large dose of adenovirus 12 in a relatively resistant strain of mice (*C3H*). These effects are presumably caused by the decreased efficiency of cell-mediated immune responses after administration of ALS. In keeping with this view are recent findings of Jooste (personal communication) that injections of ALS in *CBA* mice on the day of birth and day 11 greatly prolong survival of skin grafted across an H-2 barrier on day 21. It has also been found<sup>12</sup> that delayed hypersensitivity to protein antigens is abolished or depressed by injections of ALS into newborn mice. Injections of rabbit antisera prepared in a different way against rat thymus were reported by Anigstein *et al.*<sup>13</sup> to increase the size of sarcoma 180 implants subsequently made in mice. Although these tumours are not known to contain specific antigens, they could have been antigenic through carriage of viruses or for other reasons. Thus the potentiation of sarcoma growth may have been caused by the immunosuppressant effects of anti-thymus serum rather than by stimulation of tumour growth itself.

Usually the use of ALS has certain advantages over surgical thymectomy. The dosage and timing of the immunosuppressant effect can be better controlled with

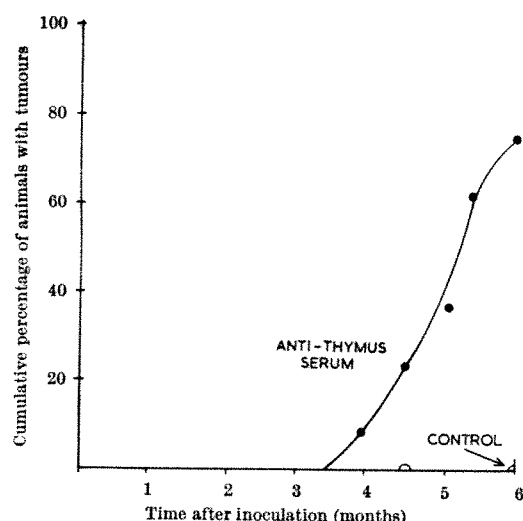


Fig. 3. Appearance of tumours after inoculation of newborn C3H/He mice with a large dose of adenovirus type 12.

antisera, and simple injections are easier than surgery, especially with hamsters and rabbits. Doses of ALS that do not produce runtting are sufficient to potentiate tumour formation. The effectiveness of relatively small doses of antiserum in newborn animals may be the result of induced immunological unresponsiveness of the recipients to the rabbit  $\gamma$ -globulins involved, which allows the latter to exert a prolonged action. Furthermore, when antisera are used, the thymus is left *in situ*, which may be important in certain cases, notably with the leukaemias, in which the thymus is a target organ for oncogenic viruses. Further experiments along these lines have given promising results which will be presented for publication shortly.

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### Iron Resorption in Fish-induced Anaemia in Mink

ANAEMIA is a common condition in mink kits and may cause serious economical losses, for the development of good quality fur depends on a normal haemoglobin concentration in the blood. Anaemia in mink induced by fish leads to achromotrichia, cotton fur, and may be described as a hypochromic microcytic iron deficiency anaemia.

The particular anaemic condition considered in this investigation occurs when the dietary protein for kits consists mainly of raw fish such as whiting (*Gadus merlangus*), coalfish (*Gadus virens*) and hake (*Merluccius vulgaris*). The percentage of kits which develop anaemia, however, varies widely<sup>1-5</sup>.

Development of anaemia and light underfur can be prevented by replacing the raw fish in the diet with boiled fish of the same species. Supplementing the diet of raw fish with vitamins and trace elements in the growing period failed to give any response<sup>3,6</sup>.

There are two fundamental routes through which this form of anaemia may develop: (1) the absorption of iron from the intestine into the intestinal mucosa may be obstructed; (2) the transport of iron from the intestinal mucosa to the iron stores and to the haemoglobin synthesizing tissues may be disturbed or reduced in one or more steps.

We have studied the absorption of iron in mink kits fed raw and boiled coalfish, to discover how and where the disturbances take place. We have used the iron isotope <sup>59</sup>Fe as a tracer. Comparative studies were performed with anaemic and non-anaemic animals.

Iron absorption studies were made on animals which had become anaemic after a diet of raw fish. The animals which were tested with boiled fish were fed this diet 2 days before the administration of iron.

All experiments were carried out with two animals in each series, one fed raw and the other boiled fish. A dose of 6  $\mu$ c. ferrie <sup>59</sup>Fe was administered, as suggested by preliminary studies, together with 1.5 mg inactive ferrie iron as a carrier, all in a volume of 15 ml. The solutions were slightly acidic (HCl) and were administered through a stomach tube. A blood sample from the tail vein was transferred to a counting plate, weighed and air-dried.

The weight of blood samples was kept at approximately 0.5 g, which gave a reasonable counting rate from a thin film of blood. The dry blood was tested with a Geiger-Müller tube, and the activity is given as c.p.m./g blood. To keep the error due to self-absorption at a minimum, the weights of the blood samples were kept as equal as possible, within the limits of 0.48–0.52 g. To see whether there was a good proportionality between sample weight and activity within these limits, a curve was drawn, giving the counting rate as a function of weighings of one active blood sample. A fairly good proportionality was found (Fig. 1) and the activities of the blood samples

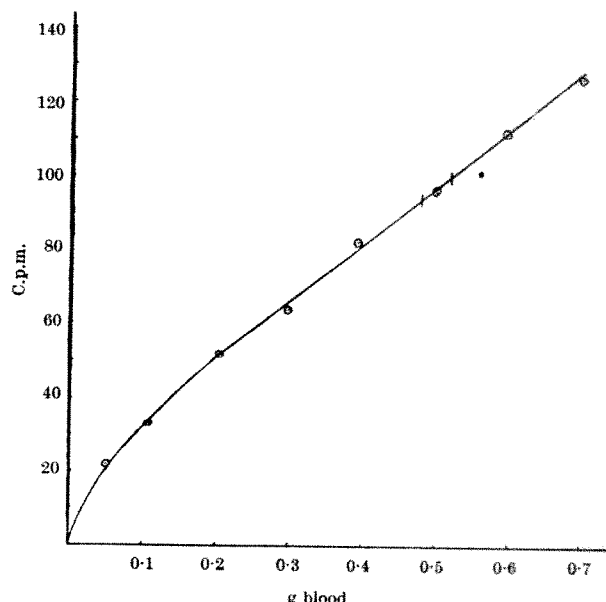


Fig. 1. Activity of different sample sizes of one active blood sample.

Table 1a

	Animal No.	Hb (per cent)	Cell volume (per cent)	Date of tracer application	Dates of collecting and counting rate of blood samples (c.p.m./g)						
					1/4	28/4	2/5	12/5	19/9	21/9	
Boiled fish	183	7.6	28	24/3	298						
	295	6.5	21	24/3	174						
	31	5.6	20	26/4		210	192				
	295a	8.8	27	26/4		180	304				
	122	6.3	26	27/4		331	565				
	106	6.5	22	9/5				210			
	31*	8.1	27	18/9					120	304	
Raw fish	153	7.6	26	24/3	40						
	106	7.8	27	24/3	62						
	124	5.3	22	26/4		88	92				
	123	8.8	29	27/4		86	87				
	45	7.6	27	27/4		38	84				
	376	6.5	19	9/5				99			
	83*	5.3	21	18/9					77	98	

\* For animals Nos. 31 and 83 the tracer was incorporated in the diet.

Table 1b

	Animal No.	Hb (per cent)	Cell volume (per cent)	Date of tracer application	Dates of collecting and counting rate of blood samples (c.p.m./g)						
					28/4	30/4	2/5	20/5	23/5	27/5	
Boiled fish	266	16.2	53	26/4	90	97	95				
	1,002	17.6	56	26/4	28	34	36				
	53	18.7	60	18/5				112	124	123	
	212	18.0	60	18/5				113	153	133	
Raw fish	214	18.5	56	26/4	77	77	80				
	192	16.6	57	26/4	32	33	30				
	231	18.5	57	18/5				164	161	157	
	164	19.2	63	18/5				100	105	111	

could be recalculated to 0.50 g weighings without introducing errors worth mentioning.

The results of these experiments are given in Table 1a. Only a few of the measurements are given in the table, but none of the other readings gave controversial results. The table reveals that in anaemic animals the absorption of iron into the blood stream is higher in animals fed boiled coalfish than in the animals fed raw fish.

Table 1b shows the results from corresponding experiments with non-anaemic, normal animals. In this case the particular differences with respect to iron absorption are not observed.

The experiments seem to indicate an impediment to iron absorption. It is still possible, however, that iron may pass into the intestinal mucosa and be stored there as ferritin but that the transport of iron from ferritin to the blood is obstructed by one or more substances simultaneously absorbed from the fish. Martinssons<sup>6</sup> studied the iron absorption in anaemic animals previously fed raw fish and found that they readily absorbed iron when dosed on an empty stomach.

In order to study the absorption into the intestinal mucosa, four of the anaemic experimental animals were killed, and 1 cm long pieces were cut from their intestines at 10 cm intervals. These tube-shaped pieces were split, and the inner intestinal walls washed with water, care being taken not to destroy the mucosa. The different pieces were thereafter air-dried, weighed and tested with a Geiger-Müller tube. The results are set out in Table 2, calculated as c.p.m./g dry tissue. The activities of some organs are also given, calculated on the same basis. With respect to self-absorption, the same precautions as for blood were taken. These results also reveal a pronounced difference in iron absorption between anaemic animals fed boiled fish and those fed raw fish. The sections close to the stomach show the highest activities.

From the measured activities in the intestines, it is reasonable to conclude that part of the iron, under the

influence of one or more factors in the raw fish, is not absorbed but remains bound in the fish material.

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### Tyrosinase Activity in the Amelanotic Melanoma in Golden Hamsters

It is known that active tyrosinase (*o*-diphenol : O<sub>2</sub> oxidoreductase) occurs in melanotic melanoma; however, its presence in the amelanotic variety of these tumours is still controversial. Tyrosinase activity in pigmented melanomata was detected and investigated by means of the Warburg manometrical method using tyrosine and dihydroxyphenylalanine (DOPA) as substrates for the enzyme<sup>1,2</sup>. To the best of our knowledge, however, when this method has previously been used to investigate amelanotic melanomata, it has failed to detect any tyrosinase activity<sup>3,4</sup>. Other methods used for studying these tumours have given disparate results<sup>5-10</sup>. For this reason we decided to use the manometrical method of Warburg to investigate amelanotic melanomata in hamsters, which have previously been described, on the basis of histochemical investigations, as "tyrosine-positive".

This tumour occurred in 1962 as a result of a spontaneous transformation of a transplantable melanotic melanoma and has since been maintained by serial passages. The properties of both related tumour lines have been described elsewhere<sup>8,11</sup>.

The transplants of the amelanotic melanoma used in the present investigations belonged to the passages consecutively numbered 25 to 29. Thirty tumours were used for assays. One month after subcutaneous implantation, transplants which measured 3-5 cm in diameter were removed and their tissue was separated from necrotic masses. The tissue was frozen and kept at -15° C for use within the next 2 weeks (we have observed that after this period a gradual decrease in activity occurs). Immediately before measurements were made, the tissues of two or three tumours were joined together and mixed with 0.1 molar phosphate buffer, pH 6.8 (10 g of frozen tissue/100 ml. of buffer). The mixture was then homogenized at 0° C and 14,000 r.p.m. Tyrosinase activity was determined in the homogenate thus prepared as well as in the sediment and the supernatant obtained from it by centrifugation at 18,000g for 10 min. Before any measurements were made, the sediment was washed with buffer, centrifuged once more and resuspended in a quarter of the volume of buffer originally used.

Table 2

	Animal No.	Date of tracer application	Date of death	Vena portae blood	Liver	Kidney	Spleen	Counting rate (c.p.m./g)						Ash of intestine	Femoral marrow
								Intestine, close to stomach	Intestine, 20 cm from stomach	Intestine, 40 cm from stomach	Intestine, 60 cm from stomach	Intestine, 80 cm from stomach	Close to rectum		
Boiled fish	122	27/4	3/5	503	200	134	506	58	61	47	55	80	49	20	182
	106	9/5	12/5	—	60	69	197	85	40	26	9	8	13	8	—
Raw fish	123	27/4	3/5	74	24	32	51	34	28	0	0	9	0	3	95
	376	9/5	12/5	—	35	43	311	23	25	0	0	0	17	7	—

The substrates used were *dl*-DOPA and *l*-tyrosine. The inhibitors were sodium diethyldithiocarbamate (DIECA) and hydroxylamine. The uptake of oxygen was measured in a Warburg apparatus according to the method devised by Lerner *et al.*<sup>12</sup> (37° C, pH 6.8). 1.3 ml. of a preparation from the neoplastic tissue and 1.0 ml. of 0.1 molar phosphate buffer were added to the main compartment of the Warburg flask. 0.2 ml. of a 10 per cent solution of potassium hydroxide was then added to the central well. 0.5 ml. of the solution of one of the substrates (DOPA at a concentration of 6 mg/0.5 ml. of water, or tyrosine at a concentration of 0.45 mg/0.5 ml. of water plus trace amounts of DOPA) was placed in the sidearm. If the inhibitors were used, 0.3 ml. of them was added to the main compartment in place of an equal volume of buffer (the concentration of DIECA was 10<sup>-3</sup> moles/l. and that of hydroxylamine was 10<sup>-2</sup> moles/l.). The uptake of oxygen was read during the first hour of measurement and the results were expressed as  $Q_{O_2} = O_2$  uptake in  $\mu$ l./h/mg of protein. The content of protein was determined by the Kjeldahl method.

For comparative purposes we assayed, under the same conditions, homogenates of the melanotic melanoma as positive controls and homogenates of liver and kidney from normal hamsters as negative controls.

The results given in Table 1 were obtained after corrections had been made for the endogenous respiration. Each value represents a mean of three measurements performed for the same preparation.

Table 1. COMPARISON OF TYROSINASE ACTIVITY AGAINST DOPA AND TYROSINE OF PREPARATIONS OF THE AMELANOTIC MELANOMA, MELANOTIC MELANOMA, LIVER AND KIDNEYS

Material and preparation	Substrate			Substrate		
	$Q_{O_2}$	DOPA Inhibition by hydroxylamine (per cent)	DOPA Inhibition by DIECA (per cent)	$Q_{O_2}$	Tyrosine Inhibition by hydroxylamine (per cent)	Tyrosine Inhibition by DIECA (per cent)
Amelanotic melanoma, homogenate	3.5	54.5	100.0	1.4	35.0	100.0
Amelanotic melanoma, sediment	3.0	50.0	100.0	1.7	29.0	100.0
Amelanotic melanoma, supernatant	7.8	49.0	100.0	4.4	77.0	100.0
Melanotic melanoma, homogenate	28.0	36.0	98.0	20.0	88.0	100.0
Liver, homogenate	2.1	74.0	75.0	—	—	—
Kidneys, homogenate	2.2	66.0	71.5	—	—	—

It can be seen from Table 1 that the preparations from the amelanotic melanoma oxidize tyrosine and DOPA and that this is completely inhibited by DIECA. These findings demonstrate that in the amelanotic melanoma studied tyrosinase activity is present in an amount detectable by the manometric method. The level of activity, however, is significantly lower than that in the melanotic tumour. The partial inhibition of oxidation by hydroxylamine and the activity against DOPA observed in the homogenates of the liver and kidneys indicate that non-specific oxidases also contribute to the uptake of oxygen. During the determinations of oxidase activity against tyrosine in the homogenates of the liver and kidney the gas liberated as a result of side-reactions exceeded the eventual oxygen uptake and rendered the calculation of  $Q_{O_2}$  impossible. Tissue preparations of the amelanotic melanoma obtained by homogenization at less than 13,000 r.p.m. did not reveal any tyrosinase activity.

In the course of our measurements, we observed a gradual darkening of the reaction mixtures containing preparations of the amelanotic melanoma and tyrosine or DOPA. The homogenates of the liver and kidneys developed no pigmentation in the presence of tyrosine and slight pigmentation only when mixed with DOPA. The homogenates of the melanotic tumours were always black in colour.

The next experiments suggested that the tyrosinase activity in the material from the amelanotic melanoma may be increased by incubation with trypsin according to the method used by Trojanowski and Szarkowski<sup>13</sup>. The homogenates of the tumour tissue were centrifuged at 18,000g and the sediments were resuspended in 0.05 molar phosphate buffer (pH 7.8). The suspensions were incubated for 18 h at room temperature with trypsin at a concentration of 0.25 per cent and in the presence of antibiotics (2,000 units of penicillin and 2,000 $\gamma$  of streptomycin). After incubation the activity, as measured against tyrosine and at pH 6.8, appeared to have increased in comparison with the control samples by 50 per cent for the whole suspension and by 30 per cent for the supernatant obtained after centrifugation of the suspension. The level of tyrosinase activity in the amelanotic melanoma was low compared with that in melanotic tumour and it seems likely that this is due in part to the presence of a specific inhibitor which can be removed by the digestion with trypsin. Trypsin, however, does not restore the full tyrosine activity in the tissue examined. This suggests that other factors such as the repression of the synthesis of the enzyme protein or other inhibitors may play a part in the loss of the ability of amelanotic melanomata to produce melanin.

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### Disturbed Proliferation of Erythropoietic Cells in Pernicious Anaemia

THERE is considerable evidence that in many types of mammalian cells DNA synthesis (*S*) occupies only a part of the intermitotic time. In diploid cells the *S* period is preceded by an interval of time (*G*<sub>1</sub>) when the nucleus has a 2c DNA content and is followed by a pre-mitotic interval (*G*<sub>2</sub>) when the nucleus has a 4c DNA content<sup>1,2</sup>. Conventional autoradiography of a cell population labelled briefly with tritiated thymidine can show the cells in *S*, but it is not possible to be certain whether an unlabelled cell is in *G*<sub>1</sub> or *G*<sub>2</sub>. This difficulty can be overcome when autoradiography is combined with quantitative cytochemical measurement of the DNA content<sup>3</sup>. Furthermore, this technique can be used to examine cells the morphology of which has been identified using classical staining techniques<sup>4</sup>. We have used this technique in the investigation of the relation of the morphology of



erythropoietic cells and their position in interphase. The distribution in normal bone marrow has been compared with that in severe pernicious anaemia to locate the site in the maturation sequence at which ineffective erythropoiesis becomes predominant.

Freshly aspirated bone marrow was labelled *in vitro* with 1–3  $\mu$ c. of tritiated thymidine/ml. for 30 min at 37° C. The bone marrow fragments were smeared and stained by the May–Grünwald–Giemsa method and photographic maps prepared. The position and type of the erythropoietic cells were recorded. Erythroid cells were classified morphologically into pronormoblasts, basophilic normoblasts and polychromatic normoblasts according to nuclear size and structure and the tinctorial characteristics of the cytoplasm. Polychromatic cells have been sub-divided into early cells capable of division and late non-dividing cells. The stain was leached out and the preparation was re-stained by the Feulgen method. The absorbance of the Feulgen stained nuclei was measured with a Deeley pattern microdensitometer<sup>5</sup> and the cells in *S* were identified by autoradiography.

In normal erythropoiesis both pronormoblasts and basophilic normoblasts were observed in all stages of interphase. Thus we cannot support Lajtha and Oliver<sup>6</sup>, who suggested that the basophilic normoblast is the 2c and the pronormoblast is the 4c stage of one cell cycle.

On examination the data showed that the percentage of pronormoblasts in *G*<sub>1</sub>, *S* and *G*<sub>2</sub> was similar to the percentage of basophilic normoblasts in these compartments of interphase. The results for pro-erythroblasts and basophilic erythroblasts have therefore been grouped together. The relative distribution of erythropoietic cells in the different phases of the cell cycles in normal bone marrows is shown in Table 1. Among 638 nuclei of basophilic cells, only one unlabelled cell was observed with a DNA content clearly outside the normal range for the spread about the 2c and 4c modes.

Table 1. PERCENTAGE DISTRIBUTION OF ERYTHROPOIETIC CELLS IN THE VARIOUS PHASES OF INTERPHASE

Pronormoblasts and basophilic normoblasts					Early polychromatic normoblasts				
Percentages				<i>S/G</i> <sub>2</sub>	No. of nuclei assessed	Percentages			No. of nuclei assessed
<i>G</i> <sub>1</sub>	<i>S</i>	<i>G</i> <sub>2</sub>	<i>U</i>			<i>G</i> <sub>1</sub>	<i>S</i>	<i>G</i> <sub>2</sub>	
34	60	6	<1	10.0	638	9	82	9	490

Combined results for three normal marrows. Column *U* represents unlabelled cells with DNA contents lying between the 2c and 4c values.

The point of change in the morphology of a polychromatic cell that indicates that it can no longer undergo further division cannot be defined precisely at present. The point of cut-off between the dividing early polychromatic cells and the non-dividing late polychromatic cells was therefore based on an arbitrarily selected nuclear size. This method showed that cells classified as late polychromatic cells had a 2c DNA content. It must be emphasized that this point of cut-off between the dividing and non-dividing compartments was quite arbitrary and that small changes in the criteria defining the cut-off point would significantly alter the percentages of early polychromatic cells in *S* and *G*<sub>2</sub>. On the other hand, there is no doubt that those polychromatic cells that were in *S* or *G*<sub>2</sub> are part of a dividing population. The ratio *S/G*<sub>2</sub> is therefore a more reliable parameter of the dividing polychromatic compartment, because it is unaffected by any uncertainty in morphological classification.

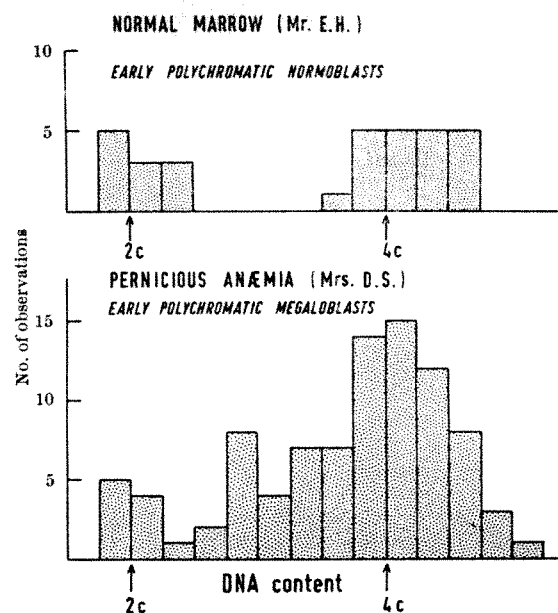


Fig. 1. The distribution of Feulgen absorption values for cells not labelled with tritiated thymidine.

In untreated pernicious anaemia there was evidence of a departure from the normal cell kinetics. The pattern of the abnormality remained constant although its severity varied from patient to patient. Table 2 shows the relative distribution of cells in different phases of the cell cycles in five cases of pernicious anaemia. The significant feature emerges that there was a decreased *S/G*<sub>2</sub> ratio in the early polychromatic megaloblasts caused by a relative increase in the number of cells in *G*<sub>2</sub> and there was an increased proportion of unlabelled cells with DNA contents that were between the 2c and 4c modes. These changes were less pronounced in the promegaloblasts and basophilic megaloblasts. Fig. 1 shows a histogram of the distribution of the DNA contents of unlabelled early polychromatic cells in a marrow from a normal subject and in a patient with pernicious anaemia. This increase of cells in *G*<sub>2</sub> in pernicious anaemia has been previously reported by Menzies *et al.*<sup>7</sup>, who did not identify the cell types concerned and did not focus their attention on the relation between the perturbation of the cell kinetics and the cell maturation.

Our findings taken independently suggest several interpretations, but complementary information is available from an investigation of erythropoietic cell kinetics in patients with pernicious anaemia, after *in vivo* labelling with tritiated thymidine. Cronkite *et al.*<sup>8</sup> have shown that the number of non-dividing polychromatic cells (*E*<sub>5</sub> cells) produced each hour/1,000 proliferating erythropoietic precursors was significantly lower than normal in two cases of pernicious anaemia. Flidner *et al.* (personal communication) have demonstrated a disappearance of erythropoietic cells which was maximal in the dividing polychromatic cell compartment (*E*<sub>4</sub>). It seems reasonable to assume that the loss of cells detected by these

Table 2. PERCENTAGE DISTRIBUTION OF ERYTHROPOIETIC CELLS IN THE VARIOUS PHASES OF INTERPHASE  
Promegaloblasts and basophilic megaloblasts      Early polychromatic megaloblasts

Case	Clinical diagnosis	Hb g/100 ml.	<i>G</i> <sub>1</sub>	<i>S</i>	<i>G</i> <sub>2</sub>	<i>U</i>	<i>S/G</i> <sub>2</sub>	No. of nuclei assessed	<i>G</i> <sub>1</sub>	<i>S</i>	<i>G</i> <sub>2</sub>	<i>U</i>	<i>S/G</i> <sub>2</sub>	No. of nuclei assessed
C. B.	PA	3.6	10	67	17	6	3.9	157	13	59	16	12	3.7	127
D. S.	PA	4.5	9	77	12	2	6.4	139	7	34	46	12	0.7	138
M. R.	PA	6.1	19	69	6	6	11.5	180	38	28	11	22	2.5	114
H. K.	Partial gastrectomy, low B <sub>12</sub>	7.7	13	76	5	6	15.2	126	17	44	11	27	4.0	81
H. P.	PA	8.5	25	32	19	24	1.7	243	20	21	39	20	0.5	148

Column *U* represents unlabelled cells with DNA contents lying between the 2c and 4c values. PA, Pernicious anaemia.

*in vivo* investigations is directly related to the failure of DNA synthesis and the build-up of cells in  $G_2$  shown in Table 2. It would appear, therefore, that the death of these arrested cells is responsible for the ineffective erythropoiesis seen in megaloblastic anaemia caused by vitamin  $B_{12}$  deficiency<sup>9,10</sup>.

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## Inhibition of Fatal Anaphylaxis in Mice inoculated with a Virus-induced Leukaemic Tumour Extract

THE salutary effect of intensive parenteral treatment with erythropoietin-containing rabbit serum in mice infected at 2 days of age with a leukaemia virus has been reported<sup>1</sup>. During the investigation ten of twenty-two mice that developed signs of neoplastic disease as determined by palpation of spleen and lymph nodes later showed evidence of regression of these phenomena. To exclude the possibility that the splenomegaly and lymphadenopathy were a hyperplastic response to repeated inoculation of a foreign antigen, the effect of rabbit serum on uninfected mice was tested using the same regimen as that used for infected mice. Splenomegaly and lymphadenopathy were minimal and transient in these circumstances, but a proportion of the uninfected mice died from anaphylactic shock during the course of treatment whereas infected mice did not. The present communication is concerned with this observation.

Inbred *BALB/c* mice were randomly bred. The  $F_1$  progeny were inoculated when 2 days old (except where noted) subcutaneously and intraperitoneally, 0.05 ml. at each site, with 10 per cent cell-free extracts of neoplastic spleens either directly virus-induced or after 110 transplant generations. The virus-induced disease was obtained in 1958 in *BALB/c* mice inoculated when newborn with a cell-free extract of the Ehrlich ascites tumour<sup>2</sup>. At that time it was considered to be primarily a reticulum cell sarcoma; cases of lymphosarcoma, however, also occasionally appeared. The average latency of the disease was eventually reduced from about 1 yr to 3 months, and lymphosarcomata were no longer seen. A continuing investigation of its cytopathology is in progress<sup>3,4</sup>. As a result of these investigations it appears more appropriate now to refer to the leukaemia as a myeloproliferative syndrome, exhibiting either (or both) abnormal myeloid or erythroid differentiation.

The extracts were prepared from spleens which had been stored in a carbon dioxide freezer as follows: after thawing, the tissue was suspended in phosphate buffered saline and homogenized for 2.5 min at the fastest speed of the 'Virtis 45' (45,000 r.p.m.) apparatus in a vessel surrounded by ice; the supernatant obtained after centrifugation at 800g for 10 min was stored in the carbon dioxide freezer until use. Normal or erythropoietin-containing rabbit serum<sup>1</sup> was used for both sensitization (beginning at 5–6 weeks of age) and shock; weekly doses of 0.5 ml. were administered through the subcutaneous route. Most instances of fatal anaphylaxis occurred after the third weekly dose; a few occurred after each of the next two doses, but none at an earlier time. Only mice that died of classical anaphylactic shock<sup>5</sup>, which generally occurred within 1 h after administration of the serum, were taken into account in the data to be presented.

There was no difference in allergic response to the normal or erythropoietin-containing rabbit serum, and so these data were combined and are presented in Table 1. It is apparent that a proportion of female mice which received a single dose of leukaemic spleen extract at 2 days of age were protected from fatal anaphylaxis. These mice were not detectably leukaemic at this time. The difference in incidence of fatal anaphylaxis, from the point of view of sex, in mice which have not been inoculated with leukaemic spleen extract was notable.

The response of females to inoculation of spleen extract containing both *Eperythrozoon coccoides*, a splenomegalogenic agent<sup>6</sup>, and the lactic dehydrogenase-elevating (LDH) virus indicated that the leukaemic spleen extract was specifically responsible for inducing resistance to fatal anaphylaxis.

The leukaemic agent consistently induces neoplasia (in an average of 75 per cent of infected mice) in about 100 days if the mice are infected within a few days of birth. The incidence of the disease is rapidly reduced if mice are infected at a later age<sup>2</sup>. It was interesting to determine whether the capability of a virus-containing extract to induce anaphylactic tolerance was correlated with its capability to induce neoplasia. The results are given in Table 2. The data suggest such a relationship, because the incidence of fatal anaphylactic shock increased when mice were infected at later ages.

Anaphylaxis is a complex phenomenon, consisting of an immunological phase followed by a series of events which may terminate in death, the end point used in these experiments. Whether the leukaemic extracts affect the initial immunological response or a later stage remains to be determined. An increasing body of evidence indicates that infection of mice with several different leukaemogenic viruses remarkably depresses production

Table 1. RESISTANCE OF MICE INOCULATED WITH LEUKAEMIC SPLEEN EXTRACT TO FATAL ANAPHYLACTIC SHOCK

Preliminary treatment of mice*	Sex	No. died/ No. sensitized†	Per cent
None	Male	0/41	0
None	Female	16/57	28
Virus-induced leukaemic spleen extract	Male	0/59	0
	Female	0/117	0
Series I transplanted leukaemic spleen extract‡	Female	2/64	3
Spleen extract containing <i>E. coccoides</i> and LDH virus	Female	7/30	23

\* In all examples the treatment consisted of a single inoculation of a 10 per cent extract, 0.05 ml. given subcutaneously and intraperitoneally at 2 days old.

† With rabbit serum beginning at weaning. See text for regimen used.

‡ A transplantable tumour originally virus-induced<sup>2</sup> and now in its 110th transplant generation.

Table 2. INDUCTION OF ANAPHYLACTIC TOLERANCE AS A FUNCTION OF AGE OF INOCULATION WITH A LEUKAEMIC EXTRACT

Age of mice at inoculation* (days)	No. died/ No. sensitized†	Per cent
2	0/15	0
21	3/28	11
42	7/25	28

\* Females were inoculated with a virus-induced leukaemic spleen extract.

† The mice were sensitized 5–6 weeks after infection in all cases.

of antibody to exogenous antigens administered subsequent to infection<sup>7-11</sup>. It is possible, therefore, that the induction of anaphylactic tolerance is the result of an abnormality at the immunological level rather than at a later stage in the anaphylactic reaction.

If the leukaemia virus was responsible for the induction of anaphylactic tolerance, it was surprising that the extract from the transplantable tumour was also effective (Table 1). This particular tumour arose from a virus-induced case in 1962 (ref. 2) and is now in its 110th transplant generation. Attempts to demonstrate the presence of the leukaemia-inducing virus in this type of material by ordinary means as early as in the third and fifth transplant generations have failed. In only one case was virus demonstrated: before the transplantation of the nineteenth generation, recipient mice were treated for several weeks with cortisone. An extract of a resulting neoplastic spleen induced two cases of leukaemia with a very long latency. It can be concluded, therefore, that virus is still present in this transplantable leukaemia. Its considerable activity in inducing anaphylactic tolerance suggests that the tolerance-inducing agent in the transplantable tumour could be an attenuated (non-oncogenic) form of the leukaemic agent. Such a possibility was also considered in the case of a culture of the Friend agent which was also extremely low in oncogenicity but active in inducing resistance to infection of mice with potent Friend virus<sup>12</sup>. It is possible that inhibition of fatal anaphylaxis or depression of antibody to exogenous antigens may be found useful to detect potential leukaemogenic viruses whether or not they are actually oncogenic in the test host.

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## CYTOLOGY

### DNA Content of Monotremes

INTEREST has recently been focused on the DNA content of the different classes of vertebrates. Although the number and size of chromosomes in placental mammals vary considerably, biochemical determinations have shown that the DNA content is relatively constant<sup>1</sup>. Atkin *et al.*<sup>2</sup>, using the microdensitometer method, found that the placental mammals constitute a fairly uniform group with regard to the total genetic content, as do birds

which have a DNA content about half (44-59 per cent) that of eutherians. Reptiles, however, fall into two distinct categories<sup>3</sup>: the order *Squamata* has a DNA value 60-67 per cent that of placental mammals, while *Crocodylia* and *Chelonina* have 80-89 per cent. These percentages agree with those obtained by Ohno<sup>3</sup>, who measured chromosomal areas of colchicized metaphases.

The difference between the DNA content of the placental mammals and birds suggests that these two classes of warm-blooded vertebrates have evolved from different lineages. It would be interesting to know where the other two groups of mammals, the marsupials and monotremes, belong. No information is available for the monotremes, but Ohno<sup>4</sup> found by estimation of the chromosomal area that the American opossum (*Didelphis virginiana*) has a value of 94 per cent of that of placental mammals.

Using the biochemical method of Schmidt and Thannhauser<sup>5</sup>, we measured the DNA content of two monotremes, the platypus (*Ornithorhynchus anatinus*) and the echidna or spiny ant-eater (*Tachyglossus aculeatus*); the marsupial, potoroo (*Potorous tridactylus*); and the rat (*Rattus norvegicus*) as a representative of placental mammals (Table 1). The values agree well with an assay of the DNA content by the method of Schneider<sup>6</sup> which we used for comparison.

Table 1. DNA CONTENT EXPRESSED IN MG/100 G OF FRESH HEART TISSUE

	$\bar{x} \pm S\bar{x}$	Relative content (per cent)
♂ Rat	29.9 ± 0.24	100
♂ Potoroo	24.2 ± 0.20	81.0
♂ Echidna	27.9 ± 0.18	93.3
♀ Platypus	29.5 ± 0.27	98.6

The higher DNA value for the female platypus compared with the male echidna probably results from the presence of the extra sex chromosome. Each value given in Table 1 is the mean of four separate estimations, using a Unicam spectrophotometer for the ultra-violet readings and the same standards and chemicals throughout the experiments. Heart tissue was adjudged the most suitable for the purpose because the heart is one of the organs least likely to be affected by variations in the condition of the animal, and the ratio of the nucleus to the cytoplasm remains fairly constant even for animals in poor condition; moreover, heart tissue does not present the problem of polyploidy as does the liver. In the case of the rat, two hearts were used for each determination in order to minimize the difference in weight. The heart tissue was kept at -10° to -15° C for 24 h, which facilitated the subsequent homogenization.

The results show that the DNA content of the potoroo is about 10 per cent lower than that of the American opossum, apparently the only other marsupial which has been examined<sup>4</sup>. The difference may be associated with the fact that the potoroo has only about half the number of chromosomes (12 in female; 13 in male) of the opossum (2n=22), and one may compare it with the 10 per cent difference in DNA value between the creeping vole (*Microtus oregoni*, 2n=17-18) and other placental mammals<sup>2</sup>. Atkin *et al.* have suggested that the low number of chromosomes in the vole is associated with the loss of a number of centromeres with their adjacent heterochromatic chromosomal material<sup>2</sup>. In the case of the potoroo, Sharman and Barber<sup>7</sup> have shown that autosomal material has been incorporated in the sex system of the animal.

The monotremes have been compared with the reptiles because of certain anatomical and physiological similarities in these two groups of vertebrates<sup>8,9</sup>. The chromosome complements of the monotremes have been described as resembling those of snakes and birds because of the presence of macro-elements and micro-elements<sup>10</sup>. Our work on the chromosome numbers of the monotremes<sup>11,12</sup>, however, has shown that their karyotype can be regarded as an extreme mammalian type rather than having close affinity with those of *Aves* and *Ophidia*.

In a preliminary survey of the genetic content of the three groups of mammals using the technique of Ohno<sup>3</sup>, we have found, by comparing chromosomal areas of leucocytes in tissue culture, a value of 92.1 per cent for the echidna compared with that of man. This is in good agreement with our biochemical estimations of the DNA content which gave a value of 93.3 per cent for the echidna compared with that of the rat.

Thus in this respect also the monotremes seem to show a closer affinity to other mammals than to birds or reptiles.

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### Mitogenic Effect of Phytohaemagglutinin at Different Ages

WHILE investigating the effect of myelotoxic drugs on the mitotic potential of phytohaemagglutinin-stimulated lymphocytes from peripheral blood, we found that the mitotic index (number of mitoses/1,000 cells) and percentage blast transformation was frequently lower in elderly individuals than in younger subjects. This report presents the results of standardized investigations of cell cultures, from 293 apparently healthy individuals ranging in age from 1 to 98 years after stimulation with phytohaemagglutinin. To avoid circadian influences all work was carried out at the same time of day, beginning with venipuncture at 9.00 a.m. To test the reproducibility of the method, duplicate cultures were made from the same individuals at the same time. The mean difference in mitotic index in duplicate cultures from ten subjects was  $0.9 \pm 4.4$  (standard deviation). The mean difference in blast transformation of duplicate cultures was  $5.1 \pm 5.5$  per cent. In seven subjects, ages 21–30 yr, cultures were repeated eight to thirty-five separate times during several weeks. The coefficient of variation ( $S.D./X$ ) for mitotic index (Fig. 1) in any given subject ranged from 25 per cent to 40 per cent. The value of mean  $\pm 1$  S.D. for mitotic index was the same for each individual so tested in this age group.

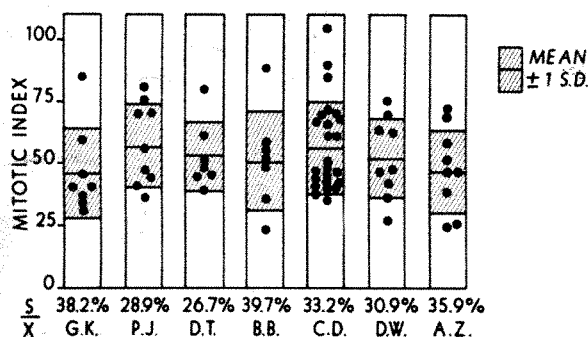


Fig. 1. Variations in mitotic index.

Fig. 2 shows the mitotic index of cultures stimulated with phytohaemagglutinin as a function of age of the cell donor. Analysis of these data yielded a linear regression coefficient ( $r$ ) of  $-0.330$ . With a sample of 289 and a value of  $r$  greater than 118, a significant correlation was found at the 95 per cent level of confidence. The values obtained for children of less than 12 yr old deviated somewhat from the regression curve. Prepubertal children showed a mitotic index considerably smaller than that which developed at adolescence, so that a pubertal peak in the mitotic index was observed. Regression analysis for the mitotic index versus age from age 13 on revealed a value for  $r$  of  $-0.427$ , which confirms an increase in slope when the first decade is eliminated. This correlation coefficient was also significant at the 95 per cent level. The critical value for  $r$  was 0.126 with a sample size of 254.

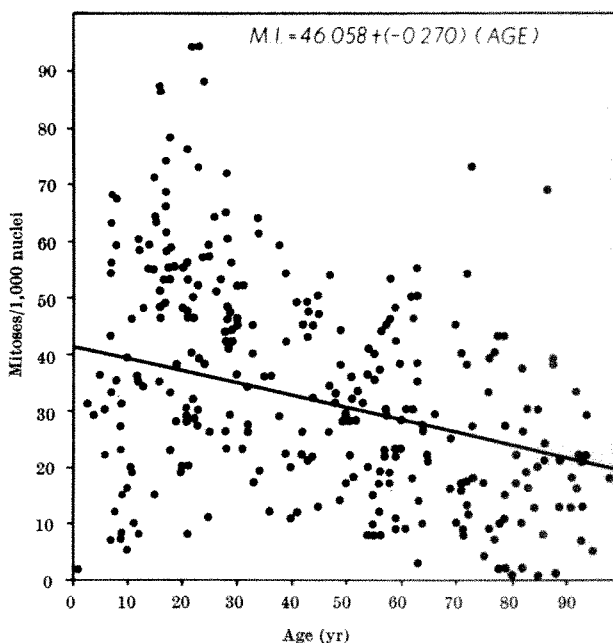


Fig. 2. Mitotic index versus age.

Blast transformation (mitoses + blasts) also varied universally with age (Fig. 3). The regression coefficient which represents the entire sample was  $-0.273$ , significant at the 95 per cent level with a critical value of 0.117 and a sample of 293, while that for age 13 and older showed a value for  $r$  of  $-0.485$ , also significant at the 95 per cent level with a critical value of 0.125 and a sample of 259. Data on ages 1–12 showed a positive value, but the sample analysed was too small to be significant.

The coefficient of determination showed that 23.5 per cent of the variation in blast transformation was accounted for by age, from age 13 onwards. This is significantly different from zero at the 95 per cent level of confidence. Sex and race did not play a significant part in these variations throughout our work.

The mean and standard deviation of mitotic index and blast transformation was compared in all subjects between 21 and 30 yr and between 71 and 80 yr of age (Table 1). A significant statistical difference was found between these age groups ( $P < 0.001$ ).

Interchange of plasma from old and young individuals demonstrated that the chief factor in diminishing mitotic potential with age is a function of the cell rather than the plasma.

Limited proliferative function of lymphocytes may be the result of acquired errors in cell division. Jacobs



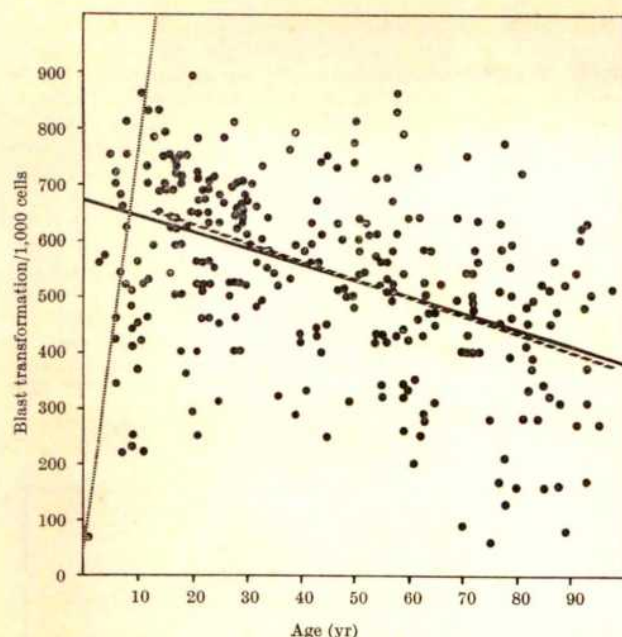


Fig. 3. Blast transformation versus age. —, Mitosis plus blasts for all ages was  $678.335 + (-3.022)$ ; ···, mitosis plus blasts, ages 1–12 yr, was  $44.045 + 73.012$ ; ---, mitosis plus blasts, aged 13 yr onwards, was  $690.324 + (-3.240)$ .

*et al.*<sup>1,2</sup> have shown increasing aneuploidy in leucocyte cultures of ageing humans. In some cases this is associated with a loss of one chromosome from the 6–12-X series<sup>3</sup>. The large frequency of mitotic non-disjunction in germ cells of older women is well known, but definitive data to explain the refractoriness of lymphocytes from older people to phytohaemagglutinin are not yet available.

Table 1. COMPARISON IN MITOTIC INDEX AND BLAST TRANSFORMATION BETWEEN SUBJECTS IN THE THIRD AND EIGHTH DECADES

	Age range	
N	21–30	71–80
	46	29
Mitotic index (No. of mitotic figures/1,000 cells)		
Mean	46	22
SD	18	18
t		5.513
P		< 0.001
Percentage of mitoses + blasts		
Mean	59.3	45.0
SD	11.9	17.6
t		4.354
P		< 0.001

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## Hepatic Response to Lysosomal Effects of Hypoxia, Neutral Red and Chloroquine

THE histochemical detection of early changes in the characteristics, distribution and enzyme contents of lysosomes, as well as their cycles of appearance and disappearance, affords a sensitive index of toxic effects on the cell. Hypoxia is known to labilize lysosomes<sup>1</sup> and might be expected to render them more susceptible to adverse influences. Selective uptake of neutral red by lysosomes<sup>2,3</sup> and the stabilization brought about by chloroquine<sup>4</sup> constitute further experimental manipulations to which lysosomes can be subjected. We have made detailed observations of lysosomal behaviour in these conditions as a preliminary to the study of lysosomal changes in the damaged liver of the rat.

Individual male rats (Carworth Farm E strain, 130–150 g, 5–6 weeks old) were sealed in 1 gal jars for 60 min. Animals (three in each group) were killed at 0, 1, 3, 6, 9, 12, 18 and 24 h after removal from the jars. One rat in each group had been given 125 mg/kg chloroquine daily for 12 days by tube; a second rat was injected intraperitoneally with neutral red (400 mg/kg) immediately following hypoxia; the third underwent no further treatment. Comparisons were made with similar rats not subjected to hypoxia. Liver slices were fixed in formol sucrose (acid phosphatase) or formol chloral hydrate ( $\beta$ -glucuronidase) for 18–24 h and transferred to hypertonic gum sucrose (18–24 h). Sections 8  $\mu$  thick were cut on a freezing microtome. Acid phosphatase was demonstrated by the techniques of Gomori<sup>5</sup> (used shortly after incubating  $\beta$ -glycerophosphate medium for 1 h at 37° C and filtering<sup>6</sup>) and Barka and Anderson<sup>6</sup>. For  $\beta$ -glucuronidase the methods of Hayashi *et al.*<sup>7</sup> and Fishman and Goldman<sup>8</sup> were used. Some of the liver slices were also fixed in neutral buffered formalin and Lillie acetic acid alcohol (18–24 h), embedded in paraffin and then stained with haematoxylin and eosin and Feulgen nuclear stain, respectively.

The results are summarized in Table 1. Hypoxia has two striking effects: an initial general increase in hepatic lysosomes and in the intensity of enzyme-staining reactions (Fig. 1), followed later by labilization of lysosomal membranes as manifested by loss of particulate enzyme activity, appearance of diffuse activity and residual perinuclear distribution (Fig. 2). This increase in lysosomes and their subsequent labilization profoundly disturbs the integrity of the cells. The release of hydrolytic enzymes into the cell cytoplasm results in cell damage, reflected in intracellular oedema seen at 3–12 h following hypoxia. The nuclei are also affected by the lysosomal disturbance; many of them enter into prophase and cell division follows.



Fig. 1. Liver of rat subjected to hypoxia for 1 h and killed 3 h later. Section of fixed tissue stained for acid phosphatase by Gomori technique, showing the variety of lysosomes ( $\times 1,000$ ).



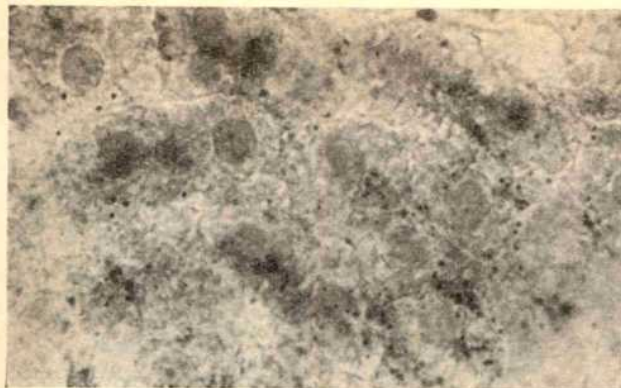


Fig. 2. A section similar to that shown in Fig. 1, but taken from a rat killed 6 h after hypoxia. Particulate enzyme activity is reduced, remaining principally in the perinuclear region; some diffuse cytoplasmic activity is present ( $\times 1,000$ ).



Fig. 3. Almost total loss of acid phosphatase in a rat subjected to hypoxia as before, treated with neutral red, and killed after 3 h ( $\times 1,000$ ).

Neutral red introduced after hypoxia brings about a great reduction in lysosomes, followed by an almost total loss of enzyme demonstrable histochemically in liver cells (Fig. 3). In comparison with the effects of hypoxia alone, this premature and abrupt release of hydrolytic enzymes into the cytoplasm causes more extensive damage to cells. Many more are swollen, and in addition most are vacuolated (Fig. 5). Moreover, abnormal mitotic figures are a feature at this stage, with cell division taking place earlier. The process of cell recovery is associated with an altered pattern of lysosomal enzymes. Acid phosphatase activity appears first in the periportal areas in sites from which neutral red has disappeared, while  $\beta$ -glucuronidase is seen in centrilobular lysosomes in which neutral red is still present. In time the normal pattern of enzyme activity is restored.

The morphology of liver lysosomes is altered in rats treated with chloroquine. The organelles are enlarged and aggregated together but remain in their normal sites.

Their membranes stain much more intensely than the inner matrix. By 6–9 h after hypoxia a greater intensity of staining reaction is visible. Over the interval 12–18 h, in addition to the existing enlarged lysosomes, fresh enzyme sites appear much smaller in size, more homogeneously stained and probably representing a new generation of lysosomes (Fig. 4). The cytoplasmic vacuoles seen in sections stained with haematoxylin and eosin seem to correspond with enlarged lysosomes. Feulgen preparations reveal no signs of cell division, except at 12 h after hypoxia where a few nuclei are seen in prophase and even fewer in any other stage of mitosis.

Besides the suggested responsibility of lysosomal enzymes for cellular alterations in damaged or autolysing



Fig. 4. Lysosomal effects of 12 day feeding of chloroquine followed by exposure to hypoxia in a rat killed 18 h after hypoxia. Large lysosomal aggregates are present, together with small foci of acid phosphatase activity, probably representing new lysosomes ( $\times 1,000$ ).

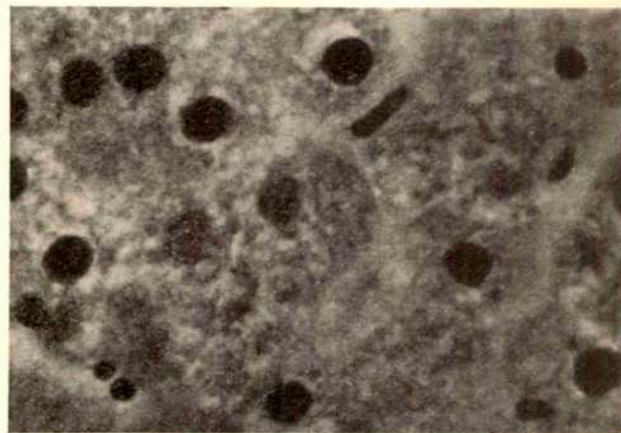


Fig. 5. Intracellular oedema and vacuolation in the liver of a rat subjected to hypoxia for 1 h, treated with neutral red, and killed 9 h later ( $\times 1,000$ ).

Table 1. LYSOSOMAL AND CELLULAR CHANGES IN RAT LIVER UNDER VARIOUS CONDITIONS

Time after hypoxia (h)	0-3	Hypoxia 6-9	12-24	1-6	Hypoxia + neutral red 9-12	18-24	Hypoxia + chloroquine 1-6	9-24
Lysosomes	+	-	+	2-	-	+	+	+
Number*	Autolysosomes	Perinuclear	Periportal	Diffuse	Diffuse†	Particulate†	Aggregated	Small lyso- somes present
Distribution,	Particulate	Diffuse nuclear	Particulate	Diffuse	Diffuse†	Particulate†	Particulate and diffuse	Particulate
characteristics		staining						
Enzymes								
Cellular changes*								
Intracellular oedema	+	2+	0	2+	+	0	0	0
Cells in mitosis‡	+	+	2+	2+	+	+	0	0
		Prophase		Abnormal mitoses				

\* Changes in comparison with controls: greatly increased, 2+; increased, +; 0, no change; decreased, -; greatly decreased, 2-.

† Lobular distribution of enzymes: acid phosphatase periportal,  $\beta$ -glucuronidase centrilobular.

‡ Maximum incidence of mitoses: hypoxia, 18 h; hypoxia + neutral red, 3 h.



tissue<sup>9</sup>, a role in mitosis has been proposed more recently by Allison and Mallucci<sup>10</sup> and by Hirschhorn and Hirschhorn<sup>11</sup>. According to these authors, release of lysosomal enzymes in some way initiates cell division. Our results show that in rat liver hypoxia results in an increase in the number of lysosomes, apparently of a more labile character, the release of the enzymes of which seems to initiate cell division and also causes cell damage. The action of neutral red provokes a more rapid release of lysosomal enzymes and accelerates the process of cell division associated with abnormal mitosis and clumping of chromosomes. Evidence for abnormal mitosis and nuclear disorganization has been presented<sup>3,12</sup>. On the other hand, stabilizing the lysosomal membranes by means of chloroquine prevents the post-hypoxia nuclear changes.

The importance of the cycle of changes undergone by lysosomes in relation to hepatocellular morphology emerges from these studies. Superimposed on the background of minimal morphological alterations induced by hypoxia, the effect of neutral red is striking in terms of intracellular oedema and vacuolation. These manifestations closely correspond to the lysosomal changes. Chloroquine, on the other hand, preserves lysosomal integrity after hypoxia, without evidence of liver damage.

Our observations emphasize that effects on lysosomes are reflected in both cytoplasmic and nuclear changes. They offer the hope that a "provocative" test for hepatic susceptibility to damage may be evolved along these lines.

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### Nuclear Bleb Formation in Human Bone Marrow Cells during Cytosine Arabinoside Therapy

ABNORMAL nuclear blebs have recently been reported in the bone marrow cells of patients undergoing 5-fluorouracil treatment for neoplastic disease<sup>1</sup>. Ultrastructural examination of these blebs revealed that they enclose areas of cytoplasmic material within their bulging projection and are unlike the nuclear invaginations described in some malignant cells<sup>2</sup> or the projections of nuclear material reported in certain chromosome abnormalities<sup>3</sup>. Investigation in our laboratory of the bone marrow of patients undergoing treatment with cytosine arabinoside revealed identical nuclear blebs containing cytoplasmic material in immature granulocytes. The condition was first noted in bone marrow samples 45 h after a single intravenous administration of the agent, 1,200 and 1,500 mg/m<sup>2</sup> body surface.

The bleb formations were frequently found to contain a varied assortment of cytoplasmic organelles (Fig. 1). Characteristically they occur as oval or round protrusions on the outer periphery of the nucleus. A thin sheet of nucleoplasm, with the exterior nuclear membrane intact, appears to be thrown out in loop-like fashion from the nuclear surface. The cytoplasmic material enclosed within the bleb is, in turn, segregated from the nucleus by double membranes (Fig. 2).

In some of these segregated areas of cytoplasm distinct cytoplasmic organelles were not observed in the sections examined (Fig. 3). The presence in these blebs of particulate matter of greater density than in the surrounding cytoplasm suggests that the blebs are isolated, and that hydrolytic activity might possibly be present within the isolated inclusions.

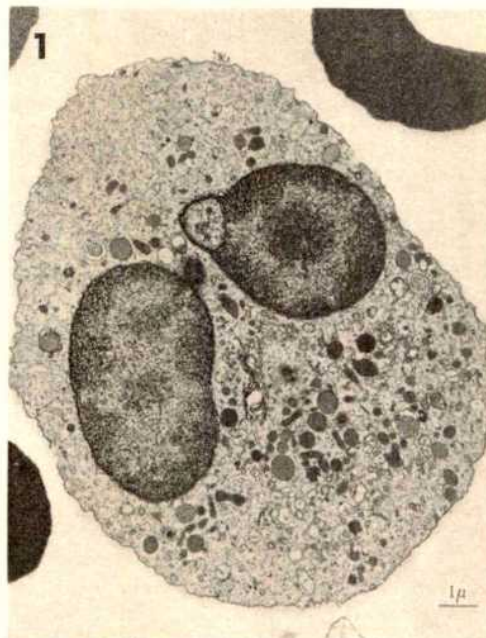


Fig. 1.



Fig. 2.





Fig. 3.

It is of particular interest to note that this nuclear abnormality, induced by two similarly acting chemotherapeutic agents, resembles the projections of the nuclear envelope which were recently demonstrated to be a naturally occurring attribute of the Burkitt lymphoma cells<sup>4</sup>.

More detailed studies are at present under way in an attempt to elucidate the mode of formation of this phenomenon as well as its functional significance in the cell.

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## PHYSIOLOGY

### Predominantly Electrochemical Nature of Biological Power-producing Reactions

THE food taken in by a human being on a normal diet is equivalent, if all is converted to heat, to about  $3 \times 10^6$  cal/24 h. If this energy was spent at a constant rate, with 100 per cent efficiency of conversion to mechanical work, the power provided would correspond to about 160 W.

For calculating the actual power available to the human organism there are, in principle, only two ways of converting the energy of chemical reactions to mechanical work. The first of these is that of the heat engine, and

the efficiency for this conversion is given by the well known Carnot cycle fraction

$$\frac{T_{\text{high}} - T_{\text{low}}}{T_{\text{high}}}$$

where the  $T$ s are, in °K, the high and low points of the temperature of the "working fluid". For a human organism at room temperature, this fraction has a value of about 1/60, so that if the principle of the heat engine is assumed in estimating the fraction of the energy conversion of the chemical reaction between food and oxygen to water and carbon dioxide available to power a human organism, the (average) power would correspond to  $160 \times 1/60 \approx 2.5$  W.

This value is much less than that which the human organism is able to make available to itself, because the circulatory system alone operates under a roughly constant power of about 10 W.

It seems, therefore, that at least a substantial part of the energy conversion of the human organism must take place by the (less well known) principle of the electrochemical conversion of the energy of chemical reactions to work, in which the Carnot efficiency expression does not limit the degree of this conversion ("cold combustion").

This simply made conclusion seems to have a broad significance. Thus it has often been stated that electron transfer reactions play a part in certain biological processes. It has not yet been accepted that only the electrochemical path of conversion of the energy of chemical reactions to mechanical work is consistent with the observed efficiency in the use of chemical energy in the body. This is possibly because of the lack of availability in English of books on more recently discussed electrokinetic principles.

In the normal concept of the electrochemical engine, an electric motor receives energy after electrochemical interfacial reactions in a fuel cell have occurred, and is assumed to convert this to mechanical work at nearly 100 per cent efficiency. The existence of an analogue of this conversion of electric energy to mechanical power is indicated in the electrical activity of muscle function.

Thus the present work supports electrochemical reaction mechanisms as those by which biological cells feed on organic materials and oxygen. A model of biological cell action which is similar to that of an electrochemical fuel cell was formulated by Del Duca and Fuscoe<sup>1</sup>.

It is noteworthy that if a biological organism converts the energy of the reaction between organic compounds and oxygen to available mechanical energy by an electrochemical mechanism, heat may be evolved or absorbed in the reaction. If the entropy of the overall reaction ( $\Delta S$ ) is sufficiently positive, a net cooling effect during production of energy by the organism could occur (and would so occur if the sum of the overpotentials associated with the hypothetical electrochemical processes of the biological cell were less than  $T\Delta S$ ). An organism could conceivably then extract some electrical energy from the heat energy of its surroundings.

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## Effect of a Branching Capillary Network on the Differential Flow Velocities of Plasma and Corpuscles

MEASUREMENT of the relative velocities of corpuscles and plasma by timing the travel of traceable materials between different points in the living body<sup>1,2</sup> shows that the mean transit time of the cells is appreciably less than that of the plasma and this is generally attributed to the effect of axial drift of the red cells in the minute vessels. Groom, Morris and Rowlands<sup>3</sup> showed that the whole of the cell arrival curve was displaced in time ahead of the plasma arrival curve. Thus some of the red cells apparently advanced bodily ahead of the plasma with which they were injected. Whitmore<sup>4</sup> compared these results with those of a theoretical hydrodynamic model, which he developed, and concluded that axial accumulation of cells in streamline flow should only affect the shape of the later portion of the cell-concentration curve; the initial rise of both curves should occur at the same moment and at a comparable rate. Thus such a model did not account for the observed time displacement of the whole cell curve ahead of the plasma curve and the advancement of the leading cells ahead of the plasma with which they were injected.

Whitmore further analysed the effect of pulsatile flow and flow deformation of the red cells and concluded that these could not account for the observed effect either. He suggested that the discrepancy could be explained by postulating a mode of flow in which the cells are constrained within an axial core, but move about freely within this core while the plasma moves freely and randomly between the core and all, or a portion, of the plasmatic layer. There is some support for Whitmore's theory of random movement in the observations of Bloch<sup>5</sup>, but I wish to direct attention to regular plasma and cell movements between the axial core and the peripheral zone which must occur in a capillary network at the sites of bifurcation because of displacement of cells by the partition wall of the branch.

Fig. 1 illustrates the effect of axial drift of red cells in blood flowing through a capillary network. The lines show approximate paths of red cells and the proximity of the lines represents the cell concentration. In region A there is axial accumulation of the cells such as develops in vessels of diameter less than about 250 $\mu$ . The diagram shows a progressive increase in cell concentration from the wall to the axis in conformity with the results of our experiments using slit-like glass channels<sup>6,7</sup>. At the bifurcation the stream is split through the central core so that in region B there is at first a concentration of cells near the inner wall of each branch. As the blood moves along each branch the cells migrate towards the axis and, if the branch is long enough, form an axial accumulation as indicated in region C. Immediately distal to the confluence the distribution of cells approximates to that shown in region D with a central core, with few cells,

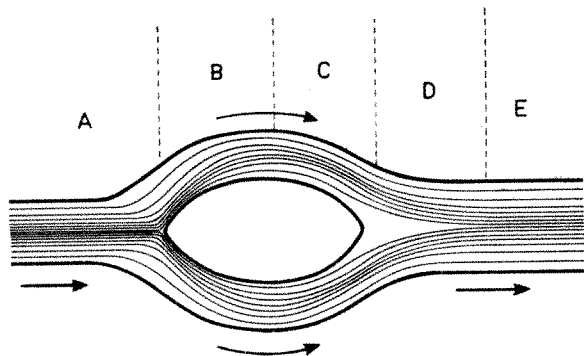


Fig. 1.

separating the cell-rich axial streams coming from each branch. Again, in region E, given sufficient length of narrow bore vessel, the cells will drift axially to re-form a central accumulation. For simplicity only one division and one confluence are shown and the cell paths are illustrated in one plane only, but in an actual network there are many successive divisions at the arteriolar end, and many confluences at the venular end. The planes of branches in general vary successively so that the effects illustrated occur repeatedly and in many planes.

If the progressive increase in cell concentration from wall to axis does not obtain *in vivo*, significant transverse movements may not occur near confluences, but such movements must occur at sites of branching because, as Maude and Whitmore<sup>8</sup> have pointed out, a particle must be displaced radially if it enters a tube along a streamline which comes to lie at a distance from the wall less than the radius of the particle and this would apply near the dividing walls of a branch. Furthermore, it is well known from microscopic observation of the capillary circulation that there is a zone of plasma containing few or no cells near the wall of most small vessels.

Thus in a capillary network there will occur reciprocal transverse movements of cells and plasma across the stream, with progressive separation of cells and plasma which were originally associated. The movements will be greater if there is axial drift of the cells as well as simple displacement by dividing walls.

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## Role of Intraluminal Precipitation in Secretion of Small Intestinal Calcium and Phosphate in the Dog

IN previous investigations of small intestinal calcium metabolism<sup>1</sup> it has been assumed that calcium in the lumen of the small bowel is entirely in solution. We examined the hypothesis that calcium exists in more than one phase, and investigated (a) the calcium composition of endogenous intestinal secretions, and (b) the calcium and phosphate content in the lumen 30 min after a dilute calcium solution had been introduced into closed segments of small intestine. We prepared two adjacent jejunal and two adjacent ileal segments *in situ* in seventeen fasted, anaesthetized dogs; each segment was approximately 20 cm long and contained two arcades of mesenteric vessels. The jejunal segments commenced 15 cm distal to the ligament of Treitz and the ileal segments ended 10 cm proximal to the ileo-caecal junction. We prepared the segments individually by tying 5 mm plastic cannulae, fitted with plugs, into each end. They were thoroughly flushed with saline at 37° C, and when the effluent was clear were flushed with air. When all were prepared, the four segments were flushed as quickly as possible with 200 ml. of saline followed by air. In five dogs no solution was introduced, the "empty" segments were left *in situ* for 1 h and the endogenous secretion was collected by washing with 25 ml. of 5 per cent mannitol. In twelve dogs we immediately introduced 10 ml. of a solution containing 1 mg of calcium (as calcium chloride) and 100 mg of

inulin. After 30 min the luminal contents were collected by drainage and analysed for calcium (using an Eppendorf flame photometer), phosphate<sup>2</sup>, and inulin<sup>3</sup>. All samples contained a small proportion of precipitate, and they were centrifuged as soon as they were obtained and the supernatant was analysed immediately for calcium and phosphate. The remainder of the sample was acidified with 0.2 ml. of concentrated hydrochloric acid and the mixed sample was reanalysed immediately for calcium and phosphate. This latter result was corrected for volume effects (acid added and samples removed), and concentration was calculated for the original volume. The quantity of calcium secreted into "empty" segments was calculated directly from the concentration of calcium in the washings and the wash volumes. When the calcium solution was introduced it was necessary to correct sample volume, for recovery by drainage is incomplete. Inulin was virtually unabsorbed during a 30 min period. The ratio of initial to final inulin concentration multiplied by the initial volume therefore gives total sample volume. This corrected volume was used to calculate total calcium and phosphate content of the segments.

Table 1 shows that endogenous calcium entered "empty" segments and was not homogeneous: calcium was present both in solution in the supernatant (S) and in the form of an acid-soluble precipitate (difference between S and T). The insoluble form was dissolved at pH 1.

Table 2 shows that 30 min after the calcium solution was introduced, the intraluminal contents were also in two

phases. Both calcium and phosphate were present in the precipitate. The proportion of precipitated calcium was much lower than in the "empty" segments. Table 2 also shows phosphate content of the samples where exogenous calcium was introduced. Unlike the calcium, most of the phosphate was in the precipitated form. Thus the distribution of phosphate resembled more closely the distribution of calcium in those experiments where no exogenous calcium was added (Table 1). In these cases, intestinal pH had no apparent influence on precipitation, for it was as common in jejunal (mean pH 6.90) as ileal (mean pH 7.65) segments.

We did not identify the acid-soluble precipitate. The phosphate was present in both phases, and so these data suggest the calcium and phosphate were precipitated in combination, although they may have precipitated independently.

On acidification, bubbles of gas were evolved, which suggests that the precipitate was at least in part a calcium carbonate or bicarbonate complex. Calcium and phosphate precipitation within the lumen of the small intestine should be evaluated in investigations of endogenous and exogenous calcium and phosphate metabolism. It represents an appreciable fraction of endogenous calcium and phosphate secretion.

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## Neurosecretory System of Heteroptera (Hemiptera) and Role of the Aorta as a Neurohaemal Organ

It is well known that the generalized pattern of the neurosecretory system of an insect consists of the medial and lateral groups of neurosecretory cells in the protocerebrum, the medial and lateral neurosecretory pathways and the corpora cardiaca<sup>1,2</sup>. The A- and B-materials elaborated by these groups of cells are transported along their respective pathways to be stored in the corpora cardiaca, from where they are released into the general blood circulation to fulfil specific physiological functions. In an insect, therefore, the corpora cardiaca are considered to form the storage-and-release (neurohaemal) organ for the two types of materials.

The neurosecretory systems of five species of Heteroptera, *Dysdercus koenigii* (Pyrrhocoridae), a species of *Graptostethus* (Lygaeidae), *Ranatra elongata*, *Nepa cinerea* (Nepidae) and *Belostoma indica* (Belostomatidae), examined with the aid of paraldehyde-fuchsin and Humberstone's performic acid-victoria blue (PAVB) *in situ* techniques<sup>3</sup> and routine histological methods<sup>4</sup>, shows an anatomical pattern which varies considerably from the basic pattern for the hexapods. In these insects, while

Table 1. ENDOGENOUS CALCIUM SECRETION INTO "EMPTY" SEGMENTS

Segments:	Jejunum				Ileum			
	S	T	S	T	S	T	S	T
Calculated on weight of segment*	21	39	18	28	18	33	12	19
Calculated on surface area of segment†	6-37	16-74	9-28	12-58	9-35	13-47	5-25	8-49
	9	16	5	12	6	11	4	6
	2-16	6-32	3-13	5-22	3-12	7-18	2-8	3-14

The data are based on wet weight and serosal surface area, and represent the mean and range for each.

S, Dissolved calcium; T, total calcium.

\* Units were mg/h/g segment  $\times 10^4$ .

† Units were mg/h/cm<sup>2</sup> serosal surface  $\times 10^4$ .

Table 2. CALCIUM AND PHOSPHATE CONTENT (MG) OF CLOSED INTESTINAL SEGMENTS, 30 MIN AFTER THE INTRODUCTION OF 1 MG OF CALCIUM AS CHLORIDE

Segment:	Jejunum				Ileum			
	Ca	P	Ca	P	Ca	P	Ca	P
Experiment 1								
S	1.000	—	1.252	0.121	0.508	0.000	0.478	0.000
T	1.112	—	1.345	0.279	0.709	0.048	0.678	0.093
Experiment 2								
S	1.247	0.040	1.035	—	0.495	—	0.327	—
T	1.329	0.050	1.138	—	0.532	—	0.353	—
Experiment 3								
S	0.848	0.220	1.010	0.230	0.609	—	0.479	—
T	1.010	0.245	1.148	0.289	0.626	—	0.507	—
Experiment 4								
S	1.359	0.060	1.064	0.052	0.676	0.000	0.644	0.000
T	1.359	0.095	1.118	0.082	0.827	0.049	0.755	0.000
Experiment 5								
S	1.189	—	1.244	—	0.692	—	0.580	—
T	1.215	—	1.296	—	0.730	—	0.610	—
Experiment 6								
S	0.946	0.050	1.152	0.026	0.736	0.000	0.718	0.000
T	0.983	0.081	1.204	0.045	0.745	0.006	0.728	0.006
Experiment 7								
S	0.952	0.073	1.159	0.221	0.655	0.011	0.566	—
T	0.965	0.100	1.202	0.266	0.678	0.012	0.588	—
Experiment 8								
S	1.790	0.199	1.650	0.230	0.813	—	0.610	—
T	1.829	0.346	1.720	0.324	1.059	—	0.760	—
Experiment 9								
S	1.160	0.012	1.327	0.091	0.731	—	0.756	0.027
T	1.380	0.087	1.702	0.204	1.145	—	0.864	0.061
Experiment 10								
S	1.490	0.562	1.272	0.257	0.623	0.018	0.704	0.087
T	1.887	0.786	1.445	0.360	0.641	0.056	2.860	0.827
Experiment 11								
S	0.974	0.437	1.395	0.780	0.983	0.058	1.000	0.075
T	1.300	0.623	1.650	0.907	1.039	0.000	1.288	0.218
Experiment 12								
S	1.558	0.478	1.156	0.085	1.341	0.276	1.005	0.129
T	1.670	0.645	1.231	0.125	1.453	0.356	1.057	0.151

S, Quantity of dissolved calcium (mg) and phosphate (mg).

T, Quantity of total calcium (mg) and phosphate (mg).

	Statistical analysis			
	1	2	3	4
	Jejunum	Jejunum	Ileum	Ileum
	Mean $\pm$ SE	Mean $\pm$ SE	Mean $\pm$ SE	Mean $\pm$ SE
S Ca	1.21 $\pm$ 0.08	1.23 $\pm$ 0.06	0.738 $\pm$ 0.066	0.655 $\pm$ 0.058
T Ca	1.34 $\pm$ 0.09	1.35 $\pm$ 0.23	0.848 $\pm$ 0.075	0.921 $\pm$ 0.190
S P	0.213 $\pm$ 0.060	0.174 $\pm$ 0.069	0.030 $\pm$ 0.041	0.045 $\pm$ 0.020
T P	0.306 $\pm$ 0.081	0.240 $\pm$ 0.076	0.044 $\pm$ 0.052	0.193 $\pm$ 0.106

P values calculated from t test significant at 0.001 for all calcium data and at 0.01 for dissolved phosphate (SP) and total phosphate (TP) in jejunum 1, at 0.05 in jejunum 2 and not significant for ileum.

the neurosecretory cells and pathways are always positive to these tests, the corpora cardiaca do not stain at all.

There are two groups of between eight and ten A-cells, which stain deep purple with the paraldehyde-fuchsin and these are present in the pars intercerebralis medialis of the protocerebrum. With the PAVB histo- and cytochemical technique specific for cystine and/or cysteine an equal number of cells are stained greenish-blue in the same locus, suggesting that the cystine rich neurosecretory material is elaborated by the A-cells (Fig. 1, and *ns c* in Fig. 2). Humberstone's PAVB technique<sup>3</sup> is a modification of Adams and Sloper's performic acid-alcian blue technique<sup>5</sup> for the histochemical demonstration of cystine and/or cysteine and, therefore, like its predecessor this technique is also specific for cystine and cysteine. The axons of the cells of each group converge to form the neurosecretory pathway (*ns p* in Fig. 2). The two pathways after crossing each other in the upper equatorial half of the protocerebrum run along its anterior and ventral surfaces, then along the deuto- and trito-cerebrum and emerge from the tritocerebrum as the nervi corporis cardiaci I (NCC I, Fig. 2). It is worth mentioning here that the emergence of the NCC I from the tritocerebrum is unique to these hemipteran species, which is not so in the case of the basic pattern where the NCC I emerge from the protocerebrum<sup>1,2</sup>. The NCC I after running for some distance between the aorta and the alimentary canal become attached superficially to either the ventral or to the dorsal surface of the corpora cardiaca (*c c*,

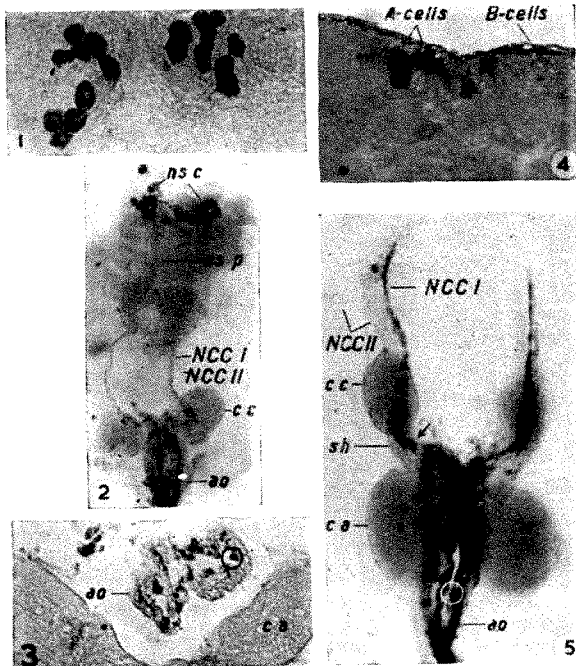


Fig. 1. Dissected protocerebral parts of the bug, *D. koenigii*, showing two groups of nine A-cells of the pars intercerebralis medialis. (Paraldehyde-fuchsin *in situ*,  $\times c. 114$ .)

Fig. 2. Neurosecretory system of *R. elongata*, mounted with dorsal surface facing upwards, showing the median neurosecretory cells, their pathways, the NCC I by-passing the corpora cardiaca and entering the aorta wall, and the NCC II (clearly visible on the right) entering the corpus cardiacum apically. The corpora allata have been removed. (PAVB, *in situ*,  $\times c. 50$ .)

Fig. 3. Cross-section ( $7\mu$ ) of a PAVB bulk-stained preparation of *R. elongata*, showing cystine-rich neurosecretory material in the aorta wall. ( $\times c. 135$ .)

Fig. 4. Cross-section ( $12\mu$ ) of a series of the bug, *D. koenigii*, routinely stained with paraldehyde-fuchsin showing A-cells and B-cells in the protocerebrum. ( $\times c. 114$ .)

Fig. 5. Magnification of the postcerebral endocrine organs of *R. elongata*, showing fanning out of the fibres of NCC I (arrow) before they enter the ventral wall of the aorta. The sheath (*sh*) of the NCC I is quite distinct from the corpora cardiaca. Absence of the material in the corpora cardiaca is as noticeable as in the corpora allata. (PAVB, *in situ*,  $\times c. 81$ .)

Figs. 2 and 5) and after passing along the surface of the glands enter the aorta wall ventrally or laterally. The corpora cardiaca and the NCC I are easily separated without damaging either structure; there seems to be no connexion between them and even the neurilemma covering them are separate (*sh*, Fig. 5). Before they penetrate the aorta wall the fibres of the NCC I fan out considerably (arrow, Fig. 5) and run for about half the length of the aorta where they gradually disappear. There is always abundant neurosecretory material in the aorta wall, as in the corpora cardiaca of orthopteroid insects, but it is not present in the lumen of the aorta, as reported by Herlant-Meewis and Paquet<sup>6</sup>. The material is distributed uniformly among the entire circumference of the aorta wall (Fig. 3). The bulk-stained preparations (circled in Fig. 5) and the sections (circled in Fig. 3) suggest that the material is localized within the axons. This contrasts with the corpora cardiaca of the Orthopteroid species where the material is stored in the intercellular spaces, and it is an interesting feature revealed by this work. It is possible, therefore, that the aorta wall acts as the storage organ for the neurosecretory material made by the A-cells. From the aorta wall it is probably released into the general blood circulatory system for specific physiological purposes. Thus the aorta wall appears to be the neurohaemal organ for the A-material.

Between four and six B-cells, stained with the light green of the counterstain of the paraldehyde-fuchsin technique, are present in the pars intercerebralis lateralis of each protocerebral lobe (Fig. 4). B-cells, being negative to PAVB, can be said to be devoid of cystine. The axons of these cells converge to form lateral neurosecretory pathways which, after passing through the proto- and deuto-cerebrum of its side emerge from the tritocerebrum just outside the NCC I, as the NCC II. Distally, the NCC II join the corpora cardiaca (NCC II, Figs. 2 and 5). In this way the neurosecretory material elaborated by the B-cells, transported by the lateral neurosecretory pathways and the NCC II, is stored in the corpora cardiaca from where it is probably also released. For B-material therefore the corpora cardiaca serve as neurohaemal organs.

We have shown clearly that there may be two neurohaemal organs in some insects, that is, the aorta wall which is responsible for the storage and release of A-material and the corpus cardiacum for the B-material.

The presence of material in the aorta wall of heteropteroid insects, at the level of the corpora cardiaca, has been noted several times<sup>7-9</sup>, but its presence has been interpreted in different ways. According to Nayar<sup>7</sup> and Ewen<sup>8</sup>, in *Iphita limbata* (Pyrrhocoridae) and *Adelphocoris lineolatus* (Miridae) the corpora cardiaca are the storage organs, like the neurohaemal organ of the Orthopteroid insects<sup>2,11</sup>, but these authors interpreted the presence of A-material in the aorta wall as a result of the release of the neurosecretory material from the storage organ. Johansson<sup>9</sup> has also observed material in the aorta wall of *Oncopeltus fasciatus* (Lygaeidae) but has not identified the exact pathways to the aorta wall. *Rhodnius prolixus* (Reduviidae) is perhaps the only hemipteran so far investigated for which it has been categorically mentioned that the axons of the NCC I end in the corpora cardiaca with no information about the presence of neurosecretory material in the aorta wall<sup>10</sup>.

There is evidence of intrinsic secretion of A-material in the corpora cardiaca<sup>11,12</sup>. If this is so, then the presence of the material in this organ observed by Nayar<sup>7</sup> and Ewen<sup>8</sup> could possibly be explained as secretion in the glands and not the stored material elaborated by the A-cells. The failure of such an intrinsic secretion in the present work could be because the insects were not in a physiological state for such a secretion. For the same reason Johansson<sup>9</sup> may have failed to observe the material in the corpora cardiaca. The above discussion, however,

suggests that the aorta wall, but possibly not the corpora cardiaca, is the storage and release organ for A-material in Hemiptera.

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### Effect of Saxitoxin on the End Plate of Frog Muscle

TETRODOTOXIN and saxitoxin are neurotoxins with very similar biological actions, though they are chemically distinct. Tetrodotoxin reduces the amplitude of the end plate potential elicited by neural stimulation. At the same time, however, the end plate membrane remains entirely responsive to acetylcholine<sup>1-3</sup>. Kao and Nishiyama<sup>4</sup> found that the actions of saxitoxin on the neuromuscular system are similar to those of tetrodotoxin. The blockade of neuromuscular transmission by saxitoxin affects the motor axon and muscle membrane, while the end plate receptor is not affected.

In order to confirm our previous results, the action of both toxins on the end plate potential and the iontophoretically elicited acetylcholine (Ach) potential was studied in this experiment. Isolated sciatic nerve-sartorius preparations of frog were used in all these experiments.

Fig. 1 shows typical records of alternate end plate and Ach potentials both before and after the application of saxitoxin and tetrodotoxin. Both toxins always reduced the amplitude of the end plate potential after a time, but

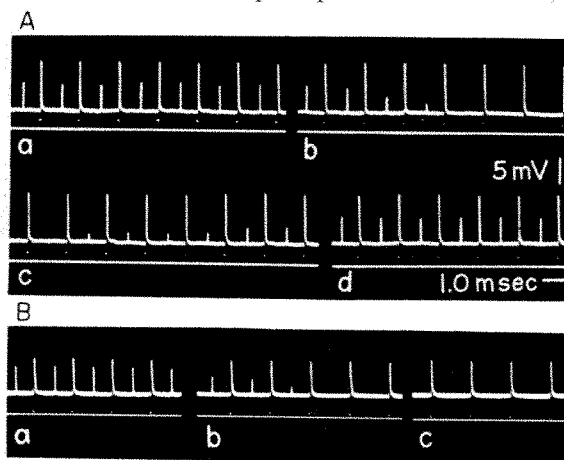


Fig. 1. End plate and Ach potentials before and after application of 0.01 µg/ml saxitoxin (A) and of 0.1 µg/ml tetrodotoxin (B). Upper traces: alternate end plate potentials and Ach potentials. Upward deflexions in lower traces: strength of current through the Ach pipette. The concentration of curare was  $2.5 \times 10^{-4}$  g/ml. in A and  $2.0 \times 10^{-4}$  g/ml. in B. 3-6 mmolar calcium in saline solution was present in both. In A: a is the control; b, 20 sec after perfusion with solution containing saxitoxin; c, 140 sec; d, 260 sec after perfusion with normal saline solution. In B: a is the control; b, 80 sec; c, 210 sec after perfusion with solution containing tetrodotoxin.

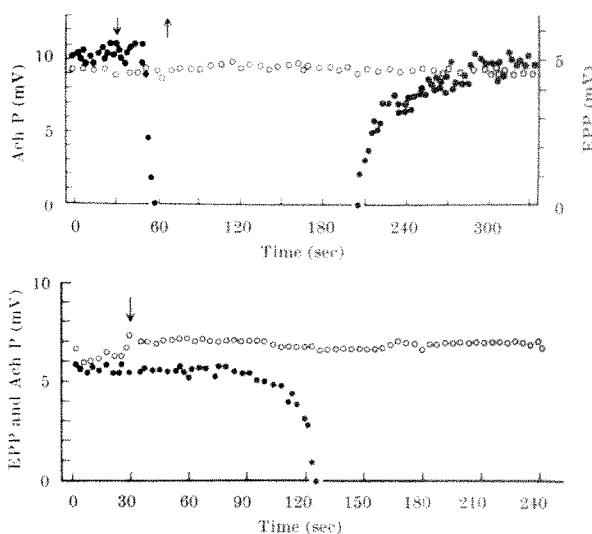


Fig. 2. Time course of the effect of 0.01 µg/ml saxitoxin (upper graph) and 0.1 µg/ml tetrodotoxin (lower graph). The same experiments as in Fig. 1. ●, End plate potentials (EPP); ○, Ach potentials. At arrows the toxins were applied, and at the inverted arrow the toxin was washed out.

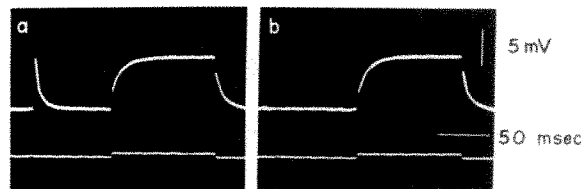


Fig. 3. Alternate records of end plate potentials and catelectrotonic potentials on the neuromuscular junction before and after application of 1.0 µg/ml saxitoxin. a, Control; b, 25 min after application of the toxin. The preparation was previously treated with  $2.5 \times 10^{-4}$  g/ml curare. The current for constant rectangular pulses across the muscle membrane was monitored in the lower traces.

they had no effect on the amplitude of the Ach potential. An almost complete block of neuromuscular transmission occurred less than 30 sec after the first sign of reduction in the end plate potential with both toxins, but even in such a completely blocked state, the amplitude of the Ach potential was not affected. The time course of the effect of both toxins is shown in Fig. 2.

The effects of both toxins on the passive electrical properties of the muscle membrane in the region of the end plate were also examined. A rectangular outward current of constant intensity was applied through a second intracellular microelectrode and both end plate and catelectrotonic potentials were recorded alternately (Fig. 3). Even when a high concentration of saxitoxin, sufficient to block the neuromuscular transmission completely, was applied, the electrical properties of the end plate region remained unchanged. Saxitoxin, therefore, does not alter the effective membrane resistance. A similar effect of saxitoxin on the crayfish neuromuscular junction was recently reported<sup>5</sup>.

This experiment shows the similarity between the actions of saxitoxin and tetrodotoxin on the frog neuromuscular junction. Both toxins block the generation of spike potentials in nerve and muscle fibres by interfering selectively with the initial increase in sodium permeability, but they do not affect the end plate receptor. This finding is a welcome addition to the evidence showing that conduction in nerve and muscle membrane is fundamentally different from transmission across synapses. This evidence was recently reviewed by Kao<sup>6</sup>. Kao and Nishiyama<sup>4</sup>, however, found discrepancies between the actions of saxitoxin and tetrodotoxin on the miniature end plate potentials. In some cases, saxitoxin produced a transient increase in the frequency of these miniature end plate potentials and the amplitude of these potentials



became somewhat smaller after a long period of immersion. This problem awaits further experiment.

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### Effect of $\alpha$ -Methyl Dopa on Acetylcholine Content of Rat Brain, Heart and Intestine

RESERPINE, guanethidine and syrosingopine, which are potent hypotensive drugs, have been shown to produce side effects suggestive of increased parasympathetic activity. Some of these effects, such as bradycardia and diarrhoea, are attributable to an increased acetylcholine content of heart and intestine<sup>1,2</sup>.

$\alpha$ -Methyl dopa, a potent inhibitor of dopa-decarboxylase, is a widely used hypotensive drug of clinical value. Its hypotensive action is believed to be caused by the inhibition of dopa-decarboxylase and subsequent depletion of the catecholamines and 5-hydroxytryptamine of the tissues. Nevertheless, recent studies suggest that this effect is not the result of decarboxylase inhibition<sup>3,4</sup> but probably results from an action more like that of reserpine and guanethidine. Like reserpine and guanethidine,  $\alpha$ -methyl dopa produces symptoms such as postural hypotension, bradycardia and diarrhoea<sup>5</sup>. It has already been shown that reserpine, which causes a depletion of 5-hydroxytryptamine and catecholamines, increases the acetylcholine content of central and peripheral nervous tissues, while its side effects are caused by an increase of acetylcholine content<sup>6</sup>. It was therefore considered worth while to study the effect of  $\alpha$ -methyl dopa on the acetylcholine content of brain, heart and intestines.

A group of eight male albino rats (weighing 100–150 g) were injected twice daily with  $\alpha$ -methyl dopa (100 mg/kg) intramuscularly for three days. An equivalent amount of normal saline was injected in a similar group of six control rats. The animals were killed on the third day, 2 h after the last injection. Extracts of brain, heart and a piece of intestine were prepared by using buffered saline (pH 4.0) and eserinated Ringer solution after the method of Richter and Crossland<sup>7</sup>. The tissues were not frozen beforehand but were placed in a cooled extractant solution to avoid reducing the yield of acetylcholine by freezing. The extractant solution was cooled by immersion in an ice salt mixture. The acetylcholine content was assayed on frog rectus muscle bathed in eserinated (10<sup>-6</sup>) Ringer solution.

The results (Table 1) show that  $\alpha$ -methyl dopa causes an increase in the acetylcholine content of brain, heart and intestine.

The increase in acetylcholine content of heart and intestine may account for the side effects of  $\alpha$ -methyl dopa such as bradycardia and diarrhoea.  $\alpha$ -Methyl dopa is reported to produce sedation when used in hypertension in human subjects<sup>8,9</sup> and in animals<sup>10</sup>. The sedation may be responsible for an increase in the total brain acetyl-

choline because it has been shown that the acetylcholine content of the brain bears an inverse relation to the degree of prevailing activity; an increase in the acetylcholine content of the brain after administration of a number of central depressants may be a result of the decreased utilization of neurohormone in the depressed brain<sup>7,11,12</sup>.  $\alpha$ -Methyl dopa was also found not to possess any anticholinesterase activity, because it failed to potentiate the acetylcholine response of frog rectus muscle. Thus it appears that the action of  $\alpha$ -methyl dopa, like that of reserpine, involves not only depletion of sympathetic transmitter but probably also facilitation of parasympathetic activity.

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### Decreased Concentration of Tissue Cortisol in the Menopause by a New Micromethod

DETERMINATION of plasma cortisol (hydrocortisone) with spectrophotofluorometric techniques has been possible as routine for the last 4–5 yr. No method has been available for the quantitative measurement of cortisol in small tissue samples, however. In our research on drug effect on cortisol metabolism and distribution we have recently devised a spectrophotofluorometric micromethod which allows quantitative determination of cortisol in tissue samples of 40–70 mg. This method may be readily adapted to material obtained by needle biopsy from skin and subcutaneous connective tissue.

This is a preliminary account of the procedure, which involves obtaining skin and subcutaneous tissue from the gluteal region by needle biopsy. The tissue is homogenized with sand, washed with petroleum benzene and transferred to alkalized dichloromethane. After evaporation at 43°–45° C with nitrogen, water and tetrachloromethane are added. The tissue is then washed twice with tetrachloromethane for quantitative removal of corticosterone and the cortisol phase is transferred to dichloromethane and finally to the fluorescent reagent, a mixture of ethanol and sulphuric acid. The fluorescence is measured using microcells (50  $\mu$ l.) in an 'Aminco Bowman' spectrophotofluorometer (excitation wavelength, 468 m $\mu$ ; emission wavelength, 524 m $\mu$ ). The amount of cortisol is calculated from a standard curve obtained by measurements of standards similarly treated.

To avoid variations caused by the considerable diurnal fluctuations known to be present in the plasma and possibly in the tissue, plasma and tissue were sampled at approximately 7.30 a.m. The samples were obtained from thirty female and thirty-two male patients selected from a general medical ward. Obese patients, those with endocrine disorders, and those undergoing hormonal

Table 1. ACETYLCHOLINE CONTENT OF DIFFERENT TISSUES OF RATS  
(Results are expressed in  $\mu$ g/g tissue, mean  $\pm$  S.D.)

	No. of experiments	Brain	Ileum	Heart
Control	6	2.38 $\pm$ 0.56	5.9 $\pm$ 1.41	3.08 $\pm$ 0.56
Treated with $\alpha$ -methyl dopa	8	4.67 $\pm$ 0.86	9.48 $\pm$ 0.65	5.09 $\pm$ 1.57

**Table 1.** CONCENTRATION OF CORTISOL IN PLASMA (ng/ml.) AND TISSUE (ng/g) OBTAINED BY NEEDLE BIOPSIES IN THE GLUTEAL REGION OF PATIENTS

Age in years	Females		Males	
	Plasma	Tissue	Plasma	Tissue
22-36	123.1 (8) 85-225	81.3 (8) 31-180	129.4 (8) 69-221	59.1 (8) 40-72
52-66	102.8 (9) 71-169	56.9 (10) 26-101	137.6 (11) 65-254	76.8 (11) 0-159
22-36 and 52-66	112.4 ± 10.4 (17)	67.7 ± 9.2 (18)	134.2 ± 12.6 (19)	69.4 ± 7.3 (19)
37-51	117.5 ± 13.8 (12)	37.8 ± 10.4 (12)	140.5 ± 15.7 (13)	53.4 ± 10.6 (13)
<i>P</i>	> 0.7	< 0.05	> 0.7	> 0.2

In the upper part of the table are given mean values and range, in the lower part mean ± standard error of the mean. Figures in parentheses indicate number of experiments.

therapy were excluded from this study. The results are presented in Table 1. The average concentration of plasma cortisol showed no significant variation with age or sex, but individual variations were considerable. These data are in agreement with the results of other investigators. In contrast, the tissue concentrations of cortisol show a marked decrease in females aged 37-51, that is, the menopausal years. Only a few patients in this group had "normal" or high tissue concentration of cortisol. There was a statistically significant difference between this group and the other two groups of females ( $P < 0.05$ ). No such difference was noted in males.

The present work offers evidence that tissue levels of cortisol are decreased in a state of presumed oestrogen deficiency. It has been known previously (for references see Plager, Schmidt and Staubitz<sup>1</sup>) that the exogenous administration of oestrogens and the increased oestrogenic activity of pregnancy are associated with increased plasma cortisol levels, protein bound as well as unbound. There have been no signs of hypercorticism, however, and consequently concentrations of cortisol in tissue were presumed to be within normal concentrations. As stated by Plager *et al.*<sup>1</sup> the theory of a relation between unbound plasma cortisol and tissue cortisol is not consistent with all observations, and the proportion of unbound to total plasma cortisol may be of greater importance, but there have been no direct investigations of concentrations of cortisol in tissue. The present observations do suggest that concentrations of cortisol in tissue are dependent on oestrogenic stimulation and this requires further investigation. The mechanism is obscure; our results, however, confirm that measurement of the total concentration of plasma cortisol does not give a correct impression of the concentration of cortisol in peripheral tissues; for example, in the connective tissue of the skin.

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## PSYCHOLOGY

### A Singular Lack of Incidental Learning

MORE than 200 people have been asked to recall the positions of the letters on a telephone dial, including all the staff of the Applied Psychology Research Unit (APRU), and not one has succeeded in performing the task. 151 of these people were tested formally and will accordingly be termed "subjects". The subjects were

first given a sheet of paper on which were inscribed ten circles arranged in the pattern of the telephone dial and were requested to fill in the digits. Following this they were given a sheet with a further dial with the digits correctly inscribed. On this they were requested to fill in the letters. If, as many subjects claimed, they had no idea at all where the letters were, they were requested to guess, or, in the extreme, to design their own telephone dial. Finally, a recognition test was given comprising twelve dials which had either been popular responses in preliminary tests or seemed plausible. All but one of these were required to be eliminated. The subjects, mostly members of the APRU Subject Panel, were divided into two groups, the smaller group consisting of people who had recently lived in London or had employment as switchboard operators. This sub-group was supplemented by a group from Birmingham and constituted a sample of forty-five "experienced" subjects who frequently use the letters when making local or trunk calls. The "normal" group contained 106 subjects, forty-seven of whom had no telephone in their homes.

Thirty-five of the normal group (30 per cent) and five of the experienced group (11 per cent) reproduced the digits incorrectly. Twenty-two of these began with 0 in place of 1 and moved all the digits up, and eighteen put the digits clockwise, eleven with 0 in the correct place. A slightly higher proportion of those without telephones in the normal group mistook the digits.

No one succeeded in correctly recalling the letters. Partial learning was estimated by considering the following features of the letters on a dial:

(a) they are ordered and run anti-clockwise, largely in threes (termed hereafter "admissible"); (b) O goes with 0—(O); (c) I has no letters with it—(I); (d) Q goes with 0 (on some dials it is absent)—(Q); (e) Z is absent—(Z).

Seven of the experienced group (15.5 per cent) and forty-one of the normal group (38.6 per cent) did not fulfil condition (a) and were excluded from the further analysis. They included three subjects who wrote

① ATU; ② BSV; ③ CRW; ④ DQX; ⑤ EPY;  
⑥ FOZ; ⑦ GN; ⑧ HM; ⑨ IL; ⑩ JK; which

has a pleasant if unusual pattern, and nine subjects who had the letters running clockwise but otherwise ordered. Of the remainder the number who correctly recalled the features are given in Table 1.

Table 1

	Total No. of subjects	Admissible	No. with feature correct				Total No. of features correct
			0	1	Q	Z	
Normal	106	65	12	4	5	10	31
Experienced	45	38	16	6	4	10	36

As expected, the experienced group produced a higher proportion of admissible dials, and had a higher proportion of the features correct.

In the recognition test two of the twelve examples were termed "correct", one with and one without the Q. Only ten of the normal group selected one of the correct responses, compared with nine of the experienced group. Neither of these figures differs significantly from chance. Half the recognition dials had no letters in the digit 1 position, but only thirty-three of the normal group selected one of these. This is significantly less than chance ( $P < 0.001$ ). Twenty of the experienced group recognized this feature. Eight of the twelve dials had O with 0, but only forty-four of the normal group selected one of these dials compared with thirty-one of the experienced group. The normal group again performed worse than chance ( $P < 0.001$ ). It would seem, then, that despite any residual memory the Post Office dials are not as the population would expect.

Subjects were asked to indicate their confidence in their responses to the recognition and recall tests by writing a digit from 1 for complete confidence to 5 for certainty that the response was incorrect with three intermediate categories of variable uncertainty. Sixty-three of the normal group were certain that their responses in the recall task were incorrect; no subject wrote 1. In this group only two subjects were confident they had recognized the correct dial (both unjustifiably); seventeen were certain they were incorrect. Seven of the experienced group were confident they were correct, of whom three in fact were.

One of the observations for which proponents of reinforcement theories of animal learning have difficulty in accounting is that animals appear to learn without motivation<sup>1</sup>. Another observation which old-fashioned behaviourists find unreasonable is that monkeys will perform complex tasks merely to be allowed to look out of a window—to satisfy their general curiosity<sup>2</sup>. It is thus of some considerable theoretical interest that so many people are incapable of recalling the layout of the digits of a telephone dial and that an almost negligible proportion of those tested were able to recall even individual features of the letter distribution or to recognize these features. It is clear that memory for the letters is better among those who, as a group, most frequently use them, but even this group performs badly in view of the amount of experience they must have had. For reasons of incidental learning or curiosity, better performance might have been expected. One possible explanation of these results may lie in the amount of time it takes for the dial to return to the resting position. In this time one can search for the next letter or digit and anticipation may in fact be a hindrance.

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### Monocularly Evoked Electroencephalogram Potentials: Influence of Target Structure presented to the other Eye

THE average evoked electroencephalogram potentials (EP) in humans are reduced in amplitude if the flashing target is structured instead of uniformly illuminated<sup>1</sup>. Furthermore, if the flashing stimulus is presented to one eye, the EP is decreased when the other eye sees a steadily illuminated target instead of a dark field<sup>2,3</sup>, or when the image moves instead of remaining stationary on the retina<sup>2</sup>. These effects were attributed to the increased complexity of the presentation, which absorbs more cortical capacity and leaves less to participate in the evoked response. The question arises whether the EP elicited by monocular

flash stimulation is sensitive enough to reveal changes related to the target structure presented to the other eye.

A troposcope was used to present light flashes generated by a sound-shielded Grass stroboscope to the right eye at a rate of 3.6/sec. The flashes illuminated a circular diffusing area subtending 20° in the visual field. This stimulus remained constant for all viewing conditions, except that the lamp was operated at different intensities depending on the tolerance of each subject. The ancillary optics reduced the light intensity by a factor of 1,000. The other arm of the troposcope presented to the left eye a 20° circular target continuously illuminated by tungsten light. The luminance in the plane of the pupils was 0.3 mlamberts. The left and right target areas were aligned so that they appeared superposed. Four different circular targets (three only with some subjects) were used—a blank field, a central black dot, an erect cross, and an erect grid of seventeen equally spaced lines along each axis. The dot and the lines all subtended 5 min of arc. The subjects were instructed to fixate carefully the centre of the left target. The targets were presented in sequence, each for 45 sec, and an interval of 100 sec was allowed for recovery after each run; during this period the subject was responsible for changing the target. Each target was shown at least five times during one experimental session. The results were collected and processed for seven female subjects (22–28 years of age). All the results presented here were collected from one experimental session with each subject. During the session, the subject was alone in a darkened sound-attenuating room with a constant background of pseudo-white noise. The pupil of the right eye was maximally dilated and both eyes were adapted for 30 min to the low ambient level of illumination in the room.

Beginning 15 sec after the start of the repetitive light flashes, 100 amplified EP were averaged from parieto-occipital electroencephalogram leads from each hemisphere in two channels of a CAT computer. The averaged results were then transmitted to a digital computer for processing.

The EP averaged during bilateral stimulation can be influenced by many experimental conditions; changes of vigilance and attention on the part of the subject are important, as is the significance of the flashing target. The flash parameters can be rigidly controlled, and vigilance and attention can be maintained at a fairly constant level in co-operative subjects by complete regularity of experimental regime, shielding from extraneous influences (such as sudden noises), shortening the length of each run as much as possible, and providing the subject with an active part to play in the experiment in the rest periods between runs.

Correct subjective superposition of the two images was also found to be critical; if the two images are presented to non-corresponding retinal areas, the resulting EP is quite different from that generated when the stimulus areas are superposed (Fig. 1, A and B). Accurate fixation was

Table 1. ROOT MEAN SQUARE VALUES FOR THE AVERAGED EVOKED POTENTIALS FROM BOTH HEMISPHERES OF ALL SEVEN SUBJECTS IN THE DIFFERENT VIEWING CONDITIONS

Target	Subject 1		Subject 2		Subject 3		Left hemisphere Subject 4		Subject 5		Subject 6		Subject 7	
	RMS $\mu$ V	P	RMS $\mu$ V	P	RMS $\mu$ V	P	RMS $\mu$ V	P	RMS $\mu$ V	P	RMS $\mu$ V	P	RMS $\mu$ V	P
Blank	—		—		—		3.338		0.927		2.132		3.050	
Dot	2.808	< 0.01	3.567	0.03	2.552	< 0.01	— (< 0.01)		0.720		1.849		2.579	
Cross	2.321	0.10	3.056	0.20	1.968	0.20	2.414		0.848		1.606		2.160	
Grid	2.191		2.916		1.895		2.139		0.867		1.377		2.141	
	Right hemisphere													
Blank	—		—		—		2.033		2.376		2.410		2.556	
Dot	2.292	< 0.01	2.200	0.05	2.950	< 0.01	— (< 0.01)		2.384		1.976		2.322	
Cross	1.916	*	1.821	*	2.624	0.41	1.354		2.106		1.589		1.869	
Grid	1.938		1.864		2.594		1.147		1.833		1.476		1.277	

The entries under P indicate the *t* test significance of the decrease between neighbouring root mean square values. An asterisk marks those points where an increase occurred. The two P values in parentheses give the significance of the root mean square decrease between next neighbours. For further explanation see text.

therefore emphasized and the subjects were trained during the adaptation period. When all these controls were applied, the averaged EPs for each subject became highly reproducible (Fig. 1C).

Fig. 2 shows the mean of five averaged EPs for subject 7 in each of the four viewing conditions; the most striking feature of these curves is the overall decrease in amplitude as the amount of structure in the left image increases. We have chosen the root mean square value about the mean of the averaged EP to characterize the amplitude of each averaged EP (Table 1). Of the thirty-two neighbour-pairs which can be obtained from this table, twenty-seven show a decrease in the root mean square value as structure increases, nineteen of these being significant at the  $P = 0.05$  level or better. The five observations which show an increase are not significantly different from their neighbours at the  $P = 0.1$  level. If we were to consider decreases over intervals greater than neighbour-pairs, a much higher proportion of the observations show decreases statistically significant at the  $P = 0.05$  level. The results thus show that an increase in the structure of a steadily illuminated target presented to one eye is associated with a decrease in the amplitude of the potential evoked by a flashing light seen by the other eye.

Factors which were not stable in our experimental conditions and which might have influenced the results are:

(1) The introduction of opaque structure in the steady field decreases the mean target luminance. The maximum decrease was caused by the grid target and was equivalent to 0.15 density units. In control experiments, a similar

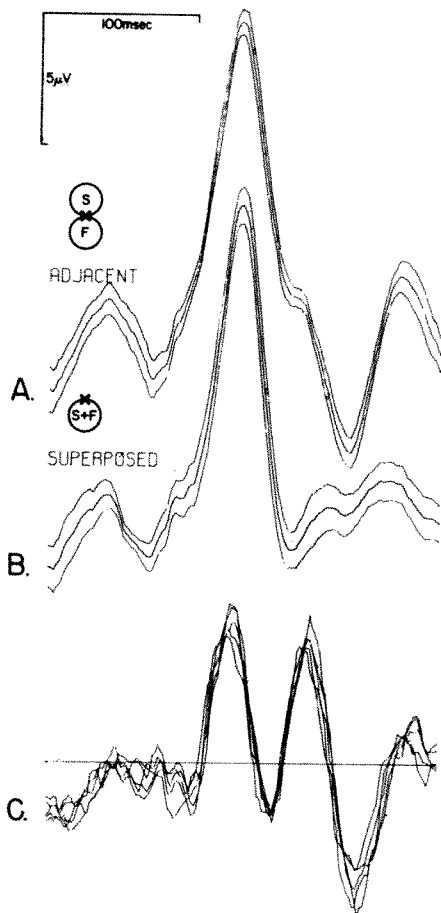


Fig. 1. Each trace represents the mean and its standard deviation of seven averaged potentials. Viewing conditions: A, Steady target (S) and flashed field (F) subjectively adjacent. B, Steady target (S) and flashed field (F) subjectively superposed. Fixation point indicated by a cross in the diagram; this point was chosen so that S fell on retinal areas of roughly equal sensitivity in both viewing conditions. C, Superimposed averaged evoked potentials from five experimental runs in subject 6 with blank target for the left eye.

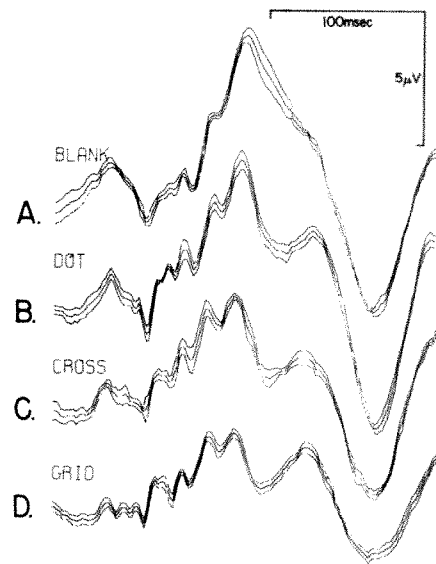


Fig. 2. Each trace represents the mean and its standard deviation of five average evoked potentials of subject 7 (left hemisphere). A, Left eye sees blank; B, left eye sees dot; C, left eye sees cross; D, left eye sees grid.

decrease in luminance of the blank target gave no consistent changes of the EP, but a further decrease of 0.40 density units resulted in increased EP amplitudes, thus exerting an effect in a direction opposite to that of the grid.

(2) Each of the steadily illuminated targets imposes a slightly different visual task on the subject. The subjects ranked the targets into two groups for ease of viewing: dot and cross were judged to be easy, while blank and grid were difficult. This would seem to arise from the problems of fixating the centre of an unstructured or a highly redundant area. If this introduced a major artefact into the results we would not expect to find the EPs for the blank target and the grid target at the extremes of the scale.

(3) Suppression and dominance between the two eyes may contaminate the results. In fact, the subjects reported spontaneous fading and reappearance of the left eye target on various occasions. However, we have not found differences between the monocular EP recorded during perceptual fade-out or visibility of a stabilized retinal image presented to the other eye<sup>2</sup>; further, it has been shown that the configuration of the evoked response is constant regardless of which eye dominates perceptually<sup>3,4</sup>.

We conclude, therefore, that the EP elicited by monocular flash stimulation is an index of the loading imposed on the visual areas by the structure of the steadily illuminated target seen by the contralateral eye.

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## BIOLOGY

**Unique Synergistic Effects produced by the  
Principal Sex Attractant Compounds of  
*Ips confusus* (LeConte) (Coleoptera:  
Scolytidae)**

THERE is a powerful assembling scent or sex attractant in the frass produced by the male of *Ips confusus* boring in ponderosa pine (*Pinus ponderosa*)<sup>1</sup>. These attractants evoke the concentration flight or mass attack by individuals of both sexes<sup>2</sup>, but the females are much more responsive than the males<sup>3,4</sup>. When the insects arrive at the source of the attractant, they take part in boring, feeding, mating and oviposition.

Recently, three terpene alcohols that are believed to be the principal components of the attractant were isolated from male frass<sup>5</sup> and identified<sup>6,7</sup> and synthesized<sup>7</sup>. These compounds are:

I: (–)-2-methyl-6-methylene-7-octen-4-ol

II: (+)-cis-verbenol

III: (+)-2-methyl-6-methylene-2,7-octadien-4-ol

In a laboratory bioassay, a mixture of compound I with either II or III evoked a response from female beetles<sup>7</sup>, as did a mixture of all three compounds. The individual compounds were not attractive.

Field tests of isolated compounds I and III and synthetic compound II were begun on June 9, 1966, at the University of California Blodgett Research Forest, Eldorado County, California. The three compounds, individually and in various combinations, were presented together with a benzene extract of frass to flying populations on packets of filter paper placed on aluminium foil in the centre of 1 ft.<sup>2</sup> masonite boards covered with 'Stickum Special'. Attractive male frass was also presented in the same way by placing it under screens of plastic mesh in Petri dishes. The packets and frass were renewed at hourly intervals to compensate for the loss of attractants by volatilization and for variations in the diurnal flight of the beetles. The boards were spaced about 25 ft. apart with no particular attention to exposure. A minimum of two replications was used for each test. All materials were presented in the amounts found in 1 g of male frass (I, 125 µg; II, 2.5 µg; III, 12.5 µg).

The combination of compounds I, II and III evoked the most potent response; the entire catch (fourteen males and eighteen females) occurred within about 20 min on the one day it was tested. Compounds I and III together attracted four females only. The beetles attracted to frass (fourteen males and forty-five females) and frass extract (twelve males and twenty-one females) were trapped over a period of 2 days. Compounds presented individually and in other combinations failed to attract a single specimen of *I. confusus*. Surprisingly, *Ips latidens* (LeConte) was attracted to compound I alone (five females) and to a combination of I and II (three males and thirty-four females). No bark beetles were trapped on the blank control boards.

After this unexpected development, *I. latidens* was exposed to all the synthetic compounds both individually and in combination, in the laboratory olfactometer. Compound I alone (20 µg), and in combination with II, definitely affected the behaviour of both sexes. The response to compound I alone is best described as an arrestant; the beetles circled very slowly in the air stream and some males eventually traversed to the receptacle containing the attractant compound. The characteristic klinotaxis<sup>8</sup> was not observed, however. Compounds I and II (2.0:0.1 µg) did evoke the typical klinotaxis, moderately for the female, weakly for the male. No response was elicited by the combination of I and III and by III alone. Compound II alone and II

with III at 10 µg each evoked at most a weak response. But when 0.1 µg of compound III was added to 2.0 µg of I and 0.1 µg of II, the attraction for *I. latidens* was eliminated. This combination proved highly attractive to *I. confusus*, however, even at one-tenth this concentration.

The unusual synergistic system obtained by adding compound III to the combination of I and II, which blocked or masked the *I. latidens* attractant and created the *I. confusus* attractant, poses some challenging chemical and biological problems. We are at present comparing the chemical fractions of *I. latidens* frass with those of *I. confusus*.

These studies demonstrate the deviations between laboratory and field evaluations and emphasize the necessity of using both bioassays to identify pheromones.

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### Volatility of Trail Marking Substance of the Town Ant

WE have found that the trail marking substance laid down by the town ant, *Atta texana* (Buckley), contains at least two components, one volatile and the other non-volatile; both are followed by workers. Both components partition into the organic phase of a methylene chloride water system.

Poison sacs from five medium-sized workers were crushed in 1 ml. of methylene chloride, and the solutions were dispensed with a pipette on circles 14 cm in diameter drawn on smooth cardboard. Polyethylene plastic sheets 0.13 mm thick were placed 0.1, 1, 2 or 4 cm over these artificial trails on solid circumferential spacers, thereby creating a closed air space between trail and plastic sheet. Holes were punched in the sheet directly above the trail with a No. 1 insect pin. For most tests, eight holes per cm were punched in each of two parallel rows 1.5 mm apart (about the distance between antennae of a minor worker).

In each test, five minor workers were released on the plastic sheet in the centre of the circle. After 10 min, actions of ants that responded were classed as (1) circling, when at least one worker travelled the entire circumference of the circle (44 cm); (2) partial circling, when a worker travelled only part of the circumference; and (3) detecting only, when workers briefly oriented toward a hole but did not follow the trail. The last two conditions were accompanied by zigzagging as the ants alternately found and lost the scent.

Height above the trail, distance between holes, and trail age affected insect behaviour. The ants circled as high as

2 cm and one partially circled at 4 cm. Workers circled when holes were spaced as far apart as 1 cm.

Response was maximum when plastic sheets were positioned immediately after trails were made. When sheets were spaced at 1 mm, workers circled a 30 min old trail in the arbitrary period of 10 min. If the sheet was laid over the trail after 45 min, they only partially circled in the same amount of time. Little if any response occurred after 60 min. Some of the volatile substance remained, however, because workers easily circled trails that were 120 min old when the plastic sheet had been placed 60 min before the bioassay. Apparently, this gave the volatile fraction time to accumulate sufficient vapour pressure under the plastic to trigger a response by the ants.

At a height of 2 cm, ants circled only if the plastic sheet was positioned within 3 min. They partially circled when the sheet was placed after 5 min, but they did not respond when it was placed after 10 min.

The non-volatile fraction gradually decomposed, probably by oxidation. Workers circled on artificial trails that had been kept in darkness at room temperature for 6 days, but only partially circled trails kept for 12 days. In contrast, trails were circled that had been held under a vacuum of 0.4 mm of mercury for 4 months.

The ants always laid a natural trail on the plastic as they detected the artificial trail below. Other ants followed both the volatile and non-volatile fractions of natural trails when sheets were removed from artificial trails. Thus, the number of workers that followed the volatile material was meaningless for statistical purposes. The trail reinforcing behaviour is in contrast to observations in the field where workers seldom touched their abdomens to the trail<sup>1</sup>.

Workers of *Trachymyrmex septentrionalis* (McCook) readily circled 1 mm above the artificial trail of *Atta texana*, and on the trail itself after it had been held in a vacuum of 0.4 mm of mercury for 3 days. Thus neither fraction is the species specific substance postulated by Blum *et al.*<sup>2</sup>. *T. septentrionalis* workers also laid a natural trail on the plastic.

In another test, five sacs of *Atta texana* were crushed in 1 ml. of methylene chloride, and the solution was thoroughly extracted with six 1 ml. portions of water. A trail prepared from the extracted solution elicited a circling response at a height of 1 mm; after 2 days under a vacuum of 0.5 mm of mercury the trail was circled by workers in direct contact with it. The first aqueous extract was in turn extracted with five 1 ml. portions of methylene chloride. A trail prepared from the aqueous solution elicited only a very feeble response from workers placed in direct contact with it and no response from workers above it.

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### Forked Tail of the Cercaria of *Schistosoma mansoni*—a Rowing Device

THE swimming motions of the cercaria of *Schistosoma mansoni* have been analysed by high-speed photography, at a rate of 500–800 pictures/sec. When cercariae swim backwards the two branches of the forked tail are spread out (Fig. 1a and b), while in forward-swimming cercariae the branches are close together (Fig. 1c and d). Without

significantly altering the stroke rate of the tail, cercariae can change from backward swimming to forward swimming, or the reverse, depending on how they align the branches of the tail. When changing from backward swimming to forward swimming the cercaria lengthens its body by 30–40 per cent (Fig. 1c and d) and at the same time makes itself narrower.

When swimming backwards the cercaria has two constrictions, which are not present when it is swimming forwards. One of the constrictions is between the fork and the stem of the tail, and the other is at the end of the body near the base of the tail (Fig. 1a and b). These two constrictions have the function of joints.

In the tail stem there are between forty and forty-eight longitudinal muscle elements with distinct transverse striations. The longitudinal muscles of the left and right side contract alternately and in turn bend the tail stem from one side to the other. The rate of oscillations of the tail in ten cercariae swimming backwards ranged from 20.7 to 23.7 sec, mean value 22.1, and in cercariae swimming forwards it was 20.9–23.6 (mean value, 22.5)/sec. The shape and poise of the body and the fork of the tail determine whether the cercaria swims forwards or backwards. Smooth muscles (also circular muscles) seem to effect these changes in shape and poise. Fig. 2 shows the behaviour of the body and the branches of the fork, which resemble oar blades, when the stem of the tail is active. Seventeen successive phases during one stroke of the tail are shown as the cercaria swims backwards. The centre line through the cercaria is shown in the position it would occupy at 1.6 mm/sec intervals. In Fig. 2 a body and its fork are in a more relaxed position after having been deflected by the resistance of the water during the preceding stroke. In Fig. 2b the fork is carried upwards at an angle of incidence which permits only slight propulsion, and in Fig. 2c the fork is driven downwards producing a considerable backward thrust. By this movement body and fork are now deflected in the opposite direction and in Fig. 2d they return to a more relaxed position.

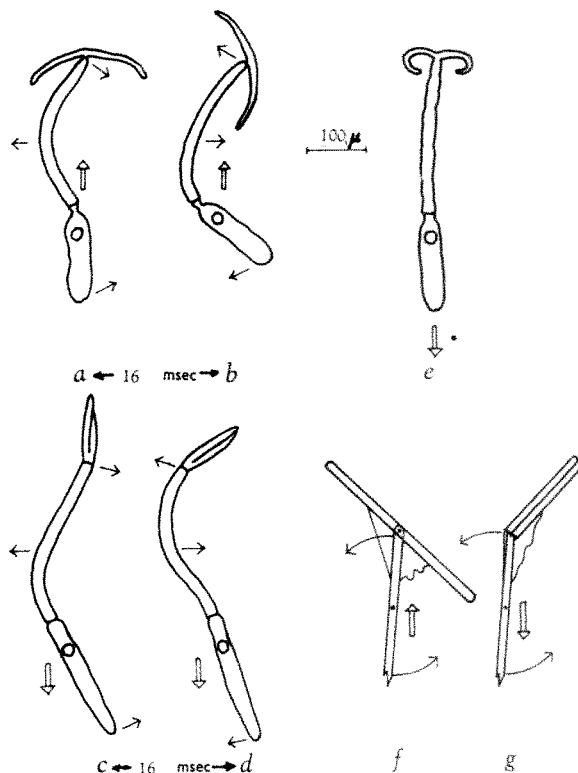


Fig. 1.

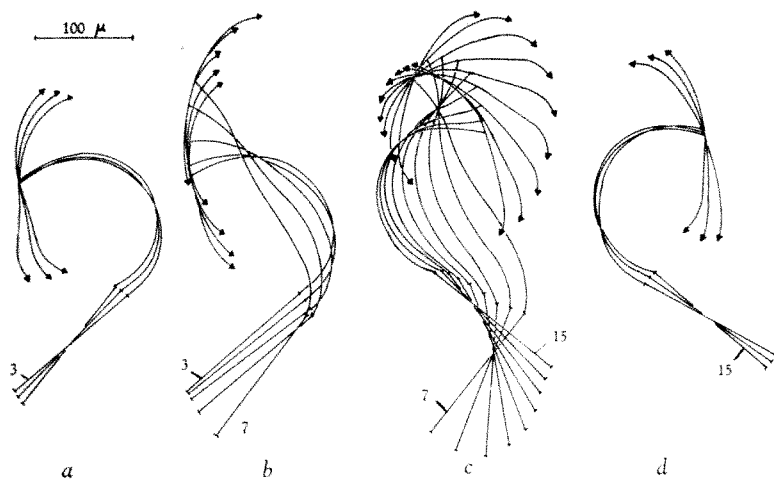


Fig. 2.

Not only the fork of the tail but also the body of the cercaria can produce a weaker propulsion and a stronger backward thrust (Fig. 2b). In this case the body works on the principle of the fish-tail, but bending is largely replaced by oscillation around a joint-like area. As the cercaria swims forward (Fig. 1c and d) the folded fork branches function like an elastic tail fin. The connexion between body and tail has lost its flexibility and is now actually rigid. As a result of new properties of resistance a wave passes along the cercaria from the body to the tail end with all striated muscles equally active (Fig. 1c and d). This results in propulsion. The maximum speeds for animals swimming backwards or forwards are 1.4 mm/sec; the distance covered in 1 sec corresponds to about three body lengths.

Swimming backward and forward by means of an adjustable forked tail has been reproduced in a model (built by H.-G. Beckmann and called "Cercaria I"). A forked tail made of rigid material with a joint as in Fig. 1f is sufficient to produce backward movement which can be converted to propulsion and vice versa, even in the model, by folding backwards the branches of the transverse oar blade and by retaining the limited range of the joint (Fig. 1g). Models of this kind could perhaps be applied to mixing tasks and driving mechanisms in engineering.

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### Decrease in Eggshell Weight in Certain Birds of Prey

THE incidence of broken eggs in nests of peregrine falcon *Falco peregrinus*, sparrowhawk *Accipiter nisus* and golden eagle *Aquila chrysaetos* in Britain has increased considerably since 1950. In 109 peregrine eyries examined in 1904-50, there were only three instances of egg breakage, compared with forty-seven in 168 eyries examined in 1951-66. Two of thirty-five golden eagle eyries examined in 1936-50 contained broken eggs, compared with twelve out of forty-eight examined in 1951-63. One breakage was found in twenty-four sparrowhawk nests in 1943-50, but eight in twenty-seven nests in 1951-60. Peregrines have been witnessed eating their own eggs<sup>1</sup>, and most recent egg breakages in all three species appeared to involve parental destruction.

Eggshell thickness was investigated by measuring weight and size of blown eggs of varying age, using

specimens cleanly emptied through a hole not exceeding 7 mm in diameter, thus rejecting heavily incubated eggs. In peregrine and sparrowhawk, eggshell weight/size ratio decreased significantly and suddenly in 1946-50, and has not recovered. Since 1947, the only "normal" peregrine eggshells were four of the seven available from the East Highlands. After 1946, sparrowhawk eggshells from Surrey were significantly lighter than those from Cumberland, Dorset and Hampshire. Golden eagle eggshells from West Scotland are slightly yet very significantly lighter but East Highland eggshells remain unchanged. Fewer golden eagle eggs were available, but change during 1945-50 is again indicated.

The eggshells as measured consisted largely of calcium carbonate (about 90 per cent in "normal" eggs), the remaining fraction being shell protein<sup>2</sup>, adherent shell membranes, and residual film of contents. The decrease in eggshell weight has involved mainly the calcium carbonate fraction. Decrease in shell thickness rather than density is implied, but this has not been measured directly.

Thickness of eggshells and production of eggs vary according to diet (especially calcium) and condition in poultry; eggshell thickness is also genetically influenced, and diminished by disease and increasing age<sup>3</sup>. Certain chemicals, for example sulphanilamide<sup>4</sup>, cause decrease in eggshell thickness, as can stress; severe fright can stop poultry laying for a time. Occasional thin-shelled eggs may be laid by any wild bird species, but before 1946, mean eggshell weight and size for clutches from the same female raptor, year by year, were fairly constant, though weight/size of eggshells within one clutch was less consistent.

Decrease in eggshell weight in peregrine and sparrowhawk was synchronous, rapid and widespread. It occurred in successive clutches of the same female peregrines at four different eyries, but three other females laid at least one clutch of normal weight after they had produced light eggs. General ageing or genetic shift in the population are thus not likely to be involved, while the synchronous effect on three raptors with different ecology and distributions, and the varying geographical response, make disease an unlikely explanation.

Decrease of calcium carbonate for eggshell formation could result from reduced food consumption, but no decline in available food supply for these raptors was known in 1940-50. Loss of hunting efficiency could cause an effective food shortage, or change in metabolic regulation could induce internal shortage of calcium, without less food being consumed. A different possibility is premature extrusion of eggs, known in poultry (personal communication from C. Tyler).

Some physiological change evidently followed a widespread and pervasive environmental change around 1945-47. Increasing radioactive contamination fails to account for geographical variations in eggshell change, whereas these match the developing regional pattern of contamination by chemical pollutants during this period. Among urban-industrial pollutants, one waste product in smoke, the persistent polychlorinated biphenyl (PCB), has been found in tissues of fish, eagles and humans in Sweden, and was first detected in 1944 in an eagle<sup>5</sup>. There was a notable boom in organic insecticides, fungicides and herbicides containing chlorine, mercury, phosphorus and sulphur after 1945, and environmental contamination by persistent residues has been widespread<sup>6</sup>. British peregrines, sparrowhawks and golden eagles have shown widespread contamination by *pp'* DDT, *pp'* DDE,  $\gamma$ -BHC, dieldrin and heptachlor epoxide<sup>7-9</sup>. The introduction of

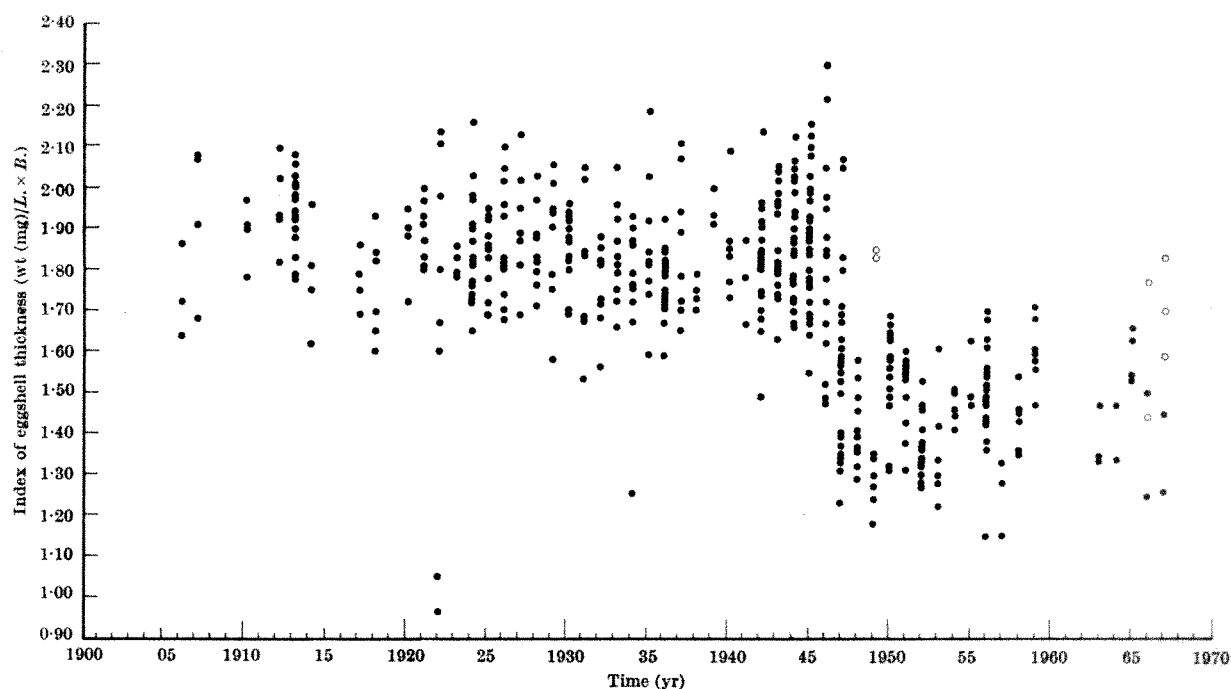


Fig. 1. Change in the ratio of weight to size (index of thickness) in eggshells of the peregrine falcon in Britain. Circles represent eggshells from the central and eastern Scottish Highlands, and dots represent eggshells from other districts (see Table 1).

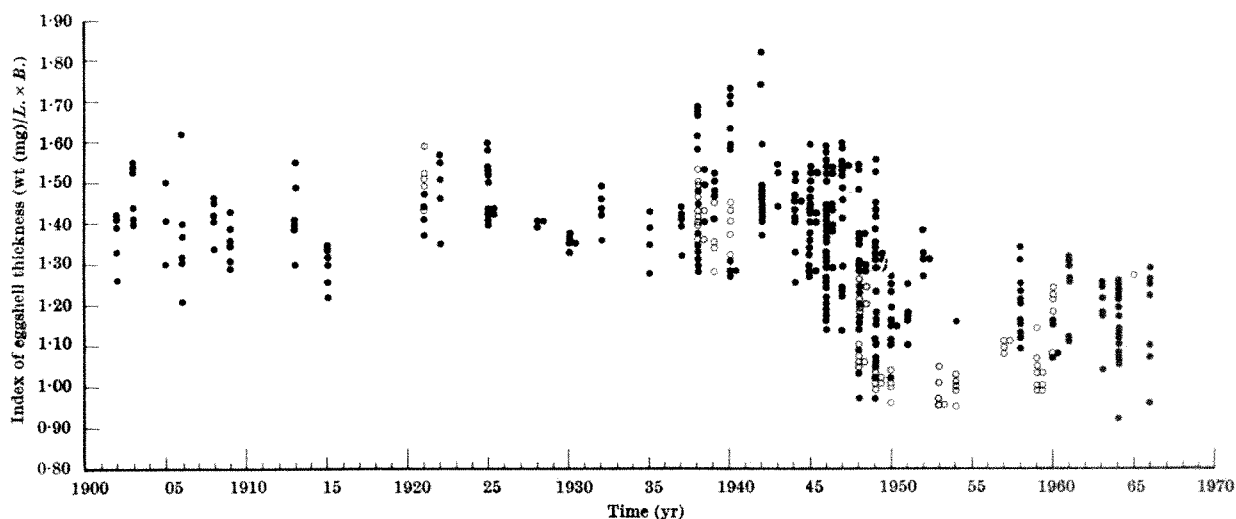


Fig. 2. Change in the ratio of weight to size (index of thickness) in eggshells of the sparrowhawk in Britain. Circles represent eggshells from south-eastern England, and dots represent eggshells from other districts (see Table 1).

DDT into general use (about 1945–46) coincided closely with onset of the eggshell change. Dieldrin, aldrin and heptachlor appeared 10 yr later and, like DDT and  $\gamma$ -BHC, have been used extensively ever since. Contamination of birds by persistent organo-mercury compounds has received little attention in Britain, but in Sweden the introduction of these as seed dressings paralleled the substantial increase in mercury content of raptor tissues during the 1940s (ref. 10).

Calcium metabolism in birds is controlled by oestrogen and parathyroid hormone<sup>3</sup>, and is thus potentially sensitive to any chemical disturbing hormone regulation. Jefferies<sup>11</sup> has demonstrated a significant correlation between intake of *pp'* DDT by the Bengalese finch *Lonchura striata* and delay in ovulation, and interprets this as a hormonal effect. One dithiocarbamate fungicide affects eggshell thickness in poultry<sup>12</sup>, but is non-persistent and of unknown relevance to raptors.

The time correlation between decrease in eggshell weight and increase in egg-breakage suggests a causal con-

nexion. In the peregrine and golden eagle there is also geographical correlation, for in the East Highlands decrease in eggshell weight is slight or unknown and egg breakage unusual. Eating eggs is probably an instinctive adaptive response to unfavourable circumstances arising after laying, and seldom invoked before 1946. Other bird species normally eat or destroy their damaged eggs<sup>13</sup>, and lighter raptor eggs are probably more prone to accidental damage than "normal" eggs. Yet, often, raptors have evidently destroyed undamaged eggs, perhaps in response to a calcium "hunger". Domestic fowl which have broken and eaten an egg often develop an incurable appetite for their eggs. While calcium is obviously implicated, in several raptor instances the egg contents had been eaten and most of the shell left.

Other bird species in which egg-breaking is unusual (hobby *Falco subbuteo*, merlin *F. columbarius*, buzzard *Buteo buteo*, carrion crow *Corvus corone*, golden plover *Charadrius apricarius*, greenshank *Tringa nebularia*, guillemot *Uria aalge*, and razorbill *Alca torda*) show no



Table 1. MEASUREMENTS OF EGGSHELLS OF THREE BRITISH RAPTORS, 1900-1967

Species	District		No. of eggs		Mean eggshell weight (g)		* Mean index of eggshell size, length (mm) × breadth (mm)		† Mean index of eggshell weight (mg) $L \times B$		Probability of significant difference between data in columns 9 and 10
	1 1900-46	2 1947-67	3 1900-46	4 1947-67	5 1900-46	6 1947-67	7 1900-46	8 1947-67	9 1900-46	10 1947-67	
Peregrine	S. England	S. England	371	158	3.81	3.09	2,072	2,102	1.84	1.47	< 0.001
	N. England	N. England									
	Wales	S. Scotland									
	Scotland										
	Ireland										
	As above	C. and E. Scottish Highlands	371	7	3.81	3.59	2,072	2,096	1.84	1.71	—
Sparrowhawk	S. England	S. England	229	188	1.83	1.54	1,293	1,293	1.42	1.19	< 0.001
	Midlands	Midlands									
	N. England	N. England									
	Wales	Wales									
	Scotland	Scotland									
	Sussex	Surrey	25	51	1.81	1.38	1,273	1,292	1.42	1.07	< 0.001
	Surrey		Included in above totals								
	Kent										
Golden eagle	Western Central and Eastern Highlands	Galloway and Western Highlands	107	26	14.37	13.19	4,553	4,563	3.16	2.89	< 0.001
	As above	C. and E. Highlands	107	14	14.37	14.45	4,553	4,630	3.16	3.12	—

\* These measurements show a sufficiently close relationship to surface areas of the eggshells to justify using the more simply derived length × breadth ( $L \times B$ ) as an index of size.

† This is presumed to indicate thickness, but it could also indicate density of eggshell.

change in eggshell weight. In kestrel *F. tinnunculus* and raven *Corvus corax*, the eggs of some individuals show a slight decrease, but not the whole population samples.

For the species examined, frequency of egg-breakage, scale of decrease in eggshell weight, subsequent status of breeding population, and exposure to persistent organic pesticides are correlated. The possibility that these phenomena are links in a causal chain is being investigated. Recovery of locally depleted populations of peregrine began in 1946 and continued for several years after eggshells became lighter and egg eating frequent, even in districts, such as Dorset, where both changes were obvious. Population "crashes" in peregrine and sparrowhawk after 1955 probably involved greatly increased adult mortality, but later factors contributing to low breeding success would tend to prevent recovery.

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### Moisture Sensitivity, Mechanical Injury and Gibberellin Treatment of *Beta vulgaris* Seeds

"RUBBING" ("shearing") is a mechanical process used to separate the fruit clusters of *Beta vulgaris* L. (sugar beet or beetroot) into more or less even-sized segments, each with one or two seeds, by grinding away some of the hard tissue surrounding the seeds with an assembly of

abrasive disks. The investigation here reported followed the almost complete failure of a rubbed sample of beetroot seeds to germinate in a field in which a different rubbed sample of the same cultivar ('Detroit Globe'), sown at the same time, germinated satisfactorily. Neither sample contained any obviously damaged seeds and no damage was found when embryos excised from imbibed seeds were examined under the microscope. Natural (unrubbed) seeds from the same sources (Lot 1, good; Lot 2, bad) were later obtained. In a series of laboratory experiments we found that: (1) Germination of unwashed seeds can be depressed by insufficient moisture (Fig. 1A). (2) When beetroot seeds are washed and then immediately placed on wet filter paper scarcely any will germinate; but if they are washed, dried and then placed on moist filter paper they germinate well (Table 1). (3) Similarly, germination can be depressed when seeds are placed on a substrate which is even slightly wetter than necessary (Fig. 1); this effect is particularly pronounced with unwashed seeds (Fig. 1A) but can be alleviated by adding small quantities of hydrogen peroxide (Table 2). Warburg respirometer results show that the respiration of beet seeds is appreciably suppressed in a slight excess of water. (4) Natural seeds from Lot 2 germinate at least as satisfactorily as seeds from Lot 1, but rubbing depresses the performance of seeds from Lot 2, though not of those from Lot 1 (Fig. 1B). (5) Shaking the seeds for 45 min in a glass bottle has an effect on germination similar to that

Table 1. EFFECT OF DRYING AFTER WASHING

Seed lot	Seed treatment	Drying (h)	ml. water per dish	
			4	6
			Per cent germination	
1	Natural	0	33	4
1	"	24	93	62
2	"	0	30	15
2	"	24	97	77
2	Rubbed	0	32	9
2	"	24	73	39

Fifty actual seeds in 9 cm diameter Petri dish on three Whatman No. 1 filter papers. Six replications. 20° C.

Table 2. EFFECT OF HYDROGEN PEROXIDE ON GERMINATION OF *Beta vulgaris* L. (DETROIT) SEEDS

Hydrogen peroxide in germination water	Per cent germination		Mean
	Natural seeds	Rubbed seeds	
0	61	39	50
2%	86	77	82†
Mean	74*	58	

\*  $P = 0.01$ .

†  $P = 0.001$ .

Fifty actual seeds were placed in a 9 cm diameter Petri dish on top of three Whatman No. 1 filter papers with 8 ml. water for natural and 6 ml. for rubbed clusters, quantities excessive for satisfactory germination of the respective type of cluster. 20° C.

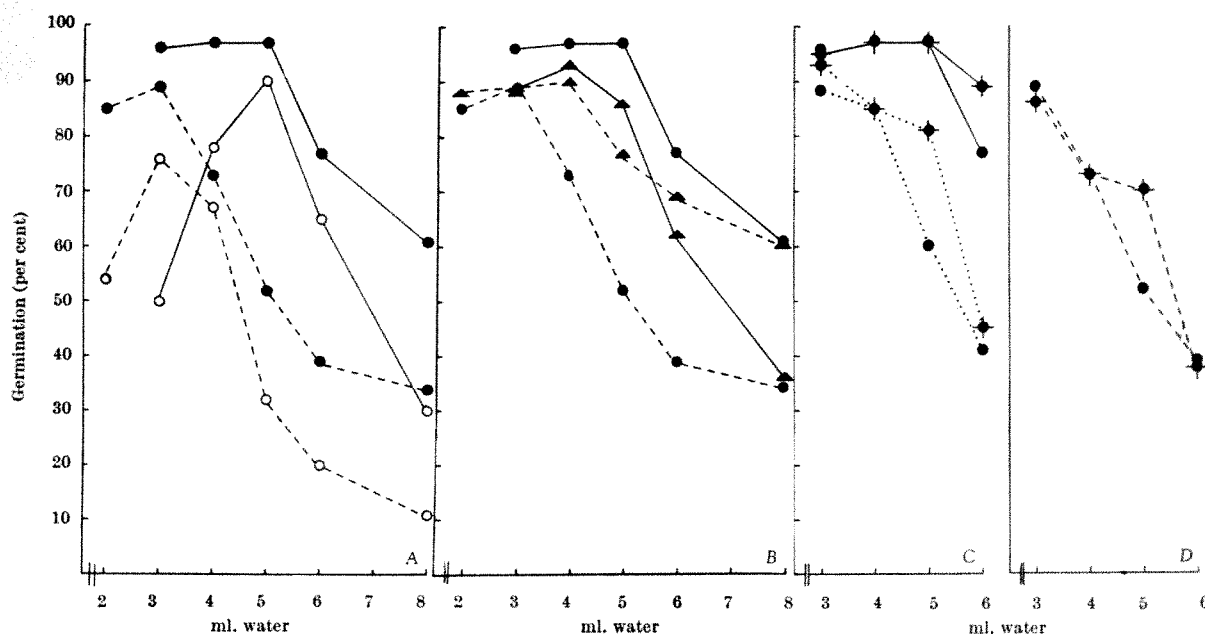


Fig. 1. Germination of individual beetroot seeds at 20°C in 9 cm diameter Petri dishes on three Whatman No. 1 filter papers in response to different treatments of seed clusters. ▲, Lot 1, washed; ○, Lot 2, unwashed; ●, Lot 2, washed; ◐, Lot 2, washed, treated with 4 p.p.m. gibberellin (Pfüzer); solid line: natural clusters; broken line: rubbed clusters; dotted line: shaken clusters. Least significant difference  $P, 0.05 = 7.3$ ;  $P, 0.001 = 13.3$ .

of rubbing (Fig. 1C). (6) Soaking the seeds in 4 p.p.m. gibberellin, followed by drying, lessens the effect of a certain level of excess moisture on germination, especially of rubbed or shaken seeds (Figs. 1C, 1D). (7) In the triphenyl tetrazolium chloride (TTC) test, in which intact cells stain red because of their dehydrogenase activity and damaged cells remain unstained, embryos from intact beetroot seeds are stained uniformly, but embryos from rubbed and shaken seeds are not. On the other hand, treatment of rubbed or shaken seeds with 4 p.p.m. gibberellin largely restores the capacity of their embryos to stain red (Table 3) in response to TTC.

Table 3. 2,3,5-TRIPHENYL TETRAZOLIUM CHLORIDE TEST OF EXCISED BEETROOT SEEDS (LOT 2)

Seed treatment	Gibberellin	No. of embryos assessed	Per cent "non-viable"
Natural	None	166	4
Rubbed	None	106	30
Rubbed	4 p.p.m.	106	18
Shaken	None	259	40
Shaken	4 p.p.m.	259	14

\* "Non-viable": embryos only partially stained or unstained.

These results confirm that natural *Beta* seed clusters contain a water-soluble inhibitor<sup>1,2</sup> the effect of which is diminished either by leaching or by removal of cluster material during rubbing<sup>3,4</sup>. But our results further suggest that: (1) Germination is impeded by excess moisture, owing to interference with respiration. (2) The effects of the soluble inhibitor in the cluster material are particularly pronounced (a) when the moisture supply is limited, but also (b) under the influence of excess moisture, even though the moisture must have diluted the inhibitor. This suggests an interaction of inhibiting principles, that is, that the restriction of oxygen supply renders the embryos more sensitive to the inhibitor in the cluster material, or *vice versa*. (3) Impact by rubbing or shaking causes damage which interferes with the dehydrogenase activity of the embryonic cells and tends to depress germination; in particular, it accentuates the detrimental effect of excess moisture. (4) Addition of gibberellin (a) restores the dehydrogenase activity, and (b) to some extent compensates for the effect of restricted oxygen supply and thereby enables the seed to germinate under

wetter conditions. The mechanisms involved are not clear at present.

We thank Dr R. G. Hiller for helpful advice.

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## Sexual Factor of the Mucorales

BURGEFF<sup>1</sup> was the first to produce evidence of the formation of a sexual factor by mated strains of the fungi *Phycomyces blakesleeanus*, *Mucor mucedo* and possibly all other members of the Mucorales. This substance, which is produced in very small amounts, is held responsible for the induction of the formation of gametangia. It appears that it is not species specific, for *M. mucedo* exhibits a positive reaction with preparations obtained, for example, from *Blakeslea trispora* as well as from *P. blakesleeanus*. Plempel<sup>2</sup> supposed that the sexual factor is a mixture of two substances that are produced by the plus and the minus strain of the fungus respectively.

We have obtained a substance showing the sexual activity described from shaken cultures of mated strains of *B. trispora* in a potato-glucose medium. It was isolated according to the method of Plempel<sup>2</sup>, identified and characterized by bioassay<sup>3</sup>, paper chromatography (Whatman No. 1; *n*-butanol saturated with 0.05 normal ammonia), and ultra-violet spectroscopy. The procedure used was as follows. The acidified medium of a 5 day old culture was extracted with diethyl ether. The extract was next fractionated by partition chromatography on a column of 'Hyflo Supercel' impregnated with 0.3 molar phosphate buffer, pH 7.7. The column was developed

Table 1. VALUES OF COMPONENTS OF AN EXTRACT OF THE CULTURE MEDIUM OF A 5 DAY OLD CULTURE OF MATED STRAINS OF *Blakeslea trispora*, PARTIALLY PURIFIED BY PARTITION CHROMATOGRAPHY ON 'HYFLO SUPERCEL'

Component	$R_F$ value*	$E_{1/1\lambda}$ †	Biological activity ‡		
			plus	towards	minus
1	0.25	-0.84	-1.50	-	-
2	0.35	-0.84	-1.50	-	-
3	0.50	-0.84	-1.50	-	-
4	0.65	-1.35§	+	+	+
5	0.90	-0.68	-0.84	-1.30	-1.50

\*  $R_F$  values of ultra-violet absorbing spots on a paper chromatogram (Whatman No. 1, *n*-butanol/0.05 normal ammonia).

† Half value potentials of polarographic waves of solutions in 1 per cent tetra-*n*-butyl ammonium hydroxide of the components that are obtained by elution with dilute alkali of the corresponding spots on the paper chromatogram. The polarographic behaviour at potentials more negative than -1.8 V was not investigated.

‡ Biological activity was determined towards the plus and minus strain of *Mucor mucedo*.

§ The polarogram of component No. 4 also has a small wave at -0.8 V and -1.50 V, attributable to impurities, for further purification on DEAE-'Sephadex' results in a biologically active preparation that gives only one wave at -1.35 V.

with hexane, benzene and diethyl ether, successively; the biologically active substance was eluted with the third solvent. Paper chromatography of this fraction separated at least five ultra-violet absorbing components of which only that with an  $R_F$  value of 0.65 was biologically active (Table 1). When this single spot was eluted with dilute alkali and rechromatographed on paper the same set of five components was obtained, so that considerable decomposition seems to take place during the isolation procedure.

It is possible to distinguish between the biologically active compound and decomposition products by d.c. polarography, using tetra-*n*-butyl ammonium hydroxide as supporting electrolyte (Table 1). This method is very suitable for relative determination of the sexual factor in partially purified extracts. It can be further purified by chromatography on DEAE-'Sephadex' with a concentration gradient of pH 9.0 *tris* buffer (Fig. 1).

The most important outcome of the experiments so far is that no indications whatsoever have been found for the occurrence of two biologically active substances, that is one active on the plus strain and the other active on the minus strain of the fungus.

More careful examination of the absorption in the near ultra-violet region revealed, however, that the isolated compound is probably a mixture of *cis* and *trans* isomers. After purification by chromatography on DEAE-'Sephadex' its ultra-violet absorption spectrum had maxima at 230 and 325 m $\mu$ . When a sample of a solution in ethanol was exposed to strong light from a fluorescent tube, the maximum at 325 m $\mu$  was much reduced and shifted towards a shorter wavelength, whereas the maximum at 230 m $\mu$  was slightly increased. If a catalytic amount of iodine was added and the sample exposed to light again there was a strong and rapid increase of the optical density at 310-325 m $\mu$ . The final absorbancy was considerably greater than that of an unexposed sample. If the latter was exposed to light in the presence of iodine, exactly the same absorbancy was obtained (Fig. 2). The reversibility of the spectral changes was a strong indication

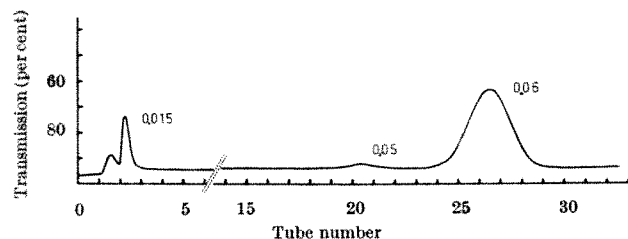


Fig. 1. Gradient chromatography on DEAE-'Sephadex' G-25 of a sexual factor isolated from the culture medium of mated plus and minus *Blakeslea trispora*. This procedure was preceded by extraction and partial purification by partition chromatography on 'Hyflo Supercel' (see text). The dimensions of the column were 47 x 1 cm. Linear concentration gradient of *tris*-hydrochloric acid buffer pH = 9.0, of ionic strength 0.01-0.1; total elution volume 1,000 ml. The concentrations at which the components were eluted are indicated in the figure. The sexual factor was eluted at the concentration of 0.065.

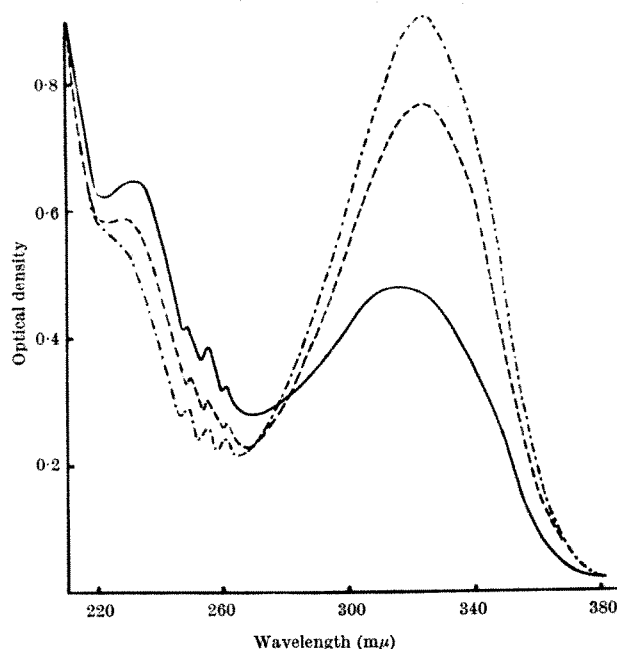


Fig. 2. Ultra-violet absorption spectrum of a solution of the sexual factor in ethanol. The factor was extracted from a culture of mated plus and minus *Blakeslea trispora* and purified by chromatography on DEAE-'Sephadex'. The eluted solution (in *tris* buffer, pH 9.0) was acidified and extracted with diethyl ether. After drying of the extract over magnesium sulphate, and evaporation of the solvent in a vacuum, the residue was taken up in ethanol. The following samples were taken from one solution: —, untreated sample; ---, sample exposed to 10,000 lux from two fluorescent tubes for 0.5 h; and - · -, untreated or illuminated sample, later exposed to the same intensity for 5 min in the presence of a trace of iodine.

of the occurrence of *cis-trans* isomerism. The spectral behaviour of the present compound is analogous to that of the carotenoids where, as described by Zechmeister<sup>3</sup>, it is caused by reversible *cis-trans* isomerization. Experiments aimed at the separation of possible isomers are in progress now.

When a solution of the sexual factor was exposed to light, biological activity towards both the plus and the minus strain was much reduced. The original activity was fully restored by illumination in the presence of iodine.

Caglioti *et al.*<sup>4</sup> isolated from mated cultures of *B. trispora* a substance said to stimulate carotenogenesis in cultures of the separate strains. This substance, named 'trisporic acid C', was characterized as a hydroxy-keto acid with three conjugated double bonds. The sexual activity of many Mucorales is coupled with increased synthesis of carotenoids, and the ultra-violet absorption spectrum of this trisporic acid is very similar to that of the substance described in this communication, and so it is possible that the two compounds are identical.

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## ANTHROPOLOGY

### Overkill at Olduvai Gorge

DR LEAKEY has replied<sup>1</sup> to my proposal<sup>2</sup> that the pattern of extinction of large mammals at the end of the Middle Pleistocene in Africa represents overkill by prehistoric

man. If one adopts all Dr Leakey's suggestions for updating or factually improving the African list of extinct mammals, the net effect is trivial—an increase in the number of living large genera from forty to forty-one, an increase in later Pleistocene extinction from more than twenty-six to more than twenty-nine genera, a decrease in earlier Pleistocene extinction from nineteen to eighteen genera, and an overall change in the amount of later Pleistocene megafaunal extinction in Africa, as computed in Table 3, ref. 2, from thirty-nine to forty-one per cent. I do not see how the omission of the genera Leakey adds to the Early Pleistocene group "... puts the whole picture out of balance".

As the richest single stratified fossil deposit representing the time interval in question the Olduvai record deserves special consideration. Leakey<sup>3</sup> reports twenty-one extinct genera of large mammals, presumably all from the living floors or camp sites of prehistoric man at Olduvai, five last recorded in the Upper Villafrancian, six in the Early Middle Pleistocene in strata associated with Chellean tools and ten surviving to the time of Bed IV, the unit associated with Acheulean artefacts of the late Middle Pleistocene (Table 1). Evidence of Acheulean association elsewhere indicates that at least four of the Bed II extinct genera from Olduvai, namely *Mesochoreus*, *Orthostonyx*, *Phenacotragus*, and a machairodont, may be considered contemporaries of the hand-axe fauna of Bed IV. Because Leakey reports that less is known about the fauna of Bed IV than about that of Bed I and II (ref. 3), they may yet be found. Clearly, generic extinction during the time of the hand-axe hunters far outstrips extinction during any earlier interval yet known in the African Pleistocene. How is it to be interpreted?

Table 1. LAST OCCURRENCES OF EXTINCT LARGE MAMMALS (> 50 KG) FROM OLDUVAI GORGE<sup>2</sup>

Upper Villafrancian (Olduvai Culture)		Middle Pleistocene	
Bed I	CHALICOTHERIIDAE	Bed II Upper (Chellean)	MACHAIRODONTINAE†
	<i>Ancylotherium</i> *		gen. indet.
	<i>Ectopotamochoerus</i>		<i>Mesochoreus</i> †
Bed II Lower	<i>Promesochoreus</i>	BOVIDAE	<i>Orthostonyx</i> †
	<i>Promotococherus</i> *		<i>Puliphaenagones</i> *
			<i>Phenacotragus</i> †
DEINOTHERIIDAE:		Bed IV (Acheulean)	
<i>Deinotherium</i>		CEROPITHECIDAE	<i>Simopithecus</i>
		EQUIDAE	<i>Stylohipparion</i>
		SUIDAE	<i>Nolochoreus</i>
			<i>Tapinochoerus</i>
			<i>Afrochoerus</i>
		GIRAFFIDAE	<i>Libytherium</i>
		BOVIDAE	<i>Thalerceros</i> *
			<i>Parmularius</i>
			<i>Xenoccephalus</i> †
			<i>Bularchus</i>

\* Rare (three individuals or less).

† Acheulean association elsewhere.

Dr Leakey regards drought as the cause of African extinction<sup>1,3,4</sup>. If so, it was the first catastrophic drought to strike the African fauna, and while one must believe it affected the entire continent, it was unique to Africa, an event that left no obvious mark on the megafauna of the time in other areas including Madagascar.

In his book on Olduvai, Leakey takes a restrained position on the palaeoclimatic meaning of extinct mammals<sup>3</sup>: "... it is not generally recognized that many of the larger mammals are remarkably adaptable... While zebra and giraffe are most commonly found in savannah and open plains with scattered thorn bush, they can also be found well within tropical forest zones such as those bordering Lake Manyara. These few examples serve to show how unwise it is to regard the usual habitat of large mammals as necessarily constant. If the habitat of large living mammals, belonging to a single species, varies so widely it is clear that the presence of extinct fossil species—even if related to the living forms—cannot be used as a basis for deducing ecological or climatic conditions." Thus in attributing the extinction of large

mammals of Bed IV to drought, Leakey ignores both his own advice and also the present distribution of East African mammals. Five of the largest species, elephant, hippopotamus, black rhino, giraffe, and buffalo, all occupy (or did in historic time) the desertic shores of Lake Rudolph, a region of under 250 mm rainfall, one of the driest parts of equatorial Africa.

I do not wish to deny the likelihood of droughts at any time during or after deposition at Olduvai. The point is that at Olduvai as elsewhere in the world a major episode of Late Pleistocene megafaunal extinction coincides with the prehistoric development of big game hunters. Unless a continent or island can be found in which a major wave of megafaunal extinction occurs other than soon after the arrival of man, or his cultural development as a stone age hunter, the possibility of overkill, will persist as a challenging "least improbable hypothesis".

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<sup>1</sup> Leakey, L. S. B., *Nature*, **212**, 1615 (1966).

<sup>2</sup> Martin, P. S., *Nature*, **212**, 339 (1966).

<sup>3</sup> Leakey, L. S. B., *Olduvai Gorge 1951-1961* (Cambridge Univ. Press, 1965).

<sup>4</sup> Leakey, L. S. B., *The Ecology of Man in the Tropical Environment* (I.U.C.N. Publ. No. 4, Morges, Switzerland, 1964).

It does not seem worth devoting more time, or space, to discussing that part of my argument with Dr Paul Martin, on the subject of Pleistocene overkill, which deals with percentages of genera that may have become extinct at a given point of time, or that have survived to the present day. My reasons for saying this are as follows: (a) there is too little agreement as to what constitutes a genus; (b) that even since the publication of the earlier notes by Martin and myself last year, *Bulcharus* has been made a synonym of *Pelorovis*, and it has been suggested that *Tapinochoerus* is generically identical with *Orthostonyx*. It must also be noted that the majority of anthropologists now treat *Telanthropus* as belonging to the genus of *Homo* and the others as *Australopithecus*. No one accepts it as a distinct genus. Similarly, *Australopithecus*, *Paranthropus* and *Zinjanthropus* are now all genus *Australopithecus*.

Then again, few zoologists agree on the subject of the generic classification of living genera in Africa. Some would list zebra as *Hippotigris*, not *Equus*, and the wild ass of Somaliland as *Asinus*: some would list *Theropithecus* as a distinct genus from *Papio*, others would not. Some would divide wildebeeste into two genera—*Gorgon* and *Connochaetes*—and so on. It thus seems clear that any arguments based on statistics and percentages of wholly unsure genera are of little value.

Dr Martin completely ignores the last paragraph of my communication<sup>1</sup> of December 31 in his present reply. I believe it is quite unscientific to invoke "overkill" by Acheulean hunters to explain the extinction of some forty or more genera at the end of the Middle Pleistocene when the much more numerous and much better equipped hunters who succeeded Acheulean man, during the Upper Pleistocene and post-Pleistocene time, had so little effect on the remaining fifty or more genera. I refer, of course, to the hunters of the Middle Stone Age, the Upper Palaeolithic, the Mesolithic, the Neolithic and also the Iron Age hunters. On the basis of Dr Martin's arguments, these people should have exterminated nearly all the genera which survived into the Upper Pleistocene. In fact we know that they did not.

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<sup>1</sup> Leakey, L. S. B., *Nature*, **212**, 1615 (1966).



## AGRICULTURE

### Carbonyl Sulphide from the Decomposition of Captan

VOLATILE sulphur compounds such as carbon disulphide and thiocarbonyl chloride are considered to be formed when the agricultural fungicide captan (*N*-trichloromethyl-thio-4-cyclohexene-1,2-dicarboxyimide) reacts with fungal spores<sup>1,2</sup>. During a general investigation<sup>3</sup> of the fungitoxicity of captan we have examined by gas chromatography the vapours released when captan reacts with the conidia of *Neurospora crassa*, macroconidial wild-type Em 5297a, at 25° C.

A solution of 375 µg of captan in 0.5 ml. of ethanol was added to a 2 ml. aqueous suspension of 840 million *N. crassa* spores in a 35 ml. bottle. After 20 min a 1 ml. sample of the atmosphere above the suspension was removed with a Hamilton gas syringe and injected into a 'Microtek GC.2000' chromatograph provided with a dual thermal conductivity detector. Helium at 90 ml./min was the carrier gas and the column, inlet, and detector were at 165° C: the stainless steel column was filled with the uncoated solid absorbent 'Porapak Q' (80/100 mesh size). Commercially available compounds were used as standards with the exception of carbonyl sulphide (COS) which was prepared from the reaction between ethyl isothiocyanate and sulphuric acid and further purified by the preparation and decomposition of potassium ethyl thiocarbonate<sup>4</sup>. Fig. 1 shows that carbonyl sulphide was evolved when captan reacted with *N. crassa* spores: there was no trace of carbon disulphide. Captan is rapidly decomposed by cell thiols<sup>1,3</sup> and when 750 µg of captan in 1 ml. of ethanol was added to an aqueous solution of excess glutathione, that is 3 mg, the resulting chromatogram (Fig. 2) of the vapours showed carbonyl sulphide but no carbon disulphide.

Infra-red gas analysis, in a 10 cm cell, with a Perkin-Elmer 237 spectrophotometer confirmed that carbonyl sulphide was produced from the reaction of glutathione and captan. The atmosphere above the solution was passed successively through acetone-solid carbon dioxide and liquid air traps<sup>5</sup>; the contents of the latter trap were then evaporated into the gas cell. The spectrum was identical to that of synthetic carbonyl sulphide with a doublet at 4.83 and 4.87 µ<sup>6</sup>. It was not possible to obtain sufficient carbonyl sulphide from the captan-spore reaction for infra-red analysis.

Lukens<sup>2</sup> confirmed his earlier supposition<sup>1</sup> that thiocarbonyl chloride is a major product of the reaction of captan with fungal spores—presumably following reduc-

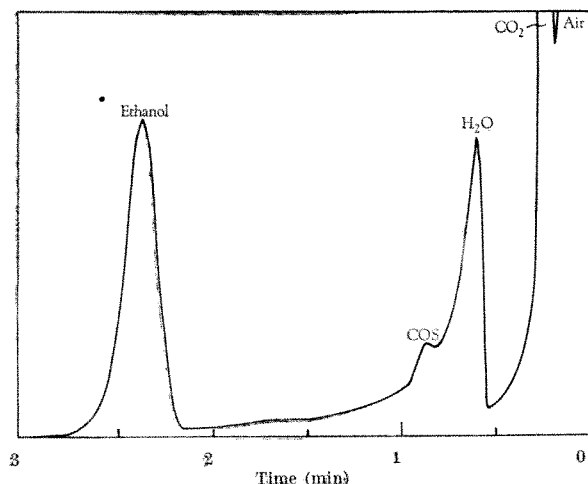


Fig. 1. Gas chromatogram of products of reaction between captan and *Neurospora crassa* conidia.

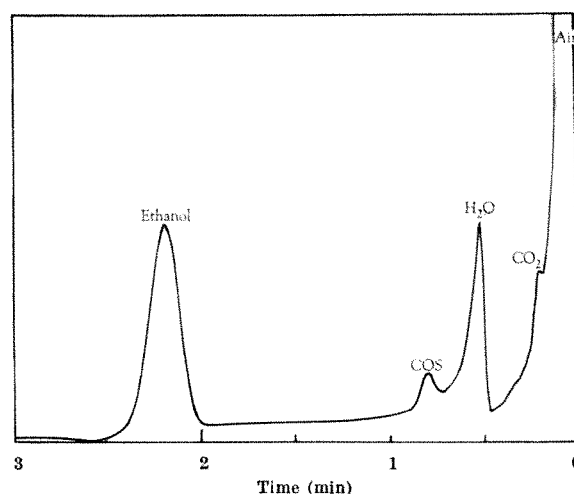


Fig. 2. Gas chromatogram of products of reaction between captan and glutathione.

tion by cell thiols. Although we were unable to resolve thiocarbonyl chloride by gas chromatography we have found that this compound is rapidly hydrolysed by excess water to form carbonyl sulphide the identity of which was confirmed, as before, by gas chromatographic and infra-red analysis. Haszeldine and Kidd<sup>6</sup> reported a similar result, but as they used a lower water-thiocarbonyl chloride ratio the hydrolysis was much slower at room temperature. The most probable explanation for the evolution of carbonyl sulphide from mixtures of captan and fungal spores is that it is a product of the rapid hydrolysis of the thiocarbonyl chloride initially formed. A more complete discussion of this reaction is in preparation.

In view of the results of Lukens and Sisler<sup>1</sup> it seemed surprising that we could obtain no evidence of the formation of carbon disulphide from the reaction of captan with either spores of *N. crassa* or the model thiol, glutathione. These authors identified carbon disulphide by trapping the gas in Viles solution<sup>7</sup> and measuring, at 440 mµ, the intensity of the yellow complex formed. We have found, however, that carbonyl sulphide also produces a yellow compound with Viles solution that gives an appreciable absorption at 440 mµ. It is apparent that this reagent is not specific for carbon disulphide and that, as Viles<sup>7</sup> has suggested, other double bonded sulphur compounds can interfere.

The formation of carbonyl sulphide from captan has biological as well as chemical interest because it has been shown<sup>8</sup> that vapours of this compound can be appreciably fungitoxic. Captan is used as a soil and seed fungicide<sup>9</sup> and the release of carbonyl sulphide after its decomposition in the soil may well give a wider area of protection than has been previously supposed.

We thank Dr G. Nickless for the use of the gas chromatograph.

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<sup>6</sup> Haszeldine, R. N., and Kidd, J. M., *J. Chem. Soc.*, 3871 (1955).

<sup>7</sup> Viles, F. J., *J. Indust. Hyg. Toxicol.*, **22**, 188 (1940).

<sup>8</sup> Kreutzer, W. A., *A. Rev. Phytopathol.*, **1**, 101 (1963).

## BOOK REVIEWS

## LEARNING FROM AMERICA

## Science and the Educated Man

Selected Speeches of Julius A. Stratton. Pp. viii + 186. (Cambridge, Mass., and London: The M.I.T. Press, 1966.) 40s.

DR STRATTON spent his life at the Massachusetts Institute of Technology. He was student, faculty member, professor, provost, chancellor and president. He was moulded by one of the great universities of the world, and he influenced it profoundly throughout his professional life.

We have become accustomed to the fact that English universities usually follow the example of America. We may protest and we may delay for twenty years, but we do so in the end. MIT has inspired many other American universities. In some ways it has been the pacemaker for the world, so Dr Stratton's thoughts about the current situation and the proposals for reform which he has enunciated in this book are likely to be important to many of us now and for several decades to come. It seems probable that American universities have solved many of the problems which are worrying us today; it is quite certain that they have encountered a whole series of problems which we have yet to face.

The whole American university system has been based traditionally on the assumption that everyone must have his chance. It is all the more interesting for an Englishman that Dr Stratton should warn his countrymen that excellence is more important than quantity, that intellectual distinction is more vital than the accumulation of factual knowledge, and that an educated man is distinguished by his attitude towards learning rather than his mastery of a particular domain of knowledge. Stratton has come to fear that Americans will degrade their standards of higher education in order to accommodate all those who aspire to a college degree. He thinks that too many students are entering American universities expecting to be taught rather than being determined to learn.

MIT is responsible for its students throughout their most formative years, when the most important experience they can have is to see new and original work in progress and study with scholars who are actively creating the subjects they teach. The faculty of this great institution is chosen because of the rugged individualism of its members and because of their interest in developing new ideas and creating a new society.

The problems of the president, who has to co-ordinate the efforts of such an undisciplined army and restrain their demands for funds, must have been terribly difficult, but Dr Stratton has studied the welfare of his students as well as encouraging his faculty. He has always believed that his institute should collaborate closely both with local industry and Washington; he sees this policy as the inevitable consequence of the Morrill Act which founded the Land Grant Universities a hundred years ago. They were expected to concern themselves with all the problems of society, whether or not those problems were numbered among the classical academic disciplines.

Engineers have transformed America in the century since MIT was founded. Techniques based on modern science will create the new industries on which the prosperity of America will depend in future. Symbiosis between universities, government and industry is more effective

and more productive in America than it is anywhere else in the world; anyone who has read Stratton's papers will understand why this has come to be so.

The American system is still not appreciated or accepted by many academics in Britain; many of the ideas which led to the Morrill Act and to the foundation of MIT came from England, although we neglected them here for a century after they had shown their promise abroad. It is tragic that we should have to import such things as business schools from America, and it will be futile for us to do so unless we accept the fundamental premise on which everything else depends—American universities have three functions which are equally important. First of all, they must be prepared to study any contemporary problem—they must be centres of innovation. The great American West was conquered in the laboratories of the Land Grant Colleges, and such places as MIT have made a fundamental contribution to the development of the whole of American industry as well as to American business. Secondly, universities must explain their discoveries to anyone of any age who can profit by them; American universities have known for a century that a professional man must be educated and re-educated throughout his working life. Thirdly, of course, universities must teach undergraduates.

Dr Stratton has tried to explain how universities should adapt themselves to these tasks in our changing society, but his main point is that technical progress has become self-sustaining, and the processes of discovery and production feed on each other. The translation of ideas into action is taking place at an ever growing pace, and the line of demarcation between scientist and engineer has almost vanished. It is the great achievement of MIT that it has known this for years, and that it has done more to create this dramatically successful machine than any other institution in the world. Everyone who is interested in our own universities should read Dr Stratton's little book. We shall be learning from it for another twenty years.

VIVIAN BOWDEN

## CHEMISTRY OF SURFACES

## Adsorption, Surface Area and Porosity

By S. J. Gregg and K. S. W. Sing. Pp. xi + 371. (London: Academic Press, Inc. (London), Ltd.; New York: Academic Press, Inc., 1967.) 95s.; \$18.

In their book Gregg and Sing have in general achieved the aim of giving "a critical exposition of the use of adsorption data for the evaluation of the surface area and the pore size distribution of finely divided and porous solids". The book at the same time serves to emphasize the great practical impact the now classic paper of Brunauer, Emmett and Teller has had on studies in surface chemistry; the most theoretical aspects of adsorption have, understandably, not been given comparable prominence. The limitations of the BET equation are discussed, but it should not be forgotten that the essential attribute of this method is its simplicity; any attempt to correct its shortcomings defeats its chief attraction.

It is particularly interesting to note that the physical adsorption of about twenty-five different gases is discussed, while G. D. Halsey expressed the view recently that so long as nitrogen is used as an adsorbate in BET measurements there is no real problem, but when other gases are used the problem is more pressing. Of course, it is not always practical to use nitrogen and this is what the book is particularly concerned with.

There are eight chapters: the first is a useful introduction, the following three deal in turn with non-porous, porous and microporous solids; while the fifth highlights Ross and Olivier's model based on a highly heterogeneous

surface where the distribution of energies is Gaussian. The sixth chapter deals with chemisorption, and I thought this the least critical part of the book. The seventh chapter deals with adsorption from solution, and the last chapter gives some experimental hints for determining isotherms.

The book is relatively free from errors, but I could not overlook some points. Pressures lower than  $10^{-5}$  torr can be measured by a suitably constructed Pirani gauge (page 309), and vacuum conditions are as important in studies of fundamental aspects of physical adsorption as for chemisorption, both phenomena being sensitive to surface topography (compare page 308, where the authors state that a vacuum of  $10^{-5}$  torr is sufficient). The well-defined point *B* reported in reference No. 89 (page 88) was actually at a very low relative pressure (0.01, not 0.1 as stated).

The book is well produced and its content is appropriate for a large number of potential users, which includes undergraduate and postgraduate students and people in industrial research laboratories. There are some 560 references and the figures have a uniform style (Fig. 6.6 excepted!).

M. W. ROBERTS

## LOW TEMPERATURE PHYSICS

### Specific Heats at Low Temperatures

By E. S. R. Gopal. (The International Cryogenics Monograph Series.) Pp. x + 240. (London: Heywood Books, for Iliffe Books, Ltd., 1966.) 70s.; \$11.50.

SOME twenty or so years ago several useful books appeared dealing with physical phenomena of importance at temperatures generally realizable using liquid nitrogen, hydrogen and helium. These books could all be given the same broad title "Low Temperature Physics" and, although the emphasis varied from one publication to another, there was in each an attempt to review a variety of topics from the theoretical and experimental aspects. The wider availability of low temperature facilities and resources since then has greatly speeded the collection of detailed knowledge about the behaviour of matter at low temperatures and this has, of course, stimulated theoretical speculation.

That a series of monographs written by specialists in limited fields is now called for to replace the general books of twenty years ago reflects the general trend of greater specialization which must accomplish greater detailed knowledge. In the circumstances, it is clear that the editors of such a series will exercise their important controlling functions in the choice of individual authors. Let it be said right away that the editors are to be congratulated on their choice of author for this volume. It is no easy task to collect within such a limited compass a sufficient theoretical background with which to compare experimental results and to make the comparisons in a way which retains the active interest of the reader. Dr Gopal has made an excellent selection of topics and dealt with these in an admirable way. The theory is sufficient to make the results intelligible in all cases and although certain readers with particular interests will want to supplement what is given in the book they will find much not requiring any additional reading; the references at the ends of chapters make good starting points for further work.

The arrangement of the book follows a conventional but logical pattern. Starting with some elementary concepts, the author goes on to a discussion of lattice heat capacity, including a very satisfactory introduction to lattice dynamics, and the occurrence of Debye  $\theta$  in other problems of the solid state. The next chapter deals with the electronic heat in metals and alloys, including superconductors, and the relationships to other properties. As

the author states, he cannot do justice to the BCS theory in the space available, but what he does clearly shows the interesting character of the results which can be followed up in the papers referred to at the end of the chapter. The fourth chapter deals with the magnetic contribution to specific heats and I found this a particularly useful chapter. After a brief outline of the thermodynamics of the problem the author deals with specific heat contributions arising from spin waves, ordering processes with reference to the Ising and Heisenberg models and specific heats near the transition temperature, Schottky effects in paramagnetic salts and those arising from nuclear magnetic moments. A useful chapter follows on liquids, most of which is given over to liquid  $^4\text{He}$  and  $^3\text{He}$ , with a brief mention of mixtures of these two liquids.

Although the chapter on the specific heats of gases has to be included for completeness, the material dealt with is readily available in many standard works elsewhere. The final two chapters deal with specific heat anomalies and miscellaneous problems such as specific heats near phase transitions, relaxation of rotational and vibrational specific heats and surface effects.

To some specialists parts of this volume may appear inadequate, but if it had been intended principally for such people it would have taken a different form and certainly not have been condensed into 240 pages. The book can be thoroughly recommended to anyone interested in calorimetry, for Dr Gopal has managed to present a great deal of factual information in a most readable style.

F. E. HOARE

## ENZYMES AND SACCHARIDES

### Methods in Enzymology

Vol. 8. Complex Carbohydrates. Edited by Elizabeth F. Neufeld and Victor Ginsburg. Pp. xxv + 759. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1966.) 208s.

THIS comprehensive volume must be regarded as a complementary volume to *Methods in Carbohydrate Chemistry* by Whistler and Wolfrom. It contains 119 articles written by more than 150 authors from many different laboratories in the world, and it is concerned with methods, including quantitative methods, for dealing with all the enzymes involved in the synthesis and degradation of complex saccharides.

The book starts with a section on analytical methods for estimating simple sugars, particularly those contained in mucopolysaccharides and mucoproteins. Some of the methods, particularly immunological methods and gas chromatography, will be particularly useful at this time.

One section deals with the methods of preparation of the substrates used in the subsequent enzyme reactions. Descriptive methods for dealing with enzymes themselves are divided into three sections: these consider sugar activation, complex saccharide synthesis and finally the utilization of complex saccharides. All the individual processes are described by acknowledged experts in the field, and so it will be found that the best available methods are provided. Readers will find the grouping of the sugars and the techniques concerning them together particularly valuable. Thus all the systems dealing with amino sugars are provided and also, for example, all those dealing with the involvement of sugar nucleotides in the important and novel transferase systems.

Each of the sections makes fascinating reading to all concerned with any aspect of carbohydrate chemistry and biochemistry. Articles on neuraminidase and the sulphatases are particularly topical.

Excellent author and subject indexes are provided, and the book is splendidly printed and can be warmly recom-

mended to all chemists and biochemists as a source of valuable working information. The editors are to be congratulated on their timely completion of a difficult task.

M. STACEY

## ENZYMES IN THE CELL

### Enzyme Cytology

Edited by D. B. Roodyn. Pp. xx+587. (London: Academic Press, Inc. (London), Ltd.; New York: Academic Press, Inc., 1967.) 140s.; \$25.

WHILE many biochemists have been increasingly concerned with the mechanism of biochemical processes at the molecular level, the hybrid subject of enzyme cytology attempts to get these processes into perspective with regard to the organelles which can be seen microscopically within the cell. *Enzyme Cytology* is an attempt to survey the entire field of this relatively new, but rapidly developing, subject. It is an ambitious undertaking and the fact that the book succeeds so well reflects great credit on the editor and the authors of individual chapters.

After a chapter on the general principles of the subject, there follow chapters on nuclei, mitochondria and peroxisomes, chloroplasts, lysosomes and phagosomes, membranes, ribosomes and cell sap. The layout of most of the chapters is similar, including a brief historical introduction and a critical discussion of preparative methods, followed by a survey of relevant work on the topic in question. One of the most useful features of this book is the close relationship between the results discussed and the experimental techniques, some of which still remain something of an art as de Duve points out in his introductory chapter.

Most of the authors attempt to survey the whole field without excessive emphasis on their own work, but despite the large amount of material dealt with most of the chapters remain very readable accounts and do not degenerate into lists of references. It is a tribute to the editor that duplication of material in chapters in which the topics overlap is kept to a minimum. The book also contains useful practical details on the most satisfactory ways of expressing results and concludes with a series of recommendations on nomenclature compiled by the editor which it is hoped will form a basis for a universally acceptable terminology in this subject.

One criticism of the book is that it attempts to cover so large a field that some aspects of the subject are treated only on a superficial level; for example, the discussion of the control of enzyme activity within the cell in the last chapter is so cursory that the book gains nothing from its inclusion. Shortage of space is probably responsible for several other omissions; for example, the list of enzymes of the cell sap is sketchy, and the otherwise excellent discussion of non-cyclic photosynthetic phosphorylation contains no discussion of the revised single light-reaction scheme now favoured by Arnon.

This criticism is, however, minor compared with the achievement that this book represents. It provides a good survey of a large field backed by an extensive collection of references, and although the enzyme aspects of cytology are the prime concern of the book, structural materials and non-protein components of cell organelles are not neglected. It also provides a series of very useful critical comparisons of the various methods used for the preparation and investigation of individual sub-cellular components, which makes the book a very useful reference work for methods. Finally, the book provides a large number of electron microscope pictures of the various components dealt with, which is useful, as it is all too easy for biochemists to forget about the structure of their starting materials.

The book may appear expensive, but this is partly justified by the excellence of the numerous electron microscope pictures. It is, on the whole, well produced, but there is a large number of printing errors; for example, the words "ultracentrifug alanalysis" occur twice in the same line.

This book represents a landmark in providing an overall picture of the state of knowledge in the subject of enzyme cytology and probably represents the last time that an adequate survey of the subject can be achieved within the space of a single volume. The overall high standard set by the authors will ensure that it will be widely used both as a reference work and as a textbook. It can be recommended not only to those working in the field but more widely as a readable, lively and highly informative survey of the current state of the subject of enzyme cytology.

K. F. TILTON

## SPECIAL SENSORY MECHANISMS

### Sensory Mechanisms

Edited by Y. Zotterman. (Progress in Brain Research, Vol. 23.) Pp. 225. (Amsterdam, London and New York: Elsevier Publishing Company, 1967.) 85s.

A LENGTHY and excellent review of the present state of knowledge of the sub-mammalian vertebrate olfactory system is followed by a shorter, and slightly less satisfactory, essay on the same system in mammals. Physiological mechanisms of olfaction are then discussed by Ottoson and Shepherd at somewhat greater length than in Ottoson's own contribution to the 1962 Stockholm symposium on "Olfaction and Taste", while in the next chapter, conversely, Zotterman compresses the information on gustation which he and his collaborators presented to the Stockholm symposium.

The first four chapters all concern the anatomy and physiology of chemical sense systems, and therefore hang together. They are followed by chapters on the clinical examination of vestibular function, the mathematical formulation of inner ear disorders, and finally a theory, poised between the twin peaks of ignorance and bigotry, of "protopathy, paresthesia and sensory suppressor zones".

Those seriously interested in the chemical senses will refer to the Wenner-Gren symposium on "Olfaction and Taste" (1963), also edited by Zotterman. The volume being reviewed is the result of a summer school held in 1964, and it is evident that there can be little new information on olfactory and gustatory physiology—which naturally received fuller treatment in the symposium entirely devoted to the subject. The first two chapters of the present volume are in the best Dutch tradition of comparative neuroanatomy, and form a valuable addition to the earlier symposium; it is indeed a pity that they could not have been included in it.

The chapter on the clinical investigation of vestibular function could be useful to physiologists working on intact human beings or animals, but it is difficult to see how or why it is included in the present volume. If such a stricture is to be applied to editorial policy with regard to this chapter, it is even more cogent to ask if an essay, however interesting, on the application of cybernetics to clinical otology has its right place here. The subject of the last chapter should, of course, concern all or any sensory mechanisms; it is a realm in which informed speculation may be of great value, but the operative word is informed.

The book here being reviewed is Volume 23 in a series entitled "Progress in Brain Research"; Volume 1 appeared in 1963, and Volume 27 is announced for this year. At a time when the nightmare prophecy in Ecclesiastes appears to be in the process of fulfilment—"of making many books



there is no end; and much study is a weariness of the flesh"—it seems justifiable to enquire into the purpose and function of the series in general and of this volume in particular.

Workers in any field of nervous system research will have recourse to original articles published in the several journals devoted to the appropriate disciplines. From time to time, experts gather together to hold a symposium on some particular topic. Although these symposia rarely provide new data, they are frequently productive of fertile new ideas—usually in direct proportion to the restriction of their terms of reference. Two superb recent examples are the symposium on "Olfaction and Taste" and the CIBA symposium on "Touch Heat and Pain" (1966). A few volumes in the "Progress in Brain Research" series may be classed in this category. The present volume most certainly cannot. If the first four chapters are at least cohesive, two of them are redundant—probably through no fault of their authors. The summer school from which this book originates is supposed to be for advanced students or young researchers, and may thus serve a useful purpose. One would have expected the authors of the second chapter to give advanced students details of their methodology rather than just a bald statement of their results; and the authors of the third chapter are being a little bold in implying that in all receptors a local potential change spreads electrotonically into the afferent nerve ending.

But why print a kind of serial textbook at the rate of seven volumes a year—volumes whose content and quality is so uneven, and which at this level must date so rapidly? Many scientific publishers now realize that now so many university and institutional libraries compulsively buy every purportedly esoteric book that comes off the presses, they have a captive clientèle and so presumably an assured profit. Just in case there should be any hesitation, or perhaps to lure the unsuspecting potential individual purchaser, a title which, though misleading, is not quite downright mendacious is chosen, and it is left to the eminent individual volume editor to explain it away as best he can in a preface whose brevity is evidence of his embarrassment. Were it not a spatial, didactic and financial waste, it would be amusing to contemplate Parkinson's law in the particular case whereby the number of books published increases as the square of the number of libraries constrained to purchase them.

DAVID BOWSHER

## POISON ON THE FARM

### Garner's Veterinary Toxicology

Third edition. Revised by E. G. C. Clarke and Myra L. Clarke. Pp. 477. (London: Baillière, Tindall and Cassell, Ltd., 1967.) 60s. net.

This is an excellent reference work and fully maintains the tradition set by previous editions by Garner, and before that by Nicholson and by Lander. A comprehensive treatment of toxicology draws from the scientific disciplines of botany, chemistry, physiology, pathology and clinical medicine, and it might be thought impossible to produce at once a textbook for the student, a ready reference work for the practitioner, and an authoritative text for the research worker. This has been achieved, however.

In the past few decades there have been vast changes in the toxic hazards to which farm animals are exposed, for example, arsenic and copper compounds for the treatment of external and internal parasites have been superseded by chlorinated hydrocarbon and organo-phosphorus compounds. The range of organic pesticides used in agriculture is continually expanding, and it is to the authors' credit, therefore, that the third edition of this

volume is only some 10 per cent larger than the previous edition, although one new section has been added and others have been revised and enlarged.

The book is divided into eight sections. The first part consists of a general thesis on toxicology including mode of action of poisons, diagnosis and treatment, and the remaining seven sections constitute an exhaustive catalogue of toxic materials encountered by farm animals. Each poison is dealt with under the general headings of "Occurrence", "Absorption", "Toxic Dose", "Symptoms", "Post-Mortem Lesions", "Diagnosis" and "Treatment". The second part deals alphabetically with mineral or inorganic materials, and organic materials are treated in the third, fourth and fifth parts, under the headings of "Drugs", "Pesticides" and "Miscellaneous Compounds". One of the co-authors, Papworth, has rewritten the section on pesticides which contains very useful tables of chlorinated hydrocarbon and organo-phosphorus insecticides listing both chemical and proprietary names. These latter are also included in the index, which greatly facilitates location in the text.

The sixth part of the book, which lists the toxic hazards of the plant kingdom, has been enlarged to include plants from other continents as well as Europe, and has been rearranged so that families appear in alphabetical order. The seventh part, on the dangers from radioactive materials, has been rewritten and enlarged by Garner, and the next part is the new addition containing short accounts of venomous stings and bites and doping of racing animals.

In all the topics covered, the literature has been reviewed up to 1964 or 1965, and, with a few exceptions, all the important contributions have been included. Selection of materials has been judicious, as has been the omission of material of waning importance in modern agriculture.

As one of the very few works on veterinary toxicology in English this new edition will be welcomed by all who are concerned with diagnosis and treatment of poisoning in animals.

J. R. TODD

## FOOD MAY BE POISONOUS

### Toxicants Occurring Naturally in Foods

(A Monograph prepared by individual scientists of the Food Protection Committee.) (Publication No. 1354.) Pp. vii + 301 (Washington, D.C.: National Academy of Sciences, National Research Council, 1966.) \$6.00.

MUCH time, money and argument is now being spent (and rightly so) in several countries to produce legislation regulating the safe levels of use for the synthetic chemical substances which are added deliberately to food for various aesthetic and technological purposes (for example, flavours, colours and preservatives) or which may occur as contaminants in food as the result of manufacturing processes, packaging or the use of pesticides in agriculture. As this book clearly shows, however, natural foodstuffs contain a large number of chemicals which are not nutrients, many of which could be toxic to man and animals if the foods in which they occur were eaten in sufficient quantity. Poisoning as the result of eating natural foodstuffs may not be uncommon, for even the humble potato has caused many outbreaks of poisoning in man as a result of its content of the solanine alkaloids in certain circumstances. Those who would insist on eating natural foodstuffs without chemical fertilizers and pesticides and without chemical additives are, like most of us, not aware usually that poisonous substances or toxicants occur naturally in their food. As the various chapters in this book show, foods contain naturally such substances as goitrogens, oestrogens, carcinogens, lathyrogens, haemagglutinins, stimulants, depressants, allergens, pressor amines, antivitamins, antienzymes,

cholinesterase inhibitors and many other potentially toxic agents.

This book constitutes an interesting and useful introduction to the occurrence of and the problems arising from toxicants naturally present in food. It contains twenty-seven short chapters, some no more than four or five pages, by twenty-five contributors, and each chapter is supplemented by a useful bibliography. But, as pointed out in the discussion at the end by Dr J. M. Coon, who was largely responsible for organizing and managing the project, it was not feasible to attempt a comprehensive coverage of what is known in the field, and the selection of specific topics was somewhat arbitrary. Nevertheless, the book has succeeded admirably in directing attention to this field. About two-thirds of the book deals with organic compounds, many of which have pharmacological and toxicological properties. A small selection of the many substances mentioned will illustrate the scope: *l*-5-vinyl-2-thioxazolidone (goitrogen) in turnips, genistin (oestrogen) in soybeans, aflatoxin (carcinogen) in peanuts, methylazoxymethanol (carcinogen) in cycads,  $\beta$ -amino-propionitrile (lathrogen) in sweet peas, vicine (haemolytic agent in favism) in broad beans, tyramine (pressor amine) in cheese, histamine (pressor amine) in wine, solanine (cholinesterase inhibitor) in potatoes, cyanogenetic glycosides, physostigmine, gossypol, coumarin, safrol, allylthiocyanate, and fish poisons. There are also chapters on excess vitamins, indispensable amino-acids and essential minerals and a whole chapter on sodium chloride which could be toxic, in certain circumstances, to hypertensive patients. Oxalates, nitrates and nitrites, and natural radioactivity each have a chapter devoted to them.

The number of toxicants occurring naturally in food must be very large and there are many which undoubtedly remain to be discovered. It seems reasonable, therefore, that the same attention should be given to these substances as has been given to deliberate additives and pesticides, without, however, any diversion of attention from the latter. Some of these substances have, of course, received considerable attention as in the case of the aflatoxins. The question may be asked: if all these toxic substances do occur in the diet, why is it that their effects are not manifest in a large proportion of the population? Here and there in the various chapters of this book the possible answers to this question are to be found. The general impression one gets from reading this book is that, although the natural sources of man's food contain many chemical substances known to be toxic, most of them are not known to be harmful to normal healthy human beings consuming what has been established as a reasonable balanced diet prepared in time-honoured ways. It is clear that the normal detoxication mechanisms of the body are able to cope with these substances in these circumstances. Poisoning, however, does occur especially in communities living on limited diets, or on unusual foods, or on foods gathered during the toxic phase of growth of a plant or animal, or foods infected by toxic fungi. Poisoning may also occur in individuals in which the normal protective mechanisms are defective, such as the young, the sick or those with inborn errors in these mechanisms. Thus in Japan, 60 per cent to 70 per cent of all food poisoning is caused by fish or sea-food because marine organisms contribute 10 per cent of the total food supply. In some African peoples who use plantains as a major article of diet, the intake of serotonin may reach 100–200 mg/day and this has been suggested as a factor in the high incidence of endomyocardial fibrosis among Africans. The unripe ackee fruit of Jamaica can cause acute toxic hypoglycaemia because of its content of hypoglycin, and quail can be toxic in the spring when they return to Europe from Central Africa where they have eaten poisonous plants. The poisoning of the Hebrews by quail is referred to in the Old Testament (Numbers, 11).

Although it appears that there is no danger in normal circumstances from toxicants occurring naturally in foods, it is pointed out that problems could arise from the simultaneous presence of two groups of materials in our food, namely, the natural toxicants and the synthetic chemicals. Such problems have already arisen, as in the case of tyramine in cheese and certain antidepressive drugs, for foods rich in pressor amines (for example, certain cheeses, certain wines, for example chianti, pickled herrings, 'Marmite', broad beans) can produce hypertensive crises in patients undergoing treatment with monoamine oxidase inhibitors. The significance, however, of many of the known toxicants occurring naturally in food to the health of man through his consumption of foods containing them has yet to be determined.

The purpose of this book, as stated in the introduction, is to "present information, however ascertained, concerning the presence of naturally occurring toxic substances in man's food". This purpose has been achieved and the book will be read with profit by all interested in food toxicology.

R. T. WILLIAMS

## MONGOOSES OF THE WORLD

### Mongoose

*Their Natural History and Behaviour.* By H. E. Hinton and A. M. S. Dunn. Pp. vii + 144 + 16 plates. (Edinburgh and London: Oliver and Boyd, Ltd., 1967.) 42s. net.

THIS is a useful and well planned account of the natural history, in the widest sense, of the small carnivores forming the sub-family Herpestinae, widespread through Africa and southern Asia, in the family Viverridae. The co-authors have provided in a simple and easily readable form an up to date survey of all reliable knowledge of this distinct group of mammals.

The range of aspects is wide: separate chapters describe the always engrossing subject of relationships between mongoose and snake; oddities of mongoose behaviour; important ecological and economic effects of the introduction of mongooses to the Caribbean and Hawaiian islands; their history in ancient times with Oriental folklore; diseases and parasites; reproduction; and a final chapter reviews briefly the systematic arrangement as currently accepted and lists the existing genera and species, together with their distribution and native names. Some of the aspects of behaviour may not be familiar to the general reader, particularly the well authenticated habit of some species of breaking the shells of eggs, snails, mussels and even nuts by flinging these food items violently either downwards or backwards against a hard surface. Such behaviour is apparently innate in certain species.

The story of the relationship between mongoose and snake provides some fascinating reading. Most mongooses seem to possess a considerable degree of immunity to normal quantities of snake venom, although massive doses injected experimentally can be lethal. In nature it often happens that mongooses and poisonous snakes ignore each other, and most of the snake remains found in mongoose stomachs or droppings have proved to belong to harmless species. In the West Indies, where control of snakes was one of the purported objects of the introduction of mongooses, there appears to have been no appreciable change in snake populations. It is even reported that in Trinidad and some other islands the local boas find the mongooses a useful addition to their diet.

Much detailed information is given about other aspects of the wide introduction of the small Indian mongoose to the Caribbean and Hawaii. Undoubtedly the spread of these terrestrial, energetic, omnivorous hunters has

had a major effect on some forms of the endemic faunas, and although the part they play in controlling the rats which are often an important pest in sugar cane plantations (a part recently diminished to a considerable extent by the disastrous results, for the mongooses, of eating thallium-poisoned rat bodies) is recognized on some islands, on others control of the mongooses has been found necessary. A graphic illustration of the biological success of the mongoose in Trinidad is provided by the figures of mongooses on which bounty was paid in 1928, 1929 and 1930: 30,026, 32,650 and 21,231 respectively. A wry commentary on the snags inherent in any bounty scheme is that it has been found necessary on certain islands to make orders prohibiting the keeping of mongooses in captivity, as it was feared that they were being bred for the reward. One further significant aspect of the mongoose problem, particularly in the West Indies, is the discovery that these large populations constitute a major reservoir of canine rabies.

The final chapter, reviewing briefly the taxonomic list, is the only one marred by errors of omission or commission. For example, on page 109 it is stated that ten genera are now recognized, but in the lists which follow thirteen full genera are tabulated. On page 112, by way of introduction to the subject of local names, there is a reference to "the common mongoose of northern Nigeria", followed by a list of known native names for it. But the subsequent generic and specific lists make no further mention of this "common mongoose" nor of any of the names quoted for it. Again, under distribution, the information for several African species is notably incomplete; for example, the distribution of *Mungos gambianus* is given solely as Gambia, yet all the native names given for this species are clearly listed as Ghanaian, and in fact the range includes both Ghana and Sierra Leone. An inexplicable error is the inclusion of Sierra Leone and Ghana in the range of *Herpestes naso*, which has never been recorded west of south-eastern Nigeria.

Apart from these few technical errors in the final chapter, the book can be thoroughly recommended as a useful introduction to all aspects of mongoose biology and history; a very full bibliography completes the work, which is fully illustrated by both photographs and line drawings.

R. W. HAYMAN

## PRIMITIVE RELIGION

### Tikopia Ritual and Belief

By Raymond Firth. Pp. 374. (London: George Allen and Unwin, Ltd., 1967.) 63s.

THE island of Tikopia is as familiar to social anthropologists as the Isle of Dogs to a Cockney, for it is the subject of the most remarkable corpus of ethnography that the present generation of anthropologists has produced. The author of this book, Professor Raymond Firth, set an unsurpassed standard of field-work with his first study of the island thirty-eight years ago. Since then he has published other books deriving from his original visit and in 1952 he returned to the island to attack the problems of social change and was fortunate (from the theoretical point of view) to arrive at a time when a typhoon had wrecked the island and faced its inhabitants with a crisis that forced them to modify many of their traditional ways. He returned there again in 1966 when he appears to have been primarily concerned with the study of religion, a subject on which he gave us *The Work of the Gods on Tikopia*. This book is now re-issued as the first volume of a trilogy of which *Tikopia Ritual and Belief* forms the second. It collects together the various essays bordering the subject which have appeared during the past thirty-five years and adds a final commentary in the light of the author's most recent visit. Yet we

must await the promised volume, *Rank and Religion in Tikopia*, to assess Firth's contribution to the study of primitive religion. In the meantime it is very convenient to be offered these essays in a single volume. In spite of their diversity and the age of some, they represent a single point of view and even the oldest does not seem dated.

JULIAN PITT-RIVERS

## BEWARE OF THE MONKEY

### Zoonoses of Primates

The Epidemiology and Ecology of Simian Diseases in Relation to Man. By Richard Fiennes. (Manuals in Biology.) Pp. ix+190. (London: Weidenfeld and Nicolson, 1967.) 168s.

THIS book is the first volume in a series of manuals in biology intended for research workers and students and edited by Dr L. Harrison Matthews and Richard Carrington. The book's author is a veterinary surgeon who has been conducting for nearly twenty years field and laboratory studies of diseases of wild and domesticated animals in East Africa. Formerly head of the veterinary section of the faculty of agriculture in the University of Ghana, he is a graduate of the Universities of Cambridge and Edinburgh and has been pathologist to the Zoological Society of London since 1956. This book certainly reflects the author's wide experience of its subject, the scope of which he outlines in a foreword, which includes a valuable history and definition of the term zoonosis, which has been, as he explains, used in different senses by the authors he quotes.

In the first part of the book Fiennes discusses the affinities and classification of the primates and the complex ecology of their zoonoses, that is, diseases transmissible to man, a valuable discussion which makes it clear that the author is well aware of the intricate interactions of the many factors which govern the transmission of primate diseases to man. These include not only their vertical transmission from the arboreal habitats of the primates, but also their lateral transmission among terrestrial species, such as the rodents, which may be the means, with the help of vectors, such as mosquitoes, of infecting man, who may, once he is infected, be the starting point of serious epidemics among human populations. This maze is to some extent clarified by references to particular diseases, such as yellow fever and the numerous viruses which may be found in primates. This section, however, cannot be fully understood or appreciated unless the book itself is read. It should, indeed, be carefully read by everyone who is in any way closely associated with primates, not only for the sake of their own health, but also for the sake of the health of human populations to which they belong.

The second, third and fourth parts of the book give a disappointingly brief sketch of the various parasites of the primates, including the bacteria (among which are the bacillus of tuberculosis and enteric organisms which can cause serious diseases of man) and also the numerous viruses found in primates. Useful features are the tables which list the organisms concerned, and the list of viruses and their classification is especially useful. But many readers, especially perhaps students, will no doubt wish that more space had been given to all these organisms, so different from one another, and to their characteristics and to the structure of life histories of, at least, the protozoan and metazoan parasites, preferably with illustrations. The book ends with the author's summary and conclusions, which warn us, among other things, that man runs serious health risks when he associates closely with monkeys, although, in the author's opinion, the New World monkeys are probably less dangerous than the Old World species are.

The book is completed by a valuable and extensive bibliography of books and research papers and an author and subject index. As it is the first complete treatment of an important subject, it will be welcomed by doctors, veterinarians and everyone else concerned with the epidemiology of the diseases involved.

G. LAPAGE

## OBITUARIES

### Dr S. P. Wiltshire

THE Commonwealth Mycological Institute (formerly the Imperial Bureau of Mycology) at Kew has had two chief architects: Dr E. J. (later Sir Edwin) Butler, the first director, who laid a firm foundation, and Dr S. P. Wiltshire, who during his sixteen years as director designed much of the edifice. Wiltshire was largely responsible for the fine "new" building, opened in 1955, to house the library and the herbarium and, in addition, he initiated most of the projects by which information on plant disease, mycotic disorders of man and animals, and other aspects of mycology are currently disseminated by the Institute.

Samuel Paul Wiltshire, who died on May 13, 1967, was born on March 13, 1891, at Burnham-on-Sea, Somerset, and in 1914, after going to the University of Bristol and Emmanuel College, Cambridge, he joined the Long Ashton Research Station, to which he returned as mycologist in 1919 after a period of war work. There he investigated infection by the apple and pear scab fungi. In 1922 he was appointed by Dr Butler to the recently founded Imperial Bureau of Mycology, where he remained for the rest of his working life, becoming later assistant director and then director. In the same year he married Violet Gertrude, daughter of Dr Dukinfield Henry Scott.

The main function of the Bureau, which is still the basic function of the Institute, was to abstract the world literature on plant diseases and to publish the results in the monthly *Review of Applied Mycology*, which is now in its forty-sixth year. Wiltshire had the temperament to become a meticulous editor, and the increasing size of the literature and the increasing need for information led him to diversify the Institute's publications. He developed the regular publication of maps showing the distribution of plant diseases, supplemented the *Review of Applied Mycology* by a newsletter, and planned, but left his immediate successor to implement, a series of descriptions of pathogenic fungi and bacteria. He also initiated a second abstracting journal, the, now quarterly, *Review of Medical and Veterinary Mycology*, the *Index of Fungi* (in which new names proposed for genera and species of fungi are listed), and an annual (now half yearly) *Bibliography of Systematic Mycology*, listing taxonomic publications by titles. Finally, *Mycological Papers* (the main outlet for the taxonomic research carried out at the Institute but which also include other contributions, particularly from Commonwealth countries) and *Phytopathological Papers* were the results of his initiative.

Wiltshire was himself well known as a mycologist, and if his output was small it was of high quality, so that his papers on *Alternaria* (published in 1933) and *Stemphylium* (1938) are minor landmarks. He was president of the British Mycological Society for 1943.

Although the author of so many novelties, Wiltshire was very conservative in editorial matters, but his reluctance to make changes was basically a reluctance to jeopardize the high standards on which he always insisted. Reserved and of a retiring disposition, Wiltshire was seldom seen at scientific meetings and he travelled little. He was, however, a man of high principles and deeply

religious. The funeral service was held at the Ebenezer Baptist Chapel, Kew, which he had attended for thirty-nine years and served as a deacon for more than twenty.

G. C. AINSWORTH

### University News:

#### Dundee

DR A. R. MITCHELL, reader in the Department of Applied Mathematics in the University of St. Andrews, has been appointed to the third chair of mathematics (numerical analysis) in the proposed University of Dundee (Queen's College, Dundee, University of St. Andrews).

#### London

DR R. G. MASON, reader in geophysics at Imperial College, has been appointed to the chair of pure geophysics tenable at that College.

#### Reading

DR F. AYLWARD, at present director of the Fruit and Vegetable Preservation Research Association, Chipping Campden, has been appointed professor of food science. Dr Aylward will take up his position on the retirement of Professor E. L. Crossley at the end of the 1967-68 session, at which time the Department of Dairying will be renamed the Department of Food Science.

### Appointments

MR FRANK GREENAWAY has been promoted to the rank of keeper at the Science Museum.

### Meetings

COMPUTER Programming, August 7-25, Technical University of Denmark, near Copenhagen (H. J. Helms, Northern Europe University Computing Center, Technical University of Denmark, Lyngby).

INTERNATIONAL Lecture Meeting on the Combined Gas Chromatograph-Mass Spectrometer, August 9, at 7 p.m., School of Pharmacy, London (Dr P. R. Masek, LKB Instruments, Ltd., 232 Addington Road, South Croydon).

ERRATUM. In Fig. 1 of the communication entitled "Proton Current Flow in Mitochondrial Systems" by Peter Mitchell (*Nature*, 214, 1327; 1967) NADH Oxidase was wrongly printed as NADP Oxidase.

ERRATUM. In Fig. 1 of the paper "In vivo Effect of an Invasive Malignant Rat Tumour on Cartilage" by A. R. Poole and D. C. Williams (*Nature*, 214, 1342; 1967), the magnification should be  $\times c. 490$  and not  $c. 140$  as stated.

ERRATUM. In the communication "Methylation in vivo of Guanine in the Nucleic Acids of Rat Testes by Methyl Methane Sulphonate" by P. F. Swann (*Nature*, 214, 918; 1967), the third sentence in the legend to Fig. 2 should read:  $\bigcirc$  —  $\bigcirc$ ,  $E_1$  cm at 260 m $\mu$ ;  $\bullet$  - - -  $\bullet$ , radioactivity in c.p.m.

ERRATUM. Throughout the communication entitled "Adrenergic Effects of Chronic Administration of Neuroleptics" (*Nature*, 214, 1210; 1967) the following substitutions should be made: stereotypy for stereotype; neuroleptics for narcoleptics; phenothiazine for phenothiazone; butyrophenone for butyrophene; haloperitol for halopentol. In line 14 of the fourth paragraph on page 1211, adrenergic should read amphetamine.

CORRIGENDUM. With reference to the article "Synergistic Toxicity and Carcinogenicity of Freons and Piperonyl Butoxide" (*Nature*, 214, 526; 1967), the authors would like to make it clear that while some 'Freons' are common pesticide propellants, 'Freons 112' and '113', which were discussed in the article, are not used for this purpose, but as selective solvents. They would also like to point out that 'Freon' is a du Pont trademark and that the anaesthetic fluorocarbon halothane is not a 'Freon'.



# CORRESPONDENCE

## Applying Research

SIR,—Professor Temperley's interesting and stimulating letter (June 24, p. 1378) raises a number of points.

Although responsible for running a sponsored research institute, I would question his initial assumption that there should be a correlation between research expenditure and increase in GNP. Research increases knowledge or potential. Usually the knowledge is free for all and the people who provide the knowledge are not often in a position to exploit it commercially. In this respect research is in the same category as accountancy, which provides information but is not itself productive. It is perhaps unfortunate that so many accountants and business administrators consider research to be justified only by profitability, but fail to apply this criterion to other fact-finding activities, including their own. Although both research knowledge and accountant's information can help to increase production and profitability, other conditions have to be right, and a study of the accounts or a satisfactory outcome of research do not lead inevitably to financial gain. However, lack of knowledge of either type must result in eventual loss. We need knowledge to maintain as well as to increase production.

I cannot share Professor Temperley's concern about a Ph.D. being employed in devising production schedules. The Ph.D. degree is, after all, only an indication of a full education and there are few activities that are not the better for an educated mind. The idea that a Ph.D. demeans himself by going into production engineering is probably one reason why we, in this country, fail to use our best brains in increasing productivity. We would do better and be more likely to apply new knowledge effectively if we had university trained people as foremen, or even at the workbench, who would both welcome and understand innovation.

Another reason for failure to apply research in Britain is that most results which emerge from universities, Government laboratories, or research associations are made freely available. In some specific cases results may be exploited commercially through NRDC, but so far the costs of exploitation have tended to equate with or exceed the returns.

Finally, we have the tendency to restrict our scientific manpower by engaging technical staff to work only in specific fields and to maintain industrial establishments or teams of scientific workers in fields where there is no immediate commercial justification. When the icy winds of economic change are experienced, or when there is a change in the attitude of management, scientific personnel are thrown on the market, often when this is at its lowest ebb. This gives British industry the reputation of not being able to use scientific manpower and tempts scientists to seek work abroad.

The efficient use and exploitation of research can be best ensured by the establishment of multi-discipline organizations, in which both research and development can be carried out, with the results kept confidential until ready for exploitation, and where the staff can switch from one project to another without being hampered by the original terms of reference under which they were engaged.

Such an organization should contain experts on industrial management, market research, and commercial intelligence, as well as staff engaged in conventional science and technology. Probably even these business experts would be the better for a basic scientific or technological training, for the techniques involved are comparatively simple and capable of development and improvement by the application of scientific principles.

Such organizations as exist in Britain for sponsored research are hampered by being small in relation to similar

institutions in the US and by being unable to offer those other services by which contact and confidence are established between industry and the scientist. Recently both Arthur D. Little and Battelle have successfully extended their activities in this direction and include many British firms among their clients. Although there has been a very marked growth of management consulting organizations in this country, few of these are science based and one suspects that they recommend reduction of research expenditure or closure of research departments, rather than suggesting alternative lines of research likely to prove profitable. Thus they emphasize rather than reduce the rift between scientific research and industry.

By all means let us question the figures for British expenditure on research and development and their implications, as Professor Temperley suggests, but I, for one, am prepared to accept them as published and to be more concerned with how to remedy the situation. In my view this lies in the enlargement and extension of contract research facilities, coupled with industrial consultancy services in the same organization.

Yours, etc.,

E. A. G. LIDDIARD

*Director of Research,*  
Fulmer Research Institute Ltd.,  
Stoke Poges.

## What Place for Engineers?

SIR,—Professor Thring's ideas about an Academy of British Engineers are attractive. Certainly they have been given much thought within the Council of Engineering Institutions but, for the present, rejected for the reasons given in your leading article (June 17, p. 1175).

To expand on some of your comments, an important difference between the British engineering institutions and the American societies, affecting their relative standing in the community, is that the former are qualifying bodies while the latter are not. The former consequently enjoy a better status as learned societies and may be thought the more effective in promoting the art of engineering. The American federal body, the Engineers' Joint Council, has not had a workable constitution, being inherently unable to offer effective advice to Government. Very recently, its constitution has had a radical change. Whatever factors may be the cause of American industrial pre-eminence, the organization of their engineering profession does not seem to be one, and the need for a National Academy arose for reasons which are not relevant to conditions in Britain.

The CEI has an excellent relationship with the Royal Society. Sixty-three Fellows of the Royal Society are Chartered Engineers. This is not far short of the 10 per cent which had been the normal level of representation of the profession during the 19th and early 20th centuries, until the "Rutherford era" created a preponderance of pure scientists. The purpose for which the Royal Society was created by Charles II would seem to have been the furtherance of science for the betterment of industry by its application, and the engineer deserves a place in such a body. Remembering the difference in scale between the United States and Britain, there would seem every reason for the strength of engineering influence to be built up in the Royal Society rather than to create some new body whose relationship with the Council of Engineering Institutions would be difficult to define. In any case, scientists and engineers are very closely linked through collaboration in industry with the numbers employed of each not greatly different.

Yours, etc.,

J. R. G. FINCH

*Secretary,*  
Council of Engineering Institutions,  
London, S.W.1.

## FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

## Monday, July 10—Thursday, July 13

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS; INSTITUTION OF PRODUCTION ENGINEERS; and the INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at the University, Nottingham)—Joint Conference on "The Integration of Design and Production in the Electronics Industry".

## Tuesday, July 11

COUNCIL OF ENGINEERING INSTITUTIONS (at the Institution of Civil Engineers, 1-7 Great George Street, Westminster, London, S.W.1), at 10 a.m.—Conference on "The Structure of the C.E.I. Examination".

## Thursday, July 13—Friday, July 14

BIOCHEMICAL SOCIETY (at the University of Oxford)—473rd Meeting. Programme will include Dr J. B. Chappell: "The Transfer of Reducing Power between Cytoplasmic and Mitochondrial Nicotinamide Adenine Dinucleotides" (Colworth Medal Lecture); Symposium on "The Metabolic Roles of Citrate", and an Ordinary Meeting for the presentation of communications.

## Friday, July 14—Saturday, July 15

SOCIETY FOR ANALYTICAL CHEMISTRY, MICROCHEMICAL METHODS GROUP (at Lincoln Hall, University of Nottingham)—Symposium on "Elemental Organic Microanalysis".

## APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

LECTURER/ASSISTANT LECTURER (preferably with a special interest in crystal structure analysis and in crystal chemistry and preferably some experience of teaching in crystallography) in CRYSTALLOGRAPHY—The Academic Registrar (LFG), University of Surrey, Battersea Park Road, London, S.W.11 (July 14).

LECTURER or ASSISTANT LECTURER in the DEPARTMENT OF CERAMICS, Houldsworth School of Applied Science—The Registrar and Secretary, The University, Leeds, 2 (July 14).

RESEARCH ASSISTANT (with an honours degree, preferably in physics, and willing to join a research group concerned with the image evaluation of optical and photographic systems) in the SCHOOL OF PHOTOGRAPHY, COLLEGE OF ENGINEERING AND SCIENCE—The Director of Education, The Polytechnic, Regent Street, London, W.1 (July 14).

SENIOR LECTURER or LECTURER (graduate in veterinary medicine, medicine or science and experience and/or an honours degree in physiology) in the DEPARTMENT OF VETERINARY PHYSIOLOGY—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh (July 14).

ASSISTANT LECTURER in the DEPARTMENT OF CHEMICAL PATHOLOGY—The Registrar and Secretary, The University, Leeds, 2 (July 15).

LECTURER and an ASSISTANT LECTURER (graduates in pharmacology, pharmacy, physiology, biochemistry or medicine) in the DEPARTMENT OF PHARMACY—The Registrar, University of Strathclyde, George Street, Glasgow, C.1, quoting Ref. 48/67 (July 15).

LECTURER (preferably with research qualifications in solid state or superfluid theory) in THEORETICAL PHYSICS—The Secretary, University of St. Andrews, College Gate, St. Andrews, Fife, Scotland (July 15).

RESEARCH ASSISTANT in the DEPARTMENT OF BIOLOGICAL SCIENCES to collaborate in one of the following projects: biochemistry, general physiology or ultrastructure, genetics, microbiology—The Staff Officer, The University of Aston in Birmingham, Gosta Green, Birmingham, 4 (July 15).

SENIOR TECHNICIAN (A.I.M.L.T., A.I.A.T. or with similar qualifications, and experience in the care and handling of laboratory animals) in the PHARMACOLOGY DEPARTMENT—The Registrar, Brighton College of Technology, Moulsecomb, Brighton 7, Sussex (July 15).

S.R.C. RESEARCH STUDENT (with a good honours degree in physics, chemistry, engineering science or forestry) in TIMBER SCIENCE in the DEPARTMENT OF FORESTRY to work on an approved topic in the field of timber technology—The Professor of Forestry, University College of North Wales, Bangor, North Wales (July 15).

ASSISTANT LECTURERS (2) (with a special interest in economic geography) in GEOGRAPHY—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (July 17).

RESEARCH CURATOR in the DEPARTMENT OF GEOLOGY to assist with the preparation of exhibits and demonstrations, curating duties, and to spend half-time in research leading to a higher degree—The Assistant Bursar (Personnel), University of Reading, Reading, Berkshire (July 17).

RESEARCH STUDENTS in the DEPARTMENT OF CHEMICAL ENGINEERING—The Academic Registrar, University of Exeter, Northcote House, The Queen's Drive, Exeter, Devonshire (July 17).

LECTURER (with a good honours degree in psychology, relevant industrial experience, and preferably some knowledge of air transport problems) in ENGINEERING PSYCHOLOGY—The Secretary, Birkbeck College (University of London), Malet Street, London, W.C.1 (July 18).

TUTORS (2) in the DEPARTMENT OF GEOGRAPHY to assist with tutorial groups involving small groups of students and to provide assistance in practical map classes—The Registrar, University College of Swansea, Singleton Park, Swansea, South Wales (July 18).

ASSISTANT EXPERIMENTAL OFFICER (with a degree or equivalent in botany, horticulture or related subject) to assist with ecological work on annual weeds of vegetable crops—The Secretary, National Vegetable Research Station, Wellesbourne, Warwick (July 21).

DIRECTOR OF COMPUTER SERVICES to be responsible for the organization and development of the central computing facility in the University which is at present equipped with an upgraded KDF 9 computer—The Assistant Registrar (S), University of Birmingham, P.O. Box 363, Birmingham, 15 (July 22).

GRADUATE PHYSICIST (preferably experienced or with a higher degree, but suitable new graduates considered) in the DEPARTMENT OF MEDICAL PHYSICS to join a team investigating electron spin resonance signals from animal tissues and develop new techniques—The Secretary, The University, Aberdeen, Scotland (July 22).

SENIOR LECTURER IN RHEUMATOLOGY—The Registrar, The University of Manchester, Manchester, 13, quoting Ref. 109/67 (July 22).

LECTURER (graduate in science or medicine with special qualifications and/or experience in virology) in VIROLOGY—The Secretary, Trinity College (University of Dublin), Dublin 2, Republic of Ireland (July 24).

LECTURER or ASSISTANT LECTURER in ELECTRON BEAM TECHNIQUES in the DEPARTMENT OF METALLURGY AND MATERIALS TECHNOLOGY, to take charge of a group concerned with electron microscopy, microprobe analysis, and related techniques—The Academic Registrar (LFG), University of Surrey, Battersea Park Road, London, S.W.11 (July 28).

SCIENTIFIC INFORMATION OFFICER (with a degree in agriculture or related subjects, a knowledge of German and flair for languages generally, and preferably with a reading knowledge of Japanese) to help production of two scientific journals of international repute—The Director, Commonwealth Bureau of Pastures and Field Crops, Hurley, near Maidenhead, Berks (July 28).

SENIOR LECTURER (with special experience either in bacteriology, immunology or virology); and a LECTURER (graduate in science or medicine) in the DEPARTMENT OF BACTERIOLOGY—The Secretary, The University, Aberdeen, Scotland (July 29).

CHAIR OF BIOCHEMISTRY at the University of Auckland, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, July 31).

CHAIR OF STATISTICS; and CHAIR OF APPLIED MATHEMATICS—The Registrar, University College of Wales, Aberystwyth (July 31).

GRADUATE RESEARCH ASSISTANT (preferably with experience in geology, palaeontology and modern taxonomy) to work on a mesozoic palynology project for one to three years—The Secretary, Department of Geology, University of Cambridge, Downing Street, Cambridge (July 31).

LECTURER/ASSISTANT LECTURER (with experience in comparative neurophysiology and/or ultrastructural research) in the DEPARTMENT OF ZOOLOGY AND COMPARATIVE PHYSIOLOGY—The Assistant Registrar (S), University of Birmingham, P.O. Box 363, Birmingham, 15 (July 31).

LECTURER (preferably medically qualified or in possession of a Ph.D. degree with a basic training in pharmacology) in the DEPARTMENT OF PHARMACOLOGY—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh (July 31).

READERS (2) in MECHANICAL ENGINEERING—The Academic Registrar (LFG), University of Surrey, Battersea Park Road, London, S.W.11 (July 31).

RESEARCH DEMONSTRATORS (2) (with a good honours degree in chemical engineering or, exceptionally, in other branches of engineering, in chemistry or in applied physics) in the DEPARTMENT OF CHEMICAL ENGINEERING—The Academic Registrar, University of Exeter, Northcote House, The Queen's Drive, Exeter, Devonshire (July 31).

SENIOR LECTURER or LECTURER in SOCIAL ANTHROPOLOGY at the University of Auckland, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, July 31).

ASSISTANT LECTURER or LECTURER in the DEPARTMENT OF INORGANIC, PHYSICAL AND INDUSTRIAL CHEMISTRY—The Registrar, The University, Liverpool, quoting Ref. RV/62/N (August 1).

UNIVERSITY LECTURER (preferably with special interests in one or more of the following: theoretical geochemistry, crystal chemistry, sedimentary geochemistry) in GEOCHEMISTRY in the DEPARTMENT OF GEOLOGY AND MINERALOGY—The Secretary of Faculties, University Registry, Oxford (August 1).

LECTURER/ASSISTANT LECTURER (with a background in the physical sciences and preferably bilingual in Malay and English) in SCIENCE METHOD; a LECTURER/ASSISTANT LECTURER (preferably bilingual in Malay and English) in GEOGRAPHY METHOD; and a LECTURER/ASSISTANT LECTURER in EDUCATIONAL PSYCHOLOGY at the University of Malaya—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Kuala Lumpur and London) (August 4).

SENIOR LECTURER or LECTURER (Clinical) in PSYCHIATRY at the University of Queensland, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, August 4).

SENIOR LECTURER or LECTURER in the DEPARTMENT OF PATHOLOGY, Monash University—The Academic Registrar, Monash University, Wellington Road, Clayton, Victoria, Australia; or The Secretary-General, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia, August 4).

READER in PHARMACEUTICS at the School of Pharmacy—The Academic Registrar, University of London, Senate House, London, W.C.1 (August 7).

LECTURER in MATHEMATICS at the University of Natal, Pietermaritzburg, South Africa—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, August 15).

GENETICIST (with an interest in the cytology of interspecific crosses); and an AGRONOMIST to work mainly on flowering control, and on agronomic characters in selection—The Secretary, West Indies Central Sugar Cane Breeding Station, Groves, St. George, Barbados (August 30).

PROFESSOR OF MATERIALS TECHNOLOGY in the DEPARTMENT OF METALLURGY AND MATERIALS TECHNOLOGY—The Academic Registrar (LFG), University of Surrey, Battersea Park Road, London, S.W.11 (August 31).

PROFESSOR OF CLINICAL SCIENCE AND DIRECTOR OF THE WELLCOME MEDICAL RESEARCH INSTITUTE, University of Otago, Dunedin, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, September 15).

BIOCHEMICAL RESEARCH ASSISTANT (Basic Grade) for studies of human muscle metabolism in health and disease (post suitable for recent graduate) at the Midland Centre for Neurosurgery and Neurology, Holly Lane, Smethwick—The Group Secretary, West Bromwich and District General Hospital, Edward Street, West Bromwich.

GRADUATE FOR BIOLOGICAL RESEARCH in the DEPARTMENT OF DERMATOLOGY—Dr I. Sarkany, The Royal Free Hospital, Gray's Inn Road, London, W.C.1.

JUNIOR LECTURER in CHEMISTRY—The Registrar, Victoria University of Wellington, P.O. Box 196, Wellington, New Zealand.

LECTURER (with a first- or second-class honours degree or equivalent, a thorough grounding in electrical engineering with a special interest in the field of electrical machines and/or electrical materials and in their applications and preferably experience in teaching, research or industry) in ELECTRICAL ENGINEERING—The Registrar, Royal Military College of Science, Shrivenham, Swindon, Wiltshire.

LECTURER (with an interest and experience in educational psychology with particular reference to learning theory and/or the methodology of teaching) in EDUCATION at the University of Malawi—The Inter-University Council, 33 Bedford Place, London, W.C.1.

**PHYSIOLOGIST or ZOOLOGIST (graduate)** for research on emotional behaviour in the rat—Dr. J. A. Gray, Institute of Experimental Psychology, The University, 1 South Parks Road, Oxford.

**POSTGRADUATE PHYSIOLOGY STUDENT** for research into ion transport across membranes—Prof. J. Anderson, Department of Medicine, King's College Hospital Medical School, London, S.E.5.

**RESEARCH ASSISTANT** in the DEPARTMENT OF PHYSICS to work with a research group supported by the Science Research Council investigating the properties of metals at high rates of loading using pulse propagation methods—Prof. John Raffle, Loughborough University of Technology, Loughborough, Leicestershire.

**TECHNICIAN** to operate and maintain analytical ultracentrifuges and other specialized instruments for research in molecular biology—The Administrative Officer (Ref. EGR), Biophysics Research Unit, Medical Research Council, 26-29 Drury Lane, London, W.C.2.

**YOUNG GRADUATE or TECHNICIAN** (preferably with experience with an autoanalyser for amino acid analysis) for the BIOCHEMISTRY RESEARCH LABORATORY—The Establishment Officer, University College London, Gower Street, London, W.C.1, quoting Ref. BC/9.

## REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

### Great Britain and Ireland

The Water Research Association. Twelfth Annual Report for the year ended December 31st, 1966. Pp. 48. (Medmenham. Marlow: The Water Research Association, 1967.) [155]

University of Strathclyde. Annual Report, 1965/66. Pp. 76. (Glasgow: University of Strathclyde, 1967.) [155]

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## IS THE AEA NECESSARY?

NOBODY needs a clairvoyant to persuade himself that the weeks ahead will be critical for the UK Atomic Energy Authority. The doubts which have hung over the AEA for several years, and which have been explicit for at least two, are plainly about to be resolved. There are a number of signs of that. Although Sir William Penney is about to retire as chairman of the authority in only a few weeks, his successor has not yet been appointed. Then the Select Committee on Science and Technology of the House of Commons has uncovered—by no particular cleverness on its own account—a great deal of confusion and even anger about the way in which the commercial exploitation of nuclear reactors is being carried out (see *Nature*, this issue, page 235). There is talk, some of it wild, about the dismemberment of the authority, and it seems as if the committee appointed to consider the future of the thermonuclear fusion laboratory at Culham has raised the possibility—unacceptable to the board of the authority as a whole—that the laboratory might with advantage be shut down altogether. From time to time, ministers in the House of Commons promise statements on the future of this or that part of the authority's activity. The departure of Sir John Cockcroft from the authority at the end of this month will be a sad occasion, and a nostalgic reminder that things will never be the same again.

The reasons why the authority has been transformed from a kind of national hero in the mid-fifties into a subject for dispirited speculation are also easy to determine. In part, of course, the AEA has worked itself out of a job, at least where research and development are concerned. A decade ago there were half a dozen types of civilian reactors on the stocks, ranging from thermal gas cooled reactors to marine propulsion units and fast reactor prototypes. It was entirely sensible that this programme should engage the efforts of a large engineering force based in Lancashire and a large laboratory engaged on basic research at Harwell. For a time, indeed, there was even some sense in the AEA's argument in favour of a reactor testing station at Winfrith Heath in Dorset. And a decade ago, of course, there was a sufficiently vigorous military programme to occupy the efforts of the large laboratory at Aldermaston. In one way or another, the steam has now gone out of most of these activities. In defence, economy has become the watchword, and there is not much for the laboratory at Aldermaston to do. On the civilian side, it is now clear that there is no real need to develop types of thermal neutron reactors other than those now coming into service with the Central Electricity Generating Board. Fast reactors have a future,

and may even begin to make a commercial contribution a decade hence, but development work on them was costing only £12 million last year, and is likely to cost only £150–£200 million between now and 1975, when fast reactors may become commercial. In the circumstances it is not surprising that the laboratory at Harwell, like that at Aldermaston, looks a little like a whale left stranded by a retreating tide. What is going to happen?

The first thing to say is that in spite of all the uncertainties, the AEA has a splendid record for getting things done. It was able to function with speed and panache in the mid-fifties, when the rest of British industry was still too inclined to feel sorry for itself. That is something to be pleased about. But the authority, like the government to which it is responsible, has been unreasonably slow to appreciate the need of change. In the past few years it has oscillated between defensiveness and a pretence that nothing has changed. Much of the trouble can be traced back to the way in which the AEA evolved out of the old atomic energy group at the Ministry of Supply. From the beginning, it was an immensely powerful organization. In its relationships with academics, the authority was rightly respected. In its dealings with industry, it was able at the beginning to act paternal or, worse, avuncular. The authority's unkindest critics may say that it was endowed with all the arrogance but none of the inhibitions of the Civil Service from which it sprang. Whatever may be the most accurate form of words, the truth is that the AEA's inclination to do all its research and development itself is one of the reasons why it is now so vulnerable to changing circumstances—and, in passing, one of the reasons why the nuclear power industry in Britain finds independence uncomfortable. There is a powerful echo here of the declaration some months ago by Professor P. M. S. Blackett (see *Nature*, 213, 755; 1967) that successive British governments may have been mistaken in choosing to concentrate research and development in publicly operated laboratories.

Muck-raking by itself will not unfortunately suggest what should be done, although a recognition of past mistakes may help to avoid a repetition. The first need is to be clear why reorganization has now become essential. In the first place, there is a need of structural reorganization of the relationships between the AEA, the CEBG and the industrial consortia in the nuclear power business, chiefly so as to ensure a more effective exploitation of nuclear power domestically and in exports. This is the problem with which the Select Committee has been principally concerned, and it is improbable that the Minister of Technology will have



the cheek to announce important decisions before the committee produces its report some time after the summer holidays. But it is also important to make sure that the fullest use is being made of the resources at the disposal of the AEA, and from this point of view it is more important that Harwell employs 6,000 of the most skilled people in the United Kingdom than that it costs £15 million or so each year to operate. Obviously it is impossible to guess at what will actually happen to this and the other laboratories in the AEA, for even if the facts were all accessible, there can be no assurance that the Minister of Technology will be guided by pure reason and nothing else. Yet it is possible to see which way the wind is blowing or should blow.

Some things are simple. The Culham laboratory, principally concerned with long-term plasma research, will obviously be exceedingly uncomfortable if it continues within the AEA, at least as long as the Ministry of Technology is preaching the virtues of quick results. But it would obviously be unwise to throw away the investment of the past decade in thermonuclear fusion, especially when the Culham laboratory has acquired a distinguished international reputation. In the circumstances, the best place for it would be within the Science Research Council, where it would be insulated from a great many of the pressures which at present afflict it, and where it could also serve more effectively to fertilize research at the universities.

But on what scale? To people at Culham, this may seem the most important question, and there has recently been some optimism because the budget of the US Atomic Energy Commission for the current fiscal year includes an extra 15 per cent for research in fusion, and because the US Atomic Energy Commission has smugly predicted that it will need an extra 15 per cent in each of the next five years. It is important therefore that the AEC won its extra funds (which amount to rather less than \$3.5 million for 1968) by emphasizing the way in which expenditure at places such as Culham has grown in recent years, and that in any case the AEC may find itself in a similar plight to that of the UK authority long before five years have gone. In practice, there is a case for letting the Culham budget find its own level within the competitive atmosphere of the Science Research Council. The chances are that the budget would settle down at less than what it is at present, but that shrinkage would be slow. The Culham laboratory should also in future be restrained from over-ambitious ventures outside fusion proper. The way in which the laboratory has hogged the centre of the stage in the construction of the ESRO satellite has hindered rather than helped the cause of European collaboration.

The problem of Aldermaston is next in order of difficulty. The laboratory is larger, with 4,000 people on the books. It stands apart from the rest of what the AEA is up to, chiefly because of its inevitable pre-occupations with secrecy. For good management alone, the Aldermaston laboratory would most comfortably be placed alongside the other laboratories

preoccupied with defence technology—Aldermaston (aircraft) and Malvern (radio) beneath the wing of the Ministry of Defence. This implies that Aldermaston, too, is at home within the Ministry of Technology, and that its people not at present fully occupied in the industry have a better chance to move to useful jobs, or at least to be fitted out for a new career in modern industry.

Harwell is a more difficult problem, partly because the laboratory does actively support reactor development and partly because it has for two years been pursuing—with the blessing and the encouragement of the Ministry of Technology—the most active programme of diversification in any government laboratory. Unfortunately it remains a fact that if Harwell did not exist, nobody would dream in present circumstances of inventing it. Direct support for reactor development, which occupies perhaps a fifth of what the laboratory does, could if necessary be done elsewhere. The programme of diversification, concerned as it is with matters as different as the development of preparative centrifuges and the desalting of water, may be too much of a hotchpotch to accommodate within one organization and is, in any case, too much detached from industry. Historically, of course, diversification derives from the palmy days in which the Labour Party, not then a government, cherished oversimple notions of strategy for technology which have now been replaced by good sense except in connexion with public day-dreaming about what is called the European Technological Community. In other words, if the Minister of Technology is tender-hearted and suggestible, he will give Harwell a year or so to see whether diversification really works. Nobody would complain if he took the only other acceptable course, and lumped it with Aldermaston into the Ministry of Technology.

But is it permissible to treat people, especially talented people, as if they were parcels of equipment? This question may have helped to hypnotize the Ministry of Technology in the past few years. In a sense, the government's reputation as an employer of scientific labour is at stake. Although the AEA has been an autonomous public corporation for more than a decade, there is no doubt that the government as such is ultimately responsible for broad policy decisions. If the laboratories at Aldermaston and Harwell were now unthinkingly transferred back to the Civil Service, the people most concerned would be more dispirited than ever.

Everybody knows that if creative people engaged in applied science have no realistic objectives at which to aim, they tend to go to seed. Uncertainty has persisted so long at Aldermaston and Harwell—and at other government laboratories as well—that it has become a considerable scandal. What the Ministry of Technology must first do is to make a realistic decision about which parts of these two laboratories would be most usefully and creatively employed within the Civil Service. Then it must squarely face the need to find outlets elsewhere for the talents now frustrated

at the two laboratories. It will not be enough to sit back and wait for the problem to solve itself—the principal result of doing nothing will be to increase the rate of emigration. In part, the Ministry of Technology must set itself up as a superior labour exchange, able to find jobs with tempting salaries in industry and elsewhere for people who are the victims of their own success. Often it could help with training programmes, and it is ironical that the computer people should be crying out for systems analysts at a time when there are plenty of able people eating their hearts out at Harwell and Aldermaston. Above all, the ministry should recognize that the characteristic meanness of the public service towards those who work for it is one of the most serious impediments to change.

There remains the problem of how the development of new reactor systems should be managed in the years ahead. Unfortunately the Select Committee was so preoccupied with trying to decide which kind of reactor system is best that it did not examine as closely as it should have done the several alternative patterns of organization which are possible. At one extreme there is something very like the present arrangements under which the AEA is largely responsible for new developments and even for the construction of sizable prototypes, in which the three consortia are kept closely informed and given opportunities to tender for whatever orders there may be, in which the CEBG acts as the final arbiter of what kinds of power reactors should eventually be incorporated into the British programme, and in which the AEA and the three consortia do their best to work hand in glove in exploring markets abroad. Most of the defects of these arrangements stem from the way in which the AEA has been able to dominate its industrial partners technically and financially, with the result that the consortia lack experience, skill and confidence. This is why some people say that it would be better if the AEA concentrated on the processing of fuel and reactor materials, the ownership and operation of central facilities such as testing reactors, long-term planning and the allocation of funds for actual developments to those industrial companies best able to make use of them. This is a pattern very much like that in the United States—and it is perhaps important that the AEA is constantly having to complain that even in the United States industrial companies are unwilling to invest much of their own money in new kinds of reactors. Fortunately most of the interested parties seem to be agreed that there should be less wasteful duplication on reactor design and development, which implies some centralized authority spending public money, but with much stronger links with industry. The need is already recognized where experts are concerned, although not much has been done so far. It would be entirely in character with the ministry's recent pronouncements if it now looked to the Industrial Reconstruction Corporation for a more comprehensive solution.

With all this preoccupation with reorganization it should not necessarily be accepted that the present

programme of reactor development should, like the more distantly oriented research programme, be dispersed. Indeed, it is entirely possible that the development of fast reactors should not be restricted to the liquid sodium type. A year or so from now, the AEA or whichever other authority is in charge may easily wish to explore the potential benefits of gas cooled fast reactors, for example. Anything is possible and one object of the reorganization now in prospect should be to tie the development of new types of reactors much more simply than at present to straightforward commercial objectives. If, for example, it seems that a development on which work is well advanced is unlikely to be profitable, work should be stopped and nobody should cry scandal. (The Dragon high temperature gas cooled reactor could easily become one case in point.) But on the other hand there should be every encouragement for the responsible authority to back two alternative horses or even more if it seems that one of them is bound to win a substantial prize but if it is also impossible in advance to predict which will be the winner. All this is entirely consistent with the view that what is coming to an end is not nuclear power as such, but the need of a greater diversity of new developments.

In the real world, of course, nothing is simple, and in this sense there will be no reason to complain if the Ministry of Technology produces a scheme of reorganization which is not as tidy as it might be. The problem of how best to organize the relationship between the nuclear power industry and the rest of the British industrial economy is also unlikely to be settled in one swoop, fell or otherwise, so that it would make sense to have one scheme now and another three or four years hence. To say this is not, however, to imply that it will be in order for the Ministry of Technology to dodge the issues which have now been raised by circumstances, and to let uncertainty persist. If the AEA may in part have worked itself out of a job, it remains one of the most competent and formidable technical organizations in Britain. The problem with which the ministry must grapple is how to redeploy some of these resources. To do so, it will have to define—for others, not just itself—a coherent picture of how these invaluable resources might otherwise be occupied. Its vision of the future must take full account of how the people most directly concerned have wives, families and mortgages on houses in frequently outlandish places. Successful reorganization could bring great benefits and make everybody feel happier. Failure could be disastrous. And this, in a curious way, is as it should be. For the past two years the British Government has been telling everybody what wonders it would accomplish if only it had a real chance to redeploy technical resources in industry. All the time there has been a splendid opportunity waiting on its own doorstep. Not merely the future of this important sector of scientific industry but its own reputation as well will be determined by its decisions in the weeks ahead. Will it be brash, or cowardly, or somewhere in between?

## NEWS AND VIEWS

### No Future for Collaboration?

THE failure of Anglo-French collaboration on a variable-geometry aircraft has been so long discounted that when it finally happened the twitching of upper lips in Whitehall was barely perceptible. The agreement, which is a polite way of describing the working truce which Mr Denis Healey and M Pierre Messmer had reached, seems to have been a model of how not to collaborate. For a start, both sides had a totally different conception of what the aircraft might be—the French wanted an interceptor to operate primarily within Europe while the British requirement was for an aircraft with much more general functions. It was to be a strike aircraft, an interceptor, a reconnaissance plane. At one stroke it would replace Canberras, Phantoms, Lightnings, conceivably even the F 111 K. At the same time there were hopes that the German Air Force would want it as a replacement for the Starfighter. Overburdened by its roles, the plane simply lay down and died; although the coup de grace was wielded by M Messmer on grounds of cost, there is little doubt that ultimately there would have been technical reasons for opting out. One of these was the development by Dassault of the Mirage 3G, a single-engined aircraft which may well fill the role the French see for the VG aircraft.

The cancellation must give the Ministry of Defence furiously to think. The 1966 Defence White Paper described the AFVG as "operationally and industrially the core of our long-term aircraft programme". For delivery in 1974, each plane was to have cost about £1.5 million. This is to be compared with the cost of the F 111 K which is being bought from the US; a ceiling price of £2.1 million for each aircraft has been agreed, but may well only cover the first fifty to be bought. After the modifications and additions which the RAF insist on, the planes will each cost £2.5 million, and will as bombers be able to carry about twice the load designated for the AFVG.

The ministers responsible, Mr John Stonehouse at Technology, and Mr Healey at Defence, are now said to be looking for alternative partners for the VG project. This may be hard indeed, because of Britain's peculiar position. With commitments in the Far East, and a defence policy which makes an uneasy compromise between the high sophistication of the US and the USSR and the down to earth practicality of a nation like Sweden, Britain's requirements are unique. All the arguments against collaboration with France apply in equal measure to collaboration with Germany, Holland, or Italy. To pretend otherwise is a delusion.

As if to rub salt in the wound (though it is doubtful if any such deviousness entered the question) the USSR last week demonstrated its own swing wing aircraft. There were apparently two of them on show, one an interceptor-fighter, the other a strike aircraft. Clearly the USSR believes that the two roles call for two aircraft. Also shown was a vertical take-off aircraft. In appearance the Russian planes were somewhat similar to the F 111.

What now is the future of Anglo-French co-operation? There are currently five projects either in prospect or in existence: Concord, the Jaguar fighter, the proposed airbus, the Martel guided missile, and a joint agreement on helicopters. Two helicopters are to be produced, the French SA 340 and the British WG 13. In the interest of mollifying the French, much of the design leadership has been ceded to them, and Mr Stonehouse (or Mr Healey) may now feel that without the VG the helicopter agreement looks rather one-sided. Attempts to adjust it in Britain's favour will doubtless be made, but the result may very well be that the helicopters will follow the VG into limbo.

### Computers Hang Fire

THE latest figures produced by the Ministry of Technology about the British computer market make cheerless reading. Between the first quarter of 1966 and the first quarter of 1967, exports of computers manufactured in Britain were substantially unchanged, and amounted to £8.3 million in the quarter. During the same period, the delivery of British computing equipment to the domestic market actually declined, from £21 million in 1966 to £18 million in 1967, largely because of a reduction of the value of computer equipment manufactured in Britain from components imported from elsewhere.

This sad state of affairs is reflected, in part at least, in the figures provided by the ministry for computers now on order from British manufacturers. Between the first quarter of 1966 and the first quarter of 1967, there was no important change in the value of export orders, which declined slightly from £28 million to £27 million. Domestic orders for computing equipment increased substantially, however, from £88 million to £133 million. There will no doubt be wry speculation in some quarters that in spite of the British Government's economic restrictions in the past year, such growth as there has been consists of domestic investment and not export. The ministry points out that there are great uncertainties in these comparisons, chiefly because the figures for 1966 do not strictly compare with those for the current year, but even so it is compelled to acknowledge that there is "cause for anxiety". The ministry is also unhappy that £55 million of the orders for the domestic market are for what is called "factored equipment".

One by-product of this statistical exercise is that the ministry now has available figures showing how many people are employed in various stages of computer development and manufacture. Altogether some 37,300 skilled people are employed in the computer industry, 11 per cent of them on design and development, 41 per cent on manufacture and 13 per cent on sales.

### Whatever Happens to Physicists?

MORE information about the distribution of PhD physicists in Britain has now been gleaned by Professor J. C. Willmott, Director of the Physical Laboratories at the University of Manchester. The survey which Professor Willmott has been carrying out was mentioned briefly in *Nature* (214, 443; 1967) and has recently been published in the *Bulletin* of the Institute of Physics and the Physical Society.

Professor Willmott's survey follows one made in 1961 by Professor A. W. Merrison, which showed that of physicists with doctorates, 26 per cent entered government service, 22 per cent went into industry, 27 per cent stayed in the universities, and 18 per cent went abroad. After some prodding, Professor Willmott persuaded 26 universities to reply to a similar questionnaire in October 1966, and produced the results shown in Table 1.

	First employment		Present employment	
	No.	Per cent	No.	Per cent
Government service	83	11.2	79	10.8
Industry	95	12.9	79	10.8
Universities	308	41.8	302	41.2
Technical colleges	18	2.4	23	3.1
Teaching	18	2.4	16	2.2
Abroad	217	29.3	234	31.9
Total	739	100	733	100

The table shows first that a substantial number of physicists have stayed in universities, a piece of conventional wisdom these days, and the fact that government laboratories are now taking fewer than in the past because of the more modest scale of their activities. What alarms Professor Willmott is the sharp increase in the numbers going abroad and the decline in the numbers going into industry. Not only have the numbers declined since 1961, but there is an actual decline between first and present employment, from 12.9 to 10.8 per cent. Professor Willmott fears that physicists take one look at industry and then turn tail and rush back to the universities as fast as they can. (It is fair to point out that the Swann report did not paint such a dismal picture—it suggested that the proportion going into industry was nearer 20 per cent.)

These figures are not, perhaps, surprising, but add to the volume of evidence which is now accumulating about the use of qualified manpower in Britain. But Professor Willmott has gone further in seeing differences between different branches of physics. While the sample was smaller, and the division between different branches of the subject sometimes blurred, the figures are revealing. Of solid state physicists, for example, 19.7 per cent take their first job in industry, while only 5.7 per cent of nuclear and elementary particle physicists do so, and only 2.6 per cent of theoretical physicists. In each case the numbers show a decline between first and present employment. The numbers staying in universities—36.7 per cent of the solid state physicists, 41 per cent of the particle physicists, and 55.3 per cent of the theorists—are almost constant between first and present employment. It is difficult to draw any conclusions from this, except the obvious one that investment in solid state research in universities is more effective industrially than investment in other forms of physics: it is still a poor investment, though.

## In and Out of Space

THIS is likely to have been a week of great importance for space research in Europe. Although Europa I, the sixth in the programme of rocket launchings being organized by ELDO from Woomera in Australia, failed to leave the ground on Monday, July 10, the chances are brighter than they have been for at least a year that there will now be some sensible marriage

of the collaborative programmes in Europe for rocket development and space research. Certainly the proposal that ELDO (European Launcher Development Organization) and ESRO (European Space Research Organization) should be merged was one of those discussed at a meeting of government delegates at Rome this week. It remains to be seen whether member nations will be prepared to sink their separate ambitions in a programme which would develop a launching vehicle and then provide something for it to do. Another of the questions to be decided is how best to provide machinery for a continuing review of policies within ELDO and ESRO, married or single. Nobody wants to repeat the unseemly public squabbles which nearly brought ELDO to an end a year ago.

European space research will also be helped along a little bit by the holding in London between July 17 and 29 of the annual ICSY meetings of the IQSY (International Year of the Quiet Sun) and of COSPAR. It is thought that the IQSY meeting will be one of the first opportunities to survey the work of that period of collaborative activity.

## Good Health

THERE has been a curious contrast in the past week between the annual report of the Ministry of Health (HMSO, 19s.), with its cosy account of how the National Health Service in Britain is developing, and some of the things which doctors have been saying at the annual conference of the British Medical Association at Bristol. Innocent observers would be hard pressed to know whether the service is making progress towards some comfortable millennium or, alternatively, will have collapsed before the year is out.

The Ministry of Health acknowledges that the National Health Service "continued to be the subject of much public discussion", and regrets that "some of this has sought to give the impression that the year has been one of disappointment and failure". The truth, says the ministry, is that there has been "a striking measure of success". In practice, £75 million is to be spent on hospitals in the current financial year, which is said to be 63 per cent more than the rate of spending (in real terms) before the war; but in any case the ministry plans to spend £100 million a year in the next decade on new hospital building. The report unfortunately does not say whether the ministry considers an increase of 63 per cent in the rate of hospital renewals is enough to cover the increased sophistication of hospital treatment since before the war.

On the productivity of hospitals, the ministry points out that the number of patients being treated in hospitals has increased by 30 per cent in the past decade even though the population as a whole has only increased by 7.6 per cent. There are also increases in the numbers of patients treated in each available hospital bed—in 1956 the average was 16.6 patients per bed per year and in 1966 it had increased to 22.6. The ministry is also able to claim that medical staffs in hospitals have increased numerically by 60 per cent since 1949, but the report admits that still more doctors are necessary if the hospitals are to function efficiently. It points out that, in the past two or three years, there has been a vigorous growth of health and welfare services provided by local authorities. One



reason for this is that health centres housing both general practitioners and local authority services are springing up in many places. The ministry seems to be convinced that this development will take an increasing proportion of the health service budget in the decade ahead.

The report also gives details of the total size of the budget and how it is divided among the competing branches of the service. In 1965/66 the Health Service cost £1,241 million, a figure expected to rise in 1966/67 to £1,367 million. Hospitals account for about half the total budget (£687 million on current expenditure and £81 million on capital expenditure in 1966/67). Drugs will cost £142 million this year, local health services £127 million, general medical services £98 million, and dental services £70 million. Income from patients in 1966/67 is expected to be about £19 million. Faced with figures like these, it is easy to understand the problems which the minister, Mr Kenneth Robinson, must face when he asks the Treasury for more. It is also easy to see why the doctors were driven to suggest in Bristol that more of the cost of the service should be carried by the patients. Ignoring the most extreme attitudes, sometimes expressed in the claim that the relationship between patients and doctors would somehow be magically transformed for the better when money is allowed to change hands, there may nevertheless be a case for saying that the Health Service will have to become more selective.

## Boom in Physics

THE Institute of Physics and the Physical Society (which is the name of a single learned society based in London) is evidently pleased with its own growth in the past year and the prospects for extending its activities into Europe in the immediate future. In the annual report of the council of the Institute of Physics and the Physical Society presented to the annual general meeting on July 4, membership is said to have increased by 10 per cent to a total of 12,350 at the end of 1966. Although this implies a more rapid increase than that of the money being spent on research in physics, the Institute of Physics and the Physical Society is disappointed that its membership includes fewer than half of those eligible for membership.

The European venture is now apparently reaching a point at which decisions will be possible, and a steering committee under Professor G. Bernadini has been circulating the draft of a constitution for a federation of physical societies on a European basis. It is hoped that reactions to the proposals will be sufficiently well known by October or November for the steering committee to settle for a final version of the constitution. By all accounts, everything is going well, and the chances are high that by the end of the year or the beginning of 1968 a federal physical society will be in being.

Commercially, the Institute of Physics and the Physical Society continues to do well. In 1966 it was able to transfer £10,674 to the reserves on a turnover of £279,000. More than a third of its income comes from the sale of publications. This year, in contrast with 1965, profit on publications has been largely swallowed up by the cost of launching the new journal *Physics Education*—a venture which in commercial

terms cannot be expected to break even for some years after its foundation. In its report, the council has included graphs which show that the numbers of words of text printed in its journals are growing less rapidly than might have been expected. With the *Proceedings of the Physical Society*, for example, bulk has increased by only 50 per cent since 1961. The *British Journal of Applied Physics* has not grown in bulk since 1964.

At the meeting on July 4, Dr A. D. I. Nicol (Cambridge) was elected a vice-president of the Institute of Physics and the Physical Society, P. T. Menzies honorary treasurer, Dr R. Press honorary secretary, and Professors S. F. Edwards and C. A. Hogarth members of council.

## Hopeful Forecast

CONSIDERABLE improvements in weather forecasting were achieved during 1966, according to the report of the Meteorological Office for that year. Two reasons are given; first the acquisition of equipment for the automatic drawing of forecast charts for 48 hour forecasts (described in *Nature*, **214**, 230; 1967) and secondly an improved method for detecting the small scale changes which are one of the hardest problems of weather forecasting. This new approach uses a sophisticated model of the atmosphere, taking account of the water vapour in the air, and predicts pressure, temperature, wind and rainfall at hundreds of places 25 miles apart. To make the best use of the new method, though, the office will need a much faster computer; for a 24 hour forecast, the Atlas takes no less than eight hours to produce results.

The report preens itself a little about the report of the Estimates Committee of the House of Commons, which examined meteorological services during the year. Apart from congratulating the Meteorological Office on its efficiency, the committee seems to have agreed with the Office diagnosis of what needs to be done. A major expansion at Bracknell to overcome the shortage of space is one suggestion warmly welcomed by Dr B. J. Mason, Director of the Office. There also seem to be staff difficulties, principally in the scientific assistant grade where the recruitment rate cannot keep up with the resignations. There is also a shortage of experimental officers trained in experimental physics, electronics and computing, and these will have to be recruited on a much larger scale.

A new departure was the establishment of a Cloud Physics Branch at Bracknell. This includes a laboratory physics group transferred *en masse* with Dr Mason from Imperial College London, a cloud dynamics group, and a radar unit. To get the best from the branch, Dr Mason hints, new aircraft will be needed for the Research Flight. The Office has also flown experiments in rockets during the year, and one to study molecular oxygen in the British satellite UK 3. During the year regular direct reception of pictures began from the weather satellites launched by NASA; these have been very useful in weather analysis and forecasting. Difficulties of recruitment are aggravated by the lack of suitable university courses in Britain, and for some years the gap has been filled by the Meteorological Office Training School. Dr Mason reports that, at last, new accommodation has been found for the school, which will move from Stanmore to Shinfield Park in Reading at the end of 1968.

## Visitors to Britain

THE British Council seems to be pleased with the way its scheme for awarding scholarships for advanced study has been building up. In the present academic year, 126 scientists from various places are studying in Britain, most of them for periods of one academic year or more. Since 1963, the numbers of scholarships awarded to scientists have increased by roughly 50 per cent. At present, the pure sciences and technology account for 49 and 50 scholarship holders respectively. There is a steady trickle of half a dozen or so each year of people anxious to have first-hand experience of school science teaching in Britain. Most of the visitors to Britain under the scheme come from European countries—in the past four years there has been an especially rapid growth of business with Czechoslovakia, Hungary, Romania and Bulgaria, and 32 scientists from these countries are now in Britain under the scheme. 39 scientists have come from elsewhere in Europe and the scientists now account for more than a quarter—or, if awards to doctors are also included, more than 40 per cent—of the scholarships awarded by the British Council each year.

The ideal British Council scholar is a man or woman between 25 and 35. Although the awards cover cost of travel, tuition and maintenance, one snag may be that they are not always generous enough to keep all applicants in styles to which some of them may have become accustomed. Another difficulty, no doubt inescapable in arrangements of this kind, is that applications must be made roughly a year before the beginning of an academic year.

## New Chairman for CEI

MR LEONARD DUCQUER is the new Chairman of the Council of Engineering Institutions. He was elected on July 13 to the post left vacant by the death of Mr H. N. Pemberton on April 6, 1967. Mr Ducquer, 65, now takes office for a period of 18 months.

Mr Ducquer was educated at Haberdashers Aske's and the London Polytechnic Engineering College, and began his career with the British Thomson Houston Company in 1920. Until 1938 he worked in outside construction and contract engineering, and then moved to selling switchgear. BTH is one of the roots from which Associated Electrical Industries Ltd sprang, and from 1958 to 1965 Mr Ducquer was divisional director and general manager of the Heavy Plant Division of AEI. Since 1965, he has been a group consultant to AEI and a member of the board of AEI Automation. Mr Ducquer has been a member of the Council of Engineering Institutions for some years, and already has experience of its internal operations; he has been chairman of the overseas relations sub-committee and the finance sub-committee.

## Women Engineers

WOMEN feel that they are an underprivileged class, at least where engineering is concerned. This is what emerged from the last day of the Second International Conference of Women Scientists and Engineers, held last week in Cambridge. Delegates felt that the difficulty lay in attracting girls into engineering, as

there are always places for the determined few who wish to make it their career. The unfortunate view of engineering as a profession for those who like to get oil under their nails gives it a particularly unfeminine image, while very few schools are prepared to encourage their girls to do practical rather than academic science. Several speakers described how they met not only with discrimination in employment (a survey in Canada revealed that women engineers are better qualified than men in equivalent jobs) but also with some social hostility from their contemporaries, both male and female. Mrs Josephine Webb produced some interesting figures to show how strong these pressures are.

Table 1. ESTIMATED RATIO OF MEN TO WOMEN ENGINEERS IN VARIOUS COUNTRIES

	Ratio of men engineers to women engineers
USSR	3
Philippines	40
France	50
Sweden	60
USA	130
Britain	300
Japan	400
India	500
Canada	500
Republic of South Africa	4,000

There seems to be less prejudice against women as engineers in some developing countries than elsewhere. Delegates described how in India and the Philippines, women engineers can command well paid responsible jobs and become respected members of society. In Nigeria, however, the attitude of men towards women engineers is just as bad as in the West. One reason suggested for the "backward" attitude of the West was that the position of a woman as a mother is more highly regarded than her position as a technologist.

Most delegates agreed that the way to improve the situation is through education. Because most women who graduate in engineering go into industry, there is a shortage of teachers of technical subjects, and this does not encourage a more liberal attitude in the schools towards engineering as a career for girls. In future perhaps women engineers who cannot work full time because of their family commitments will be able to go into teaching part time, but this is by no means the whole solution to the problem. A delegate from Nigeria pointed out that one of the few positive things many women engineers can do is to have daughters, and bring them up with a different attitude to technology.

## End of an Era?

THE UK Atomic Energy Authority has been saying goodbye to Sir John Cockcroft, the first director of the Atomic Energy Research Establishment at Harwell and a member of the UK Atomic Energy Authority since its formation out of the atomic energy research group at the Ministry of Supply in August 1954. Sir John has been Master of Churchill College, Cambridge, since 1959, and a part-time member of the board of the AEA since that time. Although Sir John does not retire officially until the end of this month, his colleagues on the AEA found his last appearance at a board meeting on July 6 something of a wrench.

Within the AEA, Sir John is most affectionately regarded for the intellectual stature which he gave to the Harwell establishment from its beginning

more than twenty years ago. For several years, the laboratory was an exceedingly unusual compromise between a university and a successful industrial laboratory. Harwell also managed under his guidance to avoid the worst excesses of too much security. To have demonstrated the artificial disintegration is obviously as much a help when dealing with policemen as with other physicists.

As is the modern custom, there seems to be no prospect that retirement will allow Sir John to do less work. For one thing, of course, being the head of a Cambridge College is enough to keep many other people fully occupied. Among the extra-mural activities on which he is engaged, however, becoming President of the Liberal Party next year will be particularly interesting, if only because it will provide an opportunity for a distinguished but never compliant public servant to say many things which have not been possible while on the government payroll. The first benefits—which will not everywhere be acclaimed as such—could come quite soon.

## More Uranium in South Africa

THE most interesting feature of the report of the Atomic Energy Board of the Republic of South Africa is that the search for uranium and thorium reserves has been renewed. The board feels that in the foreseeable future the demand for uranium will exceed the supply, and hopes to find new reserves in South Africa. Whatever the reserves of uranium, there seems to be plenty of thorium, although the report admits that as yet there is no market for it. Apparently reserves of monazite in the Karroo system exceed 200,000 tons, and contain over 7 per cent of thorium dioxide, while there are 163,000 tons of slightly poorer ore, containing between 5.5 and 7.0 per cent of thorium dioxide. The board believes that once large scale converter and breeder reactors come into use, there will be an assured market for thorium. In addition to exploration, work has been continued on the solvent extraction of uranium directly from leach liquors, cutting out the ion-exchange absorption step.

The report also conveys some idea of the reactor types which the board is studying. The main effort is being devoted to a reactor called Pelindaba, a name incorporating both the name of the laboratory—Pelindaba—and the chemical symbols for deuterium, uranium, and sodium. This reactor, as its name indicates, is fuelled with slightly enriched uranium (2 per cent), moderated with heavy water, and cooled by sodium. The lay-out of a 30 MW prototype is now in an advanced stage and is intended as a first step towards a 300 MW electrical station. The fuel will be in the form of spheres of either metallic uranium or uranium monocarbide, and sphere sizes in the range 2 to 4 mm are being contemplated. So far 1 cm spheres have been produced, but smaller ones are being produced by the lead shot technique in which molten droplets of uranium solidify as they pass through a column of fused salt. The monocarbide pellets, on the other hand, will be produced by die-pressing, arc-melting, or possibly a sol-gel process.

The third major aspect of the board's work is concerned with applications of radio-isotopes and radiation. This includes radio-isotope production, environmental radioactivity, and some fundamental

studies in nuclear physics. Biological, chemical, and waste problems have also been considered. The cost of the board's activities is divided into two accounts; one, covering salaries, clerical costs, equipment, conferences and subscriptions to international organizations, amounts to R4.46 million (£2.23 million). The other account is called the research fund, covers capital and running costs and amounts to R4.44 million (£2.22 million).

## New Look for Birmingham

THE new technical University of Aston in Birmingham will be "an urban residential university, intimately linked to the life and needs of Birmingham and the Midland region" according to the development plan now published.

The university, formerly the Birmingham College of Advanced Technology, will be expanded on a thirty acre site next to the existing four acres at Gosta Green in the centre of Birmingham. Because of the limitation in space, the university will presumably be built rather more up than out, with five new teaching blocks of seven or eight storeys dwarfing the single and double storey heavy engineering and research laboratories. These blocks will eventually house the whole of the engineering and social science faculties. The existing building will be extensively renovated for continued use, primarily by the science departments.

The university hopes to expand its student population from the present total of 2,300, with 10 per cent postgraduates, to a total of 5,500 with 27 per cent postgraduates in the late seventies. There is an intermediate target figure of about 3,300 students in 1971–72. The largest increase will be in the social sciences, from about 140 students, 6 per cent, to about 1,200, 22 per cent. The number of students in science will increase from about 760, 33 per cent, to about 1,650, 30 per cent; engineering will expand from about 1,400 students, 61 per cent, to about 2,650, 48 per cent. It is hoped that the expansion of the social science faculty will reflect the increasing need for scientists and technologists to be aware of the demands and techniques of management. Introductory management studies at undergraduate level should give an appreciation of the organization, control and economic background of modern industry.

Advisory services to industry are mentioned in the university's charter, and it is to this end that the Department of Economic Affairs has given a grant of £51,650 per annum for five years to set up a centre for the study of small businesses.

## Intricacies of Bacterial Cell Walls

from our Correspondent in Microbiology

THE cell walls of bacteria differ from those of other organisms in several remarkable ways. Subject to considerable mechanical stresses, they are, nevertheless, highly dynamic structures and their chemistry and structure are of a complexity not usual in other cells. It is in the Gram negative bacteria that the nature of the cell wall appears at its most intricate. Several chemical analyses have been made of these bacteria, but only within very recent years have useful correlations been offered between these and fine structure

data. The work of Weidel and his colleagues has been paramount in establishing the basic chemistry of the *Escherichia coli* wall, while de Petris has attempted to identify various layers seen in electron micrographs with these chemical components. Unfortunately, correlations of this kind are founded on indirect evidence alone, and various interpretations have been made.

De Petris has sought to substantiate his own views by subjecting *E. coli* cells to a variety of chemical, physical and enzyme treatments and has checked any changes which were produced by electron microscopy (*J. Ultrastruct. Res.*, **19**, 45; 1967). There is general agreement that the cell wall of this bacterium has an asymmetric, multilayered construction comprising an outer triple layered zone (the *L* membrane), an intermediate zone (the *G* layer) and a less well defined, electron transparent *M* layer. By heat treating cells and thereby denaturing autolytic enzymes, de Petris found the *G* layer could be further resolved into  $g_1$  and  $g_2$  substructures. When the cells were incubated with proteolytic enzymes such as papain or pepsin, the  $g_2$  layer separated from the external *L* membrane appeared free of other material and, significantly, maintained the original cell morphology. Subsequent digestion with lysozyme removed the  $g_2$  layer. Similarly the combined action of lysozyme and EDTA was effective in eliminating this structural entity. The obvious interpretation is that  $g_2$  is wholly or in part composed of mucopeptide (murein). Isolation of the "murein sacculus" (*sensu* Weidel and Pelzer) by successive enzyme and detergent treatments and demonstrating it to have an identical appearance to that of the  $g_2$  component is compelling confirmation of this suggestion.

Subjecting the lipid-containing *L* membrane to phenol attack reduced the total wall structure to a single electron opaque zone, possibly the equivalent of  $g_2$ ; milder detergent treatments produced a similar result, but one less frequently obtained. Lipopolysaccharides which are extractable in phenol-water mixtures had a triple layered organization but are more likely to be preparative artefacts rather than *L* membrane fragments *per se*. The nature of the *M* layer remains an enigma and it is by no means certain whether it represents free space or a solid matrix. An interesting suggestion of de Petris is that it could be a site—together with the  $g_1$  layer—of surface bound enzymes.

The model of *E. coli* walls erected by de Petris is in accord with that proposed by the Munich group (see Martin: *J. Theoret. Biol.*, **5**, 1; 1963) and is a significant refinement of it. Differences in detail are obvious, particularly in the interpretation of the lipid and protein constituents. But obvious also are the unanswered questions about the character of the *M* layer and the structural protein, the role of the latter and other materials in imparting rigidity to the murein sacculus and the dearth of really comparable data on other Gram negative species. Finally, the walls of Gram positive bacteria, generally regarded as being homogeneous in construction, are also being resolved into a complex with several layers. The elegant analysis of *Bacillus polymyxa* walls by Nermut and Murray (*J. Bact.*, **93**, 1949; 1967) demonstrates forcibly the chemical heterogeneity and structural differentiation which can occur in Gram positive cells.

## Parliament in Britain

### Sonic Booms

IN reply to a written question in the House of Commons on July 4, the Minister of State, Ministry of Technology, Mr J. Stonehouse, announced that it had been decided that during the month of July Lightning aircraft from the Ministry's Aeroplane and Armament Establishment at Boscombe Down should be permitted to fly supersonically over various parts of Southern England in such a way as to create sonic bangs. The intensities, he said, would be well below those likely to cause damage. The flights will be confined to the hours of daytime, and will be monitored by technicians from the Royal Aircraft Establishment, Farnborough.

### Costs of Technology

MR A. WEDGWOOD BENN, Minister of Technology, stated that over the last 10 years £151 million has been spent on the Atomic Energy Research Establishment, Harwell, £73 million (including £10 million contributed to the international Dragon project) on the Atomic Energy Establishment, Winfrith, £15.5 million on the National Engineering Laboratory and £36.3 million on the National Physical Laboratory (including since April 1, 1965, expenditure on the National Chemical Laboratory). (Written answer, July 4.)

### Teacher Training

MR G. ROBERTS, Minister of State at the Department of Education and Science, gave figures for students successfully completing courses of initial training in colleges of education 1953–63 as follows: 1952–53, 2,290 men and 8,165 women. For 1953–54, the corresponding figures are 2,331 and 8,119; for 1954–55, 2,361 and 8,403; 1955–56, 2,513 and 8,609; 1956–57, 2,537 and 8,891; 1957–58, 2,709 and 9,248; 1958–59, 2,991 and 9,559; 1959–60, 3,588 and 10,092; 1960–61, 4,160 and 10,360; 1962–63, 4,254 and 9,591. (Written answer, June 30.)

### Traffic Control

LORD SHEPHERD, for the Ministry of Transport, gave news of developments in the control of traffic in London by computer. He said that the ministry intended to introduce computer controlled traffic lights to phase traffic flows, and experiments would soon be beginning in West London. The Greater London Council were involved in the West London experiment, and were already considering extensions of traffic control by computer in London. He admitted that the scheme had been held up since 1963, and explained that the delay had been caused by the time taken to design and build the computer. (Answer, House of Lords, July 6.)

### Sea Bed

MR ANTHONY CROSLAND, Minister of Education and Science, in reply to a question by Mr T. Dwyer on what studies were being made of the possibility of greater exploration of the resources of the sea and sea bed by the UK, said that two initiatives had recently been taken. The Government had initiated a review which would examine what additional work would be profitable and how such work could be put into effect and co-ordinated with existing activities. Also the NERC was examining the extent to which it would be justifiable and practicable to expand its exploration of the Continental Shelf, with particular reference to economic returns and the needs of the extractive industries. (Written answer, July 3.)



# Planning for Food

by our Special Correspondent

What can science and technology contribute to what is called the world food problem? President Johnson's Science Advisory Committee has been studying this question. It has nothing new to suggest, but it does consider that if the next twenty years can be endured, the more distant future should be supportable.

SOLVING the food problems of the world is a massive problem, as President Johnson recognized when he asked his Science Advisory Committee to investigate it. The committee has now come up with a massive response, in size at least, in the shape of a report of three volumes and more than 1,000 pages. Fortunately, the bulk of the report is summarized in volume 1, *Report of the Panel on the World Food Supply* (US Government Printing Office, Washington, D.C. 60 cents).

The report is subdued, almost detached. The facts it records are not new: "We have been unable to devise any new or original statement of the world food problem". So often and so thoroughly has the problem been described that the panel feared it was becoming obscured by "rhetorical overkill". Clearly it set out with the intention of avoiding this trap, at least, and it is successful. The facts alone are alarming enough, without the need for oratorical excess.

The report first deals with common delusions about the food problem, before it gets down to discussing it in detail. Overpopulation and the shortage of food are separate but interrelated problems: "the choice is not to solve one, or the other; to solve both is an absolute necessity. The tendency to think of food production and fertility control as alternative solutions to a common problem is dangerously misleading". The panel also admits that solving the food problem can never be as exciting as other, more dramatic projects: "It is dependent on far-reaching social reforms and long range programmes of hard work which offer no promises of quick and dramatic results so helpful in maintaining enthusiasm for a concerted, difficult undertaking". There are, it says, no buildings to dedicate, no vehicles to launch into space, just toil, year in and year out. Equally, the panel has no new solutions to offer; indeed, it seems to distrust solutions because they tend to distract attention from the fundamental problem, which is that the shortage of food is only one part of the enormous problem of economic development in the poor nations.

The main panel was chaired by Ivan L. Bennett, jun., who is deputy director of the Office of Science and Technology, and thirteen sub-panels were set up to tackle all aspects of the problem. The broad philosophy of the panel was to study food production in the context of the more general questions of economic development and foreign aid. First, the report defines the scale of the problem: by 1985, the world will need 52 per cent more calories; even if the most optimistic view of birth control programmes is taken, the need will still be for 43 per cent more than today. These figures are presented in the context of a world situation in which the total amount of food in the world, both in quality (protein) and quantity (calories), is adequate. Only the uneven distribution of food between countries, within countries, and among families with different incomes has caused the problem, the report states. The panel was confident that the next twenty years would be the critical period, since after 1985 family planning programmes will ease the pressure. If the problem is solved within the next two decades, the report concludes, "it will be manageable thereafter".

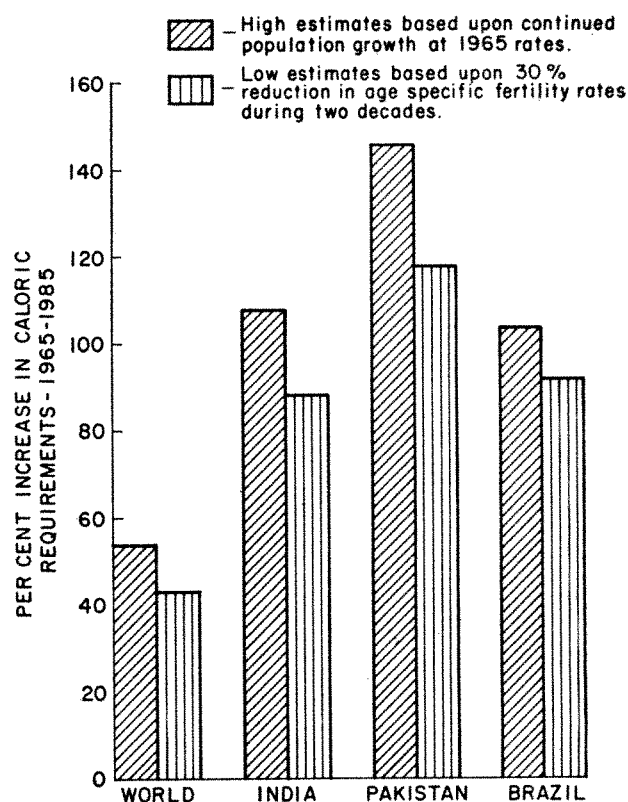


Fig. 1. Projection of calorie requirements for the world, India, Pakistan and Brazil.

The falling mortality rate among children is often quoted as a cause of the population explosion, as indeed it is, but paradoxically the panel believes that it can also reduce rather than increase the rate of population growth. This will happen when families are finally freed from the tyranny of having to have at least four children in order to ensure that one son will survive. In this view, lowering mortality rates is an essential pre-condition for the acceptance of birth-control methods, which are an irrelevance until couples have secured the desired number of living children.

Ideas about agriculture itself must also be changed, the report suggests. For centuries, farming in underdeveloped countries has been carried out at subsistence level, producing only enough food for family or local needs. Farmers in these countries must be converted to a commercial system in which production is for markets at a distance from the farming area; "fundamentally, it appears that many nations are under-utilizing the power of the market economy". Mexico, apparently, is an exception, and the panel gives it a pat on the back. Not only must farmers be expected to change, but the situation must be rigged so that the pay-off for change is enormous — "not 5 to 10 per cent, but 50 to 100 per

cent". This needs a system of farm credit enabling farmers to buy essential "inputs"—seeds, fertilizers, and pesticides—without financial risk, at least at first.

The panel is pessimistic about unconventional sources of protein, or about food which requires people to change their traditional eating habits. Habits certainly cannot be changed in the short run, and even in the long run changes must be carefully planned. As for unconventional approaches, the report declares that there are no panaceas. Single cell protein, derived from fermentation by yeasts or bacteria of carbohydrates, hydrocarbons or cellulose, seems the most promising, but there are unsolved problems of production, processing quality, consumer acceptance, and cost which remain to be worked out. Methods of extraction of protein from green leaves exist, but again there are problems, and the use of algae has so far been unrewarding because of the excessive cost of producing a product fit for human consumption. The conclusion is that the vast majority of increased food production must come from the developing countries themselves. Animal sources, including fish farming (which the panel calls aquiculture) and developments such as fish protein concentrate, can help, but most of the increase must come from agriculture.

Having worked out what will be needed, the panel goes on to estimate what it will cost. The conclusion is that to pay for an increase of food production at the compound annual growth rate of 4.0 per cent per year, gross national income in the developing countries will have to grow at 5.5 per cent per year. Laconically the report says, "The achievement of such growth rates will require massive efforts which must be more successful than history has recorded in any previous 20 year period". Current growth rates are of the order of 4.5 per cent per year, and only Mexico and Taiwan are achieving growth rates of the order necessary. The additional capital requirement if food production is to rise at the rate required will be \$300 million annually in the early years, rising to almost \$4 billion by 1985. To do this, the United States will be powerless alone, and should instead take the lead in mounting a global effort.

How will agricultural production be able to increase at this rate? First, the panel believes that an inadequate proportion of the world's surface is at present under cultivation. The amount of potentially arable land is 24 per cent of the Earth's surface, but less than half of this has ever been cultivated in the past twenty years.

In any given year, only a third of this area is harvested. In Asia, unfortunately, rain is so rare over some of the potentially arable land that even one four-month growing season is impossible, and there is no hope of increasing the area harvested. Production must be pushed up by increasing yields per unit area, or by double or triple cropping based on irrigation development. The situation in Africa and Latin America is different; here the limitations are not of potentially arable land, but are economic, institutional and social problems. Secondly, the panel favours the adoption of improved crop varieties. Some of these are mentioned: rice varieties which originated in Taiwan, and were developed at the International Rice Research Institute; dwarf wheat varieties developed in Mexico; maize varieties developed in Tropical America; and sorghum genotypes which possess wide ranges of geographic adaptation.

Although brief mention is made of the contribution which new technologies can make in food production—weather and climate modification, and weather studies by satellite—the conclusion is that the technology needed is at hand, if only it can be applied. In this connexion, AID, created in 1961 as the US umbrella organization for aid, comes in for some criticism. Since AID took over the technical assistance programme, the reports says, weaknesses and lack of quality have clearly grown worse. AID finds it hard to recruit high-quality personnel, a difficulty not shared by the Department of Agriculture, or the Department of Health, Education and Welfare. Both departments have found it harder to provide assistance overseas since AID came on to the scene. Both the short term contracts from AID and the fact that the departments are not involved in the planning stage of projects hold back effective development. "Consequently, through a combination of factors, these agencies, which . . . are best able to conduct programmes of technical assistance overseas, are largely excluded from programme planning and limited to short term activities". Another organization should be sought which could involve the Federal agencies more effectively, and, although the change would be difficult, the panel recommends a new co-ordinating and funding agency to oversee the foreign aid programme. The distinction within the Federal establishment between domestic and foreign aid should be abolished, so that the entire manpower and expertise of the agencies can be made available for foreign aid, without subterfuge or indirection.

## Parliament and Nuclear Power

by  
NIGEL HAWKES

In December 1966 the House of Commons established the Select Committee on Science and Technology. The committee, thirteen strong, began with an examination of the nuclear power programme in Britain. It has finished taking evidence, so that it is possible to guess what the committee may recommend—and whether the experiment has been a useful one.

THE Select Committee on Science and Technology has finished taking evidence on the British nuclear reactor programmes. Before the members can write their report, they must sift through a pile of detailed written memoranda and more than thirty hours of oral evidence. Is it all waste paper? Did the committee ask the right questions, or understand the answers? Is there any real hope that non-specialist committees of this sort can provide worthwhile advice?

The committee set out with a fund of goodwill, announc-

ing that it would operate in public, and would not hesitate to call ministers to give evidence, if necessary. Its first mistake was to call Sir William Penney, Chairman of the UK Atomic Energy Authority, to open the oral evidence. The position of the AEA is fundamental to the entire issue. Chronologically, it may have seemed tempting to begin with it, but it was a temptation that should have been resisted. At least one of the interviews with Sir William, who saw the committee twice, should have been held at the end of the session, when the committee was

better acquainted with the issues. In the event, Sir William was charming, lucid—and essentially uninformative.

Perhaps Mr Stanley Brown of the CEGB should have performed first. It was not, of course, that Mr Brown had anything rude to say about the AEA, or about anyone else; one of the committee's problems has been that most of the witnesses have been so appallingly good mannered that interpreting their evidence became a question of reading minutely between the lines. Only rarely did witnesses say precisely what they meant; Lord Robens, and Colonel Raby of the Associated Power Company, were the most refreshing in this respect. This makes it vital to ask the right questions, and it certainly became clear that the committee was getting better at this as time progressed.

Several red herrings were chased in connexion with technical problems. Had the change of channel diameters which the Central Electricity Generating Board had sanctioned for the Hinkley *B* contract really cost more than £10 million? And then there was doubt about whether it would be better to standardize designs for the Advanced Gas-cooled Reactor or to allow technical development free reign. On the argument between the AGR and the Boiling Water Reactor, there was doubt whether the Dungeness *B* appraisal of the CEGB truly represented the costs of the designs. The committee really made no headway with these problems.

Too often, it found, the answers rely on technical value judgments—how much more development is to be had from the AGR, or what importance is to be put on outage (the time power stations are out of action through shut-downs). On questions like these, the committee could not compete with the accountants of the CEGB or the scientists of the AEA—and nobody seriously expected anything else. In Britain, there is no organization which can argue successfully with the AEA, a situation highlighted by the frank admission to the committee by the Minister of Technology that there is nobody in his ministry who is capable of checking the figures sent to him by the AEA.

When the problems of the structure of the industry or its export possibilities were discussed, the committee was much happier, even though the political inclinations and disagreements of committee members could not be concealed. Mr Tam Dalyell, for instance, seemed at times to be suggesting that there is no need for internal competition supplied by three consortia, since General Electric and Westinghouse supplied all the competition anybody could ask for. Other members were better disposed towards the consortia, but the probable outcome will be a recommendation that there should be only two consortia, formed by mergers across the board between the existing consortia, or by breaking up one consortium completely.

As for the AEA, the committee can hardly do anything but endorse and encourage the rundown of effort which is already under way. It would, for example, be surprising if the AEA were told to become a consortium in its own right, tendering for stations and looking for overseas business. Equally, it is unlikely that the committee will have anything kind to say about the diversification upon which the AEA is somewhat breathlessly engaged. The Ceramics Centre and Non-Destructive Testing Centre recently set up at Harwell will probably be criticized. While the committee recognizes what a splendid organization the AEA has been, it is likely to take the view that for too long the AEA has been sucking in talented people who might otherwise have improved the technical quality of the consortia. The CEGB may perhaps come off best of all, although it will certainly be criticized for its handling of the Hinkley Point *B* contract. Apart from matters such as the research programme of the CEGB, it is difficult to see what the committee can recommend—Mr Duncan Burn's suggestion that the CEGB should be divided into three competing groups will probably get short shrift.

One thing is clear. The committee was thoroughly dissatisfied with the export performance of the British consortia, and is certain to suggest ways in which it could be improved. The British Nuclear Export Executive, it is likely to feel, is too small and unambitious to be effective. Either it will be told to expand, or to make way for another organization with a sales force abroad. One possibility here is that the committee will try to find a way of using the prestige of the AEA abroad as an element in the export organization.

The value of the committee's work will clearly depend on its collective judgment, which is still uncertain, but the balance of forces on the committee indicates a conservative report along the lines suggested. The chairman, Mr Arthur Palmer, is a middle of the road Labour back-bencher, who has controlled the committee without apparently antagonizing it, but without firing it with any great enthusiasm either. The most effective member has been the only Liberal, Mr Eric Lubbock, who has been particularly good on technical subjects. He has also been persistent, and emerged from a memorable clash with Lord Robens as a winner on points. Of the rest, Mr Ernest Davies and Mr Tam Dalyell have both been effective in their different ways—Mr Davies patient and eminently reasonable, Mr Dalyell abrupt. There has been little direct axe-grinding, either on constituency or party issues, although Mr David Ginsburg has taken the opportunity of digging for information about the coal-fired power station, Drax, which is being built in his constituency. Defence issues have been studiously avoided except by the indefatigable Mr Norman Atkinson, who announced at an early session that he was a member of the Campaign for Nuclear Disarmament, but later remembered that he was merely a supporter, without actually belonging. At times he has been frustrated by the good manners of the committee, which have sometimes prevented it from pinning down evasive witnesses. There have been no such inhibitions for Mr Atkinson.

The Conservative members, to be honest, have been less effective. Sir Harry Legge-Bourke, prefacing each question with detailed references to memoranda and transcripts of previous meetings, has sometimes seemed as likely to confuse the committee as the witnesses. Mr David Price, the Conservative Front Bench spokesman on science, has been lucid. In general, though, the most effective members of the committee have been those who attended most often, and in this respect the Conservatives—with the exception of Sir Harry—have a poorer record. The general attendance has been remarkably good; although the quorum for the committee is only four, there has never been an occasion when the numbers have fallen below eight or nine.

Although the committee has so far done fairly well, there are no grounds for unbounded optimism. In considering reactors and nuclear power, the committee at least had an industry into which it could get its teeth. The problems, in fact, were as much economic as they were scientific. Other issues will not be as kind to the committee as this; it is alarming to think, for instance, what would happen if the committee were to discuss highly technical subjects, such as the justification for a British investment in the CERN big machine, or the grant giving policy of the Science Research Council. These are problems in which finance and high level scientific judgments are closely interwoven. What the committee needs is much larger staff of its own, working full time on the technical problems, but unfortunately this idea is a pipe-dream. With recruitment to the House of Commons staff at present levels, it is lucky even to have a clerk to look after its business. These criticisms may seem unduly harsh, and perhaps they are; there are certainly advisory committees a good deal less competent than the Select Committee, even on technical issues. The only difference is that the Select Committee has had the courage to do its agonizing in public. It should be encouraged to continue.

# The Student's Microscope

by

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How suitable are modern students' microscopes for teaching undergraduates? Dr Baker has been investigating the ergonomics of microscope construction for several years.

THIS article is based on the opinion that the microscope provided for use by undergraduates should include all the really fundamental constituent parts of the more elaborate instruments intended for research, and should be convenient for use not only by the student but also by the demonstrator.

To find out whether the modern student's microscope fulfils these conditions, visits were paid to nine of the principal manufacturers of the world (three British, two West German, one East German, and one each from Austria, the Soviet Union and the USA). Attention was concentrated in each case on the instruments recommended by the manufacturers for use by undergraduates in the biological departments of universities. Each instrument was examined in detail in the presence of an expert representative of the firm.

It was found that with certain exceptions the instruments tended to conform rather closely to a single type. The lenses were generally of high quality, and the mechanical parts, particularly the fine adjustments, were in most cases of good design and well made. Nevertheless, the instruments did not fulfil the requirements set out in the first paragraph of this article.

In what follows, the requirements that concern the student directly will be considered first, and attention will then be directed to the needs of the demonstrator.

In the typical modern student's microscope, the illuminating apparatus is an integral part of the instrument. It usually consists of an electric bulb placed in the optical axis, with a plano-convex lens above it. The plane surface of the lens, slightly roughened, faces downwards. The distance between the convex upper surface of the lens and the lower focal plane of the substage condenser is so short that it is impossible to provide a field stop, for this would inevitably act as an aperture stop. Accurate focusing of the condenser is impossible. It is commonly recommended that this lens should simply be raised as high (or nearly as high) as possible<sup>1</sup>, or put in such a position that the roughened surface of the lamp lens does not obtrude on the field of view<sup>2</sup>. Centring screws for the condenser are not usually provided.

This method of illumination does not lead to the best possible image, partly because the absence of a field stop may result, with certain objects, in the defect technically termed 'glare', partly because an unfocused substage condenser may give too small an aperture for the effective use of an oil immersion objective, and partly because the condenser is often slightly or even markedly eccentric, so that the illumination is somewhat oblique. An even more serious objection to the method of illumination

employed in the modern student's microscope is that the student graduates without ever having learnt the basic principles that should guide him in the subsequent use of a first-rate instrument. He does not know how to focus the condenser accurately, how to centre it, or how to use a field stop to control glare. This lack of elementary knowledge, combined with an ingrained insensitivity to defects in the image, is an obstacle to progress if the student subsequently undertakes research. If he becomes a teacher, he will not encourage high standards in microscopy.

It would not be difficult to provide the student with a more satisfactory method of illumination. There is no reason why the lamp should not be an integral part of the microscope: the objection is only to its situation so close to the lower focal plane of the substage condenser that a field stop cannot be provided. The need for compactness has been greatly exaggerated, at the expense of the optical performance of the instrument and of its suitability for instruction in the basic principles of microscopy.

Köhler illumination in its usual form would be too expensive for incorporation in the student's microscope, because of the necessity to provide a transformer and resistor; but a convenient and inexpensive substitute, giving ample illumination for low-power dark ground work and for phase contrast with the 4-mm objective, is 'large-source Köhler illumination'<sup>3</sup>. In this method light is provided by an ordinary domestic electric bulb of about 100 W. The 'pearl' surface of the glass is focused by a simple plano-convex lens on the lower focal plane of the substage condenser. The field stop (an iris diaphragm or set of interchangeable stops) is placed sufficiently far from the mirror to allow its effective use. The lamp is an integral part of the microscope. It may be placed in front of or to one side of the instrument. The intensity and colour of the light are controlled by a few filters, fitting into sockets in the substage.

The substage condenser should be provided with centring screws. In microscopes that lack these screws, this lens is often markedly eccentric, as has already been mentioned; and in any case it is desirable that the student should be familiar with the process of centring the condenser, so that he may be capable of using more elaborate instruments.

Every student of biology at a university should be familiar with phase contrast. The method is invaluable in class work with living cells of all kinds. The expense can be kept quite low. There is no need to provide students with complete sets of phase contrast objectives. The phase contrast effect is more striking with objectives



of focal length about 4 mm than with those of lower or higher power. The presence of the phase annulus in the 4-mm objective need not cause any serious deterioration in the image when it is used for direct ('ordinary') microscopy, provided that the amplitude reduction in the annulus is rather high. A single objective would suffice for both purposes if enough money were not available for the purchase of two. The illuminating annulus can be held in a mount arranged to be pushed into the substage from below. It is necessary to provide centring screws for this mount (separate from those for the condenser). It is unnecessary for each student to have a telescope for centring the illuminating annulus: one can be shared among twenty or more students, as the centration will not be readily undone by slipping the mount in and out.

Every biological student should use polarized light, where appropriate, as a matter of course. Much useful work can be done with a polarizing film in the substage filter tray and another in a mount to slip over the eyepiece. With a little ingenuity it is not difficult to provide a couple of compensators (red of the first order and a quarter wave plate) and to ensure that these are correctly orientated in relation to the plane of vibration of the polarizer. With this simple equipment the student can instantly distinguish positively from negatively birefringent objects.

The angle of the microscope tube is a matter of special importance in the teaching laboratory. The stools provided in classrooms are usually of the same fixed height. In these circumstances the vertical tube is convenient for both student and demonstrator. The sitting heights of students vary greatly, and it is easier for persons of different heights to adapt themselves to vertical tubes than to sloping ones, provided that the eyepiece is not too high. A tall student has only to let the head fall slightly forwards, into what is in fact a position of rest. If a sloping tube is of the correct height for a short student, a tall one can only use the microscope by pushing his seat back, leaning far forwards, and then throwing his head backwards into a position that involves considerable strain.

It is for the demonstrator, however, that the sloping tube is particularly inconvenient. As a general rule students are unwilling to vacate their seats when the demonstrator approaches, unless for some reason it will obviously be necessary for him to sit down. The result is illustrated in Fig. 1. The student leans back in her seat; the demonstrator, standing, has to put his head where hers was. To do this necessitates the assumption of a very inconvenient attitude. He must bend low: indeed, if he is tall his back will be almost horizontal. He must turn his head to left or right (according to the side on which he is standing), and at the same time bend it strongly backwards. To do this repeatedly over a period of two or three hours is extremely irksome and tiring. With a vertical tube the demonstrator has only to lean forward. He need not turn his head to right or left, and there is no question of bending it backwards. His position involves no strain or discomfort.

If asked, many microscope manufacturers will provide a vertical tube intended for use in photomicrography, to replace the sloping one of the modern student's microscope. It cannot be too strongly emphasized that this simple change does not solve the problem. The sloping tube of the modern student's microscope is so placed that it is convenient for students of average sitting height when there is the usual difference in height between seat and bench. If the sloping tube is replaced by a vertical one, but the microscope remains in other respects unchanged, the eyepiece is far too high for comfortable vision by most students, and the instrument is actually unusable by those whose sitting height is low. The presence of the whole illuminating system below the stage makes it necessary that the latter shall be high, and this means that the eyepiece is too high if a vertical

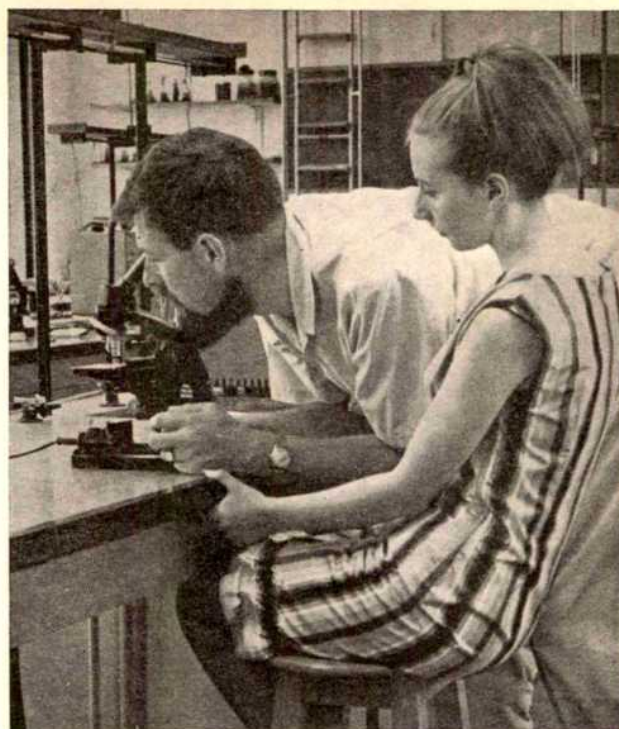


Fig. 1.

tube replaces the sloping one. A fundamental change of design is necessary if this replacement is to be made. With the system of illumination suggested in the present article there is no reason why the eyepiece should be at an inconvenient height even for short students.

If students were given adjustable seats, they could all use the sloping tube conveniently, but it would be necessary to provide their microscopes with vertical tubes in addition, for the convenience of the demonstrator. This could be achieved by the use of a beam splitter or a tilting mirror. The problem would now be to make it possible for both demonstrator and student to see the former's pointer, which is one of the most essential pieces of equipment in the teaching laboratory. The familiar pointing eyepiece could obviously not be used, unless the student were to use the vertical tube when looking for the object indicated by the pointer. The best plan would probably be to place a movable pointer in the plane of the field stop, as this plane is conjugate with the field of view when the substage condenser is accurately focused. The same part of the object would necessarily be indicated in both eyepieces. Even an ordinary Abbe condenser gives a sufficiently clear image of such a pointer when a 4-mm objective (or any lower power) is used.

It is unnecessary for the demonstrator and student to see the object at the same time, and indeed this would be rather embarrassing, from the proximity of the two heads. The only requirement is that, when the demonstrator has set the pointer by use of the vertical tube, exactly the same point will be indicated to the student when he looks through the sloping tube.

It is sometimes suggested that the demonstrator need not look through the student's microscope, because he can teach by using the projection microscope. I have already, however, listed seven reasons why this is not so<sup>4</sup>. Here it must suffice to say that the projection microscope is a useful adjunct to the lecture room, but looking at projected images is not practical work and teaches nothing about microscopy.

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# Point Holograms as Optical Elements

by

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A point hologram is formed on a photographic plate when a spherical wavefront interferes with a plane or another spherical wavefront. Such holograms behave like lenses or mirrors, but are easier and cheaper to produce and may replace them for certain applications involving coherent light. It seems to be feasible to make them very much larger than conventional optical elements.

THE principle of reconstructing wavefronts by means of holograms<sup>1</sup>, which is now practicable using laser light sources<sup>2</sup>, has usually been applied to reconstituting the wavefronts beyond finite objects, in order to reconstruct images of such objects. It should also be possible, however, to reconstitute holographically the wavefronts emerging from good quality lenses or mirrors and thus to use such holograms as optical elements, replacing some of or all the lenses in an optical system. This has been our aim, and the principle is quite independent of the object or test region to be imaged. Because the essential property of the optical elements is that they have a focus—that is, that they will transform an incident plane wavefront into one diverging spherically from a fixed centre, such replicas of optical elements would be essentially holograms of wavefronts emerging from a point; they are therefore referred to as point holograms.

A point hologram is the fringe pattern produced by the interference of the spherical wavefront to be reconstructed with a plane wavefront (see Fig. 1) or with a wavefront of different curvature (see Fig. 2). The point hologram has a focal length and behaves like any other imaging device, except that it brings about a change in the curvature of the wavefront by diffraction rather than refraction or reflexion. If its focal length is  $\pm f$ , the position of the point image, at a distance  $b$ , when the hologram is illuminated with a wavefront of radius of curvature  $a$ , is given<sup>3</sup> by

$$\pm \frac{1}{f} = \frac{1}{b} - \frac{1}{a} \quad (1)$$

Our particular field of interest is the study of such phase objects as occur in the investigation of flames or events in wind tunnels, by methods including schlieren, shadowgraphy, deflection mapping and interferometry<sup>4</sup>, and the application of laser light sources to such optical systems<sup>5,6</sup> has already proved most promising for studying phase objects at very high time resolution<sup>7</sup>. Because the cost of a schlieren mirror of the dimensions calculated later as feasible would lie in the range of several hundred to several thousand pounds and that of an equivalent lens would be quite prohibitive, there is some practical point to this study, in addition to its intrinsic academic interest.

**Making point holograms.** Point holograms of various focal lengths were made using a Mach-Zehnder interferometer by interfering a spherical wavefront emerging from a telescope objective with a planar reference wave. The essential part of the apparatus is shown in Fig. 1. The holograms were recorded on 'Ilford R52' plates, using as a light source a 1 mW helium-neon gas laser operating under single mode conditions. The intensity of the two interfering beams should be arranged so that the information is recorded on the linear portion of the photographic emulsion's amplitude transmission against exposure characteristic<sup>8</sup>. This is achieved by adjusting the intensity of one of the beams so that the transmission of the plate corresponds to the centre of the linear portion of the

characteristic: the addition of the second beam, if of sufficiently small intensity, will modulate the background by forming interference fringes the transmission of which still falls on the linear portion of the characteristic. Although it would be more economical of light to develop the hologram to a lower density, it will be shown that the intensity of solid state lasers makes a loss in the quality of reconstruction unnecessary.

It will be noted that the reconstruction process gives rise to two spherical wavefronts; one originating from a virtual point behind and the other from a real point at the same distance beyond the hologram. The distance between these points and the hologram corresponds to the focal length. The reconstructed spherical wavefront can, however, be separated from the planar wavefront illuminating the hologram only if the scene beam and reference beam are inclined to one another during the

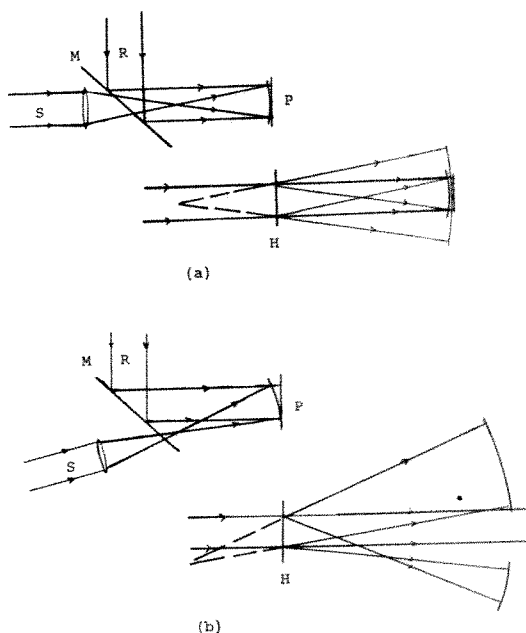


Fig. 1. R, Reference beam; P, photographic plates; S, scene beam; H, point hologram; M, semi-silvered mirror. For explanation see text.

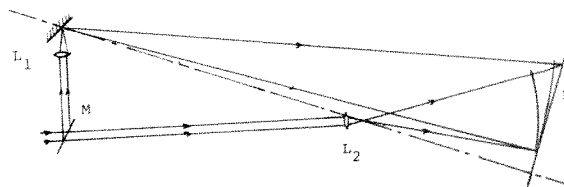


Fig. 2. — · — · — ·, Line of centres; L<sub>1</sub>, L<sub>2</sub>, lenses producing spherical wavefronts; P, photographic plate; M, semi-silvered mirror. For explanation see text.



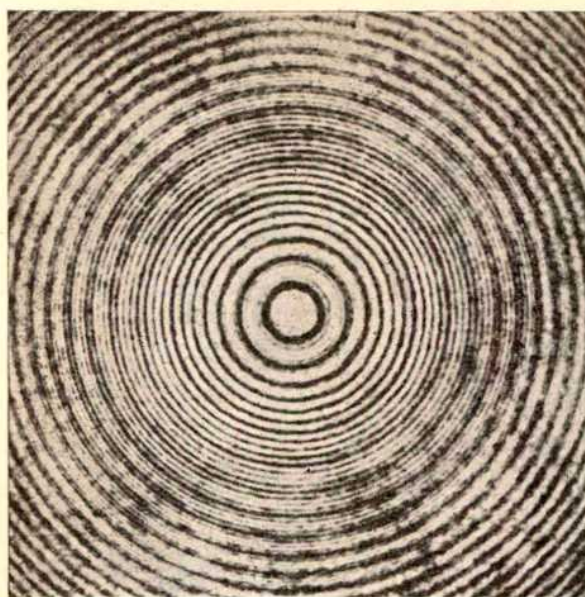


Fig. 3. Concentric circular fringe pattern.

recording process (Fig. 1b). If the emergent wavefronts are not separated they interfere with each other, forming a series of concentric circular fringes as shown in Fig. 3. Separation is essential if the hologram is to be used as an imaging device, for the image plane will otherwise be smothered by the circular fringe pattern.

In practice it is not necessary to use any device such as a Mach-Zehnder interferometer for making point holograms. This is fortunate because in order to make the large aperture point holograms mentioned later, correspondingly large planar optical elements would be needed as beam splitters and mirrors. Point holograms of any desired focal length can be made by interfering two wavefronts of different curvature. The resulting focal length is given by equation (1), where  $a$  and  $b$  now refer to the respective distances from the two centres of curvature of the wavefronts to the photographic plate. Fig. 2 shows a suitable optical system using a pair of master lenses. As before the two beams are incident on the plate at a slight angle to one another so that the reconstructed beams can be separated.

When a reconstruction is made by illuminating the hologram with a wavefront of finite curvature, two point sources are still reconstructed (one virtual and one real), but this time they are not symmetrically located on either side of the hologram. Their exact position depends on the location of the centre of curvature of the illuminating wavefront according to the foregoing principle.

**Comparison with other optical elements.** It is interesting to compare point holograms with Fresnel zone plates, because although the latter have never found much practical application in optical systems, their principle is somewhat similar. Zone plates, of course, were drawn by hand or machine and the production of a zone plate of the dimensions specified here with the detail required near the periphery is unthinkable. Apart from practical considerations, however, the photographic recording of point holograms is rather more than a simple method of making large zone plates. Thus if the photographic plate used for the hologram reproduces the intensity distributions falling on it in the manner already described, the density structure of each fringe (and each part of each fringe) must be characteristic of the two incident wavefronts. This allows, among other things, complete spatial resolution of phase detail.

In common with zone plates, point holograms can give rise to subsidiary foci. Examination of the light intensity along the optic axis does, indeed, reveal a number of

these at positions  $f/2, f/3, f/4, f/5$ , etc. This contrasts with the positions of the subsidiary foci for a zone plate, which occur at  $f/3, f/5$ , etc.<sup>9</sup> The differences between the two types of optical element and the intensities of the subsidiary foci are a function of the detailed distribution of transmission across the fringes of the hologram. If the intensities of the two wavefronts used for making a point hologram are not carefully matched as discussed, the response of the photographic emulsion will no longer be linear, resulting in "squaring-up" of the fringe structure, and giving the aberrated hologram some of the properties of a zone plate. The presence of subsidiary foci should present no practical problem as they are of relatively low intensity and their influence can easily be eliminated by using an off-axis system. Although transforming the curvature of a wavefront by diffraction rather than refraction or reflexion must always make the energy efficiency of a point hologram very much lower than that of a lens or mirror, this is offset by the availability of extremely bright, short-duration laser sources. Thus a Q-switched solid state laser in an optical system containing a few point holograms is capable of giving sufficient light to form an image (at least  $5 \text{ cm}^2$ ) on a photographic plate in 60 nsec. The efficiency is likely to improve when the technique of converting variations of density of photographic emulsions reversibly into those of phase has been fully mastered.

Point holograms differ from both lenses and mirrors on the one hand and zone plates on the other in one important practical respect. The ease of forming them suggests that it would be incomparably easier and cheaper to manufacture point holograms of large aperture than any other comparable optical elements. It can be shown that the fringe separation on a point hologram is given by

$$r_{(n+1)} - r_n \approx \left( \frac{f\lambda}{2n} \right)^{1/2}$$

where  $r_n$  is the radius of the  $n$ th (assumed  $\gg 1$ ) fringe from the centre,  $f$  is the focal length and  $\lambda$  is the wavelength of the illuminating light. Writing  $\alpha$  as the maximum emulsion resolution expressed in "fringes per centimetre", the maximum fringe radius that the emulsion can record is

$$r_{\max} \approx f\lambda\alpha$$

This corresponds with the maximum diameter of an off-axis point hologram in which the pole of the diffraction pattern falls at the edge. Table 1 shows values of  $r_{\max}$  for a number of plates based on quoted emulsion resolutions expressed in "line pairs per centimetre" and assuming a focal length of 3 m and a wavelength of  $0.63\mu$ . It can be shown that limitations imposed by lack of chromatic coherence are less stringent by at least two orders of magnitude for a stabilized helium/neon gas laser<sup>10</sup>.

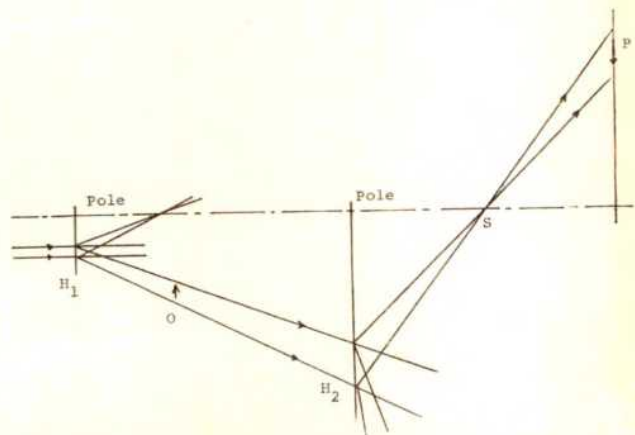


Fig. 4. — · — · — ·, Optical axis;  $H_1, H_2$ , point holograms;  $O$ , object to be imaged on  $P$ ;  $P$ , photographic plate;  $S$ , position of schlieren stop for visualizing phase object. For explanation see text.



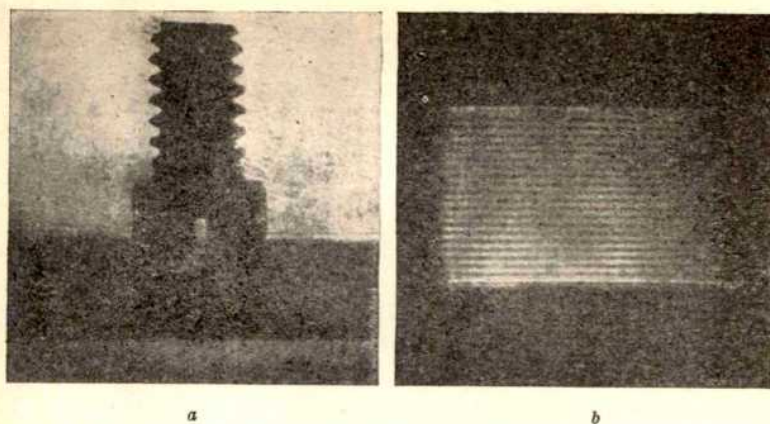


Fig. 5. *a*, Solid profile; *b*, phase object. For explanation see text.

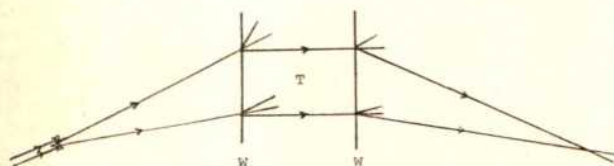


Fig. 6. *W*, Window incorporating point hologram; *T*, test region. For explanation see text.

It will be seen that when high resolution emulsions are used, point holograms of very large aperture become possible. The point holograms are suitable for imaging either by scattered or by directed light, provided it is monochromatic and has a coherence length in wavelengths at least equal to the number of fringes on the hologram.

**Using point holograms.** To test the usefulness and quality of point holograms as optical elements, a simple,

Table 1

Plate	Resolution (line pairs cm <sup>-1</sup> ) approx.	$r_{\max}$ (cm)
Ilford 'FP4'	600	11
Ilford 'RL'	800	15
Ilford 'R 52'	1,800	34
Kodak 'V 1043 D'	10,000	189
Kodak '649 F'	10,000	189

two component, off-axis, optical system was set up. This consisted of one point hologram of focal length  $\sim -20$  cm for diverging a 2 mW multimode helium-neon gas laser beam and a second point hologram of focal length  $\sim +40$  cm (Fig. 4). The latter could be used either as a simple objective for imaging a real object on to a photographic plate (Ilford 'FP4') or, for displaying phase objects, as a "schlieren lens". In this case a knife edge or other suitable schlieren stop would be placed at *S* (Fig. 4), while the point hologram was still positioned so as to make the receptor optically conjugate with the test space. Two optical records taken with this system are shown in Fig. 5:

Fig. 5*a* is the solid profile of a screw-bolt, while a phase object (Fig. 5*b*) is exemplified by the pattern of standing sound waves developed in air between a 0.5 Mc/s transducer and a reflector plate. When a gas laser is used, extended exposure times are necessary.

In view of the possibility of producing large point holograms, one possible application is to wind-tunnels of large working sections in which the optically flat viewing windows could be made to incorporate the point holograms. A suitable optical system is shown in Fig. 6; among other advantages of this scheme is that it avoids long path lengths of the parallel beam in the laboratory. For instantaneous recording of transient phenomena the ruby laser can provide sufficient illumination. A calculation based on Fig. 5*a* shows the total energy emitted during the period of exposure to be approximately 1.2 J; this is readily available from quite a modest *Q*-switched ruby in approximately 60 nsec.

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## Dehydrated Lanthanum-exchanged Type Y Zeolite

by

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Lanthanum atoms occupy different sites in the crystal structure of dehydrated type Y zeolite as the temperature changes, probably because of the absence of residual molecules for bonding at 725° C compared with their presence at lower temperatures.

ZEOLITES have recently been exploited as catalysts, especially in the petroleum industry<sup>1,2</sup>. One advantage they have compared with earlier amorphous or poorly crystalline aluminosilicate catalysts is that crystalline zeolites are amenable to standard techniques of X-ray diffraction; it is therefore possible to locate the atomic

positions and thus to test theories of catalytic processes, especially the electrostatic field theory<sup>3</sup>. Unfortunately the tetrahedrally co-ordinated silicon and aluminium atoms are usually in a disordered arrangement, and this, together with the great tenacity of exchangeable cations for sorbed molecules, makes interpretation difficult. Also



Table 1. ATOMIC CO-ORDINATES

Room temperature after activation at 350° C						725° C				
Atom	Occupancy	x	y	z	B	Occupancy	x	y	z	B
T	192	0.9652 (2)	0.8749 (2)	0.0548 (2)	1.2 (2)	192	0.965 (2)	0.866 (3)	0.054 (2)	(1.0)
O (1)	96	0.1035 (4)	0.8965	0.0	1.5 (4)	96	0.101 (3)	0.899	0.0	(2.0)
O (2)	96	0.9998 (4)	0.9998	0.8613 (5)	1.7 (4)	96	0.995 (4)	0.995	0.873 (5)	(2.0)
O (3)	96	0.9225 (4)	0.9225	0.0355 (5)	1.0 (4)	96	0.933 (3)	0.933	0.033 (4)	(2.0)
O (4)	96	0.9308 (4)	0.9308	0.6796 (5)	1.5 (4)	96	0.922 (4)	0.922	0.684 (5)	(2.0)
S(I)	13.1 (9) O <sub>x</sub> *	0.0	0.0	0.0	(2.5)	5.2 (6) La*	0.0	0.0	0.0	(1.0)
S(I')	16.0 (2) La*	0.0667 (1)	0.0667	0.0667	(2.5)	8.9 (9) La*	0.068 (2)	0.068	0.068	(1.0)
S(II)	None					5.5 (8) La*	0.227 (3)	0.227	0.227	(1.0)
S(II')	29.1 (1.2) O <sub>x</sub> *	0.1701 (7)	0.1701	0.1701	(2.5)					
U	2.9 (7) O <sub>x</sub> *	0.125	0.125	0.125	(8.0)					

Numbers in parenthesis following a parameter specify the random error; last significant figure unless decimal given. B-values in parenthesis were held constant.

\* The given occupancy was assumed for calculation: note that one lanthanum is equivalent to about 6.5 oxygens or sodiums. Full occupancies are S(I) 16, U 8, and others 32.

various technical problems make it desirable to use the less certain powder methods rather than the more demanding single crystal techniques.

The aluminosilicate framework of zeolite Y is of the faujasite type<sup>4-6</sup>. The faujasite type of structure<sup>4-7</sup> consists of linked tetrahedra lying at the vertices of the zinc blende arrangement of alternating hexagonal prisms and truncated octahedra. Truncation of an octahedron leaves eight hexagonal and six square faces. Four hexagonal prisms are attached to alternating hexagonal faces. The other four hexagonal faces are left free. Four sites in *Fd3m* are partly occupied by sodium and calcium atoms<sup>4-7</sup>: all are (*xxx*); S(I), *x* = 0, centre of hexagonal prism; S(I'), *x* = 0.07, displaced about 1 Å from the centre of a hexagonal prism face towards the centre of the truncated octahedron; S(II'), *x* = 0.18, displaced over 1 Å from the centre of a free hexagonal face of the truncated octahedron towards its centre; S(II), *x* = 0.23, displaced about 1 Å from the centre of the free face towards the large pore.

Infra-red absorption data obtained by Rabo *et al.*<sup>8</sup> on cerium and calcium exchanged zeolite Y have indicated strong retention of OH bonding even after vacuum activation at 500° C: elimination of the OH stretching frequency occurred only after activation near 700° C. Electron spin resonance for manganese (II) ion exchanged type X zeolite has indicated<sup>9</sup> that the movement of cations is controlled by temperature. We have tried to determine the effect of partial and complete dehydration on the cation positions, and to determine the positions of cations at various temperatures and under various environments.

X-ray powder data for lanthanum exchanged type Y zeolite were collected with copper radiation on a diffractometer. After the hydrated zeolite had been examined under ambient conditions, it was studied at room temperature in a stream of dry helium after vacuum activation at 350° C. Although the patterns are quite similar, the differences indicate structural changes. Subsequently another sample was examined in a furnace with controlled atmosphere. X-ray measurements were made directly at temperatures up to 725° C using dry helium, dry carbon dioxide and ordinary and humid air pulled through the system by a roughing pump which operated at about 100 μ pressure. The helium and carbon dioxide were dried by passing through a liquid nitrogen trap followed by an activated Type 4A zeolite trap. The X-ray patterns depend more on the temperature of examination than on the previous treatment and the gaseous environment (Fig. 1). Small differences at a given temperature in the ratio of the integrated intensities for the (311) and (533) reflexions, however, suggest that the structural details depend on the gaseous environment, and perhaps on the previous history. Metastable equilibrium apparently was reached for each measurement.

A detailed refinement was made of two patterns, one obtained from a sample under dry helium at room temperature after activation at 350° C, and one obtained directly at 725° C under helium. Broadening in the latter

seriously reduced the number of distinguishable peaks and interpretation of both suffered from superposition of diffractions. Refinement was made by least squares iteration in which the observed intensity of combined diffractions was split according to calculated amplitudes. The calculations were checked by difference Fourier syntheses. Because structure models with atoms in (*xxx*) give, for many reflexions, similar amplitudes to models which have atoms inverted through (0.125 0.125 0.125), we tested inverted models. Table 1 shows the final atomic co-ordinates. Chemical analysis gave: SiO<sub>2</sub>, 43.6; Al<sub>2</sub>O<sub>3</sub>, 14.9; La<sub>2</sub>O<sub>3</sub>, 14.0; Na<sub>2</sub>O, 2.2; loss on ignition, 25.7; total, 100.4 wt. per cent. Calculated to 192 T atoms, the cell contents are Na<sub>13.4</sub> La<sub>16.3</sub> Al<sub>15</sub> Si<sub>127</sub> O<sub>383</sub> (H<sub>2</sub>O)<sub>270</sub>. The analysis deviates from the condition, Al = Na + 3La and O = 384. The presence of some (LaOH)<sup>2+</sup> could account for the deviation, and is consistent with the low temperature X-ray results from which the presence of a bridging species such as hydroxyl between lanthanum ions is indicated. Recalculation on this basis gives an approximate distribution of lanthanum species in the unit cell of La<sup>3+</sup>(LaOH)<sup>2+</sup><sub>7.3</sub>. Because of the presence of both lanthanum and sodium, assignment of electron density is ambiguous; however, the greater number of orbital electrons in lanthanum (assumed to be 6.5 times greater than in sodium) restricts the possible atomic assignments. Interatomic distances (Table 2) were used in association with possible occupancies (Table 1) to deduce the more likely assignments. Table 3 shows the observed and calculated structure amplitudes for the unique reflexions. A weak (200) reflexion in the RT pattern violates the assumed space group *Fd3m*. The

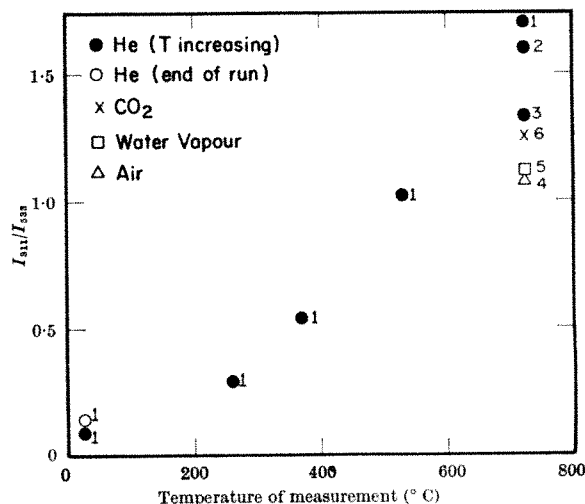


Fig. 1. Ratio of integrated intensities of reflexions 311 and 533 uncorrected for geometrical factors. The accuracy is about 10–20 per cent of the intensity ratio. The numbers specify the sequence of the measurements. Note that there is some hysteresis with the first run with helium, and that subsequent runs with helium reached different states at high temperatures: these small differences are probably significant. Detailed measurements have not yet been made with carbon dioxide, water vapour and air atmospheres, though the high temperature data indicate some small changes. Perhaps some residual molecules occupy U at 725° C.

Table 2. INTERATOMIC DISTANCES

Atoms	<i>RT</i>	725° C	Atoms	<i>RT</i>	725° C
T-0 (1)	1.58	1.6	S(I)-0 (3)	2.86 (6)	2.5 (6)
-0 (2)	1.65	1.7	-S (I')	2.87 (2)	2.9 (2)
-0 (3)	1.66	1.9	S(I')-0 (3)	2.56 (3)	2.5 (3)
-0 (4)	1.67	1.5	-0 (2)	2.94 (3)	2.6 (3)
0 (1)-0 (2)	2.72	2.7	-U	2.50 (1)	
-0 (3)	2.64	2.5	-S (I')	4.09 (3)	4.2 (3)
-0 (4)	2.70	2.7	-S (II')	2.60 (3)	4.0 (3)*
0 (2)-0 (3)	2.60	2.3	-S (II'')	4.44 (1)	6.8 (1)*
-0 (4)	2.66	2.8	S(II')-0 (2)	2.90 (3)	2.7 (3)*
0 (3)-0 (4)	2.71	2.7	-U	1.94 (1)	
			-0 (4)	2.60 (3)	2.9 (3)*

\* Replace  $S(\text{II}')$  by  $S(\text{II})$ .

Numbers in parenthesis are multiplicities with respect to first atom.

Table 3. OBSERVED AND CALCULATED AMPLITUDES FOR UNIQUE REFLEXIONS

<i>RT</i>					725° C	
<i>h</i>	<i>k</i>	<i>l</i>	<i>F</i> <sub>obs</sub>	<i>F</i> <sub>cal</sub>	<i>F</i> <sub>obs</sub>	<i>F</i> <sub>cal</sub>
2	2	0	44	29	92	133
3	1	1	75	103	127	188
2	2	2	74	96	75	65
4	0	0	96	109	96	114
3	3	1	156	149	156	183
4	2	2	70	77	24	12
4	4	0	281	295	143	140
5	3	1	29	11	53	58
4	4	2	199	187	100	104
5	3	3	397	414	286	320
6	2	2	75	51	14	14
4	4	4	107	96	25	9
6	4	2	195	185	193	191
8	0	0	278	278	34	42
7	3	3	102	102	91	95
6	4	4	145	120	130	149
6	6	2	68	115	18	33
8	4	0	236	234	277	267
8	4	2	50	54	14	86
6	6	4	243	228	269	243
9	3	1	235	222	198	196
8	4	4	113	116	158	182
9	5	3	16	31	16	8
8	6	4	56	63	17	31
10	4	2	16	27	112	39
8	8	0	490	486	426	386
10	6	0	209	199		
10	6	2	29	17		
12	4	0	35	47		
9	9	1	263	246		
12	4	4	37	13		
10	8	4	26	76		
12	6	2	92	92		
12	6	4	130	150		

*B* values of the atoms at 725° C and of the cation sites at *RT* were fixed arbitrarily; tests showed that the refined occupancies were little affected by changes in *B*.

Fig. 2 shows a section through one truncated octahedron and two of the attached hexagonal prisms. There are four positions for each of  $S(I')$ ,  $S(II')$  and  $S(II)$  in each truncated octahedron corresponding to the four triad axes passing through the centre, which is actually the  $U$  site. For the  $RT$  structure, at least 1.7 lanthanum per truncated octahedron must go into  $S(I')$  and perhaps as many

as 2.0. When more than one  $S(I')$  per truncated octahedron is occupied by lanthanum, there must be pairs of lanthanum atoms 4.1 Å apart. It seems likely that  $U$  is occupied by residual water or hydroxyl, which bridges between the lanthanum atoms. Presence of hydroxyl was indicated from the chemical analyses.  $U$  lies 2.5 Å from  $S(I')$  and 1.9 Å from  $S(II')$ . The former is typical for La-O and Na-O while the latter is very short; however it should be noticed that the observed position of  $U$  would be largely controlled by the more abundant  $S(I')$  cations obscuring the true position of  $U$  atoms bonded to  $S(II')$  cations. The observed occupancy of  $U$  was  $2.9 \pm 0.7$  oxygen atoms or equivalent, considerably less than full occupancy of this eight fold site. If the possible errors of a refinement which uses non-unique reflexions, and the likelihood of strong positional displacements are considered, this occupancy level is not inconsistent with the expected full occupancy. The observed electron count for sites I, I' and II' is greater than expected from the chemical analysis for the sodium and lanthanum. This may be related to the presence of residual water or hydroxyl. The cation distribution probably depends strongly on the distribution of silicon and aluminium. On average, each hexagonal prism has 3.4 aluminium atoms-8.6 silicon atoms. Although there may be local order it seems doubtful that a structure which was produced by rapid crystallization, with this ratio, would have long range order. Consequently some six rings should contain one aluminium atom and others two aluminium atoms (and perhaps a few with none or three).  $S(I')$  is more likely to be occupied by lanthanum when the aluminium content of the nearby six ring is high. Similar considerations apply to  $S(II')$ .  $S(I)$  should be favoured when there are three aluminium atoms in the hexagonal prism. Adding to the complexity will be a tendency for the cations to avoid each other unless water molecules or similar species intervene. Thus one can envisage a complex distribution in which the most common pattern is two lanthanum atoms in  $S(I')$ , one water or hydroxyl in  $U$ , and sodium plus water in  $S(I)$  and  $S(II')$  furthest removed from the two occupied  $S(I')$ . If a few  $S(II')$  are occupied by lanthanum the observed distribution can be explained. Such a distribution depends critically on the identification of the occupant of  $U$  as an electro-negative species capable of bridging the occupants of  $S(I')$  and  $S(II')$ .

Because such a species should be removed at high temperatures  $U$  should become unoccupied and the cations should move further apart. This prediction is consistent with the observed atomic distribution at 725° C. The 16.3

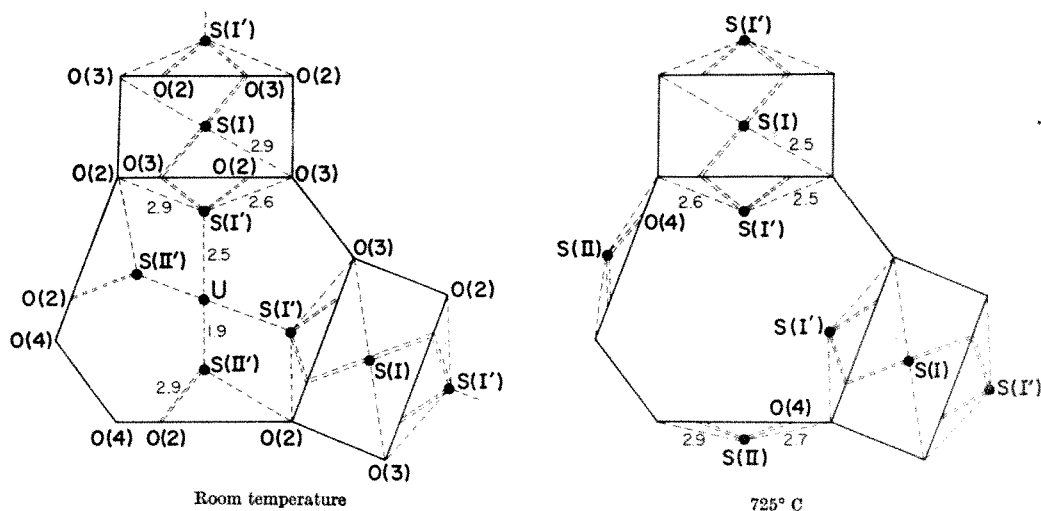


Fig. 2. Sections through a truncated octahedron and two of the attached hexagonal prisms. For simplicity the drawings are idealized, and only some oxygens are shown. Not all sites are occupied simultaneously: see text. Only the shorter bond distances are shown. The duplicated bonds are out of the plane of the drawing.

lanthanum plus 13.4 sodium given by the chemical analysis would scatter X-rays in a similar manner to 18.3 lanthanum atoms. The observed total of 19.6 is quite satisfactory considering the possible errors, and it is not necessary to invoke occupancy by residual molecules. Because the observed electron count for each of the three sites is greater than that for all the sodium atoms, all three must contain at least some lanthanum. On average at least  $1.1 \pm 0.1$   $S(I')$  are occupied in each truncated octahedron. If this were unity, there need be no short distances between lanthanum in  $S(I')$  and no need for bridging atoms in  $U$ . Perhaps there are a few undetected atoms in  $U$ ; presumably none of these atoms is hydrogen<sup>8</sup>. In contrast to the  $RT$  structure,  $S(II')$  is unoccupied; instead  $S(II)$  is occupied. The resultant displacement into the large cavity can be interpreted as the consequence of repulsion from the lanthanum in  $S(I')$ . In  $S(II)$  a cation is partly shielded by the ring of six O(2) and O(4) atoms. Although  $S(I)$  is shielded partially from  $S(I')$  by six O(2) and O(3) the cations should tend to avoid simultaneous occupancy of  $S(I)$  and  $S(I')$ . Thus  $S(I)$  is probably only partially occupied, probably mainly by lanthanum. Whatever the exact distribution of lanthanum and sodium it seems likely that most if not all of the exchangeable cations are shielded from each other by intervening oxygen atoms.

On cooling the zeolite from 725° C to room temperature under a variety of environments the  $RT$  pattern is essentially regained although there are some minor differences in the patterns (compare the starting and finishing values for a helium environment in Fig. 1). This indicates that lanthanum atoms return to  $S(I')$  and  $S(II')$ . If a bridging species does not re-occupy  $U$ , the close approach of atoms in  $S(I')$  and  $S(II')$  is unstable from an electrostatic viewpoint, although there is the possibility of attraction between adjacent metal atoms as in "metal atom clusters"<sup>10</sup>. Further measurements are required to characterize the structure obtained on return to room temperature. Perhaps the zeolite "scavenges" a suitable atom or molecule from the external environment, or perhaps water molecules are converted to oxygen with loss of hydrogen during activation near 700° C.

Whatever the details, the lanthanum atoms should occupy different sites as the temperature is changed: this change appears continuous. At lower temperatures direct contact between lanthanum atoms and large sorbed molecules should be slight unless the sorbed molecules have a greater attractive power than the occupant of

site  $U$ . At higher temperatures, lanthanum atoms at site  $S(II)$  should be in direct contact with sorbed molecules. Although detailed calculations have not been made here, it is clear that strong electrostatic fields will occur in the zeolite pores, and, even though the exchangeable behaviour of cations will be governed by an attempt to minimize the asymmetric fields caused by the silicon/aluminium distribution, conflicting demands for minimum cation-cation repulsions will cause strong residual fields. Thus the present results do not conflict with the electrostatic field theory of catalytic activity. The present results cannot be used to test the concept of three co-ordinated aluminium (and silicon) as an activated site. It is not possible, either, to test the possibility that protons are responsible for catalytic activity.

These results are consistent with those of Barry and Lay<sup>9</sup>, who concluded from electron spin resonance spectra that in manganese (II) ion exchanged type X zeolite manganese (II) ions occupied  $S(I)$  in preference to lanthanum although the lanthanum (III) ion has a strongly ionic character and prefers a high co-ordination. They suggested that lanthanum may be stabilized in  $S(II)$  if residual water or hydroxyl is present, or if there is a high activation energy for migration of lanthanum, or if the configuration of minimum energy involves the greatest separation for lanthanum atoms. The first and third suggestions appear reasonable when modified to permit occupancy of  $S(I')$  and  $S(II')$ , when used respectively for low temperature and high temperature conditions.

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## Palaeolithic Remains in Upper Egypt

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Excavations along the banks of the Nile have produced finds which indicate that Late Palaeolithic Upper Egypt was technologically less backward than it was thought to be.

THIS is a preliminary account of the recent discovery of numerous Palaeolithic remains in Upper Egypt along the west bank of the Nile River between Aswan and Luxor. Scholars have always held that few significant Palaeolithic sites would be found along the Nile in Egypt. It was believed that most of the Palaeolithic sites here either were buried deeply within the silts, or if they were near the

surface, then the sites were destroyed by the intensive cultivation of the silts during the past several thousand years. This belief was supported by the fact that north of Kom Ombo, near Aswan, *in situ* living sites of Palaeolithic age were unknown in the entire Nile Valley, in spite of the vast amount of archaeological work which had been done there with Pharaonic remains. Only a few scattered

finds of isolated and usually rolled artefacts have appeared<sup>1</sup>.

The desert areas beyond the Nile Valley have been more productive, especially Kharga and Fayum Oases, and limited occurrences of Palaeolithic materials have been reported by Caton-Thompson and Gardner<sup>2,3</sup>. Most of the Kom Ombo sites belong to the Sebilian industry which contained numerous technological and typological parallels with the Middle Palaeolithic, especially in the survival of the Levallois technique, although the Sebilian industry was found within silts of very late Pleistocene age<sup>4</sup>. The few isolated finds to the north of Kom Ombo, especially near Fayum, believed to be of Late Pleistocene age, were also strongly Levallois-like and contained many typological features reminiscent of the Middle Palaeolithic. These few finds gave a very distorted view of Egyptian prehistory.

Notably absent from the known Egyptian Late Palaeolithic materials were the long, narrow blades, burins and end-scrapers characteristic of the Upper Palaeolithic in Europe and the Levant. The survival of the technological and typological hallmarks of the Middle Palaeolithic, together with the absence of important features of the Upper Palaeolithic, led most scholars to assume that the Egyptian Late Palaeolithic cultures were culturally conservative, even retarded, when compared with adjacent areas<sup>5-7</sup>.

On a later time horizon, at the end of the Pleistocene when food production is believed to have begun, Egypt and the lower Nile Valley also are regarded as outside the main stream of these developments. Riverine environments, such as the Nile Valley, have been discounted as significant contributors to the origin of food production chiefly because of the persuasiveness of arguments supporting the "Hilly Flanks" concept, namely, that the domestication of wheat and barley occurred in the upland areas where the modern wild relatives of these plants grow today, and that the earliest known large permanent settlements based on food production are in the area of the "Hilly Flanks" of Iraq, Iran and Syria<sup>8</sup>.

Our work in Nubia has cast serious doubts on this general view of Nilotic conservatism<sup>9</sup>. Microlithic blade industries were discovered in Nubia which are dated by several radiocarbon samples as early as 17,000 B.C., as early as, if not earlier than, typologically and technologically equivalent industries elsewhere. Furthermore, several communities were discovered where there existed significant economic dependence on ground grains as early as 12,500 B.C., or nearly 3,000 years before the earliest dated evidence for a similar economic dependence in the "Hilly Flanks" area. It is not yet known if the grains used along the Nile were cultivated or wild; however, the significant use of grain at this time period, either wild or domestic, is of considerable importance because it is becoming increasingly evident that permanent, large settlements of several hundred individuals could be supported by the gathering of wild grain and hunting alone, and without the necessity of domestication<sup>10</sup>. Thus the discovery of the techniques for exploitation of this new source of food may be as important in the development of "primary village" society as any later domestication of plants, wherever that occurred.

Related to this problem of the emergence of economic dependence on ground grains is the extent of climatic change in Egypt during the Late Pleistocene. The modern arid environment of this area will not support any of the utilizable grains in a wild state. Consequently, an important change must have occurred for either wheat or barley or any other suitable grasses to have been abundant in central Egypt. Convincing evidence, however, was not available to support this hypothesis.

In order to clarify the problems of the Egyptian Late Palaeolithic, in particular the part which Egyptian prehistory played in the origin of food production, we under-

took a reconnaissance of the west bank of the Nile, beginning just north of Aswan, and working northward to Luxor (Fig. 1). The area was searched specifically for Late Palaeolithic sites dating from between 20,000 and 8,000 B.C., the critical time period involved. This survey was undertaken because during the just completed Nubian campaign, numerous Palaeolithic remains were found in southern Egypt where they were also believed to have been either buried or destroyed, and it seemed reasonable that a similar situation would be found farther north in central Egypt proper. We also postulated that one of the most promising areas to search for these remains would be along the west bank of the river where sand dunes and silts were known to interfinger in a complex sequence reflecting alternating periods of aggradation and recession.

During several previous seasons of field work in Nubia the history of the Nile Valley has been investigated in detail. From this work it is now clear that the Nile in its modern regime, deriving its main source of water from the highlands of East Africa and flowing northward to the Mediterranean, is a relatively modern feature. The oldest sediments of the Nile reflecting this regime lie about 30 m above the modern floodplain at Wadi Halfa, Sudan, and contain Late Palaeolithic artefacts dating from between 25 and 30 thousand years ago.

There seem to have been three main aggradational phases in the history of the Nile. The first and third are of limited interest here; however, the best preserved and widest spread of these is the second in the series, which, according to a number of radiocarbon dates<sup>11,12</sup>, began after 18,000 B.C. and lasted until 10,000 B.C. The maximum elevation of this silt accumulation amounted to some 20 m above the modern floodplain in Nubia and 8 m in Upper Egypt.

The majority of the deposits examined during the recent survey in Upper Egypt were identifiable with this phase of aggradation. Throughout most of this period the river continued to rise, thus building up a thicker bed in the floodplain; sand dunes were forming along the edge of vegetation on the west side and these dunes were washed and covered ephemerally during high floods. In one locality we counted more than twenty-five thin streaks of silt interfingering the dunes and following their slopes. During this period of silt accumulation the dunes were growing in an easterly direction, indicating that the prevailing winds at that time were westerlies, in contrast to the north-westerlies of today.

Towards the end of this aggradational phase there was a pause in the consistent accumulation of silts, and small ponds and lakes developed over the topographic lows in some dune fields back from the edge of the floodplain. At this time the dunes were no longer moving, but were stabilized with vegetation. At first the bottom of these ponds was un-aerated and the sediment was black and included organic matter, but then came an episode where the water became clear, the winds turned the water, and diatomites were deposited. An extensive diatomite field reaching a maximum thickness of 30 cm has been found in the dune fields west of Isna, in an area which is now being reclaimed. The ponds containing the diatomites eventually dried up, their surfaces were eroded, and, finally, they were covered by silts which were deposited during the last and highest phase of this episode.

The climatic implications of these diatomites are far-reaching, for they show that shortly before 10,000 B.C. an episode of cool and wet climate must have prevailed over Egypt. The nature of the diatomites is being investigated, but still no shallow ponds could stand for any length of time in Egypt except in cooler climatic conditions than those prevailing today. It is also significant that the development of the ponds and stabilization of dunes occurred during a recessional phase rather than during a period of aggradation of Nile silts. This observa-



tion suggests that the period of maximum pluvial conditions, when summer temperatures were at their lowest and local rainfall was greatly increased, does not correspond with the time of maximum accumulation of silt in the Nile Valley.

Associated with these deposits at several places were numerous, remarkably rich Late Palaeolithic sites containing several distinct industries, most of which were previously unreported from Egypt. The earliest industry occurs in the top of the lowermost silt and in the base of the dune which interfingered with the upper layers of the silt. It has a high frequency of Levallois technology, accompanied by a moderately high proportion of blades. The tools consist of partially backed long blades, numerous burins, denticulates and notches. This industry occurred at several sites north of Idfu, near Isna, and just south of Luxor. A developmental sequence is evident, the beginning of which probably dates before 20,000 B.C.

The next later assemblages occur within the lower dune. Two distinct industries are indicated. Levallois technology is absent in both, and both contain very high proportions of blades and blade tools. One of the industries has numerous backed blades together with double backed perforators. The other industry contains a high frequency of Ouchtata bladelets, plus occasional burins and denticulates. The geological position indicates an age for both assemblages of 14,000–16,000 B.C.

The next group of sites contain numerous backed micro-blades, truncations, lunates, trapezoids and end-scrapers. Some of them have high Levallois values and are similar to the Halfan industry in Nubia<sup>11,12</sup>; others are lacking in Levallois; some have numerous grinding stones, while

in others grinding stones are absent. Sites of this group occur at Wadi Kubbanayah and near Isna; they accompany a short interval of silt aggradation which is believed to date around 12,000–14,000 B.C.

There followed a short period of Nile recession during which permanent freshwater ponds developed in the areas beyond the Nile, and thick diatomite was deposited in these ponds. The ponds eventually disappeared, their deposits were eroded, and another level of silt was deposited over them. During this period of ponding followed by siltation there were at least three distinct industries in the area. Near Edfu there was a living site, with a typical Sebilian flake assemblage and a high frequency of Levallois technology. The second industry was represented in several sites near Isna which completely lacked Levallois technology, and yielded a moderate proportion of blades, together with numerous double backed perforators, denticulates, plus an occasional backed blade, burin, and end-scraper. Two fragmentary human skeletons were found with this group. The third industry present at this time also is very low in Levallois technology, is made primarily on flakes, and contains a very high frequency of end-scrapers and few denticulates, notches, side-scrapers and burins. All three of these industries are estimated to date between 10,000 and 12,000 B.C.

The most recent group of sites occurred again at Edfu and contain a flake industry with truncations, end-scrapers and grinding stones. They are dated with the recession of the upper silts, between 10,000 and 8,000 B.C.

The survey reported here has made four main contributions to our knowledge of North African prehistory. It

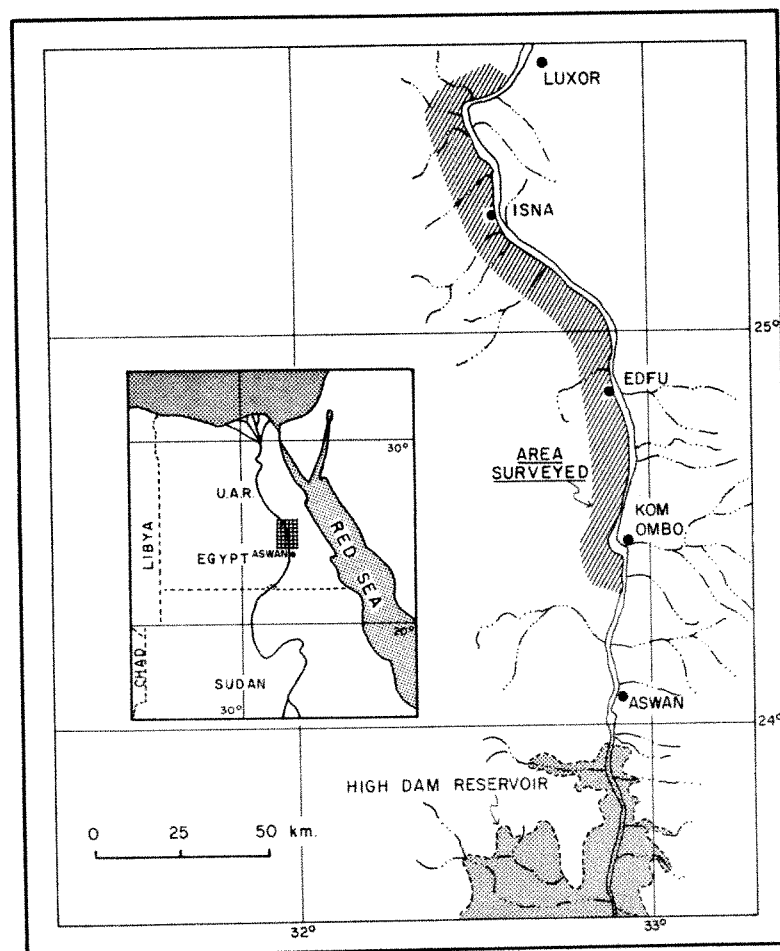


Fig. 1. Map of Upper Egypt showing area surveyed for Palaeolithic remains.

has demonstrated that: (a) Late Palaeolithic living sites do occur in Egypt in the Nile Valley; (b) the Late Palaeolithic industries from these sites are fully equivalent to the Upper Palaeolithic of the Levant and Europe in terms of developed blade technology and tool types characteristic of these areas; (c) the gathering and grinding of grain were widespread, but by no means a universal economic pursuit in Egypt during the Late Palaeolithic, apparently long before this feature appeared elsewhere; and (d) there is convincing evidence for a much cooler and wetter climate in Egypt about the time that Palaeolithic man in this area was making the first tentative steps toward the utilization of ground grain as an important source of food.

We thank Dr Bahay Issawy, Mr David Lubell, Mr James Phillips and Dr Romuld Schild for their help.

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## Phyletic Evolution in Modern Birds of the Patagonian Forests

by

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The *Nothofagus* forests of Patagonia have been isolated from other South American forests at least since before the Pliocene, and several kinds of evidence suggest that there has been little or no opportunity for speciation among the bird populations, especially the endemic ones, within the forest region.

EVOLUTIONARY change in birds and in many other organisms is of two kinds<sup>1</sup>: multiplication of species (speciation), a phenomenon involving both time and space; and phyletic change, involving mostly time. There is always phyletic change with speciation, but phyletic change is not necessarily accompanied by speciation. Speciation is usually emphasized by neontologists because they can easily demonstrate it in living organisms, but phyletic change is more frequently emphasized by palaeontologists because fossil series often illustrate linear change very well.

Investigation of the land bird fauna inhabiting the *Nothofagus* (beech) forests of western Patagonia (southern South America) showed me that the evolutionary structure of this fauna is somewhat at variance with that of other continental faunas because of its proportionately large number of endemic taxa and the lack of presently active speciation within the forest region<sup>2</sup>. This absence of species formation is of interest because the *Nothofagus* region extends over 2,000 km along the Andes from south-central Chile to Tierra del Fuego. I think that this state of affairs is a probable consequence of the ecological uniformity and the long isolation of the *Nothofagus* forest region of South America, which have favoured phyletic evolution rather than speciation (splitting).

Parts of southern Chile and south-western Argentina are covered with *Nothofagus* forests in which one or several species of *Nothofagus* are dominant. This forest occurs from the lowlands up to timber-line, and from about 36° south to the southernmost tip of South America<sup>3,4</sup>.

The birds living in these forests are quite different, as a faunal assemblage, from those of the neighbouring steppes, as well as from those of the nearest but non-adjacent forests, those of north-western Argentina (faunal resemblances were measured on the basis of Simpson's formulae<sup>5</sup>). The *Nothofagus* forest avifauna is composed of a relatively small number of taxa: there are only about forty genera and forty-four species of land birds. Endemicity is high, however, for about twenty-two species (50 per cent) are endemic to the *Nothofagus* forest region, and especially because between five and seven of these species constitute endemic monotypic genera. Three of these monotypic genera are particularly well marked.

An analysis has revealed that the *Nothofagus* avifauna, like most faunas<sup>6</sup>, is stratified into elements of different ages and into elements of different geographical origins. There is a positive correlation between the degree of differentiation of a given taxon (species or genus) and its presumed origins in time and space. More specifically, we find that the least differentiated taxa (for example, species with a barely distinguishable sub-species in the *Nothofagus* region) are also the most widespread, in other words belonging to Mayr's<sup>7</sup> Old World and Primarily North American Families. On the other hand, the most differentiated taxa are three genera which are monotypic, well marked, and belong to an old group, the ovenbird family Furnariidae (Primarily South American Family<sup>7</sup>). They are *Sylvioorthorhynchus*, *Aphrastura*, and *Pygarrhichas*.

An investigation of the speciation potential of the species of the *Nothofagus* fauna would give information about the development of the correlation mentioned above. An analysis of speciation phenomena in the birds of the *Nothofagus* fauna<sup>2</sup> showed me that only about four species out of forty-four (10 per cent) have speciated *in situ*. That is to say these four species are the result of multiplication of species within the *Nothofagus* forest. All the other species (90 per cent) are either geographically unvariable or have very slight morphological variation of a clinal nature, and show no evidence of speciation or incipient speciation within the forest region.

A close scrutiny of the affinities of the latter species with related species elsewhere in South America revealed that in about eight cases (20 per cent) the forest species originated after a stock entered the *Nothofagus* forest, and left an isolate there. It is noteworthy that the old endemics of the *Nothofagus* forest show no more trends towards speciation than more recent endemics or than the most recent, non-endemic species.

It would seem therefore that environmental conditions in the *Nothofagus* forest region are, on the whole, quite unfavourable for the formation of species through speciation. An examination of the *Nothofagus* forest shows that it is quite uniform within and lacks barriers which would be a prerequisite for species formation, and which are found in other areas comparable in size<sup>2</sup>.

I think that the information summarized above can be explained by the following hypothesis. The *Nothofagus* region has been isolated from other forest regions elsewhere in South America so that the elements which reached the beech forests did so much like insular colonists. In other words, it is likely that only a few stocks were able to colonize the *Nothofagus* forest region, and that a high faunal turnover rate existed at all times<sup>8</sup>. (The latter aspect, however, is more highly speculative than the others, and will not be considered further here.) Those stocks which succeeded in establishing themselves in the *Nothofagus* forest encountered there conditions which did not permit further evolution by speciation (splitting), and so they diverged from their ancestors mainly in a phyletic manner.

The question which immediately comes to mind is whether there is any kind of evidence in support of the hypothesis of isolation and predominantly phyletic evolution. The history of the forest fauna obviously depends upon the history of the forest itself, and so we have to turn to botanical evidence. Today's *Nothofagus* forest is well isolated from other forest tracts in South America (Andes of north-western Argentina, lowlands of southern Brazil and north-eastern Argentina) by dry to very arid zones<sup>9</sup>. Some floral resemblances between these disjunct forests exist, but they are not important. If the forests had been connected in a recent past we would expect a much greater degree of resemblance. It thus seems likely that the *Nothofagus* forest has been isolated for a long time, but how long remains an open question. We do know, however, that *Nothofagus* has existed in southern South America since the Eocene-Oligocene (personal communication from B. Vuilleumier).

Other evidence about isolation and age comes from mammals. Two living marsupials, *Dromiciops* (an opossum) and *Rhyncholestes* (a caenolestoid)<sup>10</sup>, constitute endemic monotypic genera in the *Nothofagus* forest. They both belong to groups which have been in southern South America since the early or mid-Tertiary: the caenolestoids were present in Patagonia in the earliest Eocene<sup>11</sup>, and the microbiotheriine opossums have been there since the Oligocene-Miocene<sup>12</sup>. It seems probable that these marsupials have reached the *Nothofagus* forests during an early period when the latter forests and other South American forests were, if not directly connected, at least closer to one another than they are today (personal communication from K. F. Koopman).

These considerations suggest that, as a most conservative estimate, the *Nothofagus* forest region has been isolated at least since the Pliocene. It is more likely that it has been isolated since the Miocene, or even possibly earlier. This imprecision is unfortunate, but it must be remembered that all we have are inferences and clues, not dates, showing that isolation is real and apparently rather ancient.

The second point of the hypothesis, phyletic evolution, must now be considered. Birds are notoriously poor fossil material, and so they characteristically offer no evidence whatever about the evolutionary history of the recent *Nothofagus* bird species or genera. A series of fossil frogs, however, may permit us to draw some conclusions on this point. These frogs include *Eophractus* (Lower Eocene)<sup>13</sup>, *Calyptocephalella canqueli* (Lower Oligocene)<sup>14</sup>, and *Gigantobatrachus* (Middle Miocene)<sup>15</sup>. According to Hecht<sup>16</sup> these fossils show a probable progressive evolutionary change through time towards the modern genus *Caudi-verbera*, an isolated taxon within the Leptodactylidae. By inference, it seems possible that at least some of the isolated, monotypic endemic bird genera living in the *Nothofagus* forest today (for example, *Sylviorthorhynchus*, *Pygarrhichas*) have evolved in a similar phyletic manner.

The data on which the suggestions made in this report are based will be published in full separately. I thank the Chapman Memorial Fund, Harvard University's Evolutionary Biology Committee, and Sigma Xi for financial support, and Ernst Mayr and Beryl Vuilleumier for their advice.

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## Formation of Prothrombin Converting Activity

by

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After measurement of the conversion of prothrombin to thrombin in a purified system, a model has been constructed for the kinetics of the formation of prothrombin converting activity. In the model phospholipid provides a surface on to which coagulant factors bind.

In blood coagulation prothrombin is converted to thrombin as the result of the interaction of several blood coagulation factors<sup>1,2</sup>. One of the features of blood coagulation theory, which is still controversial, is the precise identity of the prothrombin converting activity. While most

authors agree that conversion of prothrombin requires the presence of activated factor X (factor X<sub>a</sub>), factor V, phospholipid and calcium ions, the form of their interaction is still in debate. Some authors suggest that the final prothrombin converting substance is a form of factor

V modified by its interaction with factor  $X_a$  (ref. 3), while others hold the view that factor V facilitates the enzyme conversion of prothrombin to thrombin by factor  $X_a$  (ref. 4). The function of the phospholipid in this system seems to depend on its providing a suitable surface on which this reaction may take place, rather than to any specific chemical constitution<sup>4</sup>.

We describe here experiments performed with purified reagents, in which the kinetics of prothrombin activation have been investigated with a view to elucidating further the nature of the process.

Bovine prothrombin was prepared and given by Dr K. Denson<sup>5</sup>. The preparation contained 10,000 NIH u/ml. of prothrombin, and less than 10 per cent of contaminating material that had neither coagulant nor inhibitory activity. Bovine factor V was prepared according to Esnouf and Jobin<sup>6</sup>; the stock solution contained 40,000 u/ml. and had no detectable contamination. Factor  $X_a$  was prepared by the conversion of factor X (ref. 7) by the coagulant protein from Russell's viper venom<sup>8</sup>, and then separating the venom protein from factor  $X_a$  by chromatography<sup>4</sup>. Factor  $X_a$  was not contaminated with any other clotting factors.

The phospholipid was a commercial preparation of mixed phospholipids, 'Inosithin'. Emulsions of this phospholipid were prepared as described by Jobin and Esnouf<sup>4</sup>.

The experiments were carried out as follows. Phospholipid, factor  $X_a$  and factor V were added together to the final concentration desired in a medium containing 12 mmolar calcium chloride and 40 mmolar *tris*-hydrochloric acid buffer, pH 7.5. The mixture thus obtained was called the "first incubation mixture". It was kept at 37° C during the "first incubation time". Except in the experiments shown in Fig. 1 where it is plotted along the abscissa, this time was always 4–6 min. After this the first incubation mixture was put in an ice bath at 0° C which prevented appreciable decay of prothrombin converting activity for at least 30 min.

To 1.8 ml. of this "first incubation mixture" 0.2 ml. of the prothrombin solution was added at zero time of the second incubation. The concentration of prothrombin in the second incubation was thus 1,000 NIH u/ml.; this concentration represents an excess of substrate for

the prothrombin activator, and did not seem to be inhibitory.

From the second incubation mixture 0.1 ml. portions were sub-sampled into 0.1 ml. of fibrinogen solution of 100 mg/ml. (human fibrinogen Kabi). The clotting time of this mixture was measured in a water bath at 37° C. The clotting times thus obtained were converted into concentrations of thrombin by comparison with the clotting times obtained by dilutions of a standard thrombin solution, in comparable conditions of pH, ionic strength, calcium ion concentration and temperature.

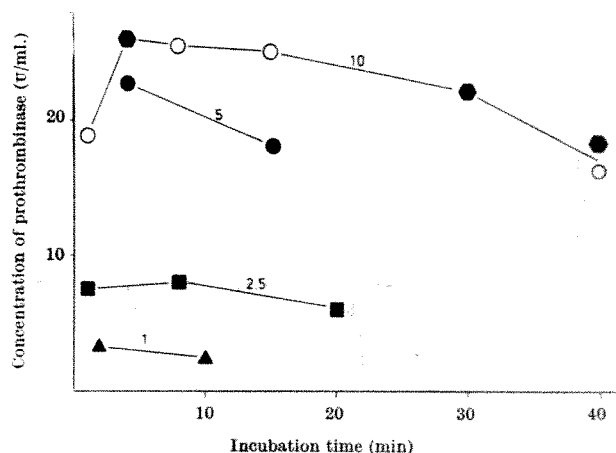


Fig. 2. Generation of prothrombinase activity in a mixture containing factor V, factor  $X_a$  and phospholipid. The final concentrations were 25 u/ml. of factor V, 10, 5, 2.5 or 1 u/ml. of factor  $X_a$  (as indicated), 5  $\gamma$ /ml. of phospholipid, 12 mmolar calcium chloride, and 40 mmolar *tris*-hydrochloric acid buffer, pH 7.5. The concentration of prothrombinase is expressed in arbitrary units, one unit being defined as the amount that causes a velocity of thrombin generation of 1 NIH u/10 min during the second incubation.

The amount of thrombin thus found was plotted against the incubation time in the second incubation mixture, and from this graph the initial velocity of thrombin formation induced by the prothrombin activator was estimated.

A representative experiment is shown in Fig. 1. In this experiment a mixture with high prothrombin converting activity was diluted 1 in 3 and 2 in 3, and thrombin generation has been followed in the original sample as well as in the dilutions. The two lower lines in the graph are drawn at 2/3 and 1/3 of the slope of the steepest line. It can be seen that: (a) the thrombin generation curve cannot be distinguished from a straight line in the initial phase; (b) the initial velocity of thrombin generation can be regarded as proportional to the amount of prothrombin converting activity present in the incubation mixture; (c) prothrombin converting activity tends to be unstable with larger dilutions, which can be deduced from the gradually declining slope of the thrombin generation curve at a low level of prothrombin converting activity; and (d) no lag period in thrombin formation is observed. It thus appeared acceptable to use the initial velocity of the thrombin generation curve obtained in the second incubation mixture, as a measure of prothrombin converting activity in the first incubation mixture.

Fig. 2 shows a typical curve of the generation of prothrombin converting activity in a first incubation mixture. After mixing the components there is a rapid rise of activity which very gradually falls off again. As will be seen below, the amount of prothrombin converting activity formed bears a relationship to the concentrations of factors V and  $X_a$ , and phospholipid. By varying the amounts of the reactants we have not been able to create conditions whereby the prothrombin converting activity rose slowly to a high value. If one of the clotting factors ( $X_a$  or V) was an enzyme which catalysed the conversion of the

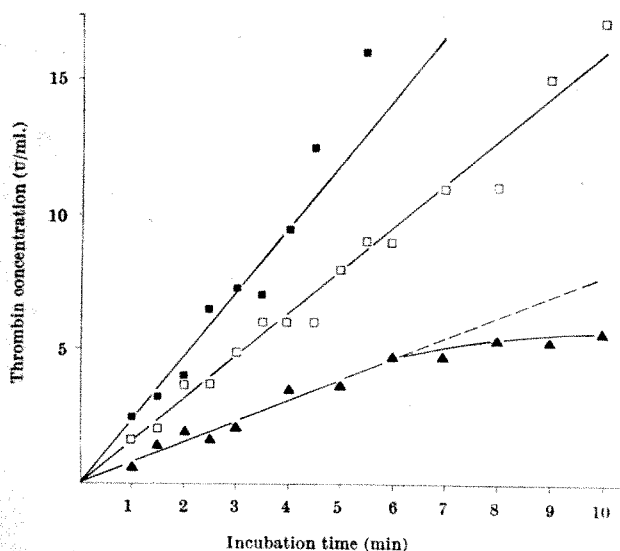


Fig. 1. Thrombin generation in a mixture containing prothrombin and prothrombinase activity. (■—■) Thrombin generation in a mixture containing 20 u/ml. of factor V, 8 u/ml. of factor  $X_a$ , 4  $\gamma$ /ml. of phospholipid, 1,000 NIH u/ml. of prothrombin, 12 mmolar calcium chloride, and 40 mmolar *tris*-hydrochloric acid buffer, pH 7.5. (□—□) Thrombin generation in the same mixture immediately after a 2 in 3 dilution with 40 mmolar *tris*-hydrochloric acid buffer, pH 7.5 containing 12 mmolar calcium chloride. (▲—▲) The same immediately after a 1 in 3 dilution.



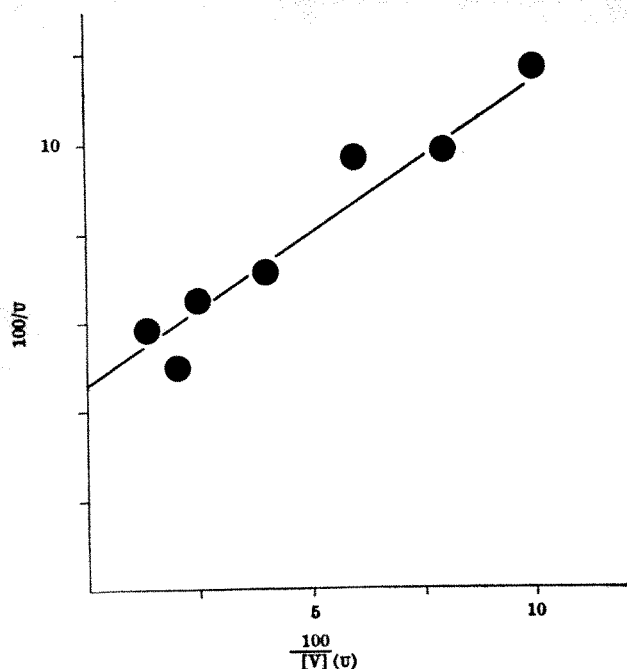


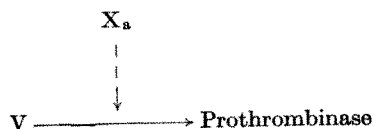
Fig. 3. Relation between concentration of factor V and prothrombinase activity. The inverse of the concentration of factor V expressed in  $\mu$  is plotted against the inverse of the prothrombinase activity expressed in  $\mu$ .

other to prothrombinase, then such a progressive increase in activity in certain conditions might be expected.

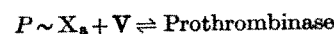
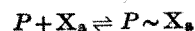
At relatively large concentrations (causing a velocity of thrombin formation of  $\sim 20$  NIH  $\mu$  of thrombin/ml./10 min), the half-life time of the prothrombin converting activity was estimated to be about 55 min; the half-life time of the prothrombin converting activity generated in a thrombin generation test (that is, in a system using normal plasma) was estimated to be about 10 min in the same conditions. Thus the prothrombin converting activity appeared to be more stable in its purified form than it is in plasma. This again emphasizes the point that an inhibitor of prothrombin conversion is likely to be present in plasma<sup>9</sup>.

Figs. 3, 4 and 5 show that a linear relationship between the reciprocal of the concentration of either of the reactants and the reciprocal of the final prothrombin converting activity is the simplest kind of relationship that can be deduced from these experiments. Essentially the same results were obtained when the amount of thrombin was estimated spectrophotometrically as *n*-benzoyl arginine methyl esterase activity.

This relationship cannot be accounted for by the reaction scheme of the type



or by any of its modifications. It can, however, be accounted for by a model based on the assumptions that: (a) there are a fixed number of factor  $X_a$  binding sites and a fixed number of factor V binding sites on each unit phospholipid micelle surface; (b) prothrombin conversion occurs when a factor  $X_a$  molecule and a factor V molecule, bound to the phospholipid surface, are in a favourable position one to the other; (c) binding of one of the factors does not influence the binding characteristics of the other one<sup>8</sup>. This model can be written in the following reaction formulae



The word prothrombinase refers to the complex which converts prothrombin to thrombin.

With these assumptions the following relationships will exist: (a) the concentration of prothrombinase is proportional to the number of bound factor  $X_a$  molecules/unit of phospholipid surface as well as to the amount of bound factor V molecules per unit of phospholipid surface; (b) the concentration of prothrombinase is proportional to the amount of phospholipid surface. This is written in a formula

$$C = K \cdot P \cdot \frac{P \sim X}{P} \cdot \frac{P \sim V}{P} = \frac{K}{P} \cdot P \sim X \cdot P \sim V$$

Where  $C$  is prothrombinase concentration;  $P$  is phospholipid concentration;  $P \sim X$  is phospholipid bound factor  $X_a$  concentration;  $P \sim V$  is phospholipid bound factor V concentration, and  $K$  is a constant. With the aid of elementary mathematics it can be shown

$$\text{from } P + X \xrightleftharpoons[k_{-1}]{k_{+1}} P \sim X; k_{-1}/k_{+1} = K'$$

$$\text{that } \frac{1}{P \sim X} = \frac{1}{X} + \frac{1}{P} + \frac{K'}{X \cdot P} \text{ and}$$

$$\text{from } P + V \xrightleftharpoons[k_{-2}]{k_{+2}} P \sim V; k_{-2}/k_{+2} = K''$$

$$\text{that } \frac{1}{P \sim V} = \frac{1}{V} + \frac{1}{P} + \frac{K''}{V \cdot P}$$

$$\text{So that } \frac{1}{C} = \frac{P}{K} \left( \frac{1}{X} + \frac{1}{P} + \frac{K'}{X \cdot P} \right) \cdot \left( \frac{1}{V} + \frac{1}{P} + \frac{K''}{V \cdot P} \right)$$

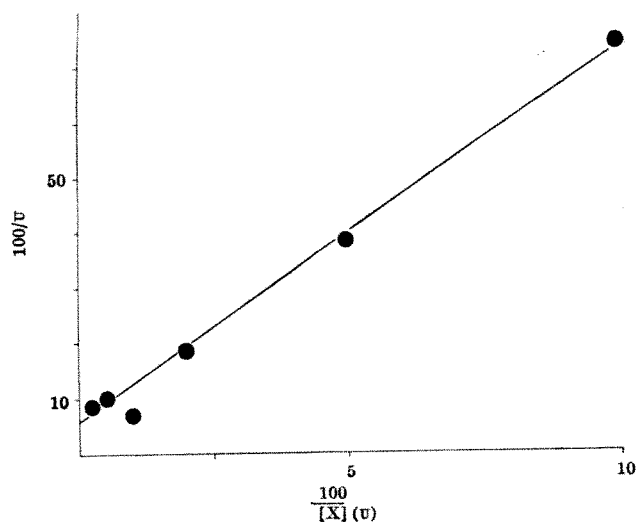


Fig. 4. Relation between concentration of factor  $X_a$  and prothrombinase activity. The inverse of the concentration of factor  $X_a$  expressed in  $\mu$  is plotted against the inverse of the prothrombinase activity expressed in  $\mu$ .

In these equations  $P$ ,  $X$  and  $V$  indicate the total concentrations of phospholipid, factor  $X_a$  and factor  $V$ , respectively. For the two protein factors this formula accounts for the relations found:  $1/C$  is linearly proportional to  $1/V$  at fixed  $V$  and  $P$  concentrations and linearly proportional to  $1/V$  at fixed  $X$  and  $P$  concentrations.

When the relationship between  $C$  and  $P$  is examined the formula is best written in the form

$$\frac{1}{C} = P + \frac{1}{P} (X + K') (V + K'') + (V + X + K' + K'') \frac{1}{XVK}$$

At small concentrations of phospholipid  $P$  will become negligible compared with  $\frac{1}{P} (X + K') (V + K'')$  and the formula will reduce to

$$\frac{1}{C} = \frac{1}{P} (X + K') (V + K'') + (V + X + K' + K'') \frac{1}{XVK}$$

which accounts for the relationship which we found.

At large concentrations of phospholipid the formula will reduce to

$$\frac{1}{C} = P + (V + X + K' + K'') \frac{1}{XVK}$$

which predicts the inhibiting effect of excess phospholipid that is often found.

This inhibitory action of excess phospholipid is not seen in the experiments shown in Fig. 5, because the range of phospholipid tested does not include the pertinent concentrations.

From these results it can be seen that near maximal velocity is often found at relatively small concentrations of the three reactants. This finding explains many of the reports in the literature, stating that further addition of one particular reactant did not increase the final yield of prothrombinase. The conclusion that this reactant may not therefore be a constituent of prothrombinase is apparently not justified.

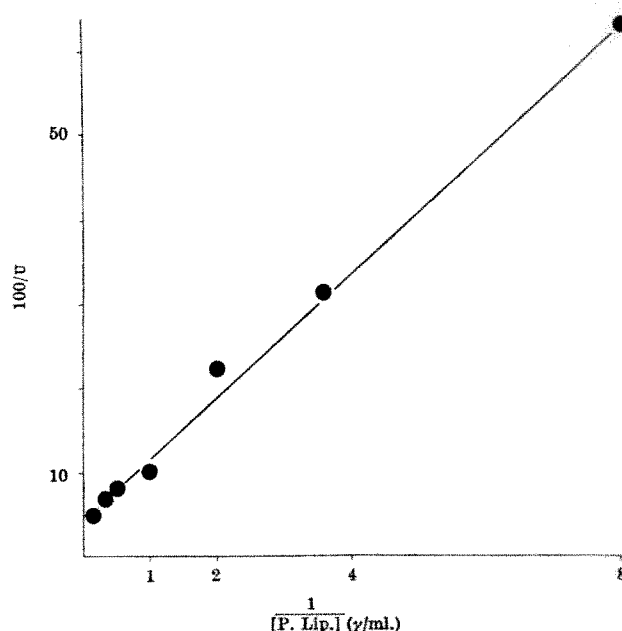


Fig. 5. Relation between phospholipid and prothrombinase activity. The inverse of the phospholipid concentration expressed in  $\gamma/\text{ml.}$

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## Changes in Visual Cortex on First Exposure of Rats to Light

It is possible that neuronal activity may produce synaptic changes, although there has so far been no direct evidence for this. Experiments with rats suggest, however, that changes in chemistry and the structure of the synapses in the visual cortex are among the consequences of the exposure of eyes to light for the first time.

### Effect on Synaptic Dimensions

It is still not clear whether synaptic changes are involved in learning<sup>1</sup>, and no functionally dependent synaptic change has been convincingly demonstrated<sup>2</sup>. It is thus reasonable to begin with the strongest possible functional

stimulus, and the first exposure of animals to light has been shown to cause changes in protein metabolism<sup>3</sup> and in the morphological development<sup>4</sup> of the visual cortex. Gyllenstein *et al.* have shown that mice reared in darkness have smaller nuclei in the cells of the upper half of the visual cortex and less internuclear material (cytoplasm

and neuropil) per nucleus. The thickness of the visual cortex is also reduced. By contrast, an increased thickness of visual cortex and increased dendritic branching have been described<sup>5</sup> in animals reared in a visually enriched environment. The first exposure of animals to light is thus a suitable stimulus to apply in looking for functionally dependent synaptic changes by electron microscopy. In the work described here particular attention has been given to the size distribution of synaptic profiles and to the density of synapses in the tissue, as well as to the qualitative appearances.

With one exception mentioned later, mother (Wistar albino) rats with their litters were kept in complete darkness from the day after birth till weaning at 3 weeks. Half of each litter was then exposed to daylight, and the rest kept in complete darkness. At biopsy, the rats were anaesthetized with pentobarbital sodium, and a pair matched for body weight and sex selected from the two groups. In later experiments, single rats with one eye covered were used, and the cortex opposite the open eye compared with that opposite the closed eye. These rats were then perfused with Pease formaldehyde fixative<sup>6</sup> and thin coronal slices of visual cortex (previously defined by retrograde degeneration)<sup>7</sup> dissected out. Each slice was cut off at the grey white boundary, and divided in half parallel to the pia. Pieces representing the upper and lower halves of the cortex were chopped into small fragments in a buffered solution of osmium tetroxide, dehydrated in acetone, block stained in uranyl acetate in acetone<sup>8</sup>, embedded in 'Araldite' epoxy resin, cut into silver sections, stained on the grid with lead citrate, and examined on a Siemens electron microscope. Fields were photographed at a fixed magnification of 14,000 regardless of their contents, and several blocks from the upper and lower halves of the visual cortex from the rat exposed to daylight and the dark-reared littermate examined. All presynaptic boutons, with or without a thickened region of apposition, were measured on photographic prints at an overall magnification of 49,000 until the diameters of about 500 profiles had been measured in blocks from each class of cortex. The latter was not identified until the measuring and counting had been completed. The number of synaptic profiles with mean diameters distributed in classes of  $0.1\mu$  was recorded. The density of the synapses in the tissue was estimated by the Abercrombie method which has previously been applied to electron microscopy<sup>9</sup>.

A rat exposed to daylight for 10 weeks after weaning differed significantly from its littermate reared in the dark: in the superficial half of the visual cortex the distribution of synaptic profiles was systematically shifted towards the larger sizes ( $0.6-1.0\mu$ ), while in the deeper half of the cortex the distribution was shifted towards the smaller sizes ( $0.4-0.1\mu$ ) compared with the dark-reared rat. The probabilities that the differences between the average diameters occurred by chance is shown in Table 1 (*t* test), together with the density of synapses in the tissue. The latter was similar in the superficial half of the cortex in the dark- and light-reared rats, but the synaptic density in the deeper half of the cortex was lower in the dark-

reared animal. This suggests that exposure to light promotes the formation of new synapses of small diameter in the deeper layers of the visual cortex, while the synapses already formed in the superficial half of the cortex grow slightly larger.

For this phenomenon to be relevant to learning one necessary condition is that synaptic changes should follow a period of exposure to light comparable in length with the consolidation time<sup>1,10</sup> (that is, something less than 1 h). The experiment was therefore repeated after shorter periods of exposure to light, as shown in Table 1. In the fifth experiment, a rat was exposed to daylight for only 3 h and then kept in the dark for 48 h before biopsy, in the expectation that processes started by the exposure to light might take longer to produce detectable morphological results. The negative result in the seventh experiment in which the biopsy was carried out immediately after exposure to light confirmed this expectation. A significant shift in the size distribution of synaptic profiles was found in each of the other pairs of rats, except in the sixth experiment. The litter used for this experiment was not put into the dark until 11 days of age (before eye opening) and it has been shown that rats react to light at this age through closed eyelids<sup>11</sup>. The synaptic dimensions and density found in this sixth experiment are compatible with both members of the pair of rats having been effectively exposed to light. In the upper half of the visual cortex, the average increase in synaptic diameter in the first five experiments was  $0.0174\mu$  (4 per cent) in the animals exposed to light, and the probability of this increase occurring by chance is less than 1 per cent (*t* test). In the lower half of the cortex, the average decrease in synaptic diameter was  $0.0634\mu$  (14 per cent) in the animals exposed to light and the probability of this increase occurring by chance is less than 2 per cent. These differences are small and can be detected only because a large number of synapses has been measured. When the minimum effective period of exposure to light has been identified a larger number of experiments must be carried out at this exposure to increase the reliability of the result. It remains to be seen how long after exposure to light biopsy should be carried out for the effect to be manifested most clearly. No qualitative difference has been noticed in the synaptic profiles, vesicles or regions of apposition between the rats reared in the light and those reared in darkness. Because the changes in synaptic size are in opposite directions in the upper and lower halves of the visual cortex, the sensitivity of the method may be increased by an exact localization in depth of the regions of maximal change. Further experiments are needed to show whether pattern vision or merely exposure to light, as suggested by the sixth experiment, is the effective stimulus. In normal animals reared in daylight, exposure to light does not generally increase the rate of neuronal firing in the visual system<sup>12</sup>, but this point does not appear to have been tested for the first exposure of dark-reared animals to light. The various effects of light exposure on the retina<sup>13</sup>, lateral geniculate nucleus<sup>14</sup> and visual cortex<sup>4,5,15</sup> of animals reared in darkness suggest that chronic deprivation of light may reduce the rate of neuronal firing

Table 1. AVERAGE SYNAPTIC DIAMETER AND DENSITY OF SYNAPSES IN THE UPPER AND LOWER HALVES OF THE VISUAL CORTEX OF PAIRS OF LITTERMATE RATS

Experiment	Time in light	Upper half of visual cortex		Density ( $\times 10^{12}/c.c.$ )	Lower half of visual cortex		Density ( $\times 10^{12}/c.c.$ )
		Average synaptic diameter ( $\mu$ )	Probability		Average synaptic diameter ( $\mu$ )	Probability	
1	10 weeks	0.439→0.456	0.01	1.28→1.19	0.493→0.397	0.001	0.847→1.35
2	2 weeks	0.417→0.445	0.05	1.50→2.07	0.500→0.435	0.01	1.25→1.43
3	1 week	0.437→0.444	0.05	1.51→1.11	0.499→0.425	0.001	0.98→1.20
4*	24 h + 48 h in dark	0.460→0.478	0.1	0.90→0.87	0.523→0.448	0.005	0.855→1.01
5*	3 h + 48 h in dark	0.363→0.380	0.02	1.88→1.81	0.394→0.387	0.1	1.40→1.50
6	4 weeks both exposed to light for 11 days	0.455→0.462	> 0.1	1.53→1.08	0.433→0.435	> 0.1	1.29→1.23
7*	3 h immediate biopsy	0.410→0.409	> 0.1	1.25→1.22	0.427→0.424	> 0.1	1.09→0.981

One rat in each pair was reared in darkness, while the other was exposed to daylight after weaning for the time shown. The figures before the arrows refer to the rats reared in the dark, and those after the arrows to the rats exposed to light. In the experiments marked with an asterisk (\*) single rats with one eye covered were used, and the figures before the arrow refer to the cortex opposite the covered eye, those after the arrow to the cortex opposite the open eye.

and that exposure of dark-reared animals to light may produce at least a temporary increase in neuronal activity.

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### Effect on Incorporation of Tritiated Lysine into Protein

A VARIETY of reports has appeared indicating that the rate of protein and RNA synthesis in the nervous system is state dependent, in that it depends on the degree of stimulation of cortical or ganglionic neurones<sup>1-6</sup>. While most reports have claimed that physiological or behavioural stimulation results in increased rates of synthesis, there are apparently some conditions under which inhibition can occur (see refs. 6-8 for reviews). One strong stimulus is the first exposure to light of animals reared in the dark, which is thought to produce changes in protein metabolism in rabbit cortex<sup>5</sup>, and increased morphological development in mouse visual cortex<sup>9</sup>. Exposure to a visually enriched environment has been claimed to result in an increase in the thickness of the visual cortex and greater dendritic branching in growing rats<sup>10,11</sup>. These results suggest that the exposure of dark-reared animals to light is a suitable stimulus to apply in looking for functionally dependent changes in rates of protein synthesis. Experiments describing such changes are reported here; an accompanying article<sup>12</sup> describes some changes in synaptic size and density observed under similar conditions.

Littermate Wistar rats were placed, with the mother, within a day of birth, in cages enclosed within light-tight boxes, the boxes being opened later, when necessary, using a photographic safety lamp as the source of light. The mother was removed at weaning and the litter remained until about 50 days of age. The rats were then divided into two groups; one group was returned, in a new cage, into the light-tight boxes, the other group was placed in a fresh cage in the open laboratory under conditions of normal illumination from fluorescent ceiling lights and windows (average illumination 25 ft.-candles). The light-tight box containing the control group was placed by the side of the experimental group during the entire period of illumination, so that random factors of extraneous sounds and changes in ambient temperature affected both groups

similarly. After varying periods of continuous exposure to light, and three hours before they were killed, each animal was injected intraperitoneally with 1.0 ml. of a solution containing 50  $\mu$ c. of generally labelled tritiated lysine, specific activity 50  $\mu$ c./mmole, in 0.9 per cent sodium chloride. The efficacy of this injection route is well established<sup>13</sup>. Control animals were returned to the light-tight box, experimental animals to their illuminated cage. No food or water was provided during this final period.

Three hours after injection the animals were killed and samples of visual and motor cortex rapidly removed, using a plastic mould with appropriate holes cut out for the dissection. The samples from each hemisphere were treated separately; thus four samples were obtained from each brain. Duplicate control samples were taken from the liver at the same time. Each tissue sample was homogenized in 0.32 molar sucrose, and portions were assayed for protein<sup>13</sup>, total radioactivity, and radioactivity bound to a thrice-washed precipitate insoluble in trichloroacetic acid (TCA). Radioactive samples were dissolved in hyamine and counted in a scintillation counter.

The results were calculated in two ways. (a) By expressing the counts found in TCA insoluble fraction as a percentage of the total counts in the sample; this represented the relative specific activity (RSA) of bound to pool lysine. Three hours after injection, some 50 per cent of the total counts found were in the fraction insoluble in TCA in the brain, and a rather higher percentage in the liver. (b) A second method of calculation was to relate the counts incorporated into bound material to total protein, corrected for variation in dose/body weight. This represented specific activity. The first method, which avoids limitations due to variations in dose, injection technique or permeability of the blood-brain barrier between different animals, gave better reproducibility in a control experiment. With ten animals the standard error of the mean was better than  $\pm 5$  per cent. Data based on this method of calculation are presented here, though either method gave essentially similar results. All tests of significance were made using Student's *t* test.

Control experiments, to check whether dark confinement of the kind described above impaired vision, were made by testing animals kept for up to 50 days in the dark in a visual cliff device<sup>14</sup>. Such animals were found to respond as well as normals on the apparatus within 15 min of first exposure to light. Continuous subsequent exposure for periods of 4 days or more to fluorescent lighting did, however, produce some impairment in response. In none of the experiments to be described were the animals exposed to continuous light for more than 4 days.

Table 1. INCORPORATION OF RADIOACTIVITY INTO TCA-INSOLUBLE MATERIAL AFTER 3 H EXPOSURE OF LITTERMATE RATS TO LIGHT. NUMBER OF DETERMINATIONS IN PARENTHESES

Region	RSA		P
	Light (8)	Dark controls (8)	
Visual cortex	51.6 $\pm$ 0.6	44.8 $\pm$ 1.8	< 0.01
Motor cortex	43.6 $\pm$ 2.0	50.8 $\pm$ 2.4	< 0.01
Liver	67.8 $\pm$ 2.4	69.2 $\pm$ 1.8	n.s.

The results of one experiment in this series are shown in Table 1. After a three hour period the relative specific activity of the visual cortex of the animals exposed to light was increased by 15 per cent over the dark controls, the RSA of the motor cortex was decreased by 14 per cent and that of the liver by 5 per cent. The increase in RSA in the visual cortex, and the decrease in RSA in the motor cortex, were significant ( $P < 0.01$ ). In four experiments, in each of which eight to ten animals were used, essentially the same results were obtained for the visual cortex; in each case the RSA was increased above the control by at least 15 per cent, even though slight procedural variations in individual experiments resulted in a range of RSA values of the control litter of from 41.1 to 52.0. In each experiment the liver showed no significant difference from controls. The increase in incorporation of lysine into acid insoluble material following 3 h light stimulation



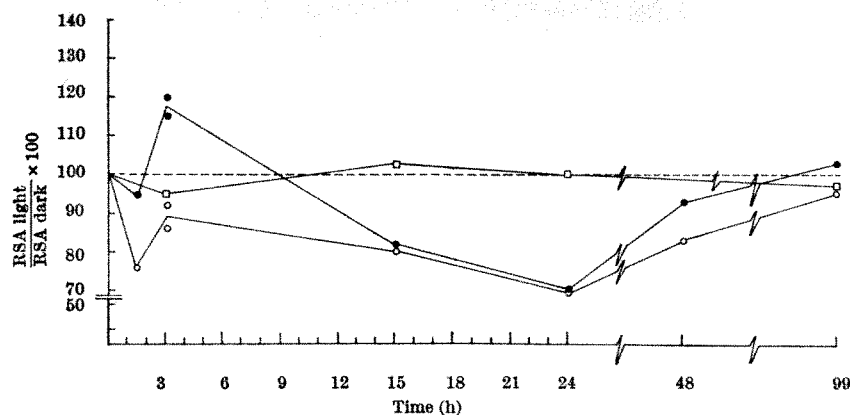


Fig. 1. Effect of exposure to light on incorporation of tritiated lysine into TCA-insoluble material in brain and liver of the rat *in vivo*. Rats were reared for 50 days in darkness and then brought into the light for varying periods of time before injection with labelled lysine and treatment as described in the text. The difference in relative specific activity between experimental and dark-control animals was calculated as described in the text. Each point represents the mean results of an experiment with at least eight littermate animals. ●—●, Visual cortex; ○—○, motor cortex; □—□, liver.

thus appeared to be confined to the visual region of the cortex (in one of the four experiments done at this time interval the motor cortex also showed a significant increase in RSA).

When light stimulation was prolonged beyond the 3 h period, however, a depression, rather than a stimulation, of incorporation resulted. These experiments are shown in Fig. 1, which shows the effects found after 1.5, 3, 12, 24, 48 and 99 h of exposure to light. Each experimental point represents the results obtained with a litter of at least eight animals; the graph is plotted by expressing the results as RSA of experimental animals as a percentage of that of controls. After 1.5 h of exposure to light there was no effect on the visual cortex, while the RSA in the motor cortex was already depressed by some 23 per cent. After 3 h the stimulation in the visual, and depression in the motor cortex, was visible, while after 12 h or more of light exposure the incorporation in both the visual and motor cortices was depressed. The greatest depression occurred at about 24 h of light exposure, when it amounted to 30 per cent. After this time, incorporation appeared to rise slowly back to the control level once more, and after 99 h of light exposure RSA in both experimental and control animals was the same. At no time during these experiments did the RSA in the liver alter significantly from control levels.

This clear, reproducible and statistically significant biphasic curve prompted several further questions. (i) Was there some permanent difference in ability to incorporate lysine between animals reared in the dark and those subject all their life to a normal animal house cycle of 12 h light/12 h darkness? Table 2 shows that there was not. For animals which had never seen the light at all, RSA in the visual cortex, motor cortex and liver was not significantly different from that of animals kept under "normal" conditions. Thus the biphasic curve represented a transient response to new experience. (ii) To what could the effects in the motor cortex be ascribed? It might be a reasonable hypothesis that if the amount of motor activity directly influenced the rate of incorporation in the motor cortex, and there was a difference in motor activity between the animals in the dark and those exposed to light, this could account for the lowered RSA. Table 3

shows the results of an experiment in which a group of normally reared 50 day old littermates were injected with tritiated lysine and placed in an activity wheel turning at 1 rev/25 sec. After 3 h of such mild but continuous motor activity, RSA in the motor cortex was increased by some 18 per cent over controls ( $P < 0.1$ ); changes in the visual cortex and liver were not significant. Thus motor activity could apparently affect incorporation into the motor cortex.

Table 3. INCORPORATION OF RADIOACTIVITY INTO TCA-INSOLUBLE MATERIAL FOLLOWING 3 h FORCED ACTIVITY ON ROTATING WHEEL. NUMBER OF DETERMINATIONS IN PARENTHESES

Region	RSA		P
	Active (6)	Inactive (6)	
Visual cortex	69.4 ± 1.8	62.7 ± 5.3	n.s.
Motor cortex	68.0 ± 2.2	57.6 ± 5.7	< 0.1
Liver	72.7 ± 3.6	72.9 ± 1.9	n.s.

Study of the relative motor activity of a group of control animals with those of their littermates brought out into the light was made by mounting both cages (one inside its light-tight box) so that they rested on pressure transducers coupled to a chart recorder. Movement of the animals inside the cages resulted in a deflexion on the recorder. Study of 6 days continuous recording showed, somewhat unexpectedly, that not only were the total numbers of movements recorded similar for both light and dark animals after the first 6 h period (2,375 movements/day in the light, 2,706 movements/day in the dark) but that both dark and light animals maintained a very regular diurnal pattern, with little or no movement between 0800–1500 h and a much higher activity at other periods of the day. During the first 6 h the number of movements in the dark was increased by some 70 per cent compared with that in the light. Thus there was only a transient difference in the pattern of motor activity of the light-exposed compared to the control animals.

(iii) Data have hitherto been presented solely on incorporation into material insoluble in TCA. To examine further which TCA-insoluble fraction increased in labelling under the experimental conditions, the TCA precipitate in one experiment was subfractionated by extracting the lipids with ethanol and 2/1 chloroform/methanol and the nucleic acids with 10 per cent TCA at 90° C for 10 min. The residue was regarded as consisting of protein. Table 4

Table 2. INCORPORATION OF RADIOACTIVITY INTO TCA-INSOLUBLE MATERIAL IN NORMAL AND DARK-REARED RATS. NUMBER OF DETERMINATIONS IN PARENTHESES.

Region	RSA		P
	Normals (6)	Dark-reared (6)	
Visual cortex	53.3 ± 3.7	57.2 ± 2.5	n.s.
Motor cortex	54.4 ± 1.7	61.7 ± 8.5	0.1 < P < 0.2
Liver	64.8 ± 2.8	67.8 ± 3.7	n.s.

Table 4. INCORPORATION INTO VARIOUS TCA-INSOLUBLE FRACTIONS FROM VISUAL CORTEX AFTER 3 h EXPOSURE TO LIGHT. NUMBER OF DETERMINATIONS IN PARENTHESES. METHOD AS DESCRIBED IN THE TEXT

RSA of	RSA		P
	Light (6)	Dark controls (8)	
Protein	40.3 ± 3.7	33.7 ± 2.9	< 0.01
Lipid	5.5 ± 2.4	7.4 ± 3.6	n.s.
Nucleic acid	5.4 ± 1.7	0.3 ± 0.3	< 0.01

shows that after a 3 h exposure to light, increases in label associated with the protein and nucleic acid were significant ( $P < 0.01$ ); changes in the labelling of the lipid fraction were not significant. About 5 per cent of the total protein, as determined using the Lowry method<sup>13</sup>, can also be extracted into the nucleic acid fraction under these conditions, and probably consists largely of nascent protein still bound to ribosomal material; amino-acyl sRNA would also be solubilized by the use of hot TCA. Either of these two constituents could account for the labelling found in the nucleic acid fraction.

What do the observed changes actually measure? The mean half-life of at least 90 per cent of cerebral protein is 10–20 days<sup>18,19</sup>, but increased incorporation over a period of 3 h need not imply net synthesis of protein. Also, perhaps, an increase in the rate of turnover of some small rapidly labelled protein fraction might be involved. While these alternatives cannot yet be distinguished, the observations none the less throw some light on the fact that although changes in the rates of synthesis of protein and RNA have previously been reported after varying types of stimulation<sup>1–8</sup>, there has been much confusion about both the specificity and the direction of the effect. The demonstration here of a biphasic response, with a stimulation in the visual cortex, followed by an apparently non-specific depression, suggests a way of resolving some of these differences. That relatively conventional biochemical techniques can be used makes some of the quantification of results simpler than that produced by autoradiography<sup>4,6</sup>. The interpretation of the phenomenon, however, is still open to doubt. It is tempting to regard the biphasic response as the sum of two different processes, one a stimulation, one a depression. One possibility might relate this to a difference in response as between neurones and glia; a second might correlate it with the inverse synaptic changes found as between the synapses of the deeper and more superficial cortical layers reported in the accompanying article<sup>12</sup>.

Other related questions that remain unresolved include the minimum period of dark exposure necessary to produce the effects, whether the effects observed are confined to the young animals used in these experiments, or can be extended also to adults, and whether such effects can only be obtained in the extreme contrast of the light/dark

situation chosen. While the stimulation conditions used in these experiments are not strictly analogous to the learning situation, there are now many reports (see, for example, refs. 1, 8, 15–17) which argue that changed rates of synthesis of macromolecules are directly associated with learning, and it would be interesting to see whether the effects described above could be obtained also in response to such, presumably less extreme, stimuli.

It is a pleasure to record the help and co-operation of Dr B. G. Cragg, of the Anatomy Department, University College, during the course of these experiments. I thank Mr A. K. Sinha for skilled technical assistance.

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## Sensitivity of Aerobic Micrococcaceae to DAQ

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The presence of small amounts of certain adhesive tapes in an incubator can give rise to marked inhibition of bacterial growth and induce the formation of small colony variants, especially of Micrococcaceae. DAQ, which is volatile, is the active agent in the tapes. Staphylococci and micrococci vary greatly in their sensitivity to DAQ—a property that may be useful as a marker for certain strains. Sensitivity to DAQ in Micrococcaceae could not be related to their quinone patterns.

SUBCULTURES of *Staphylococcus aureus*, NCTC 4163, occasionally produce variant colonies on nutrient agar. This unexpected finding was made in our early investigations when we observed colonies which were minute and translucent, with cells larger than normal and predominantly Gram-negative. The size of the variant colonies was

not increased when the concentration of carbon dioxide was raised and the minute colonies were produced only at 35°–38° C. Another culture of this strain behaved similarly. The occurrence of variants was later shown to be due to the occasional presence of adhesive masking tape on microbiological assay dishes in the incubator. Cellulose

adhesive tape was shown by Houghton and May<sup>1</sup> to contain a volatile antibacterial component, which they afterwards<sup>2</sup> identified as 2,5-di-*tert*-amyl-*p*-benzoquinone (DAQ), formed by the oxidation of the 2,5-di-*tert*-amylhydroquinone, used as an anti-oxidant in these tapes. We examined our phenomenon to see whether the same inhibitor was involved and to investigate the relative sensitivity of various bacteria.

Strains of *Staph. aureus* were found to vary markedly in their sensitivity to masking tape. Three strains, one very sensitive (NCTC 4163), one resistant and one of intermediate sensitivity, showed similar differences in sensitivity to cellulose adhesive tape, to other types of self-adhesive roll tape and also to pure DAQ (prepared by oxidation of 2,5-di-*tert*-amylhydroquinone, Santovar 4). Zinc oxide plaster strip and two brands of paper mounted adhesive label were without effect. Nutrient agar or blood agar plates, incubated at 37° C in the presence of adhesive tape, absorbed the volatile inhibitor and retained it for at least a few days during storage in the refrigerator; such plates induced variant colonies of *Staph. aureus* 4163 during subsequent incubation at 37° C in the absence of tape.

**Determination of DAQ sensitivity.** Because DAQ is volatile at 37° C the serial-dilution broth technique was found to be unsatisfactory; furthermore, the results were markedly affected by the size of the inoculum and differences in sensitivity were difficult to distinguish. The incorporation of DAQ in nutrient agar was considered but, because of the necessity to dry the agar plates before use—making it probable that DAQ would be lost by volatilization—this method was not used. We introduced the DAQ on impregnated filter paper, placed either on the dried surface of the medium or inside the lids, and then sealed the plates with 'Plasticine'. For screening the organisms were grown overnight at 37° C in liquid media and one loopful was streaked across the surface of each of three nutrient agar or blood agar plates. A strip of filter paper, 8 × 1 cm, was placed on the agar surface of each plate; two of the strips contained DAQ, 10 and 100 µg respectively, and the third was not impregnated. The plates were then sealed and incubated at 37° C overnight. Cultures of known sensitivity were included in each batch of tests. Strips impregnated with DAQ did not produce zones of inhibition; colonies over the whole plate were affected because of the distribution of volatile DAQ over the agar.

The relative sensitivity to DAQ of three strains of *Staph. aureus*, shown to differ in sensitivity by the strip method, was also determined quantitatively. Nutrient agar plates were inoculated with dilutions of overnight broth cultures by the method of Miles, Misra and Irwin<sup>3</sup>, and a circle of filter paper containing 6,000, 600 or 60 µg of DAQ was placed in each lid. The plates were sealed and incubated at 37° C overnight and the results assessed by viable counts. Lower concentrations of DAQ were tested on two of the strains of staphylococci.

**DAQ sensitivity of bacteria.** (i) *Filter paper strip method.* (Fifty-one strains.) The *Staph. aureus* strains were isolated from lesions (nineteen from humans and six from animals). The coagulase-negative staphylococci and micrococci were classified according to the scheme of Baird-Parker<sup>4</sup>.

Three sensitivity patterns could clearly be discerned by comparing the number and appearance of colonies developing on plates containing DAQ with the confluent growths on control plates (Table 1). Heavy inocula revealed a small proportion of DAQ-resistant cells in sensitive cultures after a single exposure to DAQ, these resistant cells occasionally producing a few normal sized colonies on the plates, which were discounted in the assessment.

(ii) *Quantitative determination of DAQ sensitivity of three Staph. aureus strains by viable counts.* All three concentrations of DAQ gave similar results; those obtained

with 60 µg DAQ/plate are summarized in Table 2. The colony count of W.O. 134, the resistant strain, was unaffected: the counts of the Oxford staphylococcus and strain 4163 were reduced by 180-fold and more than 20,000-fold, respectively.

Table 1. DAQ SENSITIVITY OF MICROCOCCACEAE

Group	Subgroup	DAQ sensitivity						Total strains
		No. of strains	+	No. of strains	+	No. of strains	-	
Staphylococcus 1	I ( <i>Staph. aureus</i> , coagulase + ve)	2	8	17	68	6	24	25
Staphylococcus 1	II, III, IV, VI (coagulase - ve)	0	0	5	38	8	62	13
Micrococcus 2	1-8	1	8	10	77	2	15	13
DAQ, 10 µg/plate								
DAQ, 100 µg/plate								
+ + Colonies minute and distinct.								
+ Colonies normal in appearance and reduced in numbers.								
- Growth confluent, as on control plate.								

Table 2. RELATIVE SENSITIVITY TO DAQ OF THREE *Staph. aureus* STRAINS

Strain	Lowest dilution of culture giving a countable number of colonies on DAQ plate	Number of colonies/0.02 ml. of dilution of culture	
		DAQ, 60 µg/plate	DAQ 0
NCTC 4163	10 <sup>-2</sup>	1	20,000*
<i>Staph. aureus</i> (Oxford H)	10 <sup>-3</sup>	20	3,600*
WO 134	10 <sup>-4</sup>	4	4

\* Calculated from 0.02 ml. of 10<sup>-5</sup> dilution.

With minimal concentrations of DAQ (0.06 µg/plate for strain 4163 and 0.3-0.6 µg/plate for the Oxford H strain) colonies of sensitive *Staph. aureus* were translucent and markedly reduced in size but not in numbers. With strain 4163 a high concentration (60 µg/plate) was lethal in 24 h at 37° C, but 6 µg/plate was merely bacteriostatic.

The sensitivity of thirty-three organisms in ten other genera was also studied by the filter paper strip method. None of these organisms was markedly sensitive to DAQ, but two (*Bacillus cereus* and *Corynebacterium diphtheriae gravis*) showed intermediate sensitivity (+). *Shigella sonnei*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Lactobacillus arabinosus*, *Corynebacterium pyogenes*, *Bacillus pumilus* and species within the genera *Salmonella*, *Proteus*, *Klebsiella* and *Streptococcus* were resistant.

The morphology of cells of very sensitive *Staph. aureus* strains is markedly altered in the presence of DAQ, and the colonies developing range from normal opaque and pigmented to minute translucent types. Although these are unrecognizable as staphylococci, all variants are catalase-positive and revert to normal colonial and microscopic morphology if they are re-incubated or subcultured in the absence of tape. The strains of *Staph. aureus* tested varied in bacteriophage type, antibiotic sensitivity and origin; none of these properties correlated with DAQ sensitivity. Although there was considerable variation in the degree of sensitivity to DAQ within the species *Staph. aureus*, and also within the *Micrococcus* group, most strains of *Staph. aureus* and *Micrococcus* examined were more sensitive to DAQ than were the coagulase-negative staphylococci. Organisms of extreme sensitivity to DAQ were found only in the Micrococcaceae and this property may be useful as a marker. Houghton and May<sup>1</sup> found that the colony count of the majority of their strains of *Staph. aureus* was reduced by more than 90 per cent by cellulose tape in a plate test and most coagulase-negative staphylococci were relatively resistant. Later they<sup>2</sup> used agar containing a range of concentrations of DAQ and observed the lowest inhibitory concentration that would reduce the colony count more than one hundred times. Under our conditions, in which DAQ was introduced into the air space above the agar, we observed the reduction in the colony count at a single level of DAQ (60 µg/plate). As observed by May and Houghton<sup>2</sup>, a small number of

resistant cells was revealed by a single exposure to DAQ of a heavy inoculum of a sensitive *Staph. aureus* culture, but attempts to isolate such variants from unexposed populations of *Staph. aureus* were unsuccessful. Such resistant colonies were also resistant to Santovar A but, contrary to the findings of Henriksen and Gilje<sup>6</sup>, they retained their resistance on subculture.

**Use of adhesive tapes containing DAQ in bacteriology.** Houghton and May<sup>1</sup> drew attention to the danger of using cellulose tapes containing DAQ for sealing Petri dish cultures. Our work reinforces this and emphasizes the extreme sensitivity of certain staphylococci to DAQ. We found that as little as 7 in. of tape 1.25 in. wide, with its coated surface applied to a 'Perspex' sheet in a 6.6 ft.<sup>3</sup> incubator, had a significant effect on the growth of NCTC 4163.

Thomas<sup>7</sup> used cellulose roll tapes and tacky labels on mounts for sampling the skin flora. We deposited broth cultures of *Staph. aureus*, 4163, on polystyrene and on glass surfaces, dried them and then sampled with a brand of cellulose adhesive tape (shown to be inhibitory to this strain), and also with 'Tickotab' (J. Goshon and Co., Ltd., London, W.8) labels (shown to be non-inhibitory). The tapes or labels were then used for the inoculation of agar plates. From the glass surface, more staphylococci were isolated with cellulose tape than with 'Tickotabs', whether tested immediately after taking the impressions, or after 2.5 h storage at room temperature (25° C). Conversely 'Tickotabs' were more effective than cellulose tape for the recovery of staphylococci from plastic surfaces. The degree of adhesiveness is clearly of considerable importance in this technique and the presence of DAQ in a film used for this purpose is unlikely to affect the results, because staphylococci recovered from impressions on tape containing DAQ in these experiments were found to be fully sensitive to DAQ. Furthermore DAQ in cellulose tape, although highly volatile at 35°–37° C, was not

sufficiently so at 19°–30° C to inhibit even our most sensitive strain of *Staph. aureus*. The use of such tapes would not therefore seem to present a hazard during transport of films, except perhaps under tropical conditions.

**Sensitivity to DAQ and vitamin K synthesis in bacteria.** May and Houghton<sup>5</sup> suggested that, because DAQ inhibited only bacteria containing vitamin K as the sole quinone, it antagonized the function of vitamin K. We found, however, that a menaphthone-requiring strain of *Fusiformis melaninogenicus*, when tested in a modification of the system described by Gibbons and Engle<sup>8</sup>, produced a similar response to menaphthone sodium bisulphite, both in the absence of DAQ and in the presence of a concentration of DAQ approximately ten times that producing a bactericidal effect on the highly sensitive *Staph. aureus*, 4163. Conversely, the activity of DAQ against strain 4163 was not antagonized by non-toxic equimolar concentrations of menaphthone sodium bisulphite, menaphthone or vitamin K<sub>1</sub>. These results do not support the view of May and Houghton<sup>5</sup>, and further evidence that sensitivity of bacteria to DAQ is unrelated to their quinone patterns is given in the article that follows this.

For supplying most of the cultures studied, together with information in considerable detail, we are most grateful to Drs A. C. Baird-Parker, M. T. Parker and S. P. Lapage. We also thank Dr Mair Thomas for helpful discussions during the early stages of the work.

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## Distribution of Menaquinones in Aerobic Micrococcaceae

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The previous article showed that sensitivity of Micrococcaceae to DAQ is probably not related to their quinone patterns. This article shows that several menaquinone patterns, possibly of taxonomic significance, may be found in Micrococcaceae. Distinct menaquinone patterns characterize certain species, notably *Staphylococcus aureus*.

WIDE variations have been found<sup>1</sup> in the sensitivity of certain Micrococcaceae to 2,5-di-*tert*-amyl-*p*-benzoquinone (DAQ). May and Houghton<sup>2</sup> suggested that DAQ might inhibit bacterial growth by interfering with the function of vitamin K, and we have therefore studied the distribution of the K<sub>2</sub> vitamins (menaquinones) in staphylococci and micrococci.

Menaquinones (MK) are commonly found in non-photosynthetic bacteria and are believed to be associated with oxidative phosphorylation<sup>3–6</sup>. Typical menaquinones have unsaturated side-chains containing from six to nine isoprene units (Fig. 1, *n* = 6–9) and their distribution has

been studied by many workers<sup>7–13</sup>. *Myco. phlei* has, however, been shown to contain an unusual menaquinone, an MK-9(H), containing one hydrogenated isoprene unit in its side-chain<sup>14–15</sup>. Scholes and King<sup>16</sup> have identified an MK-8(H) in *Corynebacterium diphtheriae*. Further

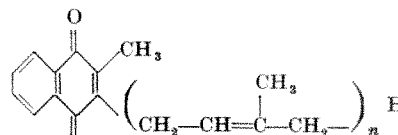


Fig. 1.



Table 1. DISTRIBUTION OF MENAQUINONES IN MICROCOCCACEAE

Classification (Baird-Parker, 1965)	Sub-group	No. of strains	Total ( $\mu\text{g/g}$ wet wt)	Menaquinones* Percentage of individual isoprenologues			
				MK-9	MK-8	MK-7	MK-6
<i>Staphylococcus aureus</i> (1)	I	15 (NCTC 4163, WO 133, C 1309, C 1341, C 2936, C 3512, C 4532, C 4601, C 5422, C 5437, C 5490, C 6828, C 6830, C 8973, Oxford (Heatley strain))	268 (127-433)	16 (10-29)	71 (66-78)	13 (5-21)	0
<i>Staphylococcus</i> (1) (coagulase negative)	II	3 (B-P 12†, B-P 14§, B-P 317‡)	72 (45-96)	0	53 (41-65)	47 (35-59)	0
	III	1 (B-P 34‡)	75	0	5	95	0
	IV	4 (NCTC 4276, NCTC 7291, B-P 19†, B-P 35‡)	141 (61-160)	<1 (0-2)	17 (9-21)	83 (78-91)	0
	VI	5 (NCTC 5955, NCTC 7944, WO 143, B-P 21†, B-P 32‡)	50 (28-80)	<1 (0-4)	14‡ (4-25)	86 (75-92)	0
<i>Micrococcus</i> (2)	2	2 (B-P 5†, B-P 16§)	9 (4-14)	0	0	100	0
	3	3 (NCTC 7292, B-P 3†, B-P 10)	37 (10-75)	0	5 (3-12)	94 (88-100)	1 (0-3)
	5	4 (NCTC 189†, B-P 45§, B-P 47§, B-P 114‡)	40 (7-102)	0	0	95 (82-100)	5 (0-18)
	6	2 (B-P 8†, B-P 24‡)	79 (7-151)	0	15 (0-29)	35 (0-71)	50 (0-100)
	7	2 (B-P 25, B-P 26)	123 (44-203)	0	0	0	100
<i>Micrococcus</i> (2)	7	Yellow-pigmented strains 6 (CCM 265†, CCM 266 (ii)†, CCM 266 (ii)†, CCM 851†, CCM 852†, CCM 853†, ATCC 9341)	61 (45-102)	MK-9 (H) 0	MK-8 (H) 75 (54-87)	MK-7 (H) 25 (13-46)	MK-6 (H)¶ <1 (0-trace)
	8	1 (B-P 97)	20	90	10	0	0

\* All values are means for the number of strains shown; the ranges are in parentheses. A further ten strains of *Staph. aureus* were examined after alkaline digestion; the MK patterns were similar to those given in the table. A further strain belonging to *Micrococcus* subgroup 5 (B-P 46) was found to yield only traces of MK-6 and MK-7, even after alkaline digestion. It was therefore not possible to determine the relative proportions of the isoprenologues in this strain.

MK figures were obtained by solvent extraction alone, except where indicated as follows:

† The bacterial mass resisted solvent extraction (values obtained by alkaline digestion).

‡ After extraction the residue was digested with alkaline and yielded either a trace of MK or none.

§ After extraction the residue was digested with alkali and yielded an appreciable amount of MK.

¶ MK-n (H) indicates a tentative identification of these compounds as MKs with partially hydrogenated side-chains (see text).

variants devoid of the 2-methyl group have been described<sup>17-18</sup>. Detailed information about the distribution of MKs in the Micrococcaceae is, however, lacking.

**Micro-organisms and cultivation.** The Micrococcaceae were classified according to the scheme of Baird-Parker<sup>19</sup>. The strains examined included the majority of those tested in the preceding article and were representative of the three degrees of sensitivity to DAQ described therein<sup>1</sup>. Twenty-eight strains of *Staphylococcus* (group 1, subgroups I-IV and VI) and twenty strains of *Micrococcus* (group 2, subgroups 2, 3 and 5-8) were stored on 'Lemco' agar slopes (Blood Agar Base, Oxoid, Ltd.) at 4° C. 'Lemco' agar plates were inoculated with peptone-water suspensions of organisms and incubated aerobically for 48 h (staphylococci at 37° C and micrococci at 30° C). The growth was collected from the surface of the agar in subdued light and placed in ethanol to remove water (about 1 g wet bacterial mass/10 ml. ethanol).

**Analytical methods.** The solid matter was allowed to settle and the supernatant liquor was decanted. Ethanol (15 ml.) containing pyrogallol (50 mg) was added to the solid and the mixture was boiled under reflux for 30 min. Water (25 ml.) was added and the combined ethanolic extracts were extracted three times with light petroleum (boiling point 40°-60° C, 25 ml.). The petroleum extract was evaporated to dryness *in vacuo*. Those bacteria found to be resistant to extraction were subjected to alkaline digestion. The bacterial mass (1 g) was boiled under reflux with a mixture of ethanol (4 ml.), pyrogallol (200 mg), and saturated aqueous solution of potassium hydroxide (2 ml.) for 30 min. After the addition of water (10 ml.) the unsaponifiable lipids were extracted with diethyl ether (3 × 25 ml.). The ether extract was washed to neutrality with water and evaporated *in vacuo*. The extracted lipids, in light petroleum solution, were chromatographed on thin layers of silica gel by the method of Horth *et al.*<sup>6</sup> to separate the crude MK fraction. This was then separated into individual MK isoprenologues by paper partition chromatography<sup>6</sup>, using 95 per cent (v/v) aqueous ethanol as developing solvent. The MK isoprenologues were identified by co-chromatography with synthetic MK markers. The individual MK bands were separately eluted with ethanol from other chromatograms run in parallel and determined spectrophotometrically by the difference in absorption at 270 nm and 285 nm (ref. 12).

$\Delta E_{1\text{cm}}^{1\%}$  values for the difference in absorption at these two wavelengths were obtained for pure specimens of MK-6, MK-7, MK-8 and MK-9 (278, 244, 238 and 222 respectively). All analytical operations, where practicable, were carried out in subdued light.

**Distribution of menaquinones in the Micrococcaceae.** All the menaquinones found in staphylococci and micrococci other than the pigmented strains in subgroups 7 and 8 were identified as MK-6, MK-7, MK-8 or MK-9, although all four isoprenologues were not present in all strains (see later). The MK patterns are shown in Table 1. The menaquinones isolated from the pigmented micrococci of subgroups 7 and 8, however, differed chromatographically from the known MKs, having  $R_F$  values of 0.275, 0.182, 0.124 and 0.086, compared with values of 0.318, 0.213, 0.143 and 0.100 found for MK-6, MK-7, MK-8 and MK-9 respectively. The ultra-violet spectra of the two series of compounds were, however, identical. The menaquinones from these micrococci were thus different from the des-methyl compounds of Baum and Dolin<sup>17</sup> and, from a calculation of their  $R_m$  values<sup>20</sup>, were tentatively identified as partially hydrogenated MKs, similar to those found in *Myc. phlei* by Gale *et al.*<sup>14</sup>. In support of this conclusion, the MKs from these micrococci could be separated from the authentic MK series by chromatography on thin layers of silica gel impregnated with silver nitrate. Ubiquinones were not found in any organism examined.

**Evaluation of analytical results.** We were unable to test directly the efficiency of extraction of the MKs from the bacteria. Of 52  $\mu\text{g}$  of MK-6 added to the ethanol used for the extraction of a 1-g sample of *Staph. aureus* (Oxford H strain)\*, however, 83 per cent was recovered. From *Staph. aureus* (Oxford H strain) only 68 per cent of the MK obtained by extraction alone could be recovered after alkaline hydrolysis. The quantitative aspects of our results are therefore to be treated with some caution, because the alkaline digestion method had to be used for a number of the organisms. On the other hand, we feel that the isoprenologue ratio found may be accepted with some confidence, because it is not likely that alkaline

\* The various strains used are identified as follows: NCTC, National Collection of Type Cultures, London; WO, Walton Oaks collection of cultures; ATCC, American Type Culture Collection, Rockville, Maryland; CCM, Czechoslovak Collection of Micro-organisms J.E. Purkyně University, Brno, Czechoslovakia; B-P, strains received from Dr A. C. Baird-Parker; C, strains received from Dr M. T. Parker, Colindale.

digestion preferentially destroys individual isoprenologues. We have found that the total MK content of a given organism sometimes varies substantially between cultures grown on different occasions. The reasons for this are still obscure. Because vitamin K forms exhibition zones in a plate test with *Fusiformis melaninogenicus*<sup>21</sup>, it is evident that at least some of the MK synthesized by Micrococcaceae grown on an agar surface is lost from the cells by diffusion into the agar. The proportion so lost may vary from one experiment to another and perhaps with different batches of medium.

*MK patterns and their correlation with systems proposed for classifying staphylococci and micrococci.* Menaquinones were detected in all the Micrococcaceae examined, but in quantities ranging from a few  $\mu\text{g}$  to more than 400  $\mu\text{g/g}$  wet weight; the highest figures were among the *Staph. aureus* strains. In strains within group I (*Staphylococcus*) of Baird-Parker's<sup>19</sup> system of classification three MK patterns were revealed. Subgroup I (*Staph. aureus*) strains were distinct and contained MK-8 as the main component, comprising about 70 per cent of the total MKs; MK-9 and MK-7 were also present in all strains. In the coagulase-negative staphylococci, comprising the remaining subgroups (except subgroup V strains, which were not available for testing), the MK pattern differed from that of subgroup I. In strains within subgroups III, IV and VI, MK-7 rather than MK-8 was the main component; subgroup II strains, however, contained similar amounts of MK-7 and MK-8.

The MK patterns of coagulase-negative variants of *Staph. aureus* would be of considerable interest, because they may form a useful addition to the list of distinguishing tests proposed by Baird-Parker<sup>19</sup>.

The second group of organisms studied, the micrococci, could be divided into two broad subgroups according to the type of MK produced. The members of subgroups 2, 3, 5 and 6, together with the two non-pigmented organisms examined in subgroup 7 (B-P 25 and B-P 26), produced normal MKs. By contrast, the yellow-pigmented strains of subgroup 7 (ATCC 9341 and the CCM strains) and the red-pigmented strain in subgroup 8 produced only MKs tentatively identified as partially hydrogenated, designated MK-n(H). The major component was MK-8(H) in all members of the yellow pigmented groups, and MK-9(H) in B-P 97. The yellow-pigmented strains were placed in group 3 by Baird-Parker<sup>22</sup>, but were later included in *Micrococcus* group 2, subgroup 7 (Baird-Parker<sup>19</sup>). MK-7 was the major component in most of the other micrococci tested, and MK-8, which was present in all the staphylococci tested, was absent from the majority of micrococci. MK-6, absent from all the staphylococci, was present in a few of the micrococci and was the only isoprenologue detected in one of the two subgroup 6 strains and in both the non-pigmented organisms of subgroup 7.

It is interesting to examine our results on the micrococci in the light of the classification proposed by Rosypal, Rosypalová and Hořejš<sup>23</sup>, who distinguished three main groups on the basis of their DNA base composition. All five of the yellow-pigmented CCM strains in Table 1 were examined by Rosypal *et al.*<sup>23</sup>, who placed them in their group 1 (guanine and cytosine (GC) content in DNA 70.8–73.3 per cent), subgroup 1a, which they regard as corresponding with subgroup 7 of Baird-Parker<sup>19</sup>, containing a variety of morphological types. Although yellow pigmentation is regarded by Rosypal *et al.*<sup>23</sup> as characteristic of strains of this subgroup, they point out that rare, non-pigmented variants do occur and indeed we found that one of their strains (CCM 266) produced not only stable yellow colonies (CCM 226) (i) but also unstable cream colonies (CCM 226) (ii), the latter giving rise to colonies with either pigment; both these variants of CCM 226 produced identical patterns. The GC base composition of the non-pigmented strains, B-P 25 and B-P 26, is not known and hence their position in the

classification of Rosypal *et al.*<sup>23</sup> is not clear, but Baird-Parker<sup>19</sup> asserted that some 18 per cent of the subgroup 7 strains he studied were non-pigmented. The organisms studied by Rosypal *et al.*<sup>23</sup> consist mainly of yellow- and pink-pigmented strains, however. Strains of subgroup 1b of Rosypal *et al.*<sup>23</sup> (of similar GC content to subgroup 1a) are differentiated by the production of pink pigment and other characters; they correspond to *Micrococcus* subgroup 8 of Baird-Parker<sup>19</sup> (*M. roseus*, Evans<sup>24</sup>). The only representative of this subgroup tested was strain B-P 97, which had a different MK pattern from the yellow strains of subgroup 1a.

The significance of MK patterns in taxonomic studies on the Micrococcaceae remains to be determined by the examination of many more strains. Our results, however, show the existence of several distinct MK patterns, one of which is characteristic of the pathogen *Staph. aureus*. The relationship between the genera *Micrococcus* and *Sarcina* is currently of interest and it has been emphasized that the genus *Sarcina* should include only the anaerobic species with fermentative metabolism<sup>25</sup>. The organism formerly referred to as *Sarcina lutea* has now been included in the genus *Micrococcus* as *M. luteus*<sup>26</sup>. These yellow-pigmented organisms can be clearly separated, on MK pattern, from the non-pigmented strains in *Micrococcus* subgroup 7, with which they were placed by Baird-Parker<sup>19</sup>.

These investigations on MKs were originally undertaken as an extension of our studies on the sensitivity of micro-organisms to DAQ. May and Houghton<sup>2</sup> suggested that in bacteria that do not synthesize ubiquinone only those producing vitamin K are sensitive to DAQ. The development of resistance to DAQ would therefore be expected to result in loss of the ability to synthesize vitamin K. A strain of *Staph. aureus* (4163), highly sensitive to DAQ, and a stable DAQ-resistant variant derived from it, produced similar amounts of total and individual MKs. Furthermore, in the bacteria listed in Table 1, there was no correlation between sensitivity to DAQ and the amount of MK synthesized by the strains.

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# LETTERS TO THE EDITOR

## PLANETARY SCIENCE

### Solar Radiation at 19.0 Gc/s during the Eclipse of May 20, 1966

SINCE the early days of radio-astronomy, several solar eclipses have been studied in detail at various radio frequencies, the results being summarized in a review by Castelli and Aarons<sup>1</sup>.

As a continuation of this work, observations of solar radiation at a frequency of 19.0 Gc/s (1.58 cm wavelength) were carried out during the annular eclipse of the Sun on May 20, 1966, at a site at Lagonissi (lat. 37° 47' N., long. 23° 53' E.) near Athens, Greece, at a distance of 3 km from the centre line of the eclipse, using an aerial 0.92 m in diameter and a conventional equatorially mounted radiometer<sup>2</sup>.

The predicted times of optical first contact, maximum phase, and fourth contact for the site were 08 h 04.5 m, 09 h 31.3 m, and 11 h 04.8 m U.T. respectively, and the predicted magnitude of the eclipse was 99.99 per cent, with a 1 sec duration for the maximum phase.

Throughout the day of the eclipse the sky in the vicinity of the Sun was cloudless, the zenith atmospheric attenuation at 19 Gc/s being  $0.23 \text{ dB} \pm 0.05$ .

The measured value for the effective disk temperature  $T_e$  of the Sun, corrected for the effects of atmospheric attenuation, beam shape, feed losses and mismatch, and allowing for maximum possible errors in these, was  $T_e = 15,300^\circ \text{K} \pm 5,000^\circ \text{K}$ , corresponding to a total flux (both polarizations) of  $1,200 \pm 400$  solar flux units. This is about 25–50 per cent greater than previous measurements at other frequencies would indicate for the quiet Sun at 19 Gc/s<sup>3</sup>, but the large possible error, resulting mainly from uncertainty in the aerial gain, must be borne in mind.

Fig. 1 shows the path of the centre of the Moon across the solar disk, together with the main optical features as given on the Fraunhofer Institute solar map for the day of the eclipse.

Fig. 2 shows the radiometer output, corrected for a 1 per cent drift, assumed linear, in the zero level over the

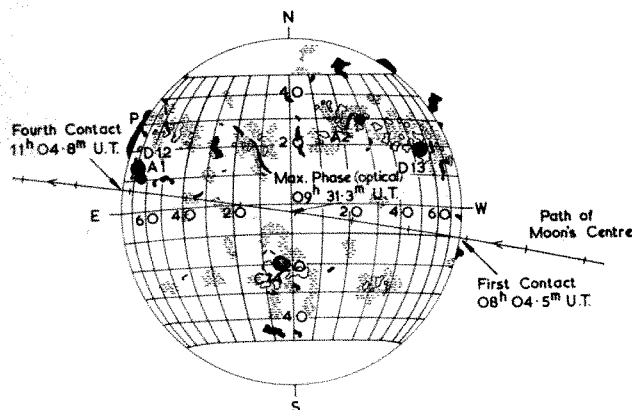


Fig. 1. Map of the Sun for May 20, 1966, showing the path of the centre of the Moon across the solar disk, with the position of the centre marked at 15 min intervals of time, together with the main sunspot regions (D12/A1, C14, A2, D13), filaments, plages and prominences as given on the Fraunhofer Institute solar map.

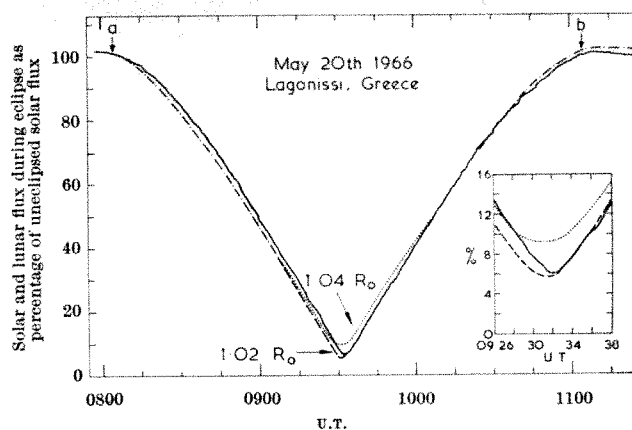


Fig. 2. The observed eclipse curve, together with curves computed for uniform disks of radii  $1.02 R_\odot$  and  $1.04 R_\odot$ , where  $R_\odot$  is the radius of the optical Sun. The times of beginning (a) and end (b) of the optical eclipse are marked. The inset shows the centre of the eclipse on record expanded scales.

duration of the eclipse, together with curves computed for a uniform distribution of radiation across disks of radii  $1.02 R_\odot$  and  $1.04 R_\odot$ , where  $R_\odot$  is the optical radius of the Sun. Although four sunspot regions were present (Fig. 1), and although sudden changes appeared on records at lower frequencies<sup>4,5</sup> (Anastassiades, unpublished report, Univ. Athens, 1966), it can be seen that the eclipse curve at 19 Gc/s nowhere differs by more than about 2 per cent from the  $1.02 R_\odot$  curve, and that there are no pronounced sudden changes in slope significantly greater than those of the general background noise on the curve, such as might be expected if the Moon occulted localized sources of high intensity within the solar disk. There is, however, a delay of 0.75–1 min in the observed time of minimum radio flux, which, as the eclipse was almost total, suggests the existence of a source of enhanced radiation extending to at least 11,000 km beyond the east limb. That this shift is genuine, and not due to random noise, is shown by the fact that during the first half of the eclipse the observed curve lies consistently above the calculated ones, whereas for the second half they are nearly coincident. It also appears more prominently on records made at lower frequencies<sup>4,5</sup>. Moreover, by replotting the eclipse curve against the angular separation of the solar and lunar centres and superimposing the two halves of the curve, there is seen to be a near-constant difference of 2.5 per cent between them, and if this is assumed to be due mainly to one localized source, its intensity is about  $1.3 \pm 0.2$  per cent of the total solar flux. It must be remembered, however, that this particular source was detected because of its effect on the time of minimum received radio flux, resulting from its position on the limb, and that if it had existed within the optical disk, as did the other sources detected at the lower frequencies, it would have produced a less noticeable effect on the record, and might even have remained undetected.

The radio flux minimum occurred at the time when the optical prominence P (Fig. 1) had just been covered by the Moon's limb, at the start of the second half of the eclipse, and it is therefore likely that there is a connexion between the two phenomena. Corresponding to the effect produced by the covering of this source, there should be another distortion of the record immediately prior to the optical fourth contact at the end of the eclipse. Assuming that the radio source has roughly the same shape as the prominence P, however, Fig. 3 shows that, due to the difference in geometry between the leading occulting edge of the Moon and the trailing uncovering edge, the source P would be covered in a relatively short period of time, producing a sudden change on the record

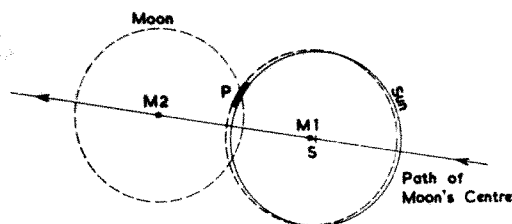


Fig. 3. Illustration of the difference between the geometries of the covering and uncovering of the east-limb prominence  $P$ .  $M1$ ,  $M2$ , position of lunar centre at times of covering, and uncovering, respectively;  $S$ , solar centre.

(the shift in time of the maximum phase), but would be uncovered much more gradually, producing a much smaller, but more prolonged, distortion in the record. Because the uncovering occurred right at the end of the eclipse its effect on the records is therefore difficult to pick out from other effects due to the Sun having drifted slightly from the centre of the beam, and to the Moon moving out towards the edge of the beam.

The total residual at the maximum phase of the eclipse was 6.2 per cent, of which about one-third was contributed by the Moon<sup>6</sup>, leaving a 4.2 per cent residual originating in the uneclipsed portion, compared with the 7.3 per cent observed by Castelli and Straka at 8.8 Gc/s<sup>4</sup>. For a uniform distribution of noise across the disk this would correspond to a radio radius of the Sun of  $1.020 R_0$ , or a maximum effective emission height of 14,000 km above the photosphere. Bearing in mind, however, that 1.3 per cent of the total radiation probably came from one localized disturbed region on the limb, these figures become 2.9 per cent,  $1.014 R_0$  and 10,000 km, respectively.

The time of first contact for the radio Sun was difficult to measure from the record with any degree of accuracy, but allowing for the fact that even with the idealized curves the initial rate of decrease in signal is very small, it appears to be between 0.5 and 1.5 min before first optical contact, corresponding to a radio radius between 1.005 and  $1.016 R_0$  (3,500–11,000 km above the photosphere). The corresponding difference for the fourth contact was masked by the effects described earlier.

This work was carried out as part of the programme of the Science Research Council's Radio and Space Research Station. We thank Professor M. Anastassiades and the staff of the Ionospheric Institute of Athens for their organization of the general eclipse programme. We also thank the manager of the Lagonissi Hotel, Athens, for permission to operate the equipment in the hotel.

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## Changes of the Earth's Magnetic Field and Radiocarbon Dating

THE radiocarbon method of dating depends on several assumptions which could not have been easily verified around 1950. Two of them seem to be of considerable importance, namely, the constant rate of the mixing of deep and surface ocean waters and the constancy of the Earth's magnetic field. This communication deals with the latter assumption.

It is well known that the radioactive isotope carbon-14 originates in the upper layers of the atmosphere as a consequence of the action of cosmic rays. Their flux as well as the rate of production of carbon-14 depends on the intensity of the Earth's magnetic field. In general, a decrease in the intensity of the field causes an increase of the cosmic ray flux and also an increase in the production of carbon-14. Higher values of the field have the opposite effect. These facts have been verified experimentally.

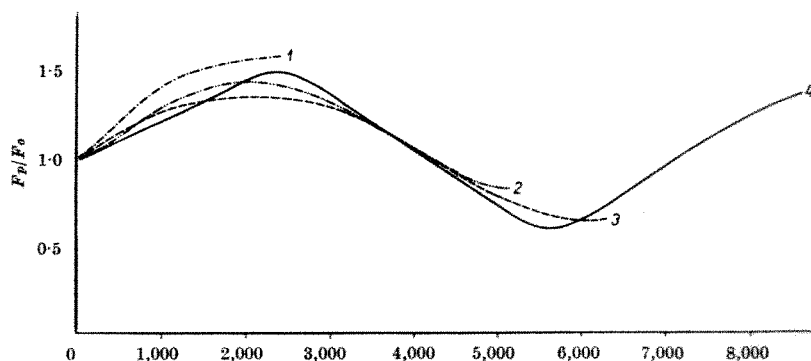


Fig. 1. Archaeomagnetic curves for France (1), the Soviet Union (2), Japan (3) and Czechoslovakia (4).

When Libby discovered the radiocarbon method of dating<sup>1,2</sup> there were no reliable methods for investigating changes in the Earth's magnetic field. It was only by means of the double heating method of E. and O. Thellier<sup>3</sup> that it became possible to investigate the intensity of the Earth's magnetic field in the remote past by measuring the remanent magnetization of burnt archaeological objects<sup>4,5</sup>. Samples from Czechoslovakia and Turkey<sup>6,7</sup>, and a number of other countries, were measured and it was found that the intensity over the past 8,500 years varied from a half to one and a half times the present value (Fig. 1). Because of this finding it was realized that the assumption concerning the intensity of the Earth's magnetic field was incorrect.

It has been shown<sup>8</sup> that the rate of production of carbon-14 can be determined from the equation

$$P(t) = \frac{C_2}{M(t)}$$

where  $C_2$  is constant and  $M(t)$  is a function representing the change of the Earth's magnetic field with time. If we substitute experimentally determined values for  $M(t)$  and solve the differential equation

$$\frac{dI}{dt} = A \exp(-\lambda_1 t) + B \frac{2.6}{\lambda_1} - C \frac{1.2 \lambda_1}{\lambda_1^2 + \left(\frac{2\pi}{8000}\right)^2} \sin \frac{2\pi}{8000} t + D \frac{1.2 \frac{2\pi}{8000}}{\lambda_1^2 + \left(\frac{2\pi}{8000}\right)^2} \cos \frac{2\pi}{8000} t$$

we obtain

$$I_t = 15.02 \lambda_1 t + 0.548 - 0.327 \sin \frac{2\pi}{8000} t - 0.106 \cos \frac{2\pi}{8000} t$$



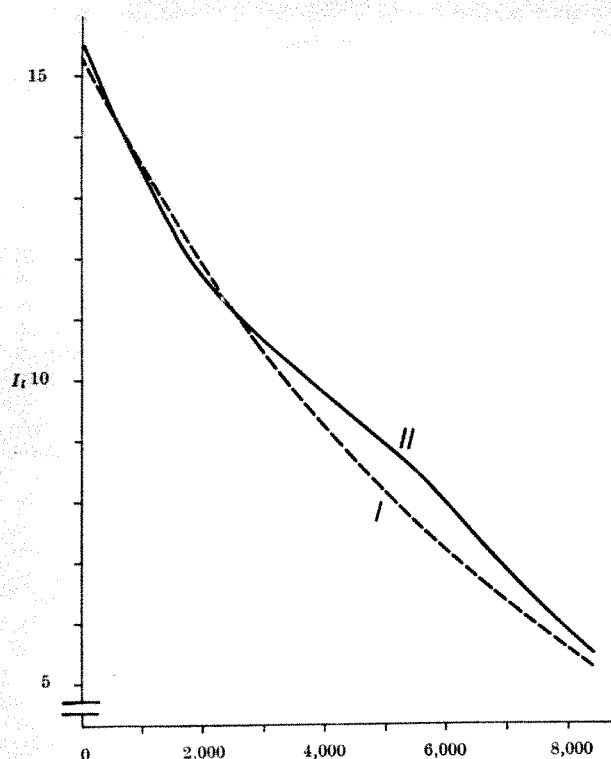


Fig. 2. Decay curves—according to W. Libby (I), with the influence of the Earth's magnetic field changes (II).

This represents the influence of the Earth's magnetic field on the temporal changes of the radioactivity of carbon-14. On the basis of this equation it is possible to investigate the course of the new decay curve given in Fig. 2 (full line) together with the known W. Libby's curve (dashed line). In Fig. 2 the horizontal distance between the full and dashed line shows the value of deviation of the radiocarbon dates from the historical ones.

Such deviations were empirically determined by measuring the radioactivity of samples the age of which could be ascertained by historical chronology and dendrochronology<sup>9</sup> (Figs. 3 and 4). The good agreement of the computed deviations with those ascertained empirically is only partly influenced by the fact that some of the constants in our equation must have been computed by substituting certain empirical values.

Table 1

	Short chronology based on archae- ological syn- chronizations (before 1950)	Uncorrected radiocarbon chronology	Radiocarbon chronology cor- rected by the factor of the magnetic field
Central-European early Neolithic (early phase Linear Pottery Culture)	2600 B.C.	4500 B.C.	Approx. 5000 B.C.
Early Chalcolithic of Central Europe (early TRB Culture)	2100 B.C.	3250 B.C.	Approx. 4000 B.C.
Middle Chalcolithic of Central Europe (classical phase Baden Culture)	2000 B.C.	2600 B.C.	Approx. 3300 B.C.

It can be seen from the shape of the curves (Fig. 2) that after zero (historical chronology) the difference between both curves represents about +30 years, around 2000 B.C. it is -400 years, around 4000 B.C. about -750 years. Then both curves approach each other again and they coincide shortly before zero and after A.D. 1000. Fig. 5 also reveals the mutual differences, the line labelled *a* (for the half-life of 5,568 years) is taken as a basis, and the line *b* represents the deviations for the half-life of 5,730 years. The curve *c* defines graphically smoothed deviations of radiocarbon dates from those based on dendrochronological investigations (compare Fig. 4), and curve *d* represents deviations (in years) between carbon-14 dates and historical dates. Finally, curve *e* includes the kind of deviations that are to be expected when the influence of the Earth's magnetic field is considered.

The agreement of curves *c* and *e* is quite evident, while curve *d* displays systematic deviations of +100 years; this may be explained by the fact that wood (usually timber) in archaeological constructions was usually about 100 years older when used for building or other purposes. All this proves the necessity of including the influence of the changes of the Earth's magnetic field in radiocarbon dating. Because the character of the field for the past 8,500 years has been investigated in relation to the ages of archaeological objects which are often open to certain errors, and because the nature of the field has been simplified, the results are only preliminary.

It is already possible, however, to apply preliminary corrections to the dates of some archaeological periods which can be reliably ascertained by the radiocarbon method (Table 1).

Several authors have already directed attention to the systematic character of the deviations of radiocarbon dates from historical and tree-ring chronology. Before the cause of these deviations was found, however, it was not possible to use them for correcting radiocarbon

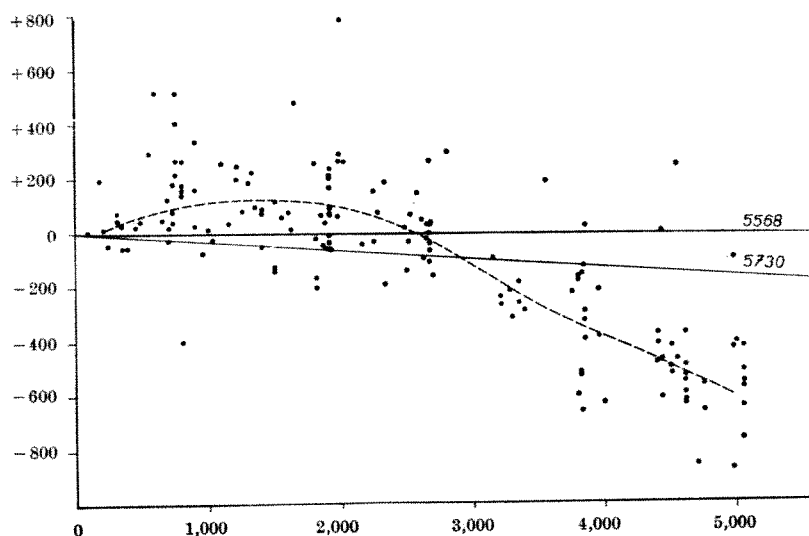


Fig. 3. Deviations between radiocarbon and historical dates. Mean values are given by the dashed line.

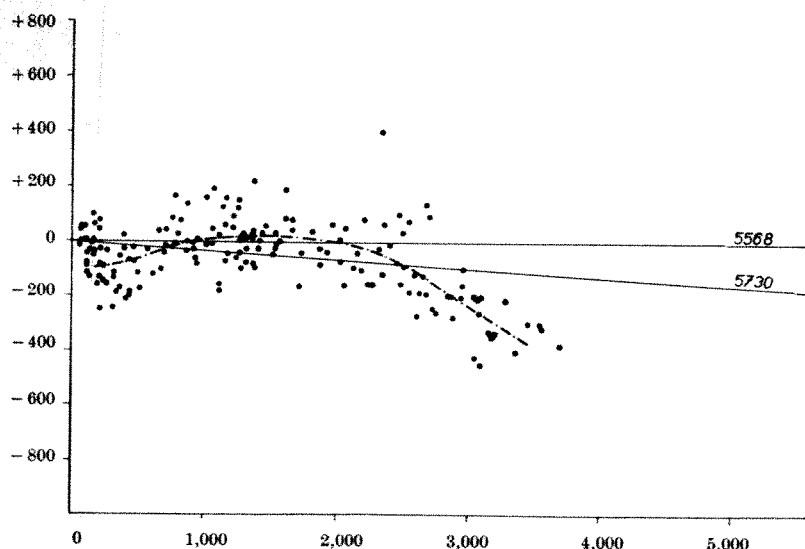


Fig. 4. Deviations between radiocarbon and dendrochronological dates (according to E. Ralph<sup>8</sup> and W. Libby<sup>1</sup>). — · — · — ·, Mean values.

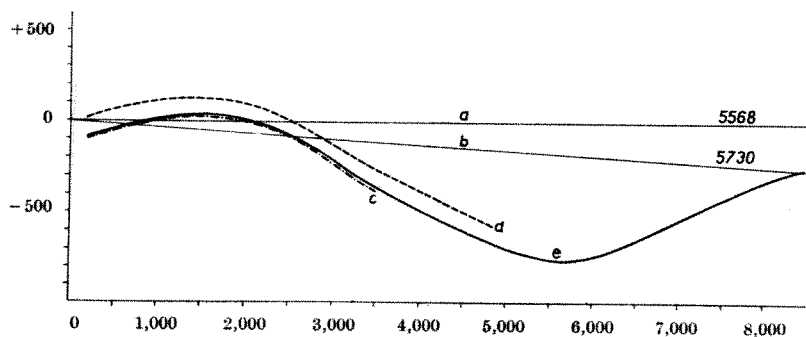


Fig. 5. Mutual differences among the deviations: *a*, the half-life of 5,568 years; *b*, the deviations for the half-life of 5,730 years; *c*, dendrochronological deviations; *d*, deviations of historical dates; *e*, deviations when the influence of the Earth's magnetic field is considered.

chronology outside the territories in which the deviations were originally ascertained.

Full details will be given in the archaeological journal *Památky archeologické*.

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## Earthquakes and Associated Faulting in Central Sudan

A SERIES of earthquakes started in the central Sudan on October 9, 1966, and was reported to be continuing into April 1967. Because the earthquakes occurred in a relatively stable part of the African land mass<sup>1</sup> away from the unstable belt of the East African and Red Sea

Rift Systems, and because of the sinistral strike-slip fault movement which was associated with the strongest shock, they are of great interest.

The strongest shock occurred on October 9 and had the following parameters according to USCGS Preliminary Determination of Epicentres: time of origin, 06:48:40.3 U.T.; epicentral co-ordinates, 12.6° N., 30.8° E.; depth of hypocentre, 11 km; magnitude (*M*), 5.1.

The nearest seismograph station, at Addis Ababa, recorded the principal shocks of the series on the first day (see Table 1).

The three stronger shocks were felt with varying intensity as far as Khartoum (a distance of 380 km at a bearing of 029°). The strongest shock was accompanied by faulting and damage to property in the region of Jebel Dumbeir (Fig. 1); this is called the Jebel Dumbeir Earthquake. Its instrumental epicentre as determined by USCGS lay within 1 km of the jebel. The frequency of shocks felt in the region dropped from more than twenty in the first 24 h to fifteen per day during the second week of activity. The intensity ranged from III to V (ref. 2). At the end of December, two to six shocks per day were reported. Later reports indicate a further fall in frequency.

Jebel Dumbeir lies at the north-eastern edge of a gentle topographical swell in the central plain of the Sudan<sup>3</sup>. It rises to a height of 260 m above the surrounding country, which stands about 500 m above sea level. Jebel Ed Dair (1,413 m) lies to the south-west. The central part of the swell is occupied by the Nuba Mountains, which are a group of steep jebels with large intervening pediments and basins; they reach a maximum height of 1,460 m. To the west and north-west the swell may merge with the volcanic hills (Tertiary and Recent) of Jebel Marra (3,088 m) and Meidob.

Jebel Dumbeir and Jebel Ed Dair are composed respectively of syenitic and granitic intrusions in the Basement Complex<sup>4</sup>. The Nawa formation (Palaeozoic-Mesozoic undifferentiated) occurs in small patches within the swell and the Umm Ruwaba formation (Tertiary-Pleistocene) surrounds the swell on all sides except the north-west<sup>5,6</sup>. The Nubian Sandstone formation (late Mesozoic) occurs to the north and flanks the Umm Ruwaba formation on the west. Superficial deposits overlie the bedrock over much of the area.

Because the Umm Ruwaba formation probably lies in two sub-parallel synclines trending N.W.-S.E. (ref. 7), the swell—in so far as it forms part of the intervening anticline—assumes a tectonic significance; it may be an offshoot of the greater swell occurring at the Sudan-Chad border<sup>8</sup>.

Jebel Dumbeir and Jebel Ed Dair are part of a N.E.-S.W. trending belt of intrusions and extrusions of unknown age; the belt extends from Jebel Hagiarat south-westwards for at least 100 km. The master joints follow two predominant trends, N.E. and N.W.

Table 1*		
Time at Addis Ababa	Estimated distance	Estimated <i>M</i>
06:06:22 U.T.	1,010 km	5.0
06:50:47	1,000	5.7
07:19:(35)	1,000	4.8
10:30:30	1,075?	5.3

\* Data from P. Gouin, personal communication.

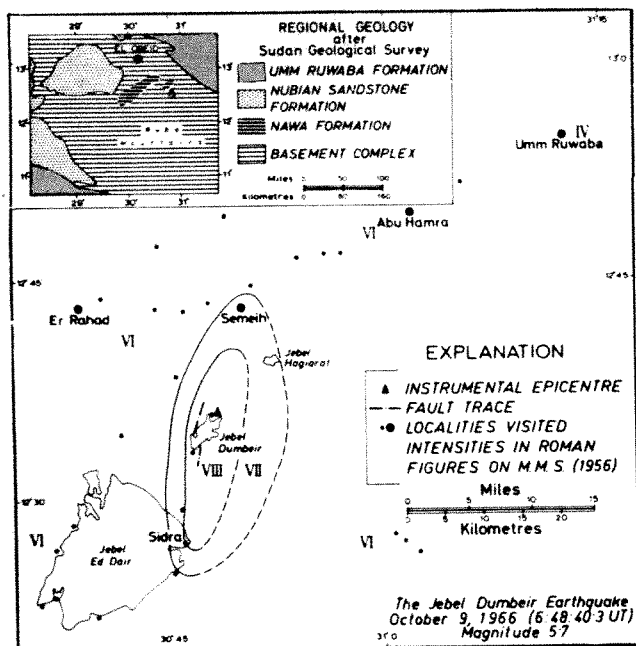


Fig. 1. Intensities and 'isoseismals' (on Modified Mercalli Scale—1956 version), and the fault trace associated with the Jebel Dumbeir Earthquake of October 9, 1966. The inset shows regional geology in the central Sudan. The epicentre of the earthquake is shown in both the maps.

The strike-slip faulting in the bedrock which took place at the time of the Jebel Dumbeir Earthquake was revealed by *en échelon* tension gashes in the overlying clayey superficial deposits (Fig. 2). North of Jebel Dumbeir the gashes formed a linear belt about 4 km long, trending about  $020^\circ$ . The gashes themselves trended at  $340^\circ$  and indicated a sinistral strike-slip movement. South of the jebel the belt swung round from an initial trend of  $343^\circ$  to a linear trend of  $012^\circ$ . High intensity shocks were felt at Semeih to the north of Jebel Dumbeir and at Sidra to the south, but it was not possible to identify or connect the gash belt with either of these areas. In all, gashes were observed for about 6 km and were not reported from anywhere outside the belt.

There was no obvious vertical displacement; because there was no linear feature crossing the belt the magnitude of horizontal displacement along the fault could not be estimated. Since the gashes showed only a little



Fig. 2. The middle part of a typical tension gash (here having a maximum width of about 4 cm) which accompanied the Jebel Dumbeir Earthquake, in ground north of the jebel.

distortion it may be assumed that the horizontal displacement was small.

The fault trace in the Jebel Dumbeir region was not so accentuated as the trace associated with the Imperial Valley Earthquake of 1940 (compare Fig. 2 with Fig. 28–12.4 in ref. 2), where the gashes were highly distorted and earth was piled up to a height of 4 ft. in a narrow belt. In contrast, the gashes associated with the Jebel Dumbeir Earthquake were long, easily recognizable and slightly distorted.

The 'isoseismals' (lines separating zones of equal intensity) for the Jebel Dumbeir Earthquake are plotted in Fig. 1 on the basis of the 1956 version of the Modified Mercalli Scale<sup>2</sup>. The intensity information in ground east and south of Jebel Dumbeir is incomplete because the area is sparsely inhabited. High intensity at Sidra is estimated on the basis of the reports of appearance of water in dry wells following the earthquake.

The 'isoseismals' show an approximate parallelism with the fault trace. Intensity appears to have been relatively higher in regions where Basement Complex formations underlie the superficial deposits than where these deposits are underlain by bedded sedimentary rocks. Consequently, El Obeid, which is underlain by Basement Complex and is situated at a distance of 90 km from the epicentre, experienced an intensity of V, while Umm Ruwaba, where a considerable thickness of the sediments of the Umm Ruwaba formation underlies the superficial deposits and which is situated at a distance of only 60 km from the epicentre, experienced an intensity of IV.

Seismological studies of the nature of sub-surface faulting show a considerable amount of strike-slip movement along faults in the African Rift Systems<sup>9–11</sup>. As a result of the analysis of two shocks (epicentres close to  $1.5^\circ$  N.,  $30.5^\circ$  E.), Sutton and Berg<sup>11</sup> deduced a component of sinistral strike-slip movement along a fault running parallel to the western wall of the Lake Albert Rift. This has not so far been supported by field evidence. A seismological study of the Jebel Dumbeir Earthquake might reveal whether the fault-plane solution accords with the surface evidence of a sinistral strike-slip movement.

The Jebel Dumbeir Earthquake is of special significance because no earthquake has been recorded the origin of which was within 700 km of its epicentre. Sieberg's and Mushketov's maps as reproduced in Gorshkov<sup>12</sup> appear to have been based on reports of earthquakes felt in the region. Instrumentally recorded shocks are confined to the south along the Western Rift and to the east along the Abyssinian and Red Sea Rift systems.

Along the Western Rift System, the earthquake epicentres for the period January 1955–March 1964 (ref. 13) show a maximum alignment along longitude  $30^\circ (\pm 1^\circ)$  E. and most of the epicentres in this belt,  $2^\circ$  wide, lie between the latitudes  $10^\circ$  S. and  $2^\circ$  N. Three epicentres lie further south, the southernmost at  $26.14^\circ$  S. Although not recorded in literature, local earthquakes have been reported frequently from Rejaf ( $4.7^\circ$  N.,  $31.5^\circ$  E.), a village in the southern Sudan<sup>14</sup>. The only earthquake recorded in this region occurred in 1915 and had the epicentral co-ordinates as  $6^\circ$  N. and  $31^\circ$  E. (ref. 1). It appears then that seismic activity associated with the Western Rift continues along longitude  $30^\circ$  E. at least as far as latitude  $6^\circ$  N. Further north along this belt, earthquakes have been reported from Dongola ( $19.1^\circ$  N.,  $30.5^\circ$  E.) (ref. 14) and from Fayum, near Cairo<sup>12</sup>. The relationship of these scattered and relatively rare events to the central active zone needs further investigation.

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### Cartier Furrow, a Major Structure along the Continental Margin of North-western Australia

A COMPILATION of recent aeromagnetic surveys in offshore north-western Australia<sup>1-4</sup> (Fig. 1) reveals a furrow in the

magnetic basement, which I propose to call the Cartier Furrow, after Cartier Island. The furrow is at least 1,250 km long, and 6 km deep, and is filled with sediment. It underlies the upper continental slope except where it crosses the Londonderry Rise, which is a spur in the continental shelf. The furrow is bounded on its south-eastern side by the offshore Bonaparte Gulf Basin in the north, by the offshore Canning Basin in the south, and, in between, by a prism of non-magnetic rocks which thin south-eastward towards the Pre-Cambrian Kimberley Block. The north-western side of the furrow is marked by the atolls of the Rowley Shoals, Scott Reef, and Seringapatam Reef, which rise from the continental slope; by the outer part of the Londonderry Rise, which is crowned by the reefs of Ashmore Island, Cartier Island and Hibernia Reef; and by the south-east slope of the Timor Trough. From a comparison with onshore surveys in the Bonaparte Gulf Basin, Veevers and van Andel<sup>5</sup> identified the magnetic basement beneath the Sahul Shelf as either a layer or layers high in the Pre-Cambrian of the Kimberley Block, or Lower Cambrian volcanics. The magnetic basement in the landward part of the Canning Basin<sup>6</sup>, as shown by drill holes, is crystalline Pre-Cambrian rock (granite or metamorphics), so that the magnetic basement offshore probably coincides also with the surface of Pre-Cambrian rocks.

The increasing thickness of the non-magnetic rocks northward and westward of Dampier Land is confirmed by surveys using conventional seismographic methods<sup>7</sup>. In these offshore areas therefore the contours of the magnetic basement probably indicate the approximate

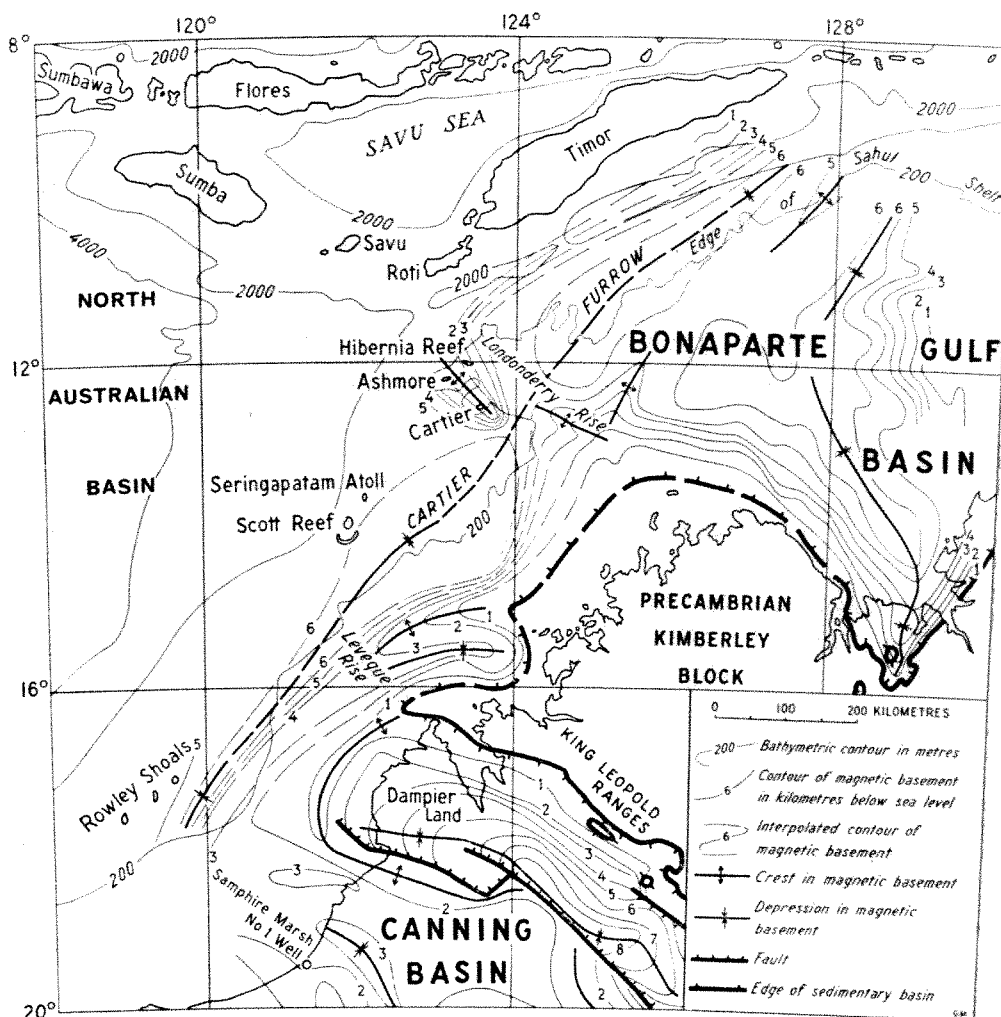


Fig. 1. North-western Australia and south-eastern Banda arcs, showing the Cartier Furrow and other structures of north-western Australia.



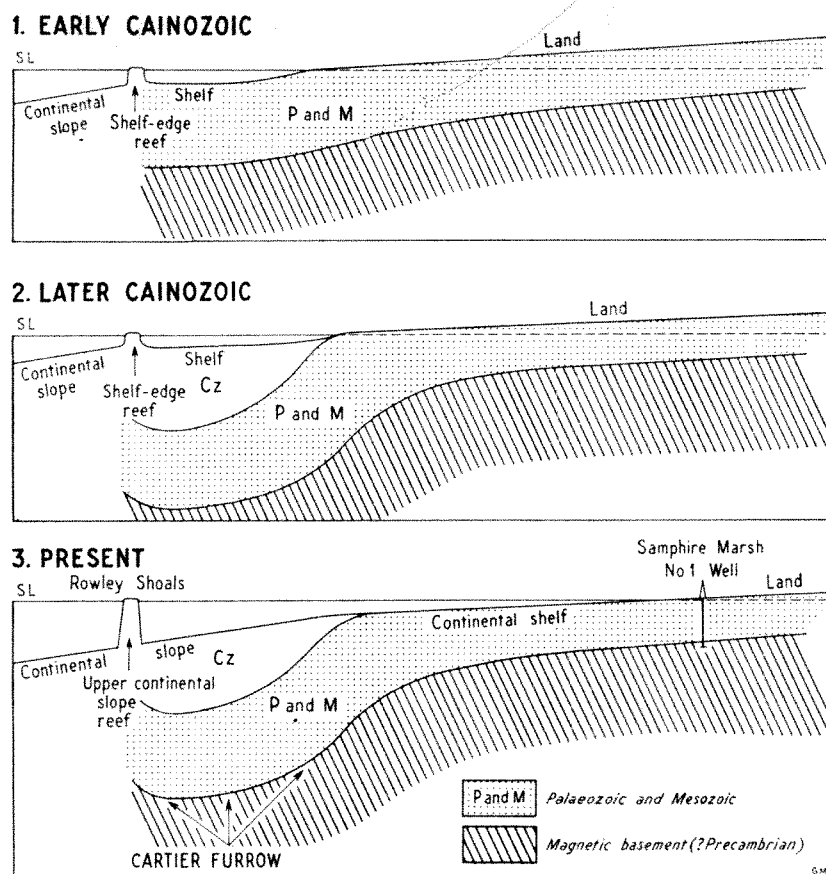


Fig. 2. Diagrammatic sections, with vertical scale much exaggerated, showing the development of the Cartier Furrow in the Rowley Shoals/Samphire Marsh area.

depth to the Pre-Cambrian rocks, or conversely the thickness of the overlying Phanerozoic rocks. In the Timor Trough, this relation does not hold because the Phanerozoic rocks of nearby Timor contain abundant volcanic and crystalline rocks, which are strongly magnetic.

The age within the Phanerozoic of the sediments that fill the Cartier Furrow can be determined directly only by drilling. Indirect methods indicate that the sediments are probably Mesozoic and Cainozoic. A reflexion seismograph traverse<sup>7</sup> shows that the sedimentary section thickens by some 3,000–3,500 m from near Samphire Marsh No. 1 Well on the shore of the Canning Basin to the Rowley Shoals, and that progressively younger horizons appear towards the north-west. The main reflector is the unconformity between the Permian and Middle Jurassic to Lower Cretaceous sediments in Samphire No. 1 Well<sup>6</sup>. Because the section thickens above the unconformity and progressively younger horizons appear north-westward, the thicker section includes sediments younger than Lower Cretaceous, probably reaching into the Cainozoic. In the Timor Sea area, van Andel and Veevers<sup>8,9</sup> deduced on geomorphological grounds that the shelf was exposed for a long period in the Cainozoic, and that thick sediments were deposited in front of the present shelf edge on the south-eastern slope of the Timor Trough. Except possibly for a brief marine incursion in the Miocene<sup>10</sup>, the Sahul Shelf was not covered by the sea until late in the Pleistocene. If the Rowley Shelf had a similar history during the Cainozoic, then the Cartier Furrow may be interpreted as a subsiding zone at the continental margin that was filled in the Cainozoic with sediment deposited behind the shelf edge, locally marked by fringing reefs (Fig. 2).

In the late Pleistocene, the region subsided some 120 m; the Cartier Furrow became the upper part of the present

continental slope, and those reefs that were able to grow upward by keeping pace with the subsidence became the present upper continental slope banks (Rowley Shoals, Scott Reef, Seringapatam Reef). According to this assumption, the continuation of the furrow across the Londonderry Rise indicates that the Rise originated late in the Pleistocene. The Londonderry Rise is the middle part of a north-west lineament that extends from the north-western coast of the Kimberley Block to the islands of Savu and Sumba. These islands rose in the 'Young Quaternary'<sup>11</sup>, possibly in response to the same forces that elevated the Londonderry Rise.

In his regional analysis of offshore north-western Australia in 1953, Fairbridge<sup>12</sup> pointed out that "depressions and rises in the shelf are arranged opposite the basins and swells of the continent. Their structural histories appear to be analogous". Offshore work since 1953 has shown that this arrangement is so for part of the Sahul Shelf<sup>6</sup> but not so elsewhere. By extrapolating the King Leopold Range (the King Leopold Mobile Zone of Traves<sup>13</sup>) seaward into the poorly surveyed offshore Leveque Rise, Fairbridge was misled into concluding that this rise was an area of shallow Pre-Cambrian basement overlain by a film of younger material, and concluded generally that the structural grain of the land, which runs at right angles to the continental margin, persisted seaward, thus overlooking the possibility of a longitudinal sedimentary trough along the continental margin. Teichert<sup>14</sup> had expressed this possibility in his concept of the "Westralian Geosyncline, which forms the southward continuation of the Timor-East Celebes geosyncline of the East Indies and thus represents the southern part of a continuous geosynclinal trough bordering the Australia-New Guinea Shield on the west". This concept was based chiefly on the similarity of some Permian, Jurassic and

Cretaceous faunas of north-western Australia to contemporaneous ones in Indonesia, and not, as Umbgrove<sup>15</sup> remarked, on sedimentological and structural similarities, which do not exist.

Twenty years later, Teichert<sup>16</sup> seemed to lose sight of his Westralian Geosyncline, and followed Fairbridge in drawing the major structural features of the region at right angles to the continental margin. Boutakoff<sup>17</sup>, reviving Teichert's early concept, made a novel interpretation of the bathymetric chart; he postulated several ridges along the outer edge of the shelf, and suggested that these are submerged folded ranges, the highest points of which are the base of the scattered reefs in the region. As noted by van Andel and Veevers<sup>9</sup>, "examination of the chart (Admiralty Chart 475) shows that, whatever the merit of the idea itself, the bathymetric data do not permit this conclusion". An example of Boutakoff's unacceptable contouring is pertinent here. "Whatever their origin, the Rowley Shoals, Scott Reef and Seringapatam Atoll have the morphology of atolls and they rise from the outer shelf. By disregarding contradictory soundings, Boutakoff has extended what the chart shows to be pinnacles into the crests of 'mountain ranges'". Nevertheless, Boutakoff's hypothesis that the sediments thicken in the general area of the postulated ridges is not entirely wrong. That the "ridges" contain folded strata seems to be disproved by seismographic surveys, and, more importantly, the existence of the "ridges" themselves is disallowed by the bathymetry.

Wider issues raised by the discovery of the Cartier Furrow—its relation with the Indonesian arcs and the deep floor of the Indian Ocean on the one side, and with the sedimentary basins of north-western Australia on the other—will be discussed in a more extensive paper to be published elsewhere<sup>18</sup>.

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## PHYSICS

### Mechanism of Radio Emission from Extensive Air Showers

THE fact that large cosmic ray air showers can generate detectable pulses of electromagnetic radiation at radio

frequencies was first established by Jelley and his collaborators at Harwell, Jodrell Bank and Dublin<sup>1,2</sup>. The pulses have been detected by several other research groups<sup>3-5</sup>, but there is as yet no clear picture of just how the radiation is produced. Theoretical treatments by Kahn and Lerehe<sup>6</sup>, Colgate (personal communication) and Allan<sup>7</sup>, all suggest that the principal mechanism should be the motion of charge due to the lateral displacement of shower particles by the Earth's magnetic field. The polarization to be expected from this geomagnetic mechanism is perpendicular both to the shower axis and to the magnetic field lines.

Alternative mechanisms can, however, be envisaged. For a shower which is not electrically neutral, but contains—as is expected—an appreciable excess of negative particles<sup>8</sup>, radiation may also occur as a result of the longitudinal motion. This pseudo-Cerenkov radiation would be polarized radially with respect to the shower axis. Two further mechanisms have been suggested recently, namely, bremsstrahlung from the delta rays associated with the shower tracks<sup>9</sup>, and acceleration of the ionization electrons in the atmospheric electric field (W. N. Charman, personal communication). These also have their characteristic polarizations; the bremsstrahlung would be unpolarized, while the polarization due to the atmospheric electric field would be typically vertical.

The experimental evidence hitherto available on polarization has been indirect and conflicting. At Jodrell Bank<sup>10</sup>, radio pulses were detected by two similar receiving channels, one directed in the general direction of the Earth's field and the other in a direction at right angles. The rate of detection in the two channels was not significantly different, a finding which disagrees with the expectations of the geomagnetic deflexion theory. On the other hand, the Moscow and Kharkov groups<sup>11</sup> state that their measurements with single antennae oriented E.-W., forming part of the large Moscow air shower array, give better internal consistency when the polarization is assumed to be that predicted by Kahn and Lerehe.

We wish to report here measurements on the amplitude and polarization of the radio pulse from an unusually large shower recorded by the Haverah Park air shower array. The direction of polarization in the horizontal plane has been unequivocally determined; as will be seen, it agrees very well with that expected from geomagnetic deflexion but disagrees with the expectations from the other mechanisms that have been mentioned.

Two receivers were used, of frequency 32 Mc/s and bandwidth 5 Mc/s. One receiver was connected to a pair of in-phase, half-wave dipoles supported  $\lambda/4$  above the ground,  $\lambda/2$  apart and oriented along the magnetic N.-S. line. The other receiver had a similar antenna system, oriented E.-W. The four dipoles made up the sides of a square. The receiver outputs were displayed on separate oscilloscopes and photographed through mirrors by a single camera whenever the Haverah Park particle array was triggered by a shower of primary energy  $\geq 10^{11}$  eV. The position on the time base (total length 10  $\mu$ sec) at which any radio pulse associated with the shower should occur could be calculated from the particle data to  $\sim 0.2$   $\mu$ sec.

It was found (contrary to our earlier experience at 60 Mc/s) that the background against which radio pulses had to be observed was very much higher than the galactic noise level. Spurious pulses with a voltage amplitude more than ten times the galactic noise appeared at frequent and erratic intervals on every time base. This made immediate visual identification of the true radio pulses difficult. Six possible candidates were selected from among 200 showers on the basis of amplitude alone, but five of these had to be rejected when checked against the timing requirement.

The sixth event was unique. Among all the pulses observed, this was much the largest, being the only one to go off the screen, and its timing in relation to the associated shower was correct to 0.2  $\mu$ sec. Because there

were only six candidates on some 4,000  $\mu\text{sec}$  of time base, there can be little doubt that this big pulse was genuinely associated with the shower. The particle analysis of the shower only strengthens this conclusion. The primary energy of  $(2^{+2}_{-1}) \times 10^{19}$  eV makes it one of the largest recorded at Haverah Park, and it is reasonable that this should be the one shower to produce a large radio pulse.

Table 1

	Relative amplitudes in N.-S. aerial	Relative amplitudes in E.-W. aerial
Geomagnetic deflexion	1.0	0.10
Radial polarization	1.0	1.85
Bremsstrahlung	1.0	0.61
Vertical polarization	1.0	2.75

The big pulse came from the pair of dipoles oriented N.-S. On the E.-W. channel the oscilloscope showed no more than the usual interference background. The relevant voltage levels at the receiver input terminals (50  $\Omega$  impedance) were about 0.5 mV for the background pulse amplitudes, 2 mV for saturation, and an estimated 5 mV for the big (off-screen) pulse. The voltage gains in the two channels did not differ by more than 10 per cent. Accordingly, we can conclude that the direction of polarization in the horizontal plane was magnetic N.-S. to a precision of about 0.1 radians or about  $5^\circ$ .

The shower which produced the radio pulse could be analysed very precisely because of the information on particle density obtained from the first of the outer (2 km) stations of the Haverah Park array to be brought into use. We are indebted to Dr R. J. O. Reid and Dr A. A. Watson of the University of Leeds for providing us with the results of their analysis. The shower, of total energy  $(2^{+2}_{-1}) \times 10^{19}$  eV, struck the ground with its axis ( $650 \pm 50$ ) m from the radio aerials at a bearing ( $25 \pm 5^\circ$ ) south of magnetic east. The zenith angle of the shower was ( $43 \pm 3^\circ$ ), and its azimuth ( $20 \pm 5^\circ$ ) south of magnetic east. The Earth's magnetic field at Leeds is directed  $9^\circ$  W. of true north, and the dip angle is  $68^\circ$ . From these figures one can calculate immediately the relative amplitudes to be expected from the N.-S. and E.-W. aerial systems for each of the four mechanisms mentioned above (Table 1).

It is clear from Table 1 that the experimental observations are decisively in favour of geomagnetic deflexion.

A further topic on which useful conclusions can be drawn is the dependence of radio pulse amplitude on total shower energy. The big pulse was obtained with an effective aerial aperture of about 50 m<sup>2</sup> and the receiver bandwidth was about 5 Mc/s at a centre frequency of 32 Mc/s. The received power level was thus  $10^{-6}$  W and, with a pulse length of 0.2  $\mu\text{sec}$ , the total energy was  $\sim 2 \times 10^{-13}$  J. In our earlier experiments at Haverah Park<sup>3</sup>, we used an aerial array of area about 100 m<sup>2</sup> and a receiver bandwidth of 2 Mc/s centred at 60 Mc/s. The increased aerial area partially offsets the effects of reduced bandwidth, and the overall sensitivity was less than in our present experiment by a factor of about 2. At 60 Mc/s we identified a number of radio pulses of amplitude two or three times the galactic noise level, from showers of primary energy a few times  $10^{17}$  eV. Only about 5 per cent of such showers gave detectable pulses, and we infer that the necessary coherence is only obtained at 60 Mc/s when there is a favourable fluctuation in the longitudinal development of the shower. Assuming that the maximum pulse amplitudes observed correspond to effectively full coherence, we may assert that showers of energy a few times  $10^{17}$  eV give pulses at about 50  $\mu\text{V}$ , and that showers of energy a few times  $10^{19}$  eV give pulses at about 5 mV at similar distances from the shower axis. The ratio of the pulse amplitudes is thus of the same order as the ratio of the primary energies, and the observations support the theoretical expectations that the pulse amplitude should be proportional to primary energy.

We thank all our colleagues at Haverah Park for their co-operation during the present experiment. Mr D. Pearce gave valuable assistance in the building of the aerial array and in the maintenance of the equipment.

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### Theory of Radio Pulses from Cosmic Ray Air Showers

THE recent results of Porter *et al.*<sup>1</sup> on detectable polarized radio pulses emitted from extensive air showers cast some serious doubt on the validity of the mechanism for coherent emission suggested by Kahn and Lerche<sup>2</sup>. This mechanism concluded, on the basis of a simple model, that the separation of positive and negative charges by the Earth's magnetic field should produce an observable polarized (east-west) radio signal of some 60,000 flux units  $\times \mu\text{sec}$  at 45 Mc/s for a shower containing some  $10^6$  electrons and positrons. The primary cosmic ray particle energy was taken to be about  $5 \times 10^{15}$  eV. The purpose of this letter is to point out several effects which are ignored in the treatment of Kahn and Lerche which may play a dominant part in modifying both the intensity and polarization of the coherent signal. These are (i) refractive index variations; (ii) Coulomb scattering; (iii) time variation of the shower front.

We shall consider the following factors in turn. The variation of refractive index with height can be shown<sup>3</sup> to be important only for distant showers observed at great height. In the experiments being performed<sup>3-6</sup> this effect does not arise.

The effect of Coulomb scattering is two-fold. If the particles in the shower front are all of the same energy, then, on the assumption that they start at the centre of the shower front at some large height, they are spread across the front as time proceeds. In a steady state, as many particles are produced per unit time in the front as are lost from the front. The total effect, however, is that we can no longer treat the front as two thin rings of opposite charge moving down through the atmosphere but must add up the electric and magnetic fields produced by a continuum of rings each weighted by the ambient number density of positive and negative charges at a given ring site. Second, when the particles in the front are distributed in energy, the effect of Coulomb collisions is different for particles of different energy. It has been calculated<sup>7</sup> that this effect can reduce the effective dipole of the shower front treated as two rings by a factor of the order of 10-100. This in turn leads to an intensity decrease of  $10^2$ - $10^4$  which may then reduce the linear polarization by a similar factor if other processes, such as charge excess<sup>8</sup>, contribute a significant amount to the

coherent radiation field. This they may do particularly when both spatial structure and distribution of energy of the particles in the shower front are allowed for.

Shower fronts are by no means constant in time. It has been shown<sup>2</sup> that a simple exponential decay of a shower front can be taken into account and that this does not seriously alter the emission of radiation from a shower in most situations; and almost certainly not in the experiments currently being performed. Whether a different growth and decay rate influences the emission characteristics significantly is difficult to say without performing the analysis, but we suspect that this is only a minor correction.

Altogether then we believe that the null results obtained<sup>1,3</sup> for the polarization experiments may possibly be accounted for by Coulomb scattering which is ignored in the Kahn and Lerche treatment. A more general theory than that already existing is clearly needed. This should incorporate several model energy and spatial distributions of particles in order to decide how sensitive is the emission of coherent radiation from showers to energy variations among the particles in the shower and, separately, spatial structure of the shower front.

One further possible explanation of the discrepancy between the Kahn and Lerche theory and observation has already been suggested<sup>1</sup>. This is that if showers contain a higher excess of negative charges than was suggested by Askaryan (> 10 per cent), then the coherent space charge emission may dominate, the general idea being to place these electrons at a lower energy than is normally recorded on a Geiger-Müller counter when shower experiments are being performed. One can still keep such electrons at a speed greater than that of light in air.

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### Random Close Packing and the Heats of Fusion of Argon at High Pressures

Using a random close packed model of the liquid state and a Lennard-Jones 6 : 12 interatomic potential function, the calculations made on the heats of fusion of the inert gases<sup>1</sup> have been extended to high pressures. Despite the difficulties discussed in ref. 1, an attempt has been made to calculate the molar volume change  $\Delta V$  on melting at pressures up to 6,000 atm.

For a change of state, using the second law

$$\Delta U = -p\Delta V + T\Delta S \quad (1)$$

where  $\Delta U$  is the initial energy change of 1 mole,  $p$  is the external pressure,  $T$  the temperature, and  $\Delta S$  the entropy change. For triple point calculations<sup>1</sup> the term  $p\Delta V$  is

negligible; for pressures an order of magnitude higher, it is not. Using the subscripts  $L$  for the liquid,  $S$  for the solid, equation 1 gives

$$(U_L + pV_L) = (U_S + pV_S) + Q \quad (2)$$

where  $Q$  is the latent heat of melting. Thus using the energy summations for a random close packed liquid as in ref. 1, an enthalpy : molar volume curve can be drawn, from which  $V_L$  can be predicted at any pressure. A similar lattice summation gives the corresponding enthalpy curve for the solid.

Unfortunately, there are very few data on  $\Delta V$  above the triple point available for experimental comparison. Bridgman's work<sup>2</sup> on the melting curve of argon is used: he gives values of  $V_S$  and  $V_L$  for pressures of 1,000, 2,000, 4,000 and 6,000 kg/cm<sup>2</sup>. Though not very extensive, these data provide an adequate quantitative test over a wide pressure range. Bridgman also tabulates the latent heat at each pressure using the Clausius-Clapeyron equation; his values are systematically lower than the generally accepted values<sup>3,4</sup>. This raises further difficulties in making experimental comparisons, which are discussed later.

Table 1. VOLUME CHANGE ON MELTING AT DIFFERENT PRESSURE

Pressure (dyne/cm <sup>2</sup> ) $\times 10^{-4}$	$V_S$ (cm <sup>3</sup> )	Ref.	Latent heat (kJ/mole)	Ref.	Implied $V_L$ (cm <sup>3</sup> )	Experi- mental $V_L$ (cm <sup>3</sup> )	Ref.
0.689	24.62	4	1.176	4	28.03 $\pm$ 0.11	28.14	4
0.980	24.84	2	1.097	2	27.93 $\pm$ 0.12	28.04	2
			1.176	4	28.27 $\pm$ 0.13		
1,961	24.49	2	1.093	2	26.04 $\pm$ 0.08	26.20	2
			1.172	2, 4	26.25 $\pm$ 0.07		
			1.176	4	26.26 $\pm$ 0.07		
3,922	24.37	2	1.078	2	25.35 $\pm$ 0.06	25.48	2
			1.157	2, 4	25.49 $\pm$ 0.06		
			1.176	4	25.53 $\pm$ 0.05		
5,882	24.25	2	1.086	2	24.95 $\pm$ 0.04	25.08	2
			1.165	2, 4	25.06 $\pm$ 0.04		
			1.176	4	25.08 $\pm$ 0.04		

Two approaches to the calculations were made: (1) Using Bridgman's<sup>2</sup>  $V_S$  for the solid, the enthalpy curves are used to predict the corresponding  $V_L$ , assuming various latent heat values. The comparison with experiment is shown in Table 1 and Fig. 1. (2) Using Bridgman's<sup>2</sup>  $V_S$  and  $V_L$  values, the heats of fusion are evaluated from the enthalpy curves. The results are shown in Table 2.

Table 2. HEATS OF FUSION AT DIFFERENT PRESSURES

Pressure (dyne/cm <sup>2</sup> ) $\times 10^{-4}$	$V_S$ (cm <sup>3</sup> )	Ref.	$V_L$ (cm <sup>3</sup> )	Ref.	Implied latent heat (kJ/mole)	Experimental latent heat (kJ/mole)	(2)	(4)
0.689	24.62	4	28.14	4	1.202 $\pm$ 0.027	1.176 $\pm$ 0.001		
0.980	24.84	2	28.04	2	1.123 $\pm$ 0.028	1.176	1.097	1.176
1,961	24.49	2	26.20	2	1.154 $\pm$ 0.029	1.176	1.093	1.172
3,922	24.37	2	25.48	2	1.152 $\pm$ 0.030	1.176	1.078	1.157
5,882	24.25	2	25.08	2	1.183 $\pm$ 0.029	1.176	1.086	1.165

(1) Using Bridgman's "low" latent heat values<sup>2</sup>, the predicted liquid volume is too low, as expected. Because Bridgman's values vary about only 2 per cent over the whole pressure range, however, it is useful to obtain  $V_L$  values for (a) the generally accepted latent heat value at the triple point<sup>4</sup> unchanged throughout the pressure range, and (b) the latent heat value<sup>4</sup> as in a, varied after Bridgman's observed variation<sup>2</sup>.

As shown in Table 1, both a and b give a good experimental fit, the differences between them being insignificant compared with the expected error. Fig. 1 shows the results in graphical form; the theoretical fit is good, and well within Bridgman's experimental variations as



## THE SOLID STATE

## Crystal Structure of Cerium (III) Exchanged Faujasite

THIS communication reports the initial results of our investigation of the crystal chemistry of the rare earth faujasite system. In this investigation a single crystal of faujasite was placed in contact with concentrated cerium nitrate,  $\text{Ce}(\text{NO}_3)_3$ , at room temperature for 6 months. The exchange data of Ames<sup>1</sup> on the cerium exchange of Linde X and our own data (H. S. Sherry, personal communication) suggest that the cerium exchange in the crystal will be approximately 60 per cent, which corresponds to a unit cell composition,  $(\text{Ce}_2\text{O}_3)_6(\text{CaO})_{7.6}(\text{Na}_2\text{O})_{3.9} \cdot 29.5 \text{ Al}_2\text{O}_3 \cdot 133 \text{ SiO}_2 \cdot 270 \text{ H}_2\text{O}$ .

The crystal was ground to a 0.27-mm diameter sphere. Diffraction data were consistent with the previously assigned space group  $Fd3m$ . Three-dimensional intensity data were collected with an integrating Weissenberg camera using copper  $K\alpha$  radiation and multiple film packs. The intensities were measured with a microdensitometer and corrected for Lorentz polarization effects and absorption. No corrections were made for the anomalous scattering of cerium.

The structure was determined using combined least squares<sup>2</sup>, Fourier and difference Fourier techniques. The framework parameters reported by Baur<sup>3</sup> were the basis of the initial structure factor—Fourier map calculations. Scattering matter was found in four non-framework positions. Fig. 1 shows the relative electron density of these non-framework sites. (Framework oxygen had a relative peak height of 17.) The small peaks at  $x = 0.22, 0.30, 0.35$  and  $0.41$  are spurious and did not appear on difference maps which contained no positive or negative peaks larger than 2 on this relative basis. Based on internuclear distances (see Table 1) we attribute the scattering matter at  $x = y = z = 0.0696, 0.1652$  and  $0.264$  to  $\text{Na}(\text{Ca}), \text{H}_2\text{O}$  and  $\text{H}_2\text{O}$  respectively. This is in agreement with Baur's<sup>3</sup> assignment. The hydrated cerium (III) ion is slightly displaced from the special position of set  $d$ , that is,  $\frac{1}{2}, \frac{1}{2}, \frac{1}{2}$ , etc. Although this displacement is not evident from the electron density map (Fig. 1), difference maps indicate a  $0.38 \text{ \AA}$  deviation from the centre of symmetry. With two partial occupancy cerium atoms at the  $SV$  site, the  $R$  value for the 263 largest  $F$ 's dropped from 0.135 to 0.127. The structural parameters of this study are given in Table 2.

The arrangement of  $\text{Na}(\text{Ca})$  and water in the six-membered rings of the sodalite cage is in good agreement with the results of Baur<sup>3</sup>. A new cation site for the faujasite system was found in this study; hydrated

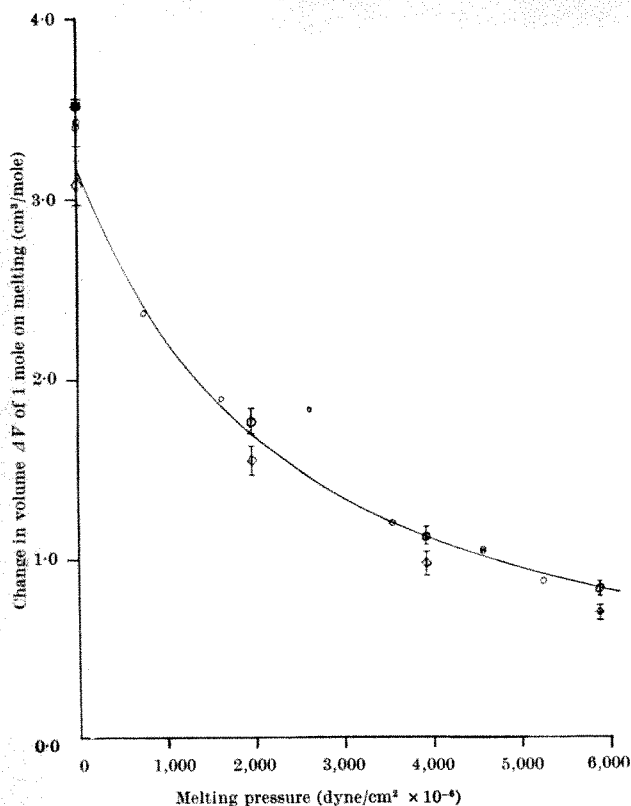


Fig. 1. Comparison of theoretical points with Bridgman's experimental data<sup>3</sup>. —, Bridgman's smoothed curve<sup>2</sup>; O, Bridgman's experimental points<sup>2</sup>; ◇, calculated using Bridgman's latent heat values<sup>2</sup>;  $\square$ , calculated using Clusius and Wiegand's latent heat value for the triple point<sup>4</sup>; ■, experimental triple point using Clusius and Wiegand's molar volumes and latent heat<sup>4</sup>.

shown by the scatter of his experimental points. Because  $a$  and  $b$  give very similar results, only values for case  $a$  are plotted.

(2) The same pattern as in (1) is evident in the second approach. The predicted latent heat value is considerably greater than Bridgman's values, but fits both the modified and unmodified Clusius/Wiegand values<sup>4</sup> within the expected error except for  $P = 0.980 \times 10^6 \text{ dyne/cm}^2$ . There is, however, a large discrepancy between Bridgman's volume figures at this pressure, and those of Clusius and Wiegand<sup>4</sup> at the triple point ( $0.689 \times 10^6 \text{ dyne/cm}^2$ ); it seems generally accepted that greater reliance can be placed on the later Clusius/Wiegand figures<sup>3</sup>.

Thus assuming a spherically symmetrical Lennard-Jones potential function and a random close packed model of liquid argon at its melting point, the energy balance between the external pressure and the internal potential energy is accounted for. At pressures up to nearly 6,000 atm., the predicted volume changes or the predicted latent heats of fusion agree with experiment within the expected error.

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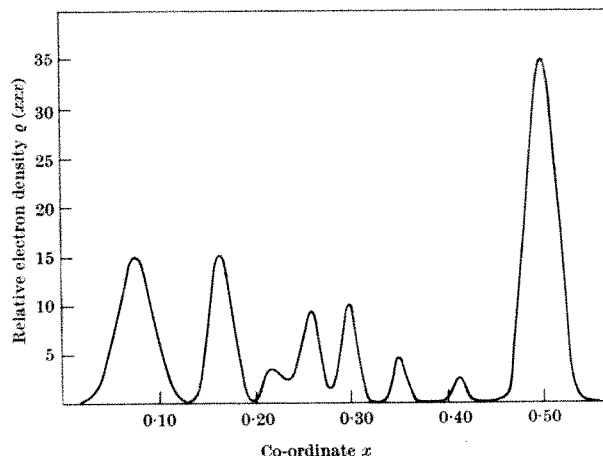


Fig. 1. Electron density map of non-framework sites for cerium (III) exchanged faujasite,  $\rho(xzz)$  versus  $x$ .

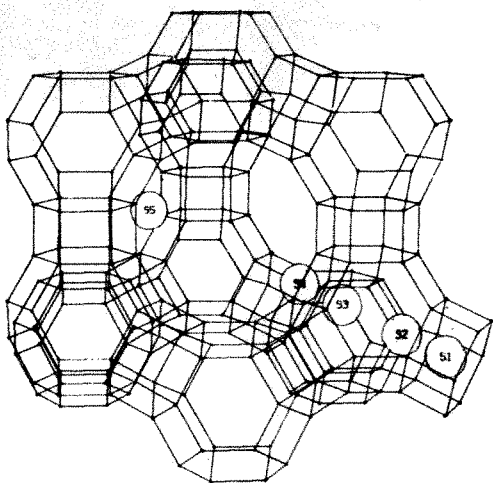


Fig. 2. Stereo pair view of faujasite framework showing non-framework sites.

cerium ions occupy the site in the centre of the twelve-membered ring, 12MR. The Ce-O framework distance of

5.0 Å is reasonable for a Ce-O  $\begin{array}{c} \text{H} \\ \diagup \quad \diagdown \\ \text{O} \end{array}$  framework arrange-

ment. On the average, six of the estimated twelve cerium ions are in the 12MR's, leaving six hydrated cations located at random in the eight super cages. Because the hydrated cerium ions are held in the 12MR's by hydrogen bonds, the observed cerium ion distribution is believed to

Table 1. PARAMETERS FOR CERIUM EXCHANGED FAUJASITE  $Fd3m$   $A = 24.00$  Å

Atom	Set	P	X	Y	Z	T
Si (Al)	i	1.00	-0.0536 (1)	0.1251 (1)	0.0362 (1)	0.57 (5)
O1	h	1.00	-0.1061 (4)	0.1061 (4)	0.0	2.1 (2)
O2	g	1.00	-0.0039 (4)	-0.0039 (4)	0.1427 (6)	2.9 (3)
O3	g	1.00	0.1745 (3)	0.1745 (3)	-0.0324 (4)	1.2 (2)
O4	g	1.00	0.1789 (3)	0.1789 (3)	0.3229 (5)	1.8 (2)
Na	e	0.57 (6)	0.0696	0.0696 (6)	0.0696 (6)	2.9 (8)
0w1	e	0.99 (9)	0.1652 (6)	0.1652 (6)	0.1652 (6)	2.9 (7)
0w2	e	0.8 (4)	0.264 (4)	0.264 (4)	0.264 (4)	23 (11)
Ce	e	0.09 (1)	0.491 (2)	0.491 (2)	0.491 (2)	3.5
C6]]	e	0.09 (1)	0.509 (2)	0.509 (2)	0.509 (2)	3.5

$R$  (all data, 636  $F$ 's) = 0.189;  $R$  (263 largest  $F$ 's) = 0.120.

$$R = \frac{\sum |F_o - F_c|}{\sum |F_o|}$$

Table 2. INTERATOMIC DISTANCES AND ANGLES FOR CERIUM EXCHANGED FAUJASITE

Atom set	Distance (Å)	Atom set	Angle (deg)
Si(Al)-O1	1.64 (1)	O1-Si(Al)-O2	109.7 (6)
Si(Al)-O2	1.64 (1)	O1-Si(Al)-O3	111.1 (5)
Si(Al)-O3	1.65 (1)	O1-Si(Al)-O4	106.6 (6)
Si(Al)-O4	1.65 (1)	O2-Si(Al)-O3	108.4 (6)
O1-O2	2.68 (1)	O2-Si(Al)-O4	108.5 (7)
O1-O3	2.71 (1)	O3-Si(Al)-O4	112.5 (6)
O1-O4	2.64 (1)	Si(Al)-O1-Si(Al)	138.3 (8)
O2-O3	2.66 (1)	Si(Al)-O2-Si(Al)	146.9 (10)
O2-O4	2.67 (1)	Si(Al)-O3-Si(Al)	140.7 (7)
O3-O4	2.74 (1)	Si(Al)-O4-Si(Al)	138.9 (8)
Na-O3	2.53 (2)	O3-Na-O3	96.4 (7)
Na-Ow1	2.42 (3)	O3-Na-Ow1	95.1
Ow1-Ow1	2.81 (4)	Ow1-Na-Ow1	71.0 (10)
Ow1-O2	3.14 (2)	O2-Ow1-O2	76.2 (6)
Ow2-O2	3.02 (12)	O4-Ow2-O4	98 (4)
Ce5-O1	5.04 (9)		

Table 3. NON-FRAMEWORK POSITIONS AND OCCUPANCIES IN FAUJASITE TYPE STRUCTURE

Site	Hydrated faujasite <sup>a,b</sup>	Anhydrous Ca <sup>++</sup> exchanged faujasite <sup>c</sup>	Hydrated Linde X <sup>7</sup>	Hydrated Ce <sup>+++</sup> exchanged faujasite
(SI)	Species Population No. ions/uc	Ca 1.0 16	Na 1.0 16	
(SII)	Species Population No. ions/uc	Ca, Na 0.5 16		Na 0.57 18
(SIII)	Species Population No. ions/uc	H <sub>2</sub> O 1.0 32		H <sub>2</sub> O 0.99 32
(SIV)	Species Population No. ions/uc	H <sub>2</sub> O 0.33 11	Ca 0.5 16	Na 1.0 32
(SV)	Species Population No. ions/uc			Ce 0.38 6

be a result of time averaging rather than the result of time invariant spatial distribution.

The position SV at the centre of the 12MR reported here is the fifth non-framework position determined for faujasite (Fig. 2, computer drawn using ORTEP<sup>4</sup>). The other four are: SI, the centre of the double 6MR; SII, within the sodalite cage just outside the double 6MR; SIII, within the sodalite cage in the 6MR opening into the super cage; SIV, in the super cage on the opposite side of the 6MR of SIII. A summary of the non-framework positions in the faujasite structure is given in Table 3. The data on hydrated Linde X would indicate that either synthetic and natural faujasite are somehow different or that in pure sodium faujasite the sodium cations take different positions. In a re-evaluation of Broussard and Shoemaker's data<sup>7</sup>, however, Baur<sup>3</sup> finds that in hydrated Linde X sites I-IV are all partially occupied with population parameters,  $P$ , between 0.3 and 0.8. This is more in line with results on the other hydrated faujasite forms.

Preliminary data obtained with dehydrated cerium (III) exchanged faujasite and fully exchanged LaX indicate that all rare earth ions move from site SV to SII.

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## Tubular Ice Crystals

It has been found that on very rare occasions long, thin ice needles grow into the air from the solid surface of water frozen in an open container<sup>1-4</sup>. In the course of supercooling experiments, Dorsey<sup>5</sup> observed a similar phenomenon in the laboratory. He assumed that growth occurred when, because of the increase in volume during solidification, water was forced through an opening in the ice covering the surface. Dorsey also suggested that a tube formed, through which water flowed and which "grew"

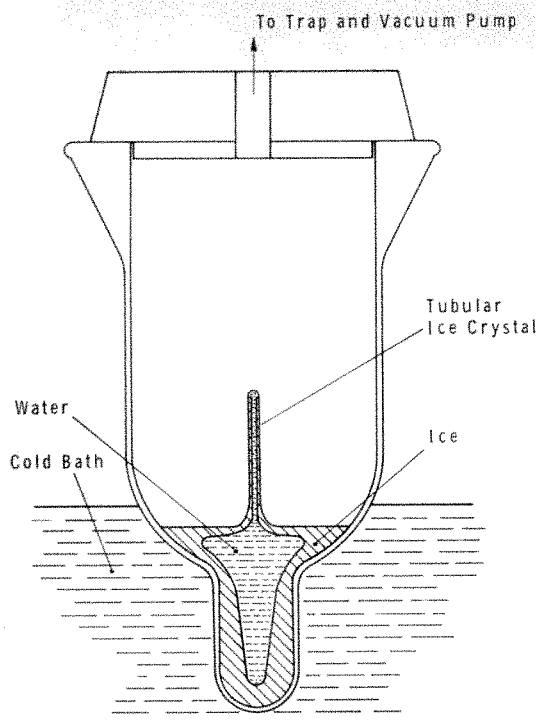


Fig. 1. Diagram of the cell showing growth of tubular ice crystal.

at its tip. Recently, Hayward<sup>6</sup> reported a method for growing such spikes and obtained experimental evidence in support of Dorsey's mechanism.

This communication describes a simple method of growing hollow tubes or needles of controlled size, suitable for physical testing; some of the crystallographic characteristics are also reported. We present evidence in support of Dorsey's mechanism, elaborate on it, and suggest a necessary extension. Finally, we indicate how these results can be used for growing tubular bi- or tri-crystals other than ice.

The apparatus used in these experiments consisted of a cell, a cold trap and a rotary vacuum pump. The cell was cylindrical in shape, with a cold finger protruding from the bottom which contained about 20 cm<sup>3</sup> water (Fig. 1). The trap was placed in a mixture of dry ice and acetone or in liquid air and the system was evacuated to a pressure of 10<sup>-1</sup> mm of mercury. Soon after the cell was immersed in the cold bath (-15° to -35° C) dendrite branches started to grow on the water surface. Before it was completely covered with a crust of ice the water surface became hemispherical in shape. Solidification then continued around the edge of the hemisphere and a short section of the tube was thus formed. The continuous flow of water maintained the droplet at the tip of the tube resulting in steady growth.

Some of the tubes were longer than 100 mm and were slightly tapered toward the tip (Fig. 2a). The average diameter varied between 1 and 3 mm with a wall thickness of about 0.2 mm. It was frequently found that the diameter decreased abruptly once or twice during growth. Both the diameter and the length of the secondary tubes were about one order of magnitude smaller than the same dimensions of the primary tubes (Fig. 2b).

Crystallographic orientation was determined in polarized light and with the etch pit technique developed by Higuchi<sup>7</sup>. The specimens were found to be single, bi-, or tri-crystals of random orientation. In all cases investigated the grain boundaries in the bi- and tri-crystals were parallel to the main axis over the entire length of the specimen. Microscope observations showed that the wall of the tube was clear, but the frozen core occasionally contained small bubbles of air or vapour.

With this technique it was possible to observe the process of growth in great detail. The formation of the ice cover, the flow of water through the tube, and growth at the tip were observed visually. The existence of a high water pressure due to freezing was demonstrated by reversing the process which supplied the water flow. When, during tube formation, the cell was lifted from the bath and the ice crust inside the cold finger wall melted, the consequent volume contraction drained the water from the tube of ice. Incidentally, this technique was used to produce hollow tubes. These observations support Dorsey's theory and provide further insight into the mechanism. In general, the rate of extraction of heat from the reservoir (controlled in the present experiments by the temperature and geometry of the bath) determines the rate of flow of water through the tube. The water transported to the tip of the tube has, however, to be frozen with an appropriate speed which is governed by the evaporation rate (pumping speed and trap temperature). The vapour pressure of the water droplet is greater than that of the ice because of its higher temperature and convex surface. Cooling by evaporation is thus concentrated on the water droplet and this method of heat extraction is preferable.

In steady state the amount of water transported from the reservoir in unit time is

$$V = r_i^2 \pi l + (r_o^2 - r_i^2) \pi l s + 2 \pi r_o^2 a \quad (1)$$

where  $r_i$  and  $r_o$  are the inside and outside radii of the tube, respectively;  $l$  is the tube length grown in unit time;  $s$  is the density of ice; and  $a$  is the amount of water evaporated from unit area in unit time. The condition of steady growth, not considering radiation and conduction, is that

$$2 \pi r_o^2 a L_e = (r_o^2 - r_i^2) \pi l s L_f \quad (2)$$

where  $L_e$  and  $L_f$  are the specific heats of evaporation and

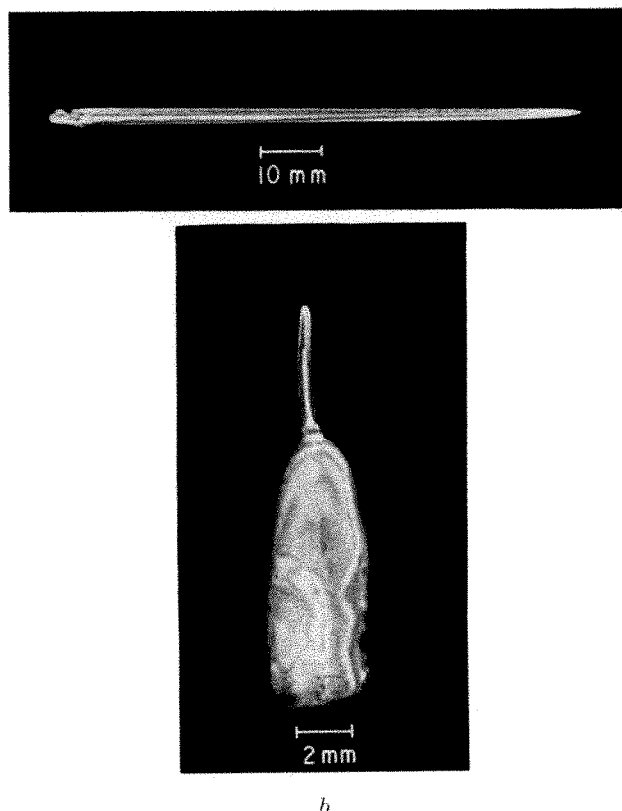


Fig. 2. a, Typical ice needle. b, Typical primary and secondary needle formation viewed in polarized light.

freezing, respectively. From equations 1 and 2 the following expression is obtained

$$\frac{V}{\pi} = r_i^2 l + 2r_o^2 a \left( \frac{L_e}{L_f} + l \right) \quad (3)$$

The relatively wide range of conditions under which growth can take place shows that the hemispherical drop at the tip of the tube can accommodate significant changes in  $V$  and in  $a$ . Thus when  $V$  becomes greater than that corresponding to the established equilibrium, the surplus amount of water increases the droplet size and growth continues with increasing  $r_o$  until the steady growth condition described by equations 1 and 2 is again satisfied.

The predictions of equations 1 and 2 were substantiated by experiments, but it is realized that in practice the process is made feasible only by the high surface tension of water and the apparently low polarity of the ice surface<sup>8</sup> which prevents overflow. It follows from our observations that a mechanism composed of water flow and heat flow alone is not sufficient to explain the process and that the introduction of a third component, the surface tension, is necessary. The realization of the essential features of the mechanism leads to the conclusion that tubes from substances other than water could be grown if an experimental technique could be devised to create a suitable mass flow and heat extraction and a balance between them. Indeed, tubes of benzene were grown with a mechanically controlled liquid flow at atmospheric pressure and  $-10^\circ\text{C}$ . In this instance, mass flow was created by artificially forcing benzene through the orifice of a syringe. The surface tension of benzene was high enough to maintain a droplet at the tip when growth was directed downward. The successful growth of benzene tubes is considered good evidence in support of the suggested mechanism. This mechanism thus explains all the previously reported tube formations under various conditions (open air<sup>1-3</sup>, vacuum<sup>5,6</sup>, undercooled<sup>6</sup>) and all the present observations. The recognition of the essential mechanism, furthermore, makes it possible to use this technique to grow tubes of a wide variety of materials.

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## CHEMISTRY

### Monomer Solvation of Ion Pairs in Anionic Polymerization

We wish to report some results obtained in a study of the anionic polymerization of styrene in benzene initiated by "living" poly- $\alpha$ -methylstyryl rubidium. Previous workers<sup>1</sup> have shown that at monomer concentrations of  $2 \times 10^{-2}$  mole l.<sup>-1</sup> and below the rate is first order with respect to both "living ends" and monomer, the rate constant for propagation ( $k_p$ ) being  $19.3 \text{ l. mole}^{-1} \text{ sec}^{-1}$  at  $19.5^\circ\text{C}$ .

We have studied the rate of disappearance of styrene dilatometrically in the concentration range  $3 \times 10^{-3}$ – $1 \times 10^{-2}$  mole l.<sup>-1</sup> for the same system at the same temperature and find somewhat more complex behaviour (see Fig. 1). In the region where we can most accurately measure the monomer concentration (above  $2 \times 10^{-2}$

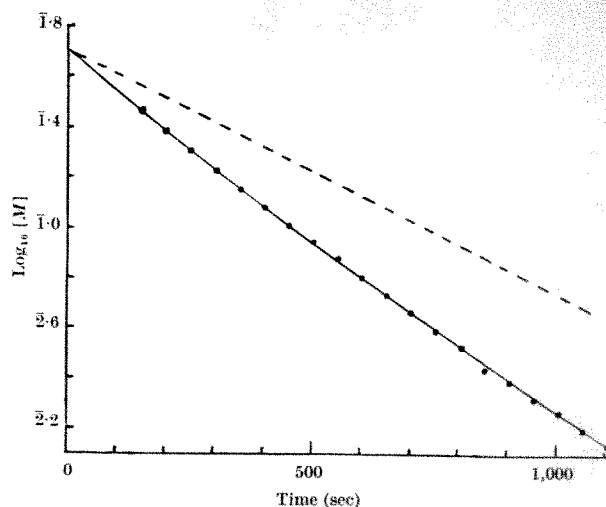
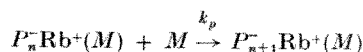
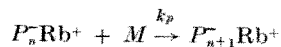


Fig. 1. A plot of  $\log_{10}$  [monomer] against time for run 71 at  $19.5^\circ\text{C}$ . The dotted line represents the predicted  $\log_{10}$  [monomer]-time line calculated from  $k_p = 19.3 \text{ l. mole}^{-1} \text{ sec}^{-1}$  and  $a_0 = 1.17 \times 10^{-4} \text{ mole l.}^{-1}$ .

mole l.<sup>-1</sup>, the reaction is always faster than the results of Roovers and Bywater would predict and is no longer first order with respect to the monomer. Below  $2 \times 10^{-2}$  mole l.<sup>-1</sup> monomer, it seems likely that the results are as predicted though we cannot be sure because the dilatometric data become increasingly unreliable in this range. After an examination of the possible experimental factors which can give rise to this discrepancy (for example, the purification of the materials and the dilatometry itself) we are forced to conclude that the data are correct and thus that a more complex mechanism is operating at monomer concentrations above  $2 \times 10^{-2}$  mole l.<sup>-1</sup>.

We suppose that at monomer concentrations above about  $10^{-2}$  mole l.<sup>-1</sup>, monomer solvated ion pairs are produced (in equilibrium with unsolvated ion pairs) and that they are active in the polymerization. The same mechanism has been proposed<sup>2</sup> to explain results obtained in the anionic polymerization of styrene in tetrahydrofuran at high monomer concentrations.

The mechanism proposed is



$K_2$  is the monomer solvation equilibrium constant.

Such a mechanism gives rise to the rate expression

$$\frac{-d[M]}{dt} = \frac{k_p a_0 [M] \left( 1 + \frac{k_p' K_2}{k_p} [M] \right)}{1 + K_2 [M]}$$

where  $a_0$  is the total concentration of ion pairs present. This equation can be integrated, but the resulting expression is difficult to test. We can rearrange the above equation, however, to the form

$$\frac{R_p}{[M]} = k_p' a_0 - \frac{(R_p - k_p a_0 [M])}{K_2 [M]^2}$$

where

$$R_p = -\frac{d[M]}{dt}$$

Measuring  $R_p$  from the contraction observed over a short time interval and using the value of  $k_p$  quoted earlier, it becomes possible to test the above expression (see Fig. 2).



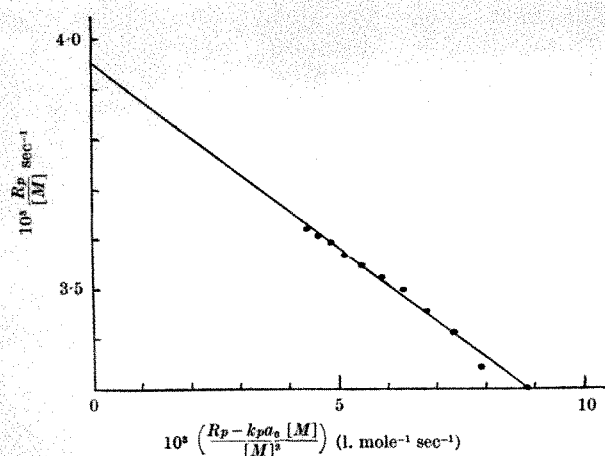


Fig. 2. A plot of  $R_p/[M]$  against  $\frac{R_p - k_p a_0 [M]}{[M]^2}$  for run 71 at 19.5° C.

Considering the errors involved, the result is satisfactory; Table 1 gives values of  $k'_p$  and  $K_2$  obtained from four runs at 19.5° C treated in the same way.

Table 1.

Run No.	$10^4 a_0$ (mole l. <sup>-1</sup> )	$k'_p$ (l. mole <sup>-1</sup> sec <sup>-1</sup> )	$K_2$ (l. mole <sup>-1</sup> )
69	0.56	36.8	15.8
71	1.17	33.7	13.6
76	2.74	37.1	6.1
77	2.02	39.3	15.2

Clearly the suggested mechanism seems to fit the data well, though more work is required to fix more definitely the values of  $k'_p$  and  $K_2$ . The value of  $k'_p$  indicates that the monomer solvated ion pair is about twice as reactive towards monomer as the ion pair which exists in the absence of monomer. The value of  $K_2$ , though less certain, does indicate that monomer solvated ion pairs should not be present to any great extent below 10<sup>-2</sup> molar styrene, in agreement with the data of Roovers and Bywater.

Because we also have data which indicate the presence of monomer solvated ion pairs when potassium is the counter-ion, and Schulz<sup>2</sup> has postulated their occurrence in tetrahydrofuran when sodium is the counter-ion, it is possible that this is a general phenomenon in all solvents and with all counter-ions. If this is so, it may well be significant in the comparison of results obtained from different systems, carried out in an attempt to understand the effect of solvent and counter-ion on the rate constant, the tacticity of the polymer produced, the interpretation of copolymerization data and the polymerization of monomers other than styrene.

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### Hydrocarbon Autoxidation followed by using the Oxygen-Hydrocarbon Charge-transfer Spectrum

It is well known that a solution of oxygen in an organic solvent produces a charge-transfer (CT) spectrum<sup>1-6</sup>, the intensity of which has in some cases been shown to be proportional to the oxygen concentration<sup>2,4,6</sup>. This spectrum is evidently "collisional" in origin, no stable complex being formed between oxygen and the organic compound<sup>2,4</sup>. We have followed the oxygen consumption in a hydrocarbon autoxidation by following the decay of

the CT spectrum. The technique is less sensitive than the traditional measurement of oxygen absorption from the gas phase into a vigorously stirred or shaken solution<sup>7,8</sup>, but it has the advantage of being a simpler technique, measuring oxygen consumption in the solution directly and requiring small amounts of materials.

Hydrocarbon at room temperature was saturated with oxygen in an optical cell which was then sealed with a perforated stopper and placed in the electrically heated cell holder of a 'Unicam SP 800 B' recording spectrophotometer. The stopper prevented oxygen from diffusing into or out of the solution, but allowed thermal expansion. The reaction temperature was reached in about 2 min. The decay of the oxygen-hydrocarbon CT spectrum with time was measured against air at a suitable wavelength. Typical results are shown in Fig. 1. Curve 1 shows the stability of the CT spectrum when oxygen is not being consumed. Curve 2 illustrates the steady depletion of oxygen in an autoxidation with constant initiation rate. Curve 3 shows how the autoxidation accelerates as a low initial concentration of retarder is consumed, attaining finally the same unretarded rate as in curve 2.

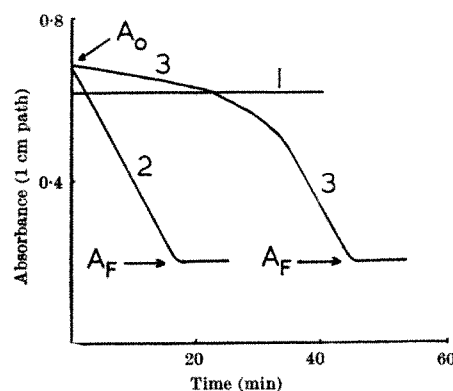


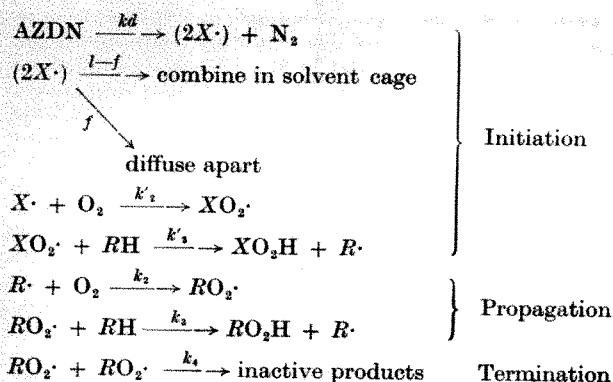
Fig. 1. Decay of oxygen-cumene CT spectrum at 60° C (measured against air at 293 mμ). Curve 1, cumene + oxygen; curve 2, cumene + oxygen + 10<sup>-3</sup> molar azo-di-isobutyronitrile; curve 3: cumene + oxygen + 10<sup>-2</sup> molar azo-di-isobutyronitrile + 10<sup>-4</sup> molar 2,6-di-*t*-butyl-4-methylphenol;  $A_0$  and  $A_F$  are initial and final absorbances.

The spectrophotometric data were converted into rates of oxygen consumption using equation (1), in which  $A_0$  and  $A_F$  are the initial and final absorbances of the solution and  $[O_2]_0$  the initial oxygen concentration. In some experiments

$$\frac{d[O_2]}{dt} = \frac{dA}{dt} \times \frac{[O_2]_0}{A_0 - A_F} \quad (1)$$

when the absorbance had fallen to  $A_F$ , the solutions were flushed with white spot nitrogen. No further change in absorbance occurred, thus confirming that all the dissolved oxygen had been used up.  $[O_2]_0$  was calculated from the solubility of oxygen in the hydrocarbon at room temperature, allowing for expansion of the hydrocarbon on heating. This solubility was measured using an adaptation of the method of Bateman, Bolland and Gee<sup>9</sup> in which oxygen was pumped from a known volume of saturated hydrocarbon and measured in a gas burette. The solubility was found to be  $8.5 \times 10^{-3}$  and  $8.6 \times 10^{-3}$  mole/l. in cumene and *p*-cymene respectively at an oxygen pressure of 760 torr.

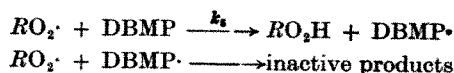
So far we have made a preliminary kinetic study of the autoxidations of cumene and *p*-cymene, followed at 293 and 305 mμ respectively, in the temperature range 56° to 74° C using azo-di-isobutyronitrile (AZDN) as thermal initiator. In the assumed mechanism<sup>10</sup> shown here,  $X$  is a radical from the initiator,  $f$  the initiator efficiency and  $RH$  the hydrocarbon.



Given stationary state kinetics, long kinetic chains and  $k_2[R\cdot][O_2] \gg k_3[RO_2\cdot][RH]$  it can be shown that

$$\frac{-d[O_2]}{dt} = R = \frac{k_3}{k_{-1}} [RH] R_{\text{eff}} \quad (2)$$

where  $R_i$  is the initiation rate ( $= 2fk_d[\text{AZDN}]$ ). In order to evaluate  $k_3/k_4$ ,<sup>1</sup> it was necessary to measure  $R_i$ . This was done by adding sufficient retarder (2,6-di-*t*-butyl-4-methylphenol, DBMP) to react with the  $\text{RO}_2\cdot$  radicals, making the termination reaction<sup>11,12</sup>



Measurements of the autoxidation rate during the retardation period were treated according to equation 3, in which  $t$  is time and  $[\text{DBMP}]_0$  the initial concentration of retarder

$$\frac{1}{(R-R_i)} = \frac{2k_5}{k_i} \cdot \frac{[\text{DBMP}]_0}{[\text{RH}]} \cdot \frac{1}{R_i} - \frac{k_5}{k_2} \cdot \frac{1}{[\text{RH}]} t \quad (3)$$

From the slope and intercept (on the  $t$  axis) of a linear plot of  $1/(R - R_i)$  against  $t$ ,  $k_5/k_3$ , and  $R_i$  were determined. The accuracy of  $k_5/k_3$  was limited by uncertainties in measuring low initial autoxidation rates.

In Table 1 our preliminary results are compared with earlier work from these laboratories; activation energies ( $E$ ) were derived from Arrhenius plots. Considering the differences in technique, agreement seems satisfactory. Howard and Robb<sup>13</sup> used a thermocouple to determine the heat liberated in an autoxidation; Melville and Richards<sup>8</sup> relied on oxygen absorption measurements.

Table 1. AUTOXIDATION OF CUMENE AND *p*-CYMENE AT 56° C

$k_3/k_4$ (l. <sup>3</sup> /mole <sup>2</sup> /sec.) <sup>†</sup>	$E_3 - \frac{1}{2}E_4$ (kcal/mole)	$k_4/k_3$ Cumene $c. 1 \times 10^4$ $6 \times 10^4$ *	$E_1 - E_2$ (kcal/mole)	Ref.
$3.3 \times 10^{-3}$	c. 7.0		$c. -4.0$	This work
$2.6 \times 10^{-3}$	6.5		$-2.5$	13
$3.2 \times 10^{-3} \dagger$	6.7			8
$1.8 \times 10^{-3}$		$p$ -Cymene $c. 6 \times 10^3$ $4 \times 10^3$ *	$c. -5.0$	This work
$1.1 \times 10^{-3}$	c. 7.0		$-3.0$	13
	6.0			

\* Values of  $k_1$  reported in ref. 13 are really  $2 \times k_1$  and have been adjusted accordingly.

† Recalculated assuming an initiator efficiency of 0.5.

Finally, a plot of  $\log (R_i/[AZDN])$  against  $1/T$  ( $^{\circ}\text{K}$ ) gave  $E_a = 31$  kcal/mole, in agreement with previous work<sup>14</sup>. A more detailed account of the method will be published elsewhere.

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## Trifluoromethyl-benzimidazoles—a New Family of Acaricides

In a previous communication, the herbicidal activity of certain 2-trifluoromethyl-benzimidazoles having a free hydrogen in the 1 position was described<sup>1</sup>. Although these compounds also possessed promising insecticidal and acaricidal properties their practical use was precluded because of their phytotoxic properties. Further chemical studies made by Newbold and his group<sup>2</sup> have shown, however, that it is possible to retain, and occasionally enhance, the insecticidal activity of the parent benzimidazoles while simultaneously reducing their undesirable herbicidal and mammalian toxicity properties by suitable *N*-substitution. This communication describes the preliminary results obtained with one of these derivatives which has shown promising acaricidal properties—namely, 5,6-dichloro-1-phenoxy-carbonyl-2-trifluoromethyl benzimidazole, to which the code number NC 5016 has been allocated.

Pure NC 5016 is a colourless crystalline solid, melting at 103° C, with a vapour pressure of  $4 \times 10^{-5}$  mm of mercury and a solubility in water at 25° C of 13 p.p.m. Its solubilities in organic solvents are: benzene, more than 40 per cent; dioxane, more than 50 per cent; acetone, more than 50 per cent; ethanol, 2 per cent; and hexane, 1.5-2.0 per cent. The compound is highly lipophilic in nature, and its partition ratio between *cyclohexane* and water is 320 : 1.

The acute oral toxicity values in mg/kg obtained by Sanderson *et al.* are as follows: mouse, 1,600; rat, 240 (female), 280 (male); hamster, 260; guinea-pig, 60; chicken, 50; rabbit, sheep, pig and calf, about 30; dog and cat, more than 50. The acute dermal toxicity to the rat of the 25 per cent W.P. formulation was much greater than 1,000 mg/kg when a 24 h contact period was used. Thus the compound may be considered to have a moderately acute oral and a low dermal toxicity. Long term dietary feeding trials still in progress with rats show a no effect level of about 250 p.p.m. at the 6 month stage.

In the initial insecticide and acaricide tests NC 5016 was found to be active against eggs and adults of the greenhouse red spider, *Tetranychus telarius*, and larvae of cabbage white butterfly, *Pieris brassicae*, as shown in Table 1.

The compound was found to be inactive at normal test rates against the bean aphid, *Megoura viciae*; confused flour beetle, *Tribolium confusum*; brown ear tick, *Rhipicephalus appendiculatus*; housefly, *Musca domestica*:

Table 1

Species	NC 5016	LD <sub>50</sub> level	Standard
<i>Tetranychus telarius</i> adults	36 p.p.m.		10 p.p.m.*
<i>Tetranychus telarius</i> eggs	34 p.p.m.		60 p.p.m.*
<i>Pieris brassicae</i> second instar larvae	$7.8 \times 10^{-3}$ g/cm <sup>2</sup>		$4.5 \times 10^{-2}$ g/cm <sup>2</sup> †

\* Dimethoate standard.  
† Diazinon standard.

yellow fever mosquito, *Aedes aegypti*; German cockroach, *Blattella germanica*; and mealworm, *Tenebrio molitor*.

Further laboratory tests indicated that a single application of 0.03 per cent active ingredient to foliage of the French bean, *Phaseolus vulgaris*, would give effective control of adult *T. telarius* and their eggs for more than 24 days. The compound was also active against dimethoate, phenkapton and chlorobenzilate resistant strains of *T. urticae*. In both soil and foliar application tests no significant systemic insecticidal activity was recorded.

Field trials since 1965 in Britain, South and East Africa, India and Australia (Q. A. Geering, unpublished work) have confirmed the promising acaricidal activity established in these laboratory trials. In particular it is worth noting that good control on apples has been obtained of phosphate resistant *Panonychus ulmi*; and of phosphate-, dicofol- and tetradifon-resistant *Tetranychus urticae* and *T. cinnabarinus*. On citrus *Aceria sheldoni* and *Panonychus citri* have been controlled as well as *Oligonychus coffeae*, *Hemitarsonemus latus* and *Brevipalpus* spp. on tea.

Laboratory tests with worker bees have shown that the compound is inactive at normal application rates as a contact insecticide on glass and as a stomach poison in honey.

No results are available at present on the activity of the compound against other beneficial species, although the spectrum of insecticidal activity is sufficiently narrow to suggest that the hazard to such species would be low. This aspect and the potential development of resistance by mites are currently under investigation.

Fungicidal tests have shown that NC 5016 lacks the activity against mildews often associated with other acaricides.

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## IMMUNOLOGY

### Structural Differences within the Gamma G Class of Immunoglobulins

A METHOD of distinguishing two populations of human IgG has been reported<sup>1</sup> recently; it is based on their differing susceptibility towards proteolysis with papain in the absence of cysteine. A papain resistant form is separated intact from the cleavage products of the papain sensitive form by gel filtration on 'Sephadex G-150'. Further evidence for the existence of these two conformational forms of human IgG has since been obtained and it has been established (using conditions of varying concentrations of ethylenediamine tetra-acetic acid) that their differing behaviour on digestion with papain is not caused, for example, by the inactivation of the enzyme by traces of heavy metal ions bound to the resistant form.

We have now investigated the IgG of species such as the rabbit and horse, and this communication describes preliminary results obtained from an investigation of IgG isolated from a group of rabbits. This work was

undertaken to determine whether the conformational characteristics, responsible for differing susceptibility to papain, were influenced by differences in the allotypic determinants located on the heavy chains of rabbit IgG. It seemed to be significant that such determinants have been identified within the Fd part of the heavy chain<sup>2</sup>, close to the site of papain cleavage of the Fc fragment.

The IgG was isolated from the rabbit sera by the DEAE cellulose batch technique<sup>3</sup>, using 0.01 molar phosphate (pH 7.5) as eluent. It was first demonstrated, with preparations isolated from pooled rabbit sera, that rabbit IgG resembled human IgG in that it comprised both papain resistant and papain sensitive forms. Thus by comparing the amount of rabbit IgG recovered undigested (from 'Sephadex G-150' filtration of papain digests performed in the absence of cysteine) with the total quantity of protein treated, the preparation from pooled sera was found to comprise 45 per cent of papain resistant molecules. In the presence of cysteine, however, the total IgG was degraded rapidly and completely to fragments. As in previous work<sup>1</sup> on human IgG therefore it appears that reduction of intra (or inter) chain disulphide bond(s) is essential for cleavage of the papain resistant form.

The results obtained by determining the relative proportions of the papain sensitive and papain resistant forms in a series of rabbit IgG of known allotype are shown in Table 1 (which also includes the results of investigations of the pooled rabbit IgG already mentioned, pooled human IgG and IgG isolated from a rabbit antiserum raised against human leucocytes).

Although all possible rabbit IgG allotypes were not examined, no clear-cut correlation between papain sensitivity and allotype is apparent from this data. Table 1 shows that varying proportions of the papain resistant form, ranging from 66 per cent to almost 100 per cent, were found in the IgG preparations isolated from the sera of the individual rabbits, which were all in the "resting state". It is interesting to note, however, that whereas 85 per cent to 95 per cent of the IgG isolated from "resting" rabbits (7,558 and 7,606) of allotype 3/4 proved to be papain resistant, only 35 per cent of this form was found in the IgG isolated from an immunized rabbit (510) of the same allotype. This animal was one of a series which had been immunized (by six weekly intravenous injections and a recent booster injection) with a suspension of human leucocytes, in an investigation of the effect of anti-leucocyte sera on DNA synthesis by peripheral lymphocytes<sup>4</sup>.

Table 1

IgG sample	Allotype	Percentage papain resistant
Rabbit 5698	1/6	70
7534	1/5	90
7558	3/4	85
532		90
7726	1, 2/4, 5	80
7654	1, 3/4, 5	74
5934	2/4, 6	70
7629	2/4	66
7606	3/4	95
510 (anti-human leucocyte serum)	3/4	35
Pooled rabbit IgG		45
Individual human IgG (type "O" serum)		45

It is significant that, although immunoelectrophoresis of the rabbit anti-leucocyte serum (Fig. 1a) revealed the usual extended IgG precipitin arc (on diffusion against goat anti-rabbit serum), the yield of IgG isolated by the batch DEAE cellulose procedure<sup>3</sup> was unusually small (only 35 per cent). Furthermore, the isolated rabbit IgG showed a restricted IgG precipitin arc of slow mobility (Fig. 1b). The finding therefore that this preparation was comprised predominantly (65 per cent) of papain sensitive molecules agreed with other evidence that electrophoretically slow (human myeloma) IgG molecules are sensitive to papain<sup>5,6</sup>, and that the proportion of



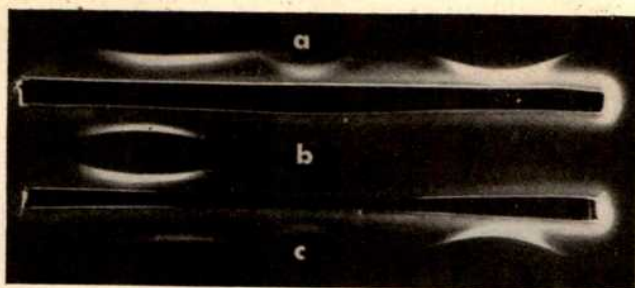


Fig. 1. Immunoelectrophoretic patterns of (a) rabbit 510 serum, (b) rabbit 510 IgG, (c) 0.5 molar sodium chloride eluate of the DEAE cellulose used to prepare (b). (Antiserum: goat anti-rabbit serum.)

papain sensitive molecules found in preparations of IgG depends on their mode of isolation. Because DEAE cellulose, under the conditions of elution which were used (that is, 0.01 molar phosphate, pH 7.5), binds molecules which are negatively charged, the IgG isolated from the rabbit anti-leucocyte antiserum (510) can be assumed to be that portion which is relatively uncharged (that is, the least negative, electrophoretically "slow", molecules). The electrophoretically faster IgG, on the other hand, would be expected to bind to the exchanger in such conditions. This was indicated by immunoelectrophoretic analysis of the IgG fraction of the rabbit anti-lymphocyte serum recovered from the DEAE cellulose by subsequent elution with 0.01 molar phosphate buffer (pH 7.5) containing 0.5 molar sodium chloride. As will be seen from Fig. 1c, the IgG thus eluted gave a relatively fast precipitin arc (on reaction with goat anti-rabbit serum).

It seems likely therefore that the serum of rabbit 510 contained a large concentration of relatively "fast" circulating antibodies, which bind to the DEAE cellulose in 0.01 molar phosphate at pH 7.5. The finding of Sela and Mozes<sup>7</sup> that a positively charged antigen elicits the production of negatively charged antibody molecules and vice versa suggests that the antigenic determinants of the human leucocytes used for immunization possess a net positive charge. This possibility is being examined further in investigations on preparations of IgG isolated from serial samples of rabbit sera, before and after immunization with leucocytes and various well defined antigens.

In the present studies on rabbit IgG, a 4 h incubation period was used in all cases, and digestion to fragments was almost complete when the incubation was carried out in the presence of cysteine. Goodman<sup>8</sup> reported, however, that rabbit IgG is heterogeneous with respect to its cleavage by papain, even in the presence of cysteine and that the most resistant molecules have a large content of carbohydrate. Other workers<sup>9</sup> have shown that human IgG is also heterogeneous with respect to carbohydrate content; the greater the content the faster its electrophoretic mobility. Thus in the light of our findings, it is possible that the relatively papain resistant molecules observed by Goodman originated from the fast mobility fraction. It is of further interest that Fleischman<sup>9</sup> and Utsumi and Karush<sup>10</sup> have concluded, from structural investigations, that there are two points of attachment of carbohydrate to the heavy chain of rabbit IgG. One moiety is recovered in the Fc fragment on papain cleavage, while the second, which is released as a glycopeptide, is located on the heavy chain in the area at which cleavage occurs. If the carbohydrate heterogeneity of the IgG molecules is expressed by the moiety attached to the latter region, then it is possible that molecules with a large content of carbohydrate possess electrostatic (and/or stereochemical) properties which exclude their cleavage by papain without previous unfolding, for example, by the reducing action of cysteine, as suggested here and in our previous work<sup>1</sup>.

In summary, no obvious relationships were observed between rabbit IgG of various allotypes and their susceptibility to papain proteolysis. Immunization seemed to influence the proportion of papain resistant to papain sensitive forms, producing further evidence that papain sensitivity is a function of the surface charge (as reflected by electrophoretic mobility and DEAE cellulose chromatography) of the IgG molecule.

Our other findings (to be reported in detail elsewhere) suggest, however, that a similar effect is not elicited by the immunization of some mammalian species such as the horse.

Dr A. Kelus kindly supplied rabbit sera of known allotype.

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### Relative Efficiency of Immune Cytolysis by IgG and IgM Antibodies

BECAUSE Humphrey and Dourmashkin<sup>1</sup> have shown IgM antibody to be more efficient than IgG antibody in causing lysis of erythrocytes in the presence of complement, we considered it of importance to investigate the relative efficiency of IgG and IgM antibodies with regard to nucleated cells.

Antibody to rat kidney was produced in rabbits immunized with rat kidney homogenate emulsified with complete Freund's adjuvant. Monolayers of rat kidney cells were grown on coverslips, the medium consisting of Parker 199 and calf serum (for details of these methods see ref. 2). Globulin was separated from the rabbit serum, which showed complement fixation with rat kidney antigen at a titre of 1:4,096, and gave immune lysis of cultured rat kidney cells in the presence of complement. Globulin components were serially eluted from 'Sephadex G-200', using 0.15 molar phosphate buffer, pH 7.4, in 0.5 molar saline. Material from the two peaks was ultracentrifuged in a sucrose gradient by the method of Kunkel<sup>3</sup>. This demonstrated 19S globulin in the first and 7S in the second 'Sephadex' peak.

The IgG and IgM fractions were diluted to give comparable protein concentrations in the tissue culture medium, after inactivation at 56° for 30 min. Fresh guinea-pig serum was added to a final concentration of 10 per cent to act as a source of complement. Tissue cultures were incubated at 37°. In the presence of 0.74 mg/ml. IgM and complement all cells were destroyed by immune lysis at 24 h after such addition, while only small numbers of cells survived at a concentration of 0.37 mg/ml. On the other hand, a concentration of 0.76 mg IgG/ml. allowed considerable cell survival at 24 h, while at 0.38 mg/ml. there was a good growth of culture cells. As shown in Table 1, complement fixation (50 per cent haemolysis reading) was several times more efficient by IgM than by IgG antibody when considered on a weight



Table 1. COMPARISON OF COMPLEMENT FIXATION (C.F.) OF IgG AND IgM ANTIBODY

'Sephadex' fraction	Protein concentration giving C.F.
IgG	14.1 $\gamma$
IgM	3.6 $\gamma$

basis. Immune cytolysis, if comparison is made on a protein weight basis, thus shows only a marginal difference between IgG and IgM. Because our efforts to produce purified specific anti-kidney cell antibody failed, we were unable to make a comparison on a molecular basis.

Humphrey and Dourmashkin<sup>1</sup>, using isolated antibody against sheep erythrocytes, concluded that two to three molecules of 19S antibody were sufficient to make a hole in the erythrocyte envelope, while of the order of 1,000 times more molecules of 7S antibody were necessary for a similar effect. Greenbury, Moore and Nunn<sup>4</sup>, working with rabbit antibody directed against human A red blood cells, concluded that 19S antibody was more avid, and on a molecular basis 750 times more efficient at agglutinating red cells than 7S antibody. Using anti-Forssman serum and dog kidney culture cells, Sell and Spooner<sup>5</sup> found a relative inefficiency of IgG as opposed to IgM antibody in complement fixation. They found, however, a relative inability of this type of IgM antibody to produce cytolysis of the dog kidney cell; IgM was a much more efficient haemolytic agent than IgG. On a protein weight basis our results also appear to show an insignificantly greater cytolytic power by IgM than by IgG antibody, although this may well indicate that each IgM molecule is a more efficient agent than a corresponding IgG molecule. On the other hand, IgM appeared to be a more efficient complement fixing antibody than IgG.

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## BIOCHEMISTRY

### Isolation of Human DNA and its Demonstration by Electron Microscopy

MANY people have purified DNA by a variety of methods and measured its length by electron microscopy<sup>1-4</sup>. The DNA of bacteria and bacteriophage have been used frequently and salmon sperm and mammalian tissues have been used too.

Our experience with isolating human cancer deoxyribonucleo-protein (DNA-P) for use as antigens in immunological experiments has led us to purify further and examine DNA from normal as well as malignant material of human origin<sup>5-11</sup>. The technique which we use for these preparations is a modification of the method of Chargaff<sup>12</sup> and is based on other original work<sup>13-15</sup>. We wanted to demonstrate the DNA isolated from these preparations using electron microscopy so that we could further establish the nature of the antigens used.

All procedures were carried out at 4° C (unless otherwise stated) using aseptic techniques, sterile glassware and instruments, and fresh triple distilled water. The specimen was trimmed of all fat and connective tissue, and weighed and minced for 1 min in a Virtis or Waring homogenizer until it was moderately fine. The homogenized tissue was

washed with two or three volumes of normal saline by centrifugation at 16,000g (10,000 r.p.m.) for 10-20 min in stainless steel cups. This was repeated until the supernatant was visibly clear of fat and red blood cells. Many of the saline soluble proteins and RNA were also removed<sup>12</sup>. All these supernatants were discarded. An equal volume of 1 molar sodium chloride glycine/saline buffer (pH 8.2) was added to the tissue particles. This buffer was prepared by adding 5.85 g of sodium chloride to each 100 ml. of Hyland's glycine/saline buffer. The suspension was put through a Ten Broeck 'Pyrex' homogenizer until it was finely ground and homogeneous. The remaining connective tissue further separated at this stage, for it tended to remain in the grinder as fibrous masses. The resultant suspension was stirred gently, to avoid foaming, with a magnetic stirrer for several hours (usually between 2 and 4) in the cold. To avoid shearing a minimal stirring force was used. This stage was continued until a good gelatinous precipitate was obtained when a small amount of the saline supernatant was added to 95 per cent ethyl alcohol. The remnants of the tissue were removed from the suspension by centrifugation and discarded. The supernatant which contained the DNA-P was precipitated in two volumes of 95 per cent alcohol, pre-cooled to -25° C to counteract the heat produced at this stage<sup>4</sup>. The precipitate appeared in the form of gelatinous strands. The DNA-P was lifted from the solution with a wooden applicator, using a "spooling" action to wind it on the stick. It was washed in 70 per cent and then in 80 per cent ethanol. The DNA-P was redissolved in 200 ml. of 1 molar sodium chloride glycine/saline buffer by stirring overnight in the cold. (For a quantitative yield of DNA a larger quantity of solution is required and a longer time of stirring may be necessary depending on the original mass of tissue.) The solution was centrifuged for 10 min at 4,150g (5,500 r.p.m.) for the separation of the DNA-P from undissolved material. To the solution of DNA-P was added one-third of a volume of a 3:1 chloroform-iso-amyl alcohol mixture. This was stirred for 150 sec and spun for 10 min at 1,465g (3,000 r.p.m.) to effect good separation of the two phases. The saline phase was removed and the previous step was repeated at least three times. The final saline phase was then slowly injected into pre-cooled (-25° C) 95 per cent ethyl alcohol. The DNA fibres that formed were spooled and lifted to drain. The mass was washed by three centrifugations at 1,020g (2,500 r.p.m.) for 7 min using first 70 per cent, then 80 per cent, and finally 100 per cent ethanol. The excess alcohol from the final wash was aspirated from the DNA mass before it was allowed to air dry at 4° C. When dry it was weighed and a 0.1 per cent solution of DNA in 0.15 molar ammonium acetate was prepared. Grids for electron microscopy were prepared by spreading DNA-cytochrome c solutions according to the method of Kleinschmidt and Zahn<sup>3</sup>.

The DNA prepared by this method from a non-cancerous kidney was relatively free from other visible contaminating substances, as can be seen in the electron micrograph (Fig. 1). The average length of the filaments obtained by this method was found to be 1.6  $\mu$  and the weight average length was 3.0  $\mu$ . There are many reasons for this degraded state, not the least of which is the vigorous stirring used when the DNA-P was redissolved in sodium chloride glycine/saline buffer. Cavalieri *et al.*<sup>16</sup> have showed that the vigorous chloroform-amyl alcohol extraction of protein results in fractionation of the larger molecules at the interface so that they are preferentially precipitated with the nucleoproteins. This may account for some of the loss of longer molecules in our preparations, for we made no attempt to prevent this.

For the immunization of rabbits and humans, the antigenic material employed is the human DNA-P extracted by the second centrifugation. Maybe the DNA is already somewhat degraded at this stage, but the length of the fibres obtained on spooling the DNA-P from the solution



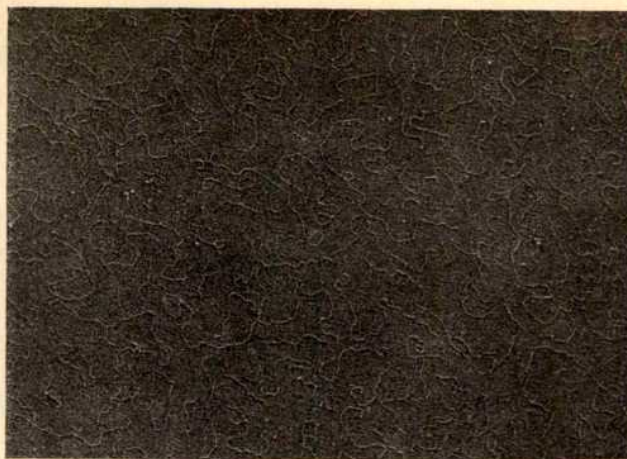


Fig. 1. Human kidney DNA, negative print. Weight average molecular length was  $3.0\mu$ . ( $\times 12,500$ .)

suggests that a high degree of degradation had not already occurred. Regardless of possible reduction of molecular size, the DNA-P retained its antigenicity. Further purification of these tissue extracts gives a purified product that resembles the DNA observed from other sources.

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### Cell Electrophoretic Mobilities in Friend Virus Disease

CHANGES in cell surface charge density have been reported several times in various types of malignancy<sup>1-4</sup>. One of the problems of applying the cell electrophoretic method to such problems is the need to obtain a quantity of single cells in suspension in a medium of known composition. The spleen, however, can be dispersed mechanically with ease, and we have chosen to investigate such spleen cell

suspensions electrophoretically during the course of Friend virus disease, where gross pathological changes occur in the spleen.

Friend virus was injected into young adult *BALB/c* mice. After various intervals, spleens from infected mice and comparable control animals were cut into small pieces and pressed gently through a nylon sieve into 0.145 molar sodium chloride buffered to pH 7.2 with 10 per cent v/v 0.1 molar phosphate buffer. After a few minutes, when debris and cell aggregates had sedimented out, the supernatant cell suspension was taken and washed twice in buffered saline by centrifugation and then resuspended to about  $2.5 \times 10^6$  cells/ml. to give a suspension suitable for electrophoresis. Viability tests were performed using lissamine green<sup>5</sup>, and suspensions containing more than 10 per cent of staining cells were discarded. Electrophoretic measurements were made in a cylindrical cell apparatus of the type described by Bangham *et al.*<sup>6</sup>.

In the saline buffered with phosphate which we used the electrophoretic mobilities of nucleated spleen cell suspensions from control animals showed a bimodal distribution. The two groups of cells had mobilities of  $-0.83 \pm 0.06$  and  $-1.17 \pm 0.08 \mu/\text{sec}/V/\text{cm}$ , respectively (Fig. 1A). In spleen cell suspensions taken from animals infected with Friend virus certain characteristic changes were seen. When the observations were grouped according to spleen weight, mobilities did not differ significantly from normal until the spleens reached about 0.3 g, that is, until about the tenth day of the disease. In larger spleens the ratio of the numbers of faster to slower moving cells increased, until in spleens of more than 0.6 g only fast moving cells were seen. A series of measurements on 187 separate cells from large spleens yielded an average mobility of  $-1.20 \pm 0.11 \mu/\text{sec}/V/\text{cm}$ . In Figs. 1B and 1C are plotted histograms of measurements made on spleens with mean weights 0.43 g and 0.86 g, respectively, which illustrate the aforementioned changes.

Sialic acid residues are important surface charge determinants in a variety of mammalian cell types<sup>7,8</sup>. Accordingly, some experiments were carried out in which spleen cell suspensions from control animals and from animals with advanced disease were incubated with neuraminidase before electrophoresis. Samples (2 ml.) of cell suspension,  $10^7$  cells/ml., were incubated for 30 min at 37° C with 500  $\mu$  of the enzyme. The cells were then washed and resuspended for measurement in buffered saline. Suspensions from normal spleens seemed to be unaffected, showing the same bimodal distribution and characteristic

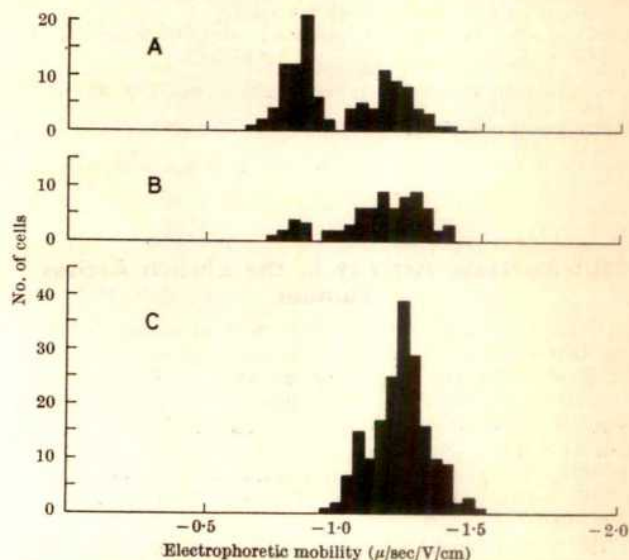


Fig. 1. (A) Spleen cells of normal mice with mean spleen weight of 0.12 g. (B) Spleen cells from mice infected with Friend virus with mean spleen weight of 0.43 g. (C) Spleen cells from Friend virus-infected mice with mean spleen weight of 0.86 g.



mobilities. In the suspensions of cells from large spleens, however, a proportion of the cells in the population suffered a considerable decrease in electrophoretic mobility. This gave rise once again to a bimodal distribution of mobilities, the two groups of cells having mobilities of  $-0.87 \pm 0.08$  and  $-1.21 \pm 0.09$   $\mu\text{sec/V/cm}$ , respectively.

The most obvious interpretation of these results is that during the course of the splenomegaly associated with Friend virus disease, cells of the normally occurring slow moving group acquire an enhanced surface charge which derives from neuraminidase-labile sialic acid residues. The initial results could equally well be interpreted in terms of a massive overgrowth of the faster moving cell type. The optical limitations of the cylindrical cell type of electrophoresis apparatus do not permit one to make any assessment of cell type on cytological grounds. We cannot therefore at present say what cells are to be found in each electrophoretic group in the normal spleen cell suspension, nor can we seek any confirmation of the foregoing interpretations of the results obtained. It must suffice to say that the cell population from the spleen of an animal with advanced Friend disease lacks an electrophoretically slow moving component present in normal populations of spleen cells, and that by treatment with neuraminidase the population from the diseased spleen can be resolved into two components with mobilities closely similar to those of the two components from normal spleens.

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### Ribonuclease Activity in the Ehrlich Ascites Tumour

PREVIOUS studies<sup>1</sup> have revealed a relationship between the distribution of intracellular and extracellular ribonucleases and the phase of growth of *Staphylococcus aureus* strain Duncan. The difficulty of distinguishing between intracellular and extracellular enzymes precludes similar studies on animal tissues, but it was considered desirable to examine such enzymes in the ascites tumour, where separation of cells and ascitic fluid presents no difficulty.

Ellem, Colter and Kuhn<sup>2</sup> have recorded the presence of extracellular and intracellular ribonuclease activity in the Ehrlich ascites tumour. With the strain of Ehrlich ascites tumour used in the present work, the situation

was found to be more complex and the results are recorded because of their relevance to studies of ribonucleases both in ascites tumours and in other animal tissues.

Tumour tissue was kindly supplied by Dr L. G. Lajtha of the Paterson Laboratories, Manchester. In most experiments suspensions of cells were withdrawn approximately eight days after implantation. In some cases, the cell suspensions were contaminated with blood and these were pooled separately. Cells were collected by centrifugation at 3,000*g* for 10 min, and washed twice with normal saline. The washed cells were suspended in 0.1 molar *tris* hydrochloric acid, pH 7.5, and homogenized in a glass homogenizer. Cell debris was removed by centrifugation at 3,000*g* for 30 min and ribonuclease activity was measured at pH 5.5 and pH 7.4, at which values activity was found to be maximal, by a modification of the method of Dickman, Aroskar and Kropf<sup>3</sup>. Enzyme activity was also determined using the ascitic fluid obtained in the first centrifugation. Assays were carried out in the presence of  $10^{-4}$  molar *p*-chloromercuribenzoate ions (PCMB), in presence of 4 molar urea, and without any addition to the incubation mixture.

In all cases, extracts of cells exhibited activity at pH 5.5 both in the presence and absence of PCMB or urea, but were devoid of activity in the alkaline range under all conditions used. In no conditions was activity observed at pH 5.5 in ascitic fluid. Measurement of activity on the alkaline side of neutrality revealed no activity in any conditions in uncontaminated fluid, but fluid contaminated with blood showed activity with an optimum pH of 7.4. This activity was increased by 80 per cent by the addition of PCMB. Contamination by blood was encountered more frequently in the later stages of the growth of the tumours, but no enzyme activity was found in uncontaminated fluid from tumours taken at any stage.

These results suggested that, in the strain of Ehrlich ascites tumour which was used, both the cells and fluid are devoid of alkaline ribonuclease and that the enzyme present in contaminated fluid is derived from the blood. Experiments showed the presence of ribonuclease activity in mouse blood plasma exhibiting maximal activity at pH 7.4. Insufficient blood was available to allow a detailed comparison of this enzyme with that of contaminated ascitic fluid and consequently a comparison was made with the enzyme from mouse pancreas.

Pancreas glands from eighty mice were frozen with solid carbon dioxide and ground in a mortar. All subsequent operations were carried out at 4° C. The powder was stirred for 30 min at 4° C with 100 ml. 0.25 normal sulphuric acid and the suspension centrifuged at 3,000*g*. The clear supernatant was submitted to fractional precipitation with ammonium sulphate. 95 per cent of the enzyme activity of the crude extract was precipitated between 60 and 70 per cent saturation. The solution of this fraction was dialysed against water and freeze-dried. The dried material (4.5 mg) was dissolved in 2 ml. of 0.05 molar *tris* hydrochloric acid (pH 7.5) and chromatographed on a column (1.2 cm × 30 cm) of DEAE cellulose using gradient elution, the initial and final eluting solutions being 0.05 molar *tris* hydrochloric acid (pH 7.5) and 0.2 molar *tris* hydrochloric acid (pH 6.8), respectively. Fractions were analysed for enzyme activity and protein was determined spectroscopically. Six peaks exhibited activity, the principal one containing approximately 49 per cent of the activity of the crude extract and showing a sixty-fold purification. Rechromatography of this fraction on DEAE cellulose gave a single protein-containing peak, possessing all the activity, and examination in the Spinco Model E centrifuge revealed only one component. Electrophoresis in the Tiselius apparatus, however, showed the material to be heterogeneous. The partially purified enzyme showed maximal activity at pH 7.4.

Contaminated cell suspensions from sixty mice were pooled and cooled to 0° C. All subsequent steps were carried out at 4° C. Cells were removed by centrifugation

and the fluid (440 ml.) was saturated with ammonium sulphate. The precipitate was collected by centrifuging and dialysed against water. The resulting solution was submitted to fractionation by the same procedures as described above. 84 per cent of the ribonuclease activity, measured at pH 7.4, was precipitated between 60 and 70 per cent saturation. Chromatography of the partially purified material on DEAE cellulose gave five fractions exhibiting activity. The fraction possessing highest specific activity contained 35 per cent of the activity of the crude protein solution, and was eluted at the same position as the principal enzyme fraction from pancreas. The material from this fraction had been purified forty times. It had maximal activity at pH 7.4 and resembled the pancreatic enzyme in its behaviour on ultracentrifugation and electrophoresis.

We consider that these results are consistent with the hypothesis that the alkaline ribonuclease of contaminated ascitic fluid in tumour-bearing mice is derived via the blood from the host tissues. With the object of obtaining experimental justification for this hypothesis, samples of ascitic fluid were taken from thirty mice, the interval after implantation varying from 4 to 11 days. Samples were individually analysed for ribonuclease activity at pH 7.4 and for iron<sup>4</sup>. The correlation coefficient calculated for these two functions was 0.93. This represents a close correlation and we conclude that the contamination by blood is responsible for the alkaline ribonuclease activity of the fluid of the strain of Ehrlich ascites tumour used.

From the results obtained, it appears that, in the strain of tumour used, both cells and fluid are normally devoid of active alkaline ribonuclease. It also appears probable that the enzyme activity found in the later stages of growth of the tumour is derived from the host tissues. In view of this, it is clear that care must be observed in evaluating the significance of levels of ribonuclease activity in animal tissues.

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### Autoradiography of Long-term RNA Metabolism in Rabbit Neurones

ATTEMPTS at studying the metabolism of RNA in neurones by the usual techniques of autoradiography have been hampered because parenteral injections of RNA precursors show very little uptake in the brain, while injections directly into the cerebrospinal fluid produce uneven distribution<sup>1,2</sup>.

We anaesthetized some 3 month old New Zealand white rabbits, weighing 2.5 kg, by intravenous injection of 30 mg of pentobarbital sodium. Puncture of the cisterna magna was then attempted, and if 1.0 c.c. of clear cerebrospinal fluid (CSF) could be removed easily, that CSF was replaced with artificial CSF in which a uridine tracer was dissolved. The tracer used in our experiment was uridine-5-H<sup>3</sup>, which cannot be incorporated into DNA as thymidylic acid unless the tritium label is replaced with a methyl group. Thus as a nucleic acid precursor, it is specific for RNA (ref. 3). No toxic effects were observed in the animals

at the doses used for as long as 50 days after injection of the tracer.

Animals were killed after anaesthesia by intracardiac perfusion with neutral formalin, their heads being packed in crushed ice. The partly fixed brains and spinal cords were then removed and immersed in Bouin fluid for 24 h of fixation. After dehydration in ethanol, representative levels of the cerebral hemispheres, cerebella, medullae oblongatae and cervical, thoracic and lumbar spinal cords were embedded in paraffin and sliced coronally in 4 $\mu$  sections.

The sections were mounted on large (1.5  $\times$  3 in.) glass slides, stained with haematoxylin and eosin, and then processed for autoradiography by the dipping technique<sup>4</sup>, using Kodak 'NTB2' emulsion. Sets of slides were exposed in the dark for 15, 45 and 135 days at 5°C in a dry atmosphere (maintained with 'Drierite' drying agent). Unstained sections were processed identically to make sure that the staining procedure had no effect on the distribution of silver grains.

The process of staining sections before coating them with emulsion evidently can cause desensitization of the latent image in an emulsion (B. M. Kopriwa and J. R. Shea, personal communication). If slides are not developed after a very short exposure time, the grain counts obtained at different intervals will not be a simple, definite function of time. Because of this, grain counts on slides with different exposure times are not comparable at absolute levels. The ratio of the nuclear to cytoplasmic grain counts, however, can be compared because the ratio is a variable that is independent of dosage, emulsion thickness, emulsion sensitivity and the thickness of the tissue section. To test the independence of this ratio from the size of the tracer dose, two animals were injected, one with 2.5  $\mu$ c., the other with 1,000  $\mu$ c. Both animals were killed 4 h later, and the values for the nuclear/cytoplasmic ratio obtained for each animal fell within 1 S.D. of the other.

Animals killed soon after injection were given the tracer in a high concentration in the hope that the labelled fraction of the total uridine incorporated in RNA during that period would be large enough to give significant grain counts. High specific activities were also given to animals to be killed a long time after injection, as it was thought there would be considerable degradation of uridine, with a parallel loss of labelled uridine (Table 1).

Table 1

Dose ( $\mu$ c.)	Time before death	Nuclear grain count (45 day exposure)
200	15 min	4,200
100	30 min	3,373
50	1 h	4,436
25	2 h	555
2.5	4 h	305
50	16 h	1,241
100	64 h	249
200	256 h	2,868
500	50 days	3,980

We found that, within an individual section, the distribution of the tracer was related to the distance from the site of the injection, the subarachnoid space, and the ventricles. The number of silver grains also varied with the type of cell beneath the emulsion. Relatively uniform distribution of the labelled uridine was found throughout the cells of the grey matter of the animals killed at intervals greater than 1 day after injection (Fig. 1) except in the lumbar cord, where there was very little activity even at 50 days. No activity greater than background was found in samples of liver or muscle taken from animals injected with up to 1,000  $\mu$ c. of tritiated uridine, where background was counted in an equal area of emulsion nearby, under which there was no tissue.

The intracellular distribution of the tracer varied characteristically with time; nuclear activity rose and



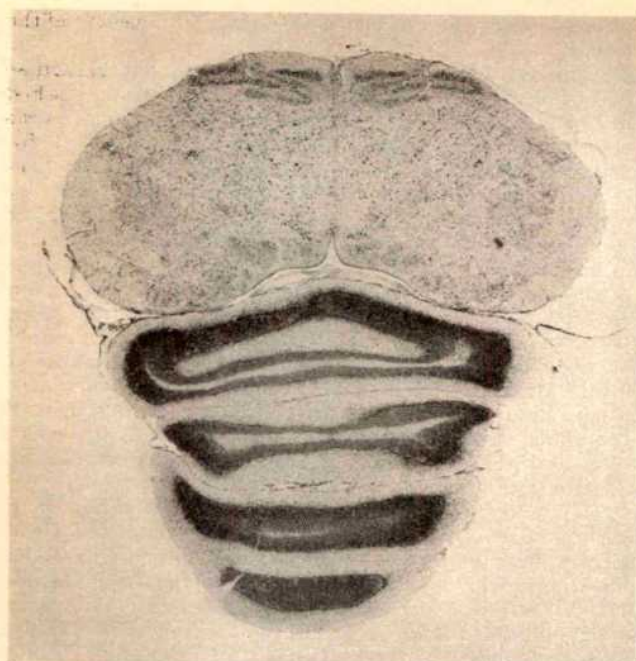


Fig. 1. Unstained 4 $\mu$  section of medulla and cerebellum from animal killed 10 days after injection of 200  $\mu$ c. uridine-5- $H^3$ : 171 day exposure.

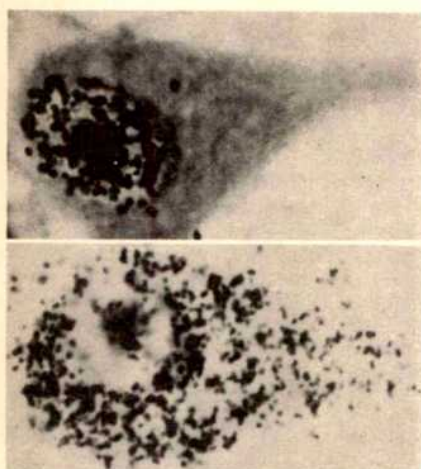


Fig. 2. Top: maximum nuclear concentration, 0.5 h after injecting 50  $\mu$ c.; 45 day exposure. Bottom: high cytoplasmic activity, 256 h after injection 200  $\mu$ c.; 135 day exposure.

fell rapidly, whereas cytoplasmic activity rose and fell more slowly. Nucleolar activity tended to be greater than the remaining nucleoplasm (Fig. 2). In choosing cells for detailed grain counting, the following criteria were required: the cytoplasmic and nuclear membranes had to be intact, the Nissl substance visible, at least two silver grains be over both nucleus and cytoplasm, and the cell be near the subarachnoid space. The variation in intracellular distribution of the tracer was studied in the neurones of the lamina pyramidalis of the area calcarina<sup>5</sup>, the hypoglossal nucleus, and the anterior horns of the cervical cord, and in the Purkinje cells of the cerebellum. The results from the Purkinje cells are presented below because these cells are the largest and most uniform of those studied, giving the smallest standard deviations. Results on the other cells were closely parallel.

Three exposure times were available for each set of slides. The set counted in each animal was the one in

which the average grain count in the nucleus or cytoplasm, whichever was greater, would be about fifty. This was felt to give enough grains so that the mistaken observation of a single grain would not greatly affect the total, yet few enough that distinguishing individual grains was not too difficult. Thirty cells were counted in each set, and independent observations on numerous cells were compared, with variations rarely greater than 2 per cent. Grains over nucleoli were included as a part of the nuclear count, as it was felt that accurate differentiation was impossible. The nuclear/cytoplasmic ratios obtained for the Purkinje cell are shown in Fig. 3.

The autoradiographs indicate only the location of uridine incorporated into large molecules. RNA precursor pools, soluble RNA and some messenger RNA were washed out of the 4 $\mu$  sections during histological processing (Shea, J. R., personal communication). Although the levels of uridine in the CSF were artificially high for a short period after the injection, this would not have accelerated the rate of RNA synthesis because the concentrations of the companion bases were not similarly altered.

Fig. 3 suggests that most of the labelled uridine was initially incorporated in RNA with a short turnover time and with a large proportion in the nucleus at any given time. The value of the nuclear/cytoplasmic ratio decreased slowly with time, but significant levels of activity were present in both nucleus and cytoplasm as long as 50 days after injection. This indicates that, as the RNA with a short turnover time is degraded, there is re-utilization of the labelled uridine within the brain. As the fraction of labelled uridine equilibrates throughout all the RNA uridine pools, the nuclear/cytoplasmic ratio approaches a constant value.

The conclusion that there is significant re-utilization of uridine in the neurone is supported by the observation that, although the tracer was initially confined to cells near the surface, after 24 h it was distributed throughout the brain. This observation suggests that most of the labelled uridine was initially picked up by cells near the surface and utilized in the synthesis of short-lived RNA. When this RNA was broken down, the uridine was released and gradually went into equilibrium with uridine in cells throughout the brain.

The absolute value of grain counts in the sections did not decrease rapidly with time. This suggests that uridine turnover in the brain, either by endogenous synthesis of uridine or by uptake from the blood, does not occur at a rapid rate. This may explain why the demon-

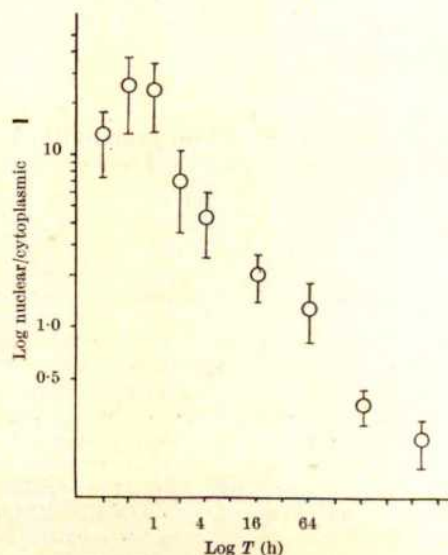


Fig. 3. Log-log relation between nuclear/cytoplasmic ratio and time.

stration of RNA metabolism by the intravenous injection of precursors is so difficult.

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### **In vitro Incorporation of Amino-acids into the Contractile Protein of Human Blood Platelets**

BLOOD platelets contain a considerable concentration of protein of which about 21 per cent can be accounted for by fibrinogen, serum albumin and a glycogen-globulin complex<sup>1</sup>. Most of the protein of the platelet consists of the specific contractile protein, thrombosthenin<sup>2,3</sup>, and other uncharacterized protein<sup>4</sup>. The origin of platelet proteins has not been established, but it is likely that albumin and fibrinogen originate in plasma, although no other plasma proteins have been detected in platelets<sup>5</sup>. Platelets are formed by fragmentation of the megakaryocyte<sup>4-6</sup> and do not contain a nucleus. It is possible that the contractile as well as other proteins are already present in the cytoplasm during the maturation of the megakaryocyte. It is also possible that certain proteins are synthesized by the platelet in which ribosomes have been observed<sup>7</sup>. This communication reports the incorporation of amino-acids labelled with carbon-14 into the contractile protein of human blood platelets.

Blood cells were separated by the method of Stefanini and Dameshek<sup>8</sup>, and contaminating erythrocytes were removed from the platelets by differential lysis in hypotonic saline as previously described<sup>1</sup>. The platelets were washed three times with 0.9 per cent sodium chloride and once with a solution of 3 per cent glucose and 0.9 per cent sodium chloride, which was the medium used for later incubations.

Washed platelets (500 mg wet weight) were suspended in 10 ml. of incubation medium containing 2  $\mu$ c. of a reconstituted mixture of labelled L-amino-acids (100  $\mu$ c./ml.). Cell suspensions were incubated at 37° C with continuous shaking. Samples of the incubation mixture were removed at 15 min intervals, chilled and 5 ml. of cold 0.6 normal perchloric acid was added. The precipitate was washed six times with 2 ml. portions of cold 0.6 normal perchloric acid and extracted at room temperature with 5 ml. of a chloroform-ethanol-ether mixture (1 : 2 : 2 v/v). The residue was heated at 70° C for 20 min with 5 ml. of 0.6 normal perchloric acid and the washed residue was dissolved in 1 ml. of 88 per cent formic acid. Samples (0.1 ml.) of the formic acid solution were pipetted on to glass fibre disks (Whatman, Gf/A, 2.1 cm diameter); the disks were dried at 65° C for 40 min and counted in a liquid scintillation counter (Packard 'Tri-Carb', Model 3000) by the method of Davis and Cocking<sup>9</sup>. Protein was determined on the neutralized formic acid samples by the method of Lowry *et al.*<sup>10</sup>. Fig. 1 shows the time course of appearance of the labelled amino-acids into material which is insoluble in hot perchloric acid.

The data presented in Table 1 show that puromycin inhibited the incorporation of labelled amino-acids into

Table 1. INHIBITION BY PUROMYCIN OF INCORPORATION OF LABELLED AMINO-ACIDS INTO MATERIAL INSOLUBLE IN HOT PERCHLORIC ACID

Treatment	Percentage inhibition*
Control	0
Puromycin, 0.69 mg/ml.	5
1.37 "	25
2.06 "	40
2.74 "	55
3.20 "	77

\* Inhibition is expressed as a percentage of the control.

material insoluble in hot perchloric acid. Platelets (250 mg wet weight cells) were incubated at 37° C for 75 min in 3 ml. of incubation medium containing 0.35  $\mu$ c./ml. of the labelled amino-acid mixture.

To determine whether amino-acids could be incorporated into a specific platelet protein, platelets were incubated for 5 h with the labelled amino-acid mixture and after centrifugation at 3,500g for 15 min the cells were washed with unlabelled incubation medium and then washed and suspended in a Weber-Edsall solution<sup>2</sup>. The contractile protein was isolated, purified and characterized as described before<sup>2,11</sup>. The contractile protein was reprecipitated four times and had a specific activity of 1,100 c.p.m./mg of protein. This is an appreciable specific activity in view of the considerable amount of the contractile protein present in these cells (15–20 per cent of the total protein). Dialysis for 48 h of 10 ml. of a 0.5 per cent solution of the contractile protein against repeated changes of 400 ml. of 0.025 molar imidazole-hydrochloric acid buffer, pH 7.0, containing 0.5 molar potassium chloride, did not decrease the radioactivity of the contractile protein. No radioactivity was removed from the contractile protein by electrophoresis on cellulose acetate strips at pH 8.6 and 4.5.

The purified contractile protein (10 mg) was treated with 5 ml. of cold 0.6 normal perchloric acid and then hydrolysed at 100° C for 15 min with 4 ml. of 6 normal hydrochloric acid. After removal of the hydrochloric acid by repeated evaporation to dryness, the residue was dissolved in 0.1 ml. of a pyridyl acetate buffer, pH 4.4 (150 ml. of acetic acid + 90 ml. of pyridine + 8 g of ethylenediamine tetraacetic acid made up to 12 l.), and subjected to high voltage electrophoresis on Whatman No. 3 paper, at 1,500 V for 80 min, followed by chromatography at a right angle to the direction of electrophoresis with 1-butanol-acetic acid-water (3 : 1 : 1 v/v)<sup>12</sup>. Fifteen fractions were detected with a ninhydrin spray. Each fraction was cut out, placed in low potassium glass bottles and

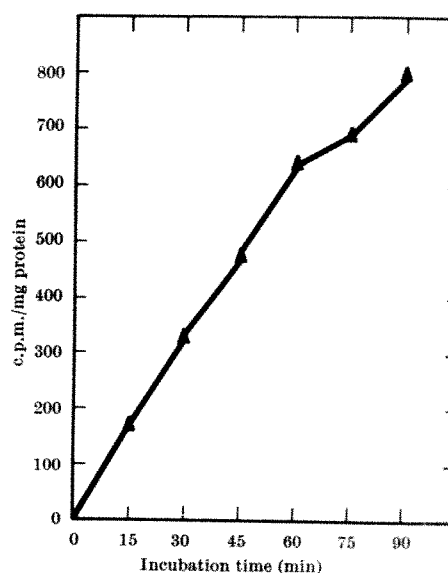


Fig. 1. Incorporation of labelled amino-acids into materials insoluble in perchloric acid. Platelets were incubated in a solution of 3 per cent glucose and 0.9 per cent sodium chloride at 37° C. Samples were removed at 15 min intervals and treated with 0.6 normal perchloric acid. The radioactivity of the washed and extracted residues was determined.



10 ml. of scintillator added (scintillation grade toluene with 0.4 per cent (w/v) 2,5-diphenyloxazole and 0.01 per cent (w/v) 1,4-bis-2-(5-phenyloxazolyl)-benzene<sup>9</sup>). All the ninhydrin-positive fractions were radioactive. Because of differential quenching by the ninhydrin chromogen, it was not possible to compare quantitatively the radioactivity recovered in the fifteen fractions with that present in the contractile protein.

In an attempt to find out more about the distribution of the labelled amino-acids in the contractile protein, the dinitrophenol contractile protein was prepared by a modification of the method of Sanger<sup>13</sup>. The DNP-protein was hydrolysed for 16 h with 6 normal hydrochloric acid at 110°C in a nitrogen atmosphere in a sealed tube. The dinitrophenol-amino-acid derivative(s) contained less than 8 per cent of the total radioactivity of the dinitrophenol-contractile protein.

We conclude from these experiments that labelled amino-acids were incorporated into the contractile protein molecule. The labelled amino-acids appear to be distributed throughout the entire molecule. We have recently reported the presence of a stable mRNA directing the *de novo* synthesis of contractile protein in platelets<sup>14</sup>. An active cell-free protein synthesizing system has been obtained from platelets. The predominant product of this system has been characterized as contractile protein. Isolation of this protein labelled carbon-14 from pulse labelled cells verifies that this is the predominant protein synthesized in platelets both *in vitro* and *in vivo*.

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Since this communication was submitted our results have been confirmed by Warshaw *et al.*<sup>15</sup>.

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### Interpretation of Subbands of Lactic Dehydrogenase Isozymes

THE lactic dehydrogenase (LDH) isozymes of tissue homogenates have been found in some cases to exhibit a regularly increasing number of subbands progressing from isozymes 1 to 5 (that is, 1 isozyme 1, 2 subbands of isozyme 2, 3 subbands of isozyme 3, etc.<sup>1-4</sup>. Such subbands have frequently been interpreted on the basis of a mutant allele, which results in the synthesis of two types

of the A subunits, of which isozyme 5 is composed (or of B subunits, of which isozyme 1 is composed, if the order of subbands is reversed). The hybridization of B subunits with two slightly different types of A subunits would account for the subband distributions observed<sup>2,3</sup>, and the presence of two allelic genes has been supported by genetic evidence<sup>3,4</sup>.

It has recently been demonstrated, however, that a regularly increasing number of subbands can be artificially produced with various human tissues, which did not originally exhibit any subbands<sup>5</sup>. This was accomplished by immersing the tissues in formaldehyde before they were homogenized. This process has now been studied further by producing subbands with formaldehyde in individual purified isozymes. The results obtained, and a consideration of their possible significance in the interpretation of subband patterns, are presented\* in this communication.

Human tissues were obtained from autopsy. The preparation of tissue homogenates, starch gel electrophoresis, and detection of LDH isozymes were conducted as previously described<sup>6</sup>. Purified isozymes were obtained by preparative starch gel electrophoresis.

Each starch gel section, containing an isolated isozyme, was saturated with water or an aqueous solution of formaldehyde, wrapped in 'Saran Wrap', and stored at 3°C for specified periods of time. The isolated isozymes, so processed, were applied to starch gels used for analytical electrophoresis by squeezing each starch section on a small piece of filter paper, and inserting this paper into the second starch gel.

Fig. 1 illustrates the production of subbands by the reaction of formaldehyde in isozymes 2 and 3. The subbands experimentally produced in this manner have a greater mobility than the original isozyme. The effect is not restricted to any particular tissue source or buffer used for the electrophoresis. (When whole homogenates were analysed with the Poulik buffer, the subbands were not clearly resolved<sup>5</sup>, possibly because of more extensive interactions with the isozymes.) The proportion of the total activity in the most rapidly migrating subband

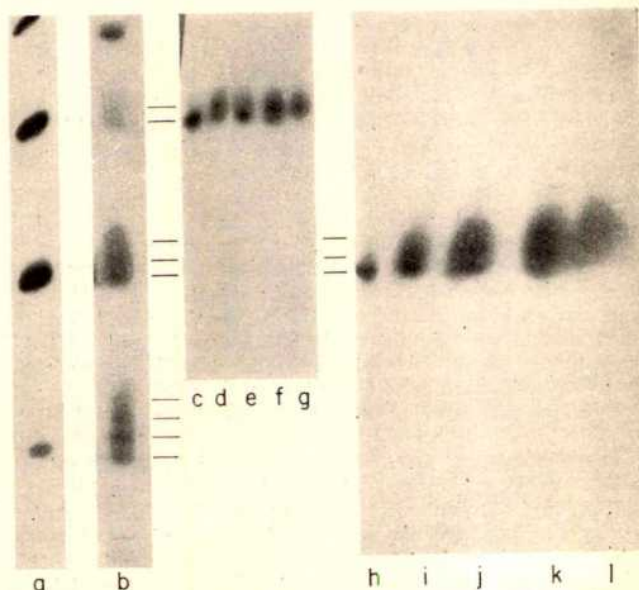


Fig. 1. Production of subbands by formaldehyde. (a) and (b) are samples of unfractionated testes; (b) but not (a) was treated with formaldehyde<sup>5</sup>. (c)–(g) are different portions of purified isozyme 2, from the kidney. (h)–(l) are portions of purified isozyme 3, from the testes. The volume per cent formaldehyde used for treatment was: (c), 0.0; (d), 0.3; (e), 0.3; (f), 1.0; (g), 2.0; (h), 0.0; (i), 0.25; (j), 0.3; (k), 1.0; and (l), 2.0. The time between formaldehyde treatment and application to the starch gel was 68 h for (d); 6 h for (e), (f), and (g); and 20 h for the others. The electrophoresis of samples (a)–(g) was conducted with a phosphate citrate buffer, pH 7.0, and of samples (h)–(l) with the discontinuous buffer of Poulik, pH 8.6 (ref. 5).



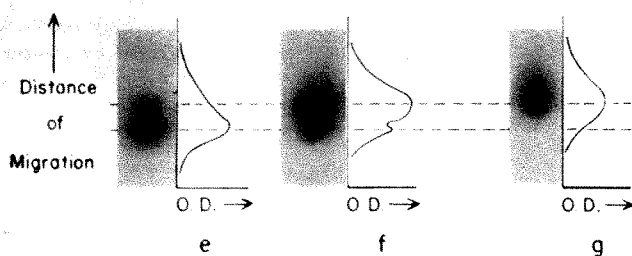


Fig. 2. Densitometer curves of samples (e), (f), and (g) of Fig. 1.

increases with increasing reaction time and with increasing formaldehyde concentration. When the concentration is sufficiently high, all of the activity is in the most forward subband.

The effect of formaldehyde concentration is also shown in Fig. 2. Samples (e), (f) and (g) of Fig. 1 were scanned by reflectance densitometry. These curves demonstrate the progressive shift in enzyme activity to the more rapidly migrating subband, as the formaldehyde concentration increases. They also indicate that the stain intensity (or enzyme activity) is of the same order of magnitude for the various samples. Because approximately the same amount of enzyme had been applied to the starch gel in each case, the production of subbands cannot be attributed to a selective inactivation of the enzyme. The production of subbands was not reversed by dialysis. Attempts to produce subbands with acetaldehyde were not successful.

The production of subbands with a purified isozyme demonstrates that no interaction with other isozymes is necessary. The simplest explanation of the results described would appear to be that formaldehyde reacts with a certain proportion of the A subunits, and that the proportion increases with time of reaction and with the formaldehyde concentration. If the A subunits which have reacted with formaldehyde have an increased charge and are designated as A', the various bands observed could be represented by the formulae BBBB for isozyme 1, BBBA and BBBA' for isozyme 2, BBAA, BBA'A' and BBAA' for isozyme 3, and so on. If the formaldehyde concentration is increased until all the subunits react, the subbands would be eliminated but the mobility of the isozymes would be increased. This appears to have occurred with sample (g) in Figs. 1 and 2. It has been reported by Fischer and Lauffer that the reaction of formaldehyde with some of the amino groups of tobacco mosaic virus is irreversible, and results in an increased electrophoretic mobility<sup>7</sup>. It is possible that the reaction of formaldehyde with LDH is of a similar nature.

Because formaldehyde can produce subbands with a greater mobility than that of the unsplit isozyme and 2-mercaptoethanol can produce subbands with less mobility<sup>1,8</sup>, it is possible that more than one type of modification of A subunits can be artificially produced. The presence of a number of subunit species may therefore be caused by either cellular synthesis, by chemical or structural modifications of portions of the subunits, or by a combination of both. (Some bands may also be caused by "nothing dehydrogenase"<sup>9</sup>, but their presence can readily be determined by the use of control gels, without substrate.)

In some cases, more subbands have been detected than can be accounted for by two types of A subunits, but not in a regular distribution pattern which would be anticipated for three or more A subunit species<sup>8,10</sup>. All of the subbands which would be anticipated for a given number of different subunits may not always be detected because of such factors as interactions<sup>8</sup>, lack of sufficient sensitivity or of sufficient resolution, or partial inactivation. Tissues are commonly stored frozen, which can cause both inactivation<sup>11</sup> and production of subbands<sup>12</sup>.

It is possible that the resistance of an LDH subunit to alterations may be affected by the inheritance of metabolic variations other than the synthesis of LDH. In such a case, the subband pattern could give a false impression of the inheritance of genes which control the synthesis of LDH subunits. Because a given number of subunit types, whether caused by cellular synthesis or by alterations after synthesis, can result in the same subband pattern, it may be concluded that the interpretation from subband patterns of the number of different LDH subunits synthesized should be made with caution.

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### Does 3,4-Dihydroxyphenylalanine Play a Part in Favism?

FAVISM is a haemolytic anaemia precipitated in some glucose-6-phosphate dehydrogenase (G-6-PD) deficient individuals by the ingestion of fava beans. Although the mechanism of haemolysis in G-6-PD deficient individuals has not been elucidated, it is generally thought that the destruction of reduced glutathione (GSH) is associated with the events which lead to haemolysis<sup>1-3</sup>. It has been shown that drugs which cause haemolysis in G-6-PD deficient individuals produce a loss of GSH in their erythrocytes *in vitro*, although it is usually necessary to use a higher concentration of the drug *in vitro*<sup>1</sup> than *in vivo*.

Fava beans contain some substances which are moderately strong reducing agents, and which could, according to the mechanism previously proposed, cause a loss of GSH in the erythrocytes<sup>4</sup>. One of these agents,  $\beta$ -(3,4-dihydroxyphenyl)-L-alanine (L-DOPA), has been detected in only a limited number of plants, but is present in relatively high concentration in fava beans, mainly in the free state<sup>5-7</sup>. We now present evidence that DOPA causes a loss of GSH in G-6-PD deficient erythrocytes.

Blood was obtained from normal individuals and from G-6-PD deficient males, Negroes and Caucasians. The red blood cells, washed three times in 0.15 molar sodium chloride, were suspended in 0.055 molar sodium chloride, 0.1 molar glycine-glycine, 0.03 molar phosphate buffer, pH 7.4, containing glucose. The suspensions of erythrocytes were incubated without and with added DOPA. Final concentrations were: glucose 10  $\mu$ M/ml. incubation mixture; haematocrit between 30-35 per cent in different experiments; DOPA 3.0, 1.5 and 0.75  $\mu$ M/ml. incubation mixtures. The mixtures were incubated in a metabolic shaker in the presence of air at 37°C for 3 h. GSH content of the incubation mixtures was determined according to the method of Beutler *et al.*<sup>8</sup>.

The results, shown in Table 1, demonstrate that a significant loss of GSH occurred in G-6-PD deficient erythrocytes when suspensions of these cells were incubated



Table 1. GSH LOSS IN NORMAL AND IN G-6-PD DEFICIENT ERYTHROCYTES INCUBATED IN THE PRESENCE OF DOPA

DOPA ( $\mu$ m/ml. inc. mix.)	GSH loss ( $\mu$ m/ml. inc. mix.)							
	Normal				G-6-PD defi- cient Negroes		G-6-PD defi- cient Caucasians	
Expt.: 1	2	3	4	1	2	1	2	
0.75				0.10	0.17	0.08	0.15	
1.50				0.17	0.23	0.20	0.23	
3.00	0.04	0.00	0.06	0.01	0.33	0.31	0.32	0.36
Maximum possible loss*	0.58	0.69	0.74	0.70	0.75	0.89	0.46	0.52

\* GSH content of control samples ( $\mu$ m/ml. incubation mixture).

in the presence of DOPA. Normal red blood cells were able to maintain their GSH content when incubated under the same conditions. Oxidation of GSH in normal erythrocytes by DOPA could be demonstrated only in the absence of glucose, that is, in the absence of a source which generated reduced nicotinamide-adenine dinucleotide phosphate.

The results reported here indicate that DOPA causes a non-stoichiometric loss of GSH in the G-6-PD deficient cells. Another agent with a similar action is divicine, the aglycone of vicine<sup>9,10</sup>. It is difficult at present to evaluate the participation of divicine and DOPA (Fig. 1) in oxidation of GSH by fava bean or fava bean extracts. Lack of information on possible synergistic effects (according to the multiple step mechanism previously described<sup>4</sup>) makes interpretation of results obtained with single compounds difficult. There are no data available on the fate of the glycosides *in vivo*, nor on the concentrations of the possible active agents in the blood.

Epinephrine causes some diminution of the glutathione content within the human red blood cell<sup>11</sup>. Non-enzymatic oxidation of DOPA and catecholamines, through reaction with oxyhaemoglobin, may play some part in normal physiology. The possibility also arises that DOPA and catecholamines contribute to the destruction of GSH under physiological conditions in G-6-PD deficient individuals, accounting in part for the shortened life span of erythrocytes found in these individuals without exposure to drugs<sup>12</sup>.

Because only some G-6-PD deficient individuals experience acute haemolysis after ingestion of fava beans, genetically determined factors may control the reaction to the active principles in fava beans<sup>13,14</sup>. The variation in the quantity and state of the potential active agents in the beans, as well as the mode of preparation, could account for the fact that individuals, suffering acute haemolysis induced by fava beans, may have eaten the beans many times previously or afterwards without

adverse reaction<sup>12</sup>. Further work is required on the active principles in fava bean and on the possible pathways for their metabolism in order to clarify the interaction between the genetic and environmental factors in favism.

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### Surface Potential of a di-Palmitoyl Lecithin Monolayer when Acetylcholine is in the [Sub-phase

Hyono and Kuriyama<sup>1</sup> described an unusual property of a monolayer of purified egg lecithin when potassium or acetylcholine ions are present in the sub-phase. They observed a plateau in the surface pressure-surface area characteristics that occurred at about 2 dynes/cm and at molecular areas greater than about 100 Å<sup>2</sup>. This type of isotherm had not been observed before in the case of potassium ions<sup>2,3</sup> and we did not find it in the case of potassium or acetylcholine ions either. We wish, however, to point out another property of these systems that may be of general interest.

In our experiments we used the same technique for measuring surface pressure, the vertical plate method (using a sand-blasted platinum plate which was easily cleaned in an oxidizing flame), but we used slightly greater concentrations of salt in the sub-phase. Our results for the surface pressure-surface area measurements at 25°C for monolayers of synthetic di-palmitoyl lecithin give an extrapolated value (from the linear region above 30 dynes/cm) for the molecular area at zero compression as 51.0 Å<sup>2</sup>/molecule. This value, as well as the general shape of the curve, agrees with values in the literature<sup>2-4</sup>. We wish to emphasize that the curve for lecithin did not change significantly when K<sup>+</sup>, NH<sub>4</sub><sup>+</sup> (CH<sub>3</sub>)<sub>4</sub>N<sup>+</sup> or acetylcholine were substituted for Na<sup>+</sup>. The observations of Hyono and Kuriyama may have resulted from the use of lecithin from egg yolk or, as they<sup>1</sup> suggest, from the spreading of the monolayer with benzene as the solvent.

Measurements of surface potential can also be used to characterize monolayers, and so we have made these measurements on monolayers of lecithin using a radium-226 air electrode and a Keithley Electrometer as described before<sup>5</sup>. Fig. 1 shows the curves for the variation in surface potential, ΔV, of a monolayer of synthetic di-palmitoyl

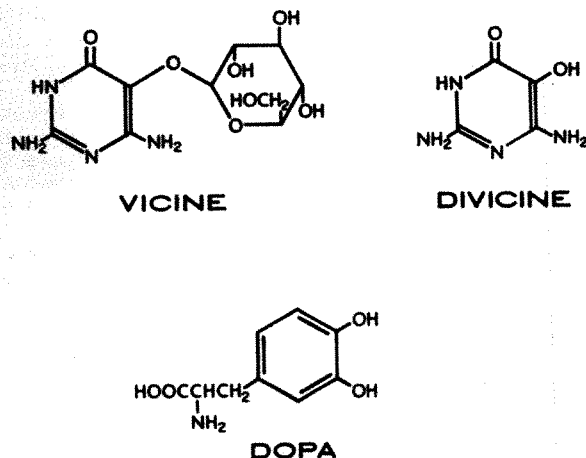


Fig. 1.

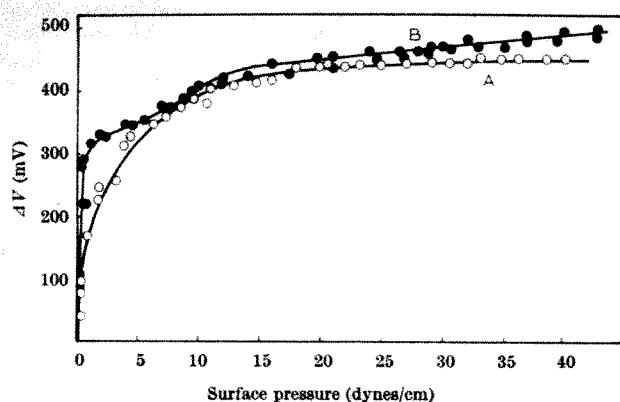


Fig. 1. The surface potential of a monolayer of lecithin as a function of the surface pressure. (A) The sub-phase contains 0.1 molar sodium chloride, and (B) the sub-phase contains 0.1 molar acetylcholine chloride.

lecithin as a function of surface pressure (curve A). These values agree with the values in the literature<sup>4,5</sup>. When acetylcholine is present in the sub-phase (curve B), the potentials are greater at all surface pressures and differ by about 50 mV at greater surface pressures. The increase in surface potential is most noticeable in the low pressure region. This change in potential was also found when  $\text{NH}_4^+$  or  $(\text{CH}_3)_4\text{N}^+$  were used instead of  $\text{Na}^+$ , but not when potassium ions were substituted. The data for  $\text{NH}_4^+$  tended to be slightly above curve B and those for  $(\text{CH}_3)_4\text{N}^+$  were slightly below. (This observation must be noted in conjunction with the known depolarizing properties of ammonium, tetraethylammonium, or acetylcholine on biological membranes.) The changes in potential occurred with no change in the surface isotherm emphasizing that the packing in the monolayer did not change appreciably at a time when the charge distribution was varying. When acetylcholine is present to a slight extent (0.01 molar) in addition to 0.1 molar  $\text{Na}^+$ , it has the same effect as 0.1 molar acetylcholine used alone. This no doubt results from the surface active nature of acetylcholine and may be a significant property in the physiological role of this substance.

These data indicate that acetylcholine somehow affects the surface properties of monolayers of lecithin but that the change in potential occurs with several agents ( $\text{NH}_4^+$ ,  $(\text{CH}_3)_4\text{N}^+$  and acetylcholine<sup>+</sup>) implying a rather non-specific type of interaction between fairly large positive ions and the amphoteric monolayers. The effects of the larger cations differ from those of  $\text{Na}^+$  and  $\text{K}^+$ , and so specific adsorption effects are considerable and the surface potential change need not be the same for all monovalent cations in the surface.

Although the changes in surface potential have been used in the past (for example, ref. 3) to analyse the orientation of dipoles in the surface, the interpretations in terms of dipoles caused by the spread molecule alone are always somewhat ambiguous, because of the unknown contribution of the water dipoles in the surface. This contribution may be considerable as has been shown in the case of the adsorption of a monolayer of benzene<sup>6</sup>, which is not even dipolar.

The data described in this communication are part of an attempt to determine the mechanical and electrical properties of monolayers of plasma membrane components as affected by the ionic environment and by substances which are known to change membrane permeability and potential. By investigating the effect of membrane depolarizing agents on monolayers of lecithin, it has been shown that depolarizing agents can affect the interfacial charge distribution or potential without altering the packing in the monolayer.

Although surface potentials have been used to investigate surface structure, their relevance to ion transport

processes in biological systems has been minimized largely because this kind of potential cannot account directly for the currents that flow across membranes. Changes in phase boundary potentials may affect the distribution and flow of other charged species, and so they may be involved indirectly in the mechanism of action of many substances on natural membranes.

In conclusion, although our measurements of surface pressure agree with those in the literature and are therefore clearly different from those reported by Hyono and Kuriyama, the measurements of surface potential indicate that acetylcholine (but not potassium) has a considerable effect on the monolayer of lecithin, especially at low surface pressure.

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### Increased Lactate Dehydrogenase 3 in Serum after Myocardial Infarction

SINCE the first report of lactate dehydrogenase (LDH) isoenzymes in human serum<sup>1</sup> a variety of different techniques has been employed to demonstrate them in serum and tissues. There is general agreement, however, that heart muscle contains predominantly the fast-moving isoenzymes lactate dehydrogenase 1 and 2 and that these are present in increased quantities in the sera of patients who have suffered a myocardial infarction, remaining demonstrably elevated for 1-3 weeks after the onset of the attack<sup>2</sup>.

While using cellulose acetate gel 'Cellogel' block as a support medium for electrophoresis of isoenzymes<sup>3</sup> we have noticed a prominent band of lactate dehydrogenase 3 in the sera of post-myocardial infarction cases, in addition to the increased bands of LDH1 and LDH2. Chromoscan reflectance scanning estimated this LDH3 to vary from 0 per cent to 30 per cent (Fig. 1) of the total content of isoenzyme. Very little, if any, LDH4 and 5 was detected, probably because samples were examined for the first 5 days only, before the liver damage, which sometimes follows myocardial infarction, had developed, and also because the inoculum was kept at concentrations compatible with accurate scanning. We felt this to be a valid finding, because we had carefully controlled the method and obtained good duplication of results. A similar degree of accuracy has been claimed for 'Strip' cellulose acetate<sup>4</sup>. We had not found these large concentrations of LDH3 when using starch or agar gels, and attributed them to the sensitivity of the 'Cellogel', because the electrophoretic run is short and heating effects at a minimum. It is well known that variations in the support media can affect the isoenzyme pattern<sup>5,6</sup>, as can buffer<sup>7</sup> and heat<sup>8</sup> which tends to inhibit the slower-moving isoenzymes.

It seemed that the increase in LDH3 could be explained in two ways. The increase could be part of a second iso-

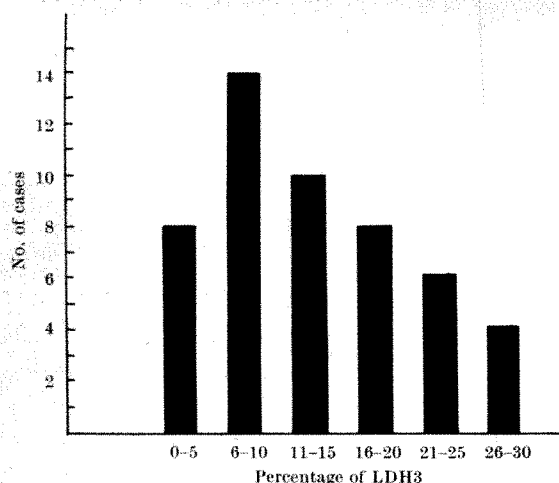


Fig. 1. Maximum activity of LDH3 found in the serum of fifty post-myocardial infarction cases. The largest number (fourteen of the fifty cases) had activities of LDH3 varying from 6 per cent to 10 per cent of the total isoenzyme activity; none had more than 30 per cent, and in one case no LDH3 at all was demonstrated.

enzyme pattern superimposed on that of myocardial infarction, which could occur with shock<sup>9,10</sup>. Alternatively, the increase could be caused by liberation from necrosed heart tissue, along with LDH1 and 2.

To investigate this finding and its possible clinical implications, we examined sera from fifty post-myocardial infarction cases, serially for the first 5 days from onset. The diagnosis had been firmly established on clinical grounds and by electrocardiographic evidence in the form of convex *S-T* segment elevation and deep symmetrical *T* wave inversion, followed by the development of *Q-S* complexes or abnormal *Q* waves (that is, of 0.04 sec or more in width). LDH3 seemed to achieve a peak excretion shortly after infarction (Fig. 2) and so we used this maximum as a parameter for comparison against the following clinical data, which included several factors associated with shock: (a) lowest blood pressure recorded; (b) pulse rate; (c) age; (d) sex; (e) site and size of infarct; (f) previous history of angina; and (g) recovery. No correlation at all with any of these features was apparent.

Several sera from cases brought to the casualty department in varying degrees of shock were examined, but a significantly elevated concentration of LDH3 was not

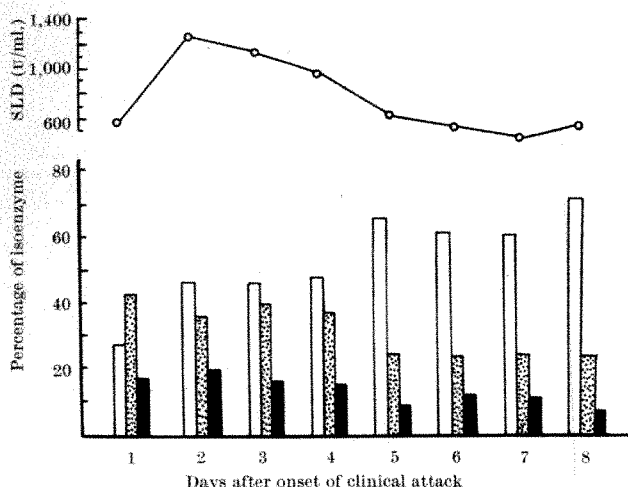


Fig. 2. Serial serum enzyme estimations from one of the fifty cases of myocardial infarction showing the relative distribution of the faster isoenzymes LDH1, 2 and 3. Peak excretion of LDH3 was on the second day, and a further infarction occurred on the seventh day. Black columns, LDH1; stippled columns, LDH2; white columns, LDH3.

found consistently. Changes were very non-specific when principal organs were not damaged and took more than 2 h to become apparent.

"Sludging" or aggregation of red blood cells in the arterioles has been described as a feature of shock<sup>11,12</sup>. The maximum content of LDH3 in the red blood cell appears to be about 15 per cent<sup>13</sup>. Our method of analysis enabled us to confirm this as an average figure, by examination of several normal red cell extracts, but we considered that if a red cell isoenzyme pattern had been present as well as that of myocardial infarction, an alteration in the ratio of LDH1:LDH2 would also occur, because of interference by the LDH1 and 2 of the red cell, which occurs in reverse ratio to those of heart muscle. This had not occurred nor had we ever detected haemoglobin absorption bands in the sera. Considering all these findings we felt the weight of evidence to be against the LDH3 being a product of shock.

We examined autopsy heart muscle from several cases of myocardial infarction. Taking sections across the necrosed, ischaemic, and normal heart tissue in close proximity, we found that the ratio of LDH1:LDH2:LDH3 was very similar for each individual section of tissue, although total activity of the enzyme was greatly reduced in the necrosed area, as would be expected (Table 1).

Table 1. RELATIVE PROPORTIONS OF LDH1, 2 AND 3 IN HEART MUSCLE TAKEN FROM AUTOPSY MATERIAL IN CASES OF MYOCARDIAL INFARCTION

Case	Necrosed tissue LDH (per cent)			Ischaemic tissue LDH (per cent)			Normal tissue LDH (per cent)		
	1	2	3	1	2	3	1	2	3
1	40	38	22	42	40	18	42	40	18
2	40	33	27	39	36	25	39	37	24
3	70	28	2	57	31	12	63	30	7

Table 2. RELATIVE PROPORTIONS OF LDH1, 2 AND 3 IN HEART TISSUE TAKEN FROM NORMAL HEARTS AT AUTOPSY

Case	Ventricle LDH (per cent)			Septum LDH (per cent)			Atrium LDH (per cent)		
	1	2	3	1	2	3	1	2	3
1	64	32	4	66	30	4	61	31	8
2	56	38	6	54	40	6	54	38	8
3	49	42	9	49	42	9	49	38	13
4	46	42	12	45	42	13	36	44	20

Tissue from normal human hearts showed variation in the ratios of LDH1:LDH2:LDH3 according to the site of the sample. Atria seemed to vary considerably (Table 2). The concentrations of LDH3 in the tissues were slightly lower than in the serum, and we attributed this to post-mortem changes and the greater instability of LDH3. We have found, for instance, that when serum from post-myocardial infarction cases stands on the bench overnight the activity of LDH3 decreases to a much greater extent than that of LDH1 and 2.

It is reasonable to suppose, therefore, that some loss of activity of LDH3 also occurs after death and, because of the relative freshness and ease of preservation of serum, the content of LDH3 in serum from post-myocardial infarction cases represents the content of this isoenzyme of heart muscle more accurately than tissue extracts. The greatest content of LDH3 in human heart muscle appears to have been given as 16 per cent<sup>14</sup>. Although there are many shortcomings in our method of analysis, it does have the advantage of permitting large numbers of specimens to be examined with only minimal loss of LDH3.

Our findings therefore indicate that (a) the source of the LDH3 band frequently found in the sera of post-myocardial infarction cases, when 'CelloGel' block is used for isoenzyme electrophoresis, is necrotic myocardium; (b) the content of LDH3 in human heart muscle is probably greater than previously reported; and (c) there is an individual variation in the content of LDH3 in human heart muscle.

Although we were unable to correlate the concentration of LDH3 with any clinical data, it is interesting to note that the only case where none of this isoenzyme was present was that of an Indian seaman who had suffered a myocardial infarction. Unfortunately, this was the only case of a non-European we examined.

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## Serum Iron Transport in the Fowl and the Mammal

It is known that in the vertebrates serum iron is transported as a transferrin complex and thus metallic toxicity in the ionic form does not occur. On the other hand, the existence of conalbumin<sup>1</sup> has been demonstrated in the serum of hens. It is a characteristic protein of egg white, the chemical and physical properties<sup>2-5</sup> of which are like those of serum iron. Immunological assay shows them to be identical<sup>5,6</sup>.

During laying periods in birds, the serum iron increases by a factor of almost five<sup>7,8</sup>. We have now observed that the serum iron values are higher than the iron binding capacity obtained by Ramsay's methods<sup>9,10</sup>. We have also observed this in other species<sup>11</sup> and we have suggested that there is an auxiliary mechanism responsible for serum transport in birds.

In the present investigation we have studied *in vitro* eighteen pooled sera from 280 fowls which had been divided into groups with similar characteristics (sex, age, laying period) and twenty-two sera from two species of mammals (lambs and bulls), in order to determine their iron transport capacities. The serum iron and the iron binding capacity were determined according to Ramsay's methods<sup>9,10</sup>.

The *in vitro* studies were carried out by the addition of known amounts of iron to the serum (70, 140, 210, 300, 400 and 500  $\mu$ g iron/100 ml. serum) and by the precipitation of the proteins by a saturated solution of ammonium sulphate. The determination of iron in the filtrate was made by Klein's technique for evaluation of the unsaturated iron binding capacity<sup>12</sup>.

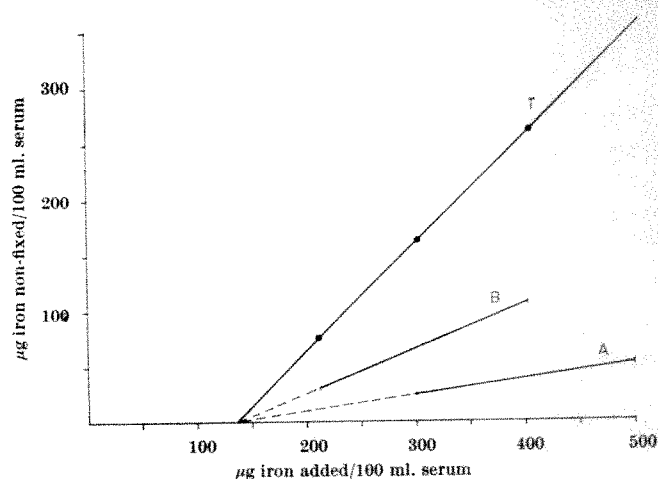


Fig. 1. Regression lines in hens (A), chickens (B) and the theoretical relation (T) between the iron added and the free iron in the serum.

The relation between the iron added (X) and the free iron (Y) in the filtrate was calculated for the chickens ( $Y = 0.426X - 61.0$ ) and for the hens ( $Y = 0.11X - 7.10$ ). The corresponding regression lines are drawn in Fig. 1. The theoretical line (T, Fig. 1) was obtained from the average values in the chickens (Table 1) by subtracting the iron binding capacity from the sum of the serum iron and the added iron. The data from laying and non-laying hens have been grouped together because they are indistinguishable. The similarities between the two sets are fallacious, however, because from Table 1 it can be seen that although the total unsaturated iron binding capacity is almost the same, the total iron binding capacity is higher in laying than in non-laying hens. This results from the clear-cut differences in the non-Ramsay iron binding capacity fraction. In the laying hens the unsaturated iron binding capacity is zero, as has previously been observed<sup>8,11,13</sup>. The unsaturated iron binding capacity of non-laying hens is similar to that of male chickens.

Table 1. SERUM IRON (SI), IRON BINDING CAPACITY (IBC), UNSATURATED IRON BINDING CAPACITY (UIBC) AND DATA ON *in vitro* FIXATION OF IRON IN HENS AND CHICKENS

Lot	No. of specimens	SI $\mu$ g iron (per cent)	IBC $\mu$ g iron (per cent)	UIBC $\mu$ g iron (per cent)	$\mu$ g free iron/100 ml. serum					
					70	140	210	300	400	500
Chickens										
I	18	90	240	150	0	0	45	105	140	
II	24	100	255	155	0	0	25	85	115	
III	24	120	260	140	0	0	25	50	90	
IV	24	100	200	100	0	0	10	40	95	
$m \pm a$		102 $\pm$ 6	239 $\pm$ 4	137 $\pm$ 6						
Hens										
I, L	18	500	370	0	0	0	0	20	25	35
II, L	20	530	300	0	0	0	0	20	50	55
III, NL	24	170	263	93	0	0	0	20	40	45
IV, NL	16	140	250	110	0	0	0	25	50	60
L. Laying; NL, non-laying.										

L, Laying; NL, non-laying.

The hen sera have the greatest capacity for fixing iron *in vitro* and this was clearly demonstrated with radioactive iron (Fig. 2). A dose of iron-59 was added (0.40  $\mu$ Ci/ml. serum) and the radioactivity was measured in samples of dry filtrate with a Philips Geiger-Müller counter. The serum proteins were precipitated with a saturated solution of ammonium sulphate.

In mammal sera the results of iron fixation *in vitro* fit theoretical predictions well (Fig. 3). The values which were obtained in these experiments showed the same unsaturated iron binding capacity in lambs and bulls (165  $\mu$ g iron/100 ml. serum), which has not been the case with



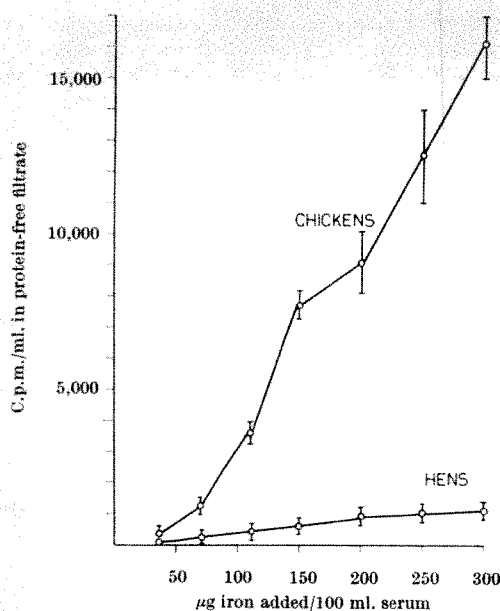


Fig. 2. Variations in the radioactivity in the protein-free serum filtrate after addition of the same amount of iron-59 and increasing concentrations of stable iron. The data are corrected to avoid the varying dilution of the radioactivity.

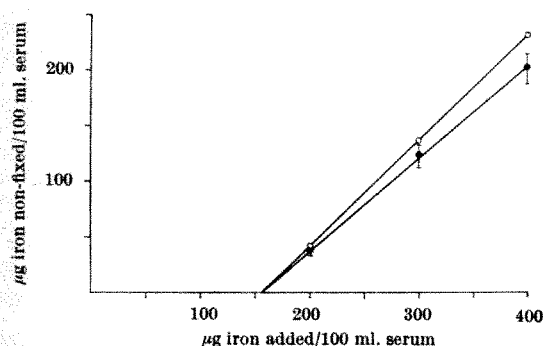


Fig. 3. Relation between the iron added and the free iron in the serum of mammals (●) against the theoretical values (○) obtained by subtracting the average values of the iron binding capacity from the sum of serum iron and µg iron added per cent.

other specimens<sup>14</sup>, and for this reason we have averaged all data from *in vitro* experiments.

Our studies on the transport of serum iron in birds point to the existence of an auxiliary mechanism in the transferrin system and suggest that the conalbumin mechanism is the more plausible. Marshall and Deutsch<sup>1</sup> have already described the relation of this protein to iron transport.

We have observed<sup>15</sup> increased values of serum conalbumin in hens, especially laying ones. The existence of the conalbumin in the serum may explain the inverted values of serum iron and iron binding capacity obtained by Ramsay's methods. The values for the iron binding capacity relate to the transferrin only, because when Ramsay's method<sup>10</sup> is applied to a conalbumin-iron solution, the magnesium carbonate used in it fixes all the iron from the complex and, in laying birds, determinations of the serum iron in serum previously treated with magnesium carbonate give values very near to those of the iron binding capacity<sup>11</sup>.

In the different species of birds studied<sup>15,16</sup>, we have found a proportionality between conalbumin serum concentration and the variations of the serum iron according to the frequency and intensity of the laying period. It has already been demonstrated<sup>17</sup> that iron metabolism is activated for egg production in laying birds.

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### Lipid Material in the Bone Marrow of a Patient with an Idiopathic Refractory Anaemia

RECENTLY, we found that bone marrow aspirated from a Nigerian boy with an unexplained refractory anaemia contained what appeared to be numerous crystals of a lipid material deposited within the fat globules.

The patient was a 10 year old boy from the Jos Plateau of Northern Nigeria. He had been suffering from general ill-health for about 4 years, during which time he had been given a large number of unspecified drugs by his father. A chronic refractory anaemia had been recognized for about 18 months. Both parents and three elder sisters were alive and well. On admission to University College Hospital, Ibadan (August 23, 1965), the boy was found to be well nourished and he showed no outward abnormality other than gross pallor.

The results of haematological investigations at this time were as follows:

Haemoglobin, 3.5 g per 100 ml.; packed cell volume, 11 per cent; mean corpuscular haemoglobin concentration, 32 per cent; reticulocyte index, 0.5; red cells were normochromic, showing great anisocytosis and slight poikilocytosis; white blood cells, 5,000/mm<sup>3</sup>; neutrophils 68 per cent; eosinophils 1 per cent; lymphocytes 29 per cent; monocytes 2 per cent; platelets less than 10,000/mm<sup>3</sup>.

Bone marrow aspirates from both anterior iliac crests and the sternum showed some hypoplastic fragments, but also many areas of erythroid hyperplasia with "megalo-blastoid" change; the white cell precursors appeared normal and the megakaryocytes were scanty. An outstanding feature of the marrow preparation was the crystalline deposit. Stainable intracellular iron was plentiful, but no ringed sideroblasts were seen. The periodic acid-Schiff staining was normal; the osmotic fragility, normal; haemoglobin electrophoresis, AA; serum vitamin B<sub>12</sub> (*Lactobacillus leichmannii*) 2,000 pg/ml. (upper limit of normal for Nigerians). The serum folic acid activity was not estimated because the patient had received folic acid before admission to the hospital. Studies of the survival of the patient's red blood cells by labelling them with chromium-51 showed a survival in the patient of 20.5 days (normal 25-35 days), but with a suggestion of two cell populations, one with a rapid destruction ( $T_{\frac{1}{2}}$  chromium-51 = 3.5 days).

The patient was observed in hospital for more than 2 months. During this time therapeutic trials of folic acid (5 mg/day), vitamin B<sub>12</sub> (500 µg/day intramuscularly



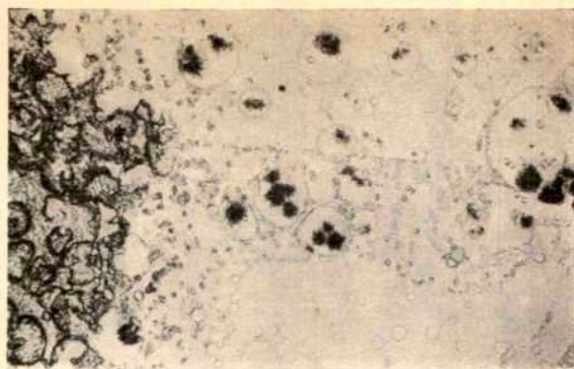


Fig. 1. Unstained smear of marrow tissue from the patient with unexplained refractory anaemia, fixed in methanol, showing numerous crystals within the spaces left by fat globules ( $\times c. 100$ ).

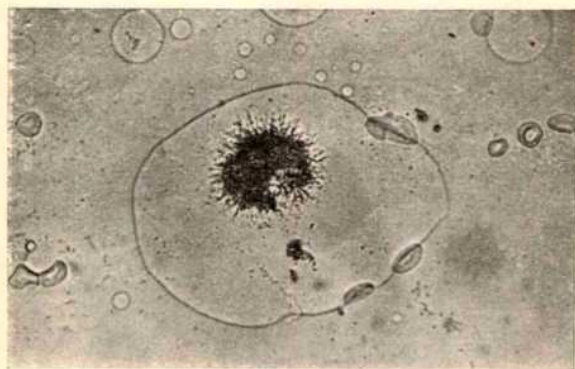


Fig. 2. A single deposit of crystals ( $\times c. 500$ ).

for 1 week) and pyridoxine (150 mg/day for 1 week) were carried out. The packed cell volume remained 9–13 per cent, the reticulocyte index 0.5–1.5, the white blood cells 700–3,400/mm<sup>3</sup>, and the platelets less than 10,000 to 23,000/mm<sup>3</sup>. After 2 months the patient was given a transfusion of fresh blood and sent home.

Bone marrow tissue removed from the boy was aspirated and smears made. As the smears dried a white efflorescence appeared and this became more obvious when the smears were fixed in absolute methanol for 20 min. Microscopically, the preparations showed irregular spherical crystals 15–25 $\mu$  in diameter within the spaces left by fat globules after fixation (Figs. 1 and 2). The material dissolved rapidly in the immersion oil, leaving behind a faint reticulum which stained light blue with May-Grünwald and Giemsa stain. It was also soluble in 70 per cent ethanol, acetone and a mixture of chloroform and methanol. It was insoluble in water, absolute alcohol and 0.1 normal hydrochloric acid. It was not stained by May-Grünwald and Giemsa, periodic acid-Schiff, ninhydrin or toluidine blue, but did take up sudan black and sudan III and IV.

On tissue culture the crystals grew out of the fat globules within 24 h. Similar crystals have been observed frequently, but only after many days of culture, and have been dismissed as unimportant artefacts of dying cultures.

Since the original observation, similar crystals showing these properties have been seen in many patients, including five out of thirteen patients with megaloblastic anaemia in pregnancy, four out of nine patients with anaemia secondary to chronic infection, and two out of seven patients with various malignant neoplasms. In all these patients the crystals were present in very small numbers.

Extracts of marrow tissue were investigated from the patient and from two patients with Hodgkin's disease and one with chronic nephritis. The three control marrows did not show a crystalline deposit. Marrow tissue which had previously been dried on glass slides was fixed in two changes of methanol for 40 min, and extracted with chloroform-methanol (2:1, v/v) according to the procedure of Folch *et al.*<sup>1</sup>. The lipid extract was washed with one-fifth volume of 0.1 molar potassium chloride and the resulting two phases separated by centrifugation. The upper aqueous phase, containing ganglioside-like substances and other water soluble substances, was siphoned off, dialysed against several volumes of water and evaporated to dryness in a rotary vacuum evaporator. The lower organic phase was washed several times with portions of the pure upper phase of chloroform-methanol-potassium chloride (3:48:47, v/v) (ref. 1). The resulting organic phase was evaporated to dryness.

The residue from the upper-phase fraction was dissolved in chloroform-methanol and subjected to thin-layer chromatography on silica gel G (Merck, A.G., Darmstadt). The chromatogram was developed in a mixture of *n*-propanol-water (7:3, v/v). Substances containing neuraminic acid were detected on the plates by spraying them with resorcinol reagent<sup>2</sup>. The residue from the lower organic phase, containing several lipid spots, was also subjected to thin-layer chromatography on silica gel G. The chromatogram was developed in a mixture of chloroform-methanol-water (65:25:4, v/v). The separate lipid components were detected by spraying the plates with 50 per cent sulphuric acid followed by heating.

Cholesterol esters, cholesterol and phospholipids were demonstrated in the lower phase fractions of all the marrow extracts examined, but no significant accumulations were observed either in the extracts from the patient with refractory anaemia or in the three controls. The upper aqueous fractions showed an accumulation of a material containing neuraminic acid in the patient with refractory anaemia, however, and only trace amounts in the three controls.

The aetiology of the anaemia in this patient remains obscure. There was a pancytopenia, and the marrow showed hypoplastic fragments and other fragments with considerable activity and abnormal normoblasts. These observations and the studies of the survival of red blood cells suggest inefficient erythropoiesis. Any part played by drugs is uncertain. The crystals of lipid material are not unusual and have been seen in more than a third of twenty-nine consecutive marrow aspirates. It is possible that they have not been reported before because they are unimpressive when only a few are present or when viewed under low-power and they dissolve in immersion oil. In this patient, crystals were very numerous and associated with an accumulation of a substance containing neuraminic acid as demonstrated by thin-layer chromatography. It seems likely that the two are the same.

A ganglioside-like substance (haematocide) is found in the stroma of erythrocytes<sup>3</sup> and further work is being carried out to ascertain the nature of the substance containing neuraminic acid found in the bone marrow of our patient. It seems possible that its accumulation may have some bearing on inefficient haematopoiesis.

We thank Professor R. J. V. Pulvertaft for carrying out tissue cultures and Dr N. C. Allan for access to haematological records and for performing red cell survival studies.

**Addendum** (April 18, 1967). A similar observation has been made in an Irish woman, aged 28 years, with refractory anaemia. She was seen in April 1966 with severe pancytopenia and a hypoplastic bone marrow picture. She has been treated with prednisone, folic acid, vitamin B<sub>12</sub> and blood transfusions with no permanent alteration in the peripheral blood picture. In August 1966 she became pregnant. A bone marrow collected in February 1967 showed both hypoplastic fragments and



areas of myeloid hyperplasia with small groups of between three and five plasma cells. The plasma protein pattern was normal. A marrow collected on April 14, 1967, showed hypoplastic fragments. In addition, this marrow showed a crystalline deposit, identical in morphology and solubility properties to the crystal described previously.

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### New Vasoconstrictor, Bovine Peptide B, released during Blood Coagulation

THE final stage of blood coagulation, the conversion of fibrinogen to fibrin by thrombin, is associated with the release of two acidic peptides designated A and B in the bovine system<sup>1</sup>. These peptides have been fully characterized by physico-chemical, chemical, and enzyme methods, and the exact amino-acid sequence has been determined<sup>2</sup>. Laki<sup>3</sup> has shown that the peptides released from fibrinogen affect the frog heart, and experiments carried out in this laboratory have shown that bovine peptide B (Fig. 1) in a concentration of  $4 \times 10^{-5}$  moles/l. produces a slow contraction of the rabbit carotid artery strip, while in a concentration of  $1.6 \times 10^{-6}$  moles/l. the peptide markedly potentiated the bradykinin-induced contraction of the isolated oestrous rat uterus. The present experiments were undertaken to ascertain whether bovine peptide B had any vasopressor effect *in vivo*.

Vascular responses were determined with a standardized bioassay technique in the rat previously described by Morris and Robinson<sup>4</sup>. In this bioassay, blood pressure in an anaesthetized rat chemically sympathectomized with pentolinium is measured by a mercury manometer connected to a carotid artery cannula. Increase in the blood pressure was measured after intravenous administration of angiotensin, oxytocin and bovine peptide B. The dose response for angiotensin is shown in Table 1. The mean increase in pressure elevation for a given dose is extremely reproducible and exhibits a small standard deviation ( $\pm 2.0$  mm of mercury). The maximum increase with intravenous angiotensin occurs in less than 1 min, and the blood pressure returns to the baseline in less than 3 min. The baseline pressure is constant within 2 mm for the duration of the experiments. The results of intravenous administration of bovine peptide B from three of the experiments are reported in Table 2. Peptide B for doses of  $4 \times 10^{-5}$ – $1.25 \times 10^{-2}$   $\mu$ moles gave a maximum increase in mean blood pressure of between 7 and 24 mm of mercury. The effect is approximately related to dose with insignificant effects noted at  $2 \times 10^{-5}$   $\mu$ moles and  $2.5 \times 10^{-2}$   $\mu$ moles and a broad maximum observed between  $8.0 \times 10^{-4}$   $\mu$ moles and  $6.2 \times 10^{-3}$   $\mu$ moles in these experiments.

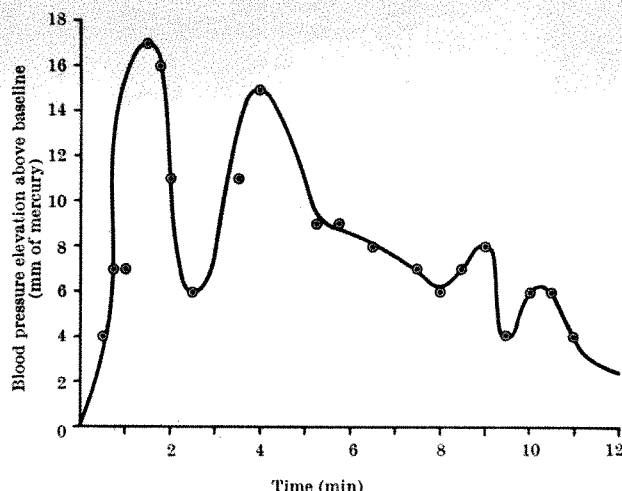


Fig. 2. Rhythmic response of blood pressure in mm of mercury above stable baseline after injection of  $8 \times 10^{-4}$   $\mu$ moles of bovine peptide B.

Table 2 shows that bovine peptide B is more active as a vasopressor in small concentrations, while in high concentrations the vasopressor effect is markedly reduced. In all cases, the duration of the vasopressor lasted longer than 8 min, and was observed to be as long as 18 min. A typical response is illustrated in Fig. 2. The pressor effect, in addition to being prolonged, has a rhythmic variation and this has been observed in all experiments.

Table 1. BLOOD PRESSURE RESPONSE TO STANDARD DOSES OF SYNTHETIC VALINE 5-ANGIOTENSIN II IN TWENTY-FIVE RATS PREPARED BY STANDARD BIOASSAY

Injected dose of angiotensin (m $\mu$ g)	Measured blood pressure above baseline in mm of mercury	Range in blood pressure response in mm of mercury	Standard deviation
100	70-25	68-73	1.42
50	52-12	49-57	1.65
25	38-0	35-42	1.80
12.5	28-08	25-32	1.70
6.25	21-42	18-24	1.68
3.125	14-60	12-18	1.63
1.560	11-33	9-14	1.43
0.780	8-38	7-10	0.27

The prolonged vasopressor effect is unlike that of any other natural vasopressor peptide. Oxytocin is the only substance which approaches the length of vasopressor effect produced by bovine peptide B. Vasopressin and angiotensin have relatively short vasopressor effects—the entire response lasts less than 5 min. This suggests that bovine peptide B is not destroyed as rapidly as other natural peptide vasopressors. The slow disappearance of the vasopressor effect of bovine peptide B may result from the fact that the "active region" of this peptide is located at a distance from the carboxy and amino terminals and therefore is less susceptible to enzyme inactivation by exopeptidases. Binding of the bovine peptide B to smooth muscle could also decrease the rate of inactivation by enzymes. This prolonged effect *in vivo* would be analogous to the prolonged action on the contraction of uterus muscle of the rat by one of the human peptides released during the blood coagulation process. The "active site" of this peptide was determined to be a small fragment from the interior of the peptide molecule<sup>5</sup>.

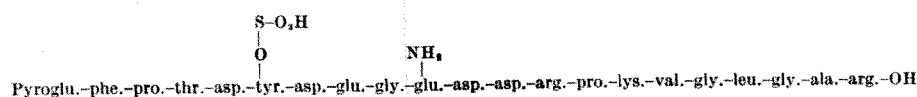


Fig. 1. The primary structure of bovine peptide B.

Table 2. VASOPRESSOR EFFECT OF BOVINE PEPTIDE-B COMPARED WITH ANGIOTENSIN

Experiment	Sample	Dose ( $\mu$ moles)	Maximum blood pressure rise (mm of mercury)	Time of maximum blood pressure rise (min)	Duration of vasopressor response (min)
1	Angiotensin	$5 \times 10^{-4}$	54	0.58	2.0
	Angiotensin	$2.5 \times 10^{-4}$	40	0.66	1.8
	Angiotensin	$1.25 \times 10^{-4}$	27	0.75	1.7
	Peptide-B	$2.5 \times 10^{-4}$	2	4.00	-
	Peptide-B	$1.6 \times 10^{-4}$	10	4.00	> 15
	Peptide-B	$1.25 \times 10^{-4}$	18	2.25	> 15
	Peptide-B	$6.2 \times 10^{-5}$	20	7.00	> 15
	Peptide-B	$6.2 \times 10^{-5}$	24	6.50	> 15
2	Angiotensin	$5 \times 10^{-4}$	52	0.58	1.9
	Peptide-B	$4 \times 10^{-4}$	14	5.00	> 15
	Peptide-B	$4 \times 10^{-4}$	15	1.50	> 15
3	Angiotensin	$5 \times 10^{-4}$	51	0.66	2.0
	Peptide-B	$2 \times 10^{-4}$	5	1.00	8.5
	Peptide-B	$4 \times 10^{-5}$	7	2.00	10.0
	Peptide-B	$2 \times 10^{-4}$	8	0.66	9.0
	Peptide-B	$4 \times 10^{-4}$	11	5.00	12.0
	Peptide-B	$8 \times 10^{-4}$	17	1.50	> 15.0
	Peptide-B	$1.6 \times 10^{-4}$	13	7.00	14.5
	Peptide-B	$3.2 \times 10^{-4}$	11	6.00	8.0

Oxytocin produces vasoconstriction in the rat bioassay with a rise of 16 mm in blood pressure that lasts 9 min after a dose of  $3.5 \times 10^{-3}$   $\mu$ moles. The vasopressor effect of bovine peptide B in a dose of  $4 \times 10^{-4}$   $\mu$ moles produces in the same bioassay preparation a rise in the blood pressure of 14 mm mercury and will last longer than 15 min. While oxytocin produces a rhythmic contraction of the uterus<sup>6</sup>, it does not produce a rhythmic vascular response. Bovine peptide B is here shown to produce a rhythmic vasopressor effect on blood pressure.

While the concentrations of bovine peptide B which produce an *in vivo* response are large, they still represent the amount of peptide ( $4 \times 10^{-4}$   $\mu$ moles) expected to be liberated during the clotting of 1 per cent of the total fibrinogen in rat plasma. If intravascular clotting is a continuous process, then this amount of endogenous peptide could be in the circulation. The concentrations producing vasoconstriction are more appropriate to the expected concentrations locally in an injured tissue, thus suggesting that the material acts physiologically.

The mechanism of action of bovine peptide B in producing vasoconstriction is unknown. Whether it acts directly on the smooth muscle of the smaller blood vessels, or through potentiation of a second substance, has not been determined. The prolonged vasopressor effect of bovine peptide B may be an *in vivo* expression of the persistent action of this substance in potentiating bradykinin *in vitro*, presumably by attaching to some smooth muscle receptor. A third possible mechanism of action is a release by bovine peptide B of a second substance which is vasopressor in action; however, this seems to be the least likely possibility.

The results demonstrating the production of a prolonged rhythmic vasoconstrictor substance at the site of clot formation during the chemical processes of blood coagulation fit well with a postulate that it is an important part of local haemostasis. Teleologically, it is reasonable to expect a vasoconstrictor substance to be present during clotting of blood *in vivo*, because this action would decrease the flow of blood and effectively prevent the washing away of a partly formed clot. Bovine peptide B would then disappear after completion of clot formation, when it is presumed that clot protector vasoconstriction is not needed. Whether chronic tissue injury with production of small concentrations of circulating bovine peptide B vasopressor has a role in the production of chronic hypertension is unknown, but remains a possibility and needs future investigation.

In conclusion, a new vasopressor substance produced during blood clotting is described. The effect of intravenous injection of bovine peptide B is a prolonged rhythmic vasoconstriction and is unlike any previously described vasopressor peptide. The possible role of bovine peptide

B is postulated as assisting in local haemostasis by vasoconstriction.

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## PATHOLOGY

### Induction of Tumours in Hamsters with Infectious Canine Hepatitis Virus

A NUMBER of adenoviruses which infect human beings and avian and other animal species have been shown to induce tumours in newborn golden Syrian hamsters. Included among these are several human adenoviruses<sup>1-3</sup>, chicken embryo lethal orphan (CELO) virus<sup>4</sup>, a number of simian adenoviruses<sup>5</sup>, and bovine adenovirus type 3 (ref. 10). We report here the induction of tumours in newborn hamsters by a canine adenovirus, the causative agent of infectious canine hepatitis (ICH). The tumour cells were shown to contain complement-fixing antigens for ICH virus.

Lederle strain 255 of ICH virus used in these studies was originally isolated in 1953<sup>11</sup> and was obtained from the American Type Culture Collection as third passage infected dog-liver suspension. A virus preparation (TCID<sub>50</sub>, titre 10<sup>7</sup>/ml.) was made by a single passage in canine kidney cells and inoculated into fourteen (three litters) newborn hamsters. Each hamster was inoculated with 0.1 ml. per site by intraperitoneal and subcutaneous routes.

Two of the fourteen inoculated hamsters developed 10-20 mm tumours at the site of inoculation, the first appearing on day 360 as a subcutaneous tumour and the



second as an intraperitoneal tumour on day 403. When the tumours reached a size of 30 mm (367 and 420 days after inoculation) they were surgically removed, minced to small pieces, and subcutaneously transplanted into newborn as well as weanling hamsters, most of which developed tumours (Table 1).

The serial subcutaneous transplants of the intraperitoneal tumour (hereafter referred to as IP line tumour) grew quite rapidly in newborn hamsters and reached sizes of 40 to 60 mm within 4–6 weeks, and the majority of these hamsters died within this time (Table 1). The growth of transplants in weanling hamsters was less rapid and some of the inoculated hamsters failed to develop tumours.

Grossly, the tumours were circumscribed pale grey masses containing small areas of haemorrhagic necrosis. The primary viral-induced subcutaneous tumour was a low grade fibrosarcoma which maintained its morphological characteristics on transplantation in hamsters. The primary intraperitoneal tumour was an undifferentiated neoplasm composed of sheets of large rounded to polygonal anaplastic cells with several foci of necrosis. Subcutaneous transplantation of this tumour through several hamster passages resulted in tumours which histologically resembled the primary intraperitoneal tumour, but which showed evidence of differentiation into whorls and bands of spindle cell sarcoma. In an occasional transplanted tumour the spindle cells were oriented about vascular

channels similar to the appearance of human adenovirus-induced hamster tumours. The basic cellular pattern was, however, that of spindle cell sarcoma in various stages of differentiation. Cell cultures established from the transplanted intraperitoneal tumour were of mixed epithelial and fibroblastic cell types. Hamster tumours induced by these cell cultures were morphologically similar to the primary viral-induced intraperitoneal tumour.

Attempts to isolate infectious ICH virus in canine kidney cells from clarified extracts of primary and transplanted tumours and tissue culture preparations of transplanted tumours repeatedly yielded negative results.

Hamsters developing large transplanted IP line tumours which survived beyond the fifth week usually developed complement-fixing serum antibodies which reacted with the homologous tumour antigen and with T antigens<sup>1,2</sup> of strain 255 and "Weybridge" strain\* of ICH virus prepared from infected canine kidney and ferret lung cultures (Tables 1 and 2). The antigen was present in titres of 1:16 to >1:80; the titre usually increased with the size and length of residence of the tumour in the hamster. Such complement-fixing antigens and antibodies were not detected in hamsters bearing the primary tumour or secondary transplants of the primary subcutaneous tumour.

The reactivity of the antigen and antibody described appeared to be specific for ICH hamster tumour system and ICH virus infected cultures; no cross reactions could be elicited with normal hamster tissue or canine kidney culture antigens or with heterologous hamster tumour antigens and antisera representative of those produced by human adenovirus types 7, 12, and 18, bovine adenovirus 3, SV<sub>40</sub>, polyoma, and Schmidt-Ruppin Rous sarcoma viruses. T antigens of human adenovirus types 7, 12, and bovine adenovirus 3 were similarly negative when tested with 4–8 units of complement-fixing antibodies from hamsters carrying the ICH virus-induced tumours<sup>5,10</sup>.

The specific reactivity of the latter sera with T antigens prepared from canine kidney cells infected with ICH virus is strong evidence that the hamster tumours observed were caused by ICH virus.

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\* This strain of ICH virus was received from Weybridge by Dr E. W. Hurst, passed twice in puppies and lyophilized in 1953; a sample of this preparation was kindly provided by Dr Trygve Berge, of the American Type Culture Collection, Washington, D.C.

Table 1. INDUCTION OF PRIMARY AND TRANSPLANTABLE TUMOURS WITH ICH VIRUS AND THE DEVELOPMENT OF COMPLEMENT-FIXING SERUM ANTIBODIES IN HAMSTERS REACTIVE WITH HOMOLOGOUS TUMOUR ANTIGEN AND ICH VIRUS INFECTED CANINE KIDNEY CULTURE ANTIGEN

Tumour	Hamster inoculated	Day observed*	No. with tumour	Complement-fixing antibody Day after inoculation	No. positive (>1:10)
Primary (intra-peritoneal)	Newborn	360	2/14†	367	0/2‡
Primary (subcutaneous)		403		420	
Transplant 1§	Newborn	29	18/18	32	4/7
	Weanling	53	7/10	60	4/7
Transplant 2§	Newborn	29	19/20	45	6/10
	Weanling	21	5/10	40	4/5
Transplant 3§	Newborn	13	10/10	32	0/5
Tissue culture grown tumour cells	Weanling	15	10/10	90	4/4
Transplant 1¶	Newborn	92	6/16	92	0/3
	Weanling	92	5/5	92	0/4

\* Primary tumour, day after inoculation when first 10 mm tumour was noted; transplant tumour, earliest day when all the recorded number of animals in the group developed tumours.

† Numerator indicates the number with tumour; denominator, the number inoculated.

‡ Numerator indicates the number with complement-fixing antibody against ICH tumour and T antigens; denominator, the number tested.

§ Serial subcutaneous transplant of primary intraperitoneal tumour.

|| Derived from second transplant passage of intraperitoneal tumour.

¶ Subcutaneous transplant of primary subcutaneous tumour.

Table 2. CHESSBOARD COMPLEMENT-FIXATION REACTIONS BETWEEN HAMSTER SERUM AND HAMSTER TUMOUR AND T ANTIGENS OF ICH VIRUS

	Antigen dilution†	Serum dilutions †				
		10	20	40	80	160
ICH hamster tumour	4	4*	4	4	1	0
	8	4	4	4	1	0
antigen ‡	16	4	4	4	2	0
	32	4	4	2	1	0
ICH tissue culture T antigen§	4	4	4	4	2	0
	8	4	4	4	3	0
	16	4	4	3	2	0
	32	1	1	1	0	0

\* Degree of complement-fixation on a scale of 0 to 4; a score of 4 means that there was no haemolysis.

† Reciprocal of dilution.

‡ Second subcutaneous transplant of intraperitoneal tumour.

§ Canine kidney tissue culture T antigen of Lederle strain 255 ICH virus.

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### Deposition of Glomerular Fibrin in the Rabbit after Infusion with Endotoxin

A WIDESPREAD deposition of fibrin in the renal glomeruli with subsequent necrosis of the renal cortex can be produced by two injections of endotoxin separated by 24 h. This mechanism of elucidation is called the (generalized) Sanarelli-Shwartzman phenomenon (SSP) (refs. 1 and 2). The single injection of endotoxin results in the deposition of glomerular fibrin in only 10 per cent<sup>3</sup> and necrosis of the renal cortex in 0.8 per cent<sup>4</sup> of the animals. In the pregnant rabbit at term, disseminated glomerular coagulation can be produced with great regularity by a single injection<sup>5</sup>, whereas fibrin thrombi in other organs, such as the heart and lung, can frequently be found after one injection<sup>6</sup>. It is believed that the time lag for deposition of fibrin in the kidney after two injections of endotoxin is essential for the SSP.

Bioassays for endotoxaemia<sup>7</sup> in patients with endotoxic shock indicated that circulating endotoxins are present for many days in these patients. It was therefore assumed that infusion with endotoxin would resemble human disease more closely than the SSP.

White albino rabbits weighing  $1.5 \text{ kg} \pm 200 \text{ g}$  were used. A polyethylene catheter was inserted through the ear vein into the superior vena cava<sup>8</sup>. Endotoxin *Escherichia coli* 025: B6 No. 462919 (Difco) was dissolved in 0.9 per cent saline (0.66 mg/100 ml.) and infused at a rate of 30–50  $\mu\text{g/kg/h}$  using a Buchler infusion pump. Blood was taken before and 2, 6, 10 and 14 h after infusion. The animals which did not die spontaneously during the experiment were killed at various times after infusion. Frozen sections (haematoxylin and eosin) as well as permanent (haematoxylin and eosin and periodic acid-Schiff) sections were prepared of liver, spleen, lungs, pituitary and kidney. The control group consisted of one single injection of 400  $\mu\text{g/kg}$  of endotoxin. Fibrinogen was assayed by the heat precipitation technique<sup>9</sup>. Platelets were counted in the phase contrast microscope using 'Unopette'<sup>2</sup>. For the thromboelastogram (TEG)<sup>10</sup> native blood was used.

The results are given in Table 1. Three control animals died spontaneously and two were killed after 6–8 h. No deposition of glomerular fibrin was found in this control group. Three animals had fibrin thrombi in the heart and lung.

In the infused group four animals died spontaneously between 0 and 6 h, and two were killed after 5 h. No fibrin was deposited in the kidneys. Twelve animals were killed or died between 8 and 14 h. All the animals of this group showed deposition of glomerular fibrin

Table 1. DISTRIBUTION OF GLOMERULAR FIBRIN DEPOSITION AND RENAL CORTICAL NECROSIS

	Injection; killed 6–8 h or died	Killed or died up to 6 h	Infused; killed or died between 8 and 14 h	Killed after 48 h	First kidney removed after 14 h	Second kidney removed after 48 h
Fibrin deposition	5/0	6/0	12/12	6/2	3/3	3/1
Cortical necrosis	5/0	6/0	12/1	6/2	3/0	3/1

and one had renal cortical necrosis. Six additional rabbits were killed after 48 h, 34 h after infusion for 14 h. Two of these animals showed severe renal cortical necrosis. Four animals showed no deposition of glomerular fibrin. This indicated lysis of the fibrin which was present before injection. In order to clarify this point, three animals were infused for 14 h after which the left kidney was removed under pentothal anaesthesia. Fibrin was deposited in the extirpated kidneys of these three animals whereas the remaining kidney of two rabbits obtained 34 h later was free of fibrin. One of these animals exhibited gross renal cortical necrosis after 34 h and moreover the fibrin was lysed.

Platelets in most of the infused group decreased in a straight line fashion to 10,000–30,000 c.c. Fig. 1 indicates the data in one animal representative for the whole group. The concentration of fibrinogen also fell and after 6–14 h it was not possible to coagulate the blood, as indicated in the TEG (Fig. 1).

These experiments indicate that deposition of glomerular fibrin and necrosis of the renal cortex can be reliably produced by intravenous infusion of endotoxin in rabbits, without a lag phase. The infusion with endotoxin resembles, so far as the widespread intravascular coagulation and the respective coagulation parameters are concerned, the infusion of thrombin in rats<sup>11</sup> and rabbits<sup>3</sup> as well as inhibitors of thrombin and protease<sup>12</sup>. The minimum time for the deposition of fibrin after infusion of this particular dose range seems to be 8 h because no animals below this infusion time showed glomerular fibrin. Platelets decreased more rapidly than fibrinogen. This agrees with previous data from Kliman and McKay<sup>13</sup>, who found that after thrombin infusion the concentration of fibrinogen rarely decreased to less than 80–100 mg per cent.

Renal cortical necrosis was defined by Margaretten *et al.*<sup>11</sup> as arising from glomerular thrombosis which persists long enough to produce necrosis of the renal cortex. The experiments on endotoxin infusion agree with this definition. Furthermore, the glomeruli in the group of animals developing renal cortical necrosis were

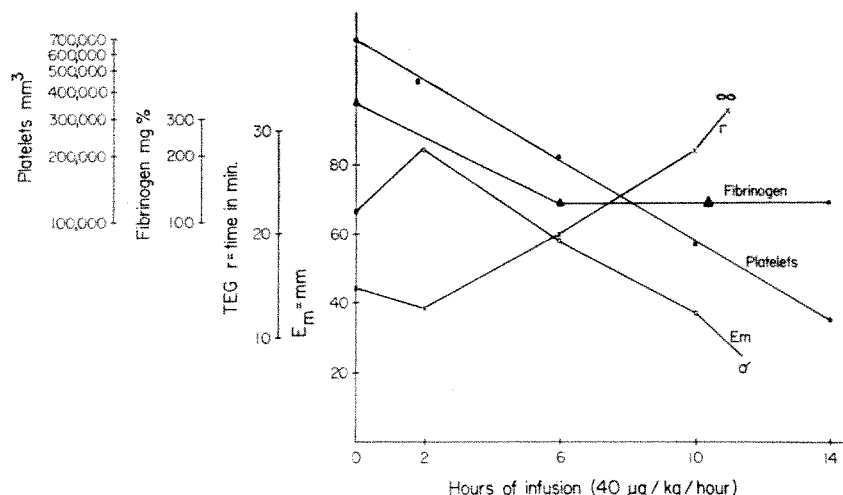


Fig. 1.

packed to nearly 100 per cent with fibrin (quantitative counting of involved glomeruli in per cent).

The four negative results in the group of animals killed after 48 h suggested that even the rabbit with its poor fibrinolytic system can lyse fibrin in the kidney after the infusion is ended. This suggestion was confirmed by the experiments in which one kidney was removed at the end of the endotoxin infusion, whereas the second kidney was obtained 34 h later after the animals had been killed. This agrees with a previous observation that glomerular fibrin produced by thrombin and protease inhibitors are lysed spontaneously after the inhibitor is metabolized from the tissue in the rabbit<sup>12</sup>. One animal which did show fibrin deposition after 14 h developed macroscopic renal cortical necrosis, but only very little fibrin was found at that time.

We suggest that this new experimental model of endotoxin infusion enables a continuous study of the biological events of endotoxaemia and the development of renal cortical necrosis as a pharmacological dose response reaction to be made.

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### Toxicity and Teratogenicity of Optical Isomers of Thalidomide

THE thalidomide molecule contains an asymmetric carbon atom, but the form of the drug which has been used therapeutically and which has produced congenital malformations in man is the optically inactive form, that is ( $\pm$ )-thalidomide. The optical antipodes of thalidomide have been synthesized<sup>1,2</sup>, and Dr A. M. Creighton has supplied samples of both (+)- and (-)-thalidomide. The (+)-isomer, melting point 240°–241° C, showed  $[\alpha]_D^{20} + 60^\circ$  ( $c=2$  in dimethylformamide) and the (-)-isomer, melting point 241°–242° C, showed  $[\alpha]_D^{20} - 58^\circ$  ( $c=2$  in dimethylformamide). ( $\pm$ )-Thalidomide has a melting point of 271° C and, of course, is optically inactive. With these samples, we have determined the acute oral toxicities of the three forms in mice, their teratogenic activity in the New Zealand white rabbit and their effect on the hypnosis induced by hexobarbitone in mice.

Acute oral toxicities were determined by administering the compounds suspended in 1 per cent (w/v) carboxymethylcellulose in water to groups of twelve male SAS ICI albino mice (20–26 g body weight) and counting the mortalities after 24 h. The results (Table 1) show that the optically active isomers are much more toxic than the ( $\pm$ )-modification. After the administration of any one of the three forms at 0.25 g/kg, the mice were sedated but were capable of being aroused. With lethal doses of the

(+)- and (-)-isomers, however, depression of the central nervous system was severe and the mice died after 4–15 h.

The teratogenicity of the compounds was determined by administering them orally in a dose of 150 mg/kg daily from days 7 to 12 inclusive of pregnancy to New Zealand white rabbits. On day 28 of pregnancy the animals were killed and the resorption sites and normal and abnormal fetuses were counted. The types of malformations produced by the (+)- and (-)-isomers were similar to those found with the ( $\pm$ )-form<sup>3</sup> and, as Table 2 shows, all three modifications were teratogenic.

Table 1. ACUTE TOXICITY OF THE OPTICAL ISOMERS OF THALIDOMIDE IN MICE

Optical form	Oral LD <sub>50</sub> (g/kg)
( $\pm$ )	> 10.0
(+)	0.4
(-)	0.7

Table 2. TERATOGENICITY OF THE OPTICAL ISOMERS OF THALIDOMIDE IN NEW ZEALAND WHITE RABBITS

Optical form administered	No. of rabbits	Implantations	Resorptions	Normal fetuses	Malformed fetuses
None	5	42	3	39	0
( $\pm$ )	6	54	19	25	10
(+)	7	67	13	46	8
(-)	8	80	15	56	9

Table 3. EFFECT OF THE OPTICAL ISOMERS OF THALIDOMIDE ON THE TIME OF SLEEP INDUCED BY HEXOBARBITONE IN MICE

Optical form administered	Mean sleeping time min $\pm$ standard deviation
None	23 $\pm$ 7
( $\pm$ )	45 $\pm$ 9
(+)	43 $\pm$ 6
(-)	49 $\pm$ 8

( $\pm$ )-Thalidomide extends the time of sleep induced by hexobarbitone in rats<sup>4</sup>. This effect was examined in male SAS ICI albino mice with the three forms of thalidomide. The compounds (200 mg/kg) were administered orally to at least ten mice, 30 min before an intraperitoneal injection of 100 mg/kg of hexobarbitone sodium. The sleeping time was taken as the time between the loss and recovery of the righting reflex. Table 3 shows that any one of the three forms, in the conditions of the experiment, approximately doubles the sleeping time.

Thus, the three optical forms of thalidomide are teratogenic in the New Zealand white rabbit, are central nervous system depressants in mice, and are able to extend the time of sleep induced by hexobarbitone in mice. But the (+)- and (-)-isomers of the drug are much more toxic when given orally to mice than the ( $\pm$ )-form. The optically inactive form is known to be less soluble than the active isomers<sup>5</sup> and this could result in smaller concentrations in the blood. Crystalline ( $\pm$ )-thalidomide is probably a racemic compound and not simply a mixture or conglomerate of the two optical isomers. The physical properties of these isomers are being investigated.

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## HAEMATOLOGY

Oxygen Binding by Haemoglobin J-Cape Town ( $\alpha_2$  92 Arg  $\rightarrow$  Gln)

AN abnormal haemoglobin with the electrophoretic mobility of a haemoglobin J has been found in Cape Town, South Africa, and subsequently shown to be  $\alpha_2$ (92 Arg  $\rightarrow$  Gln) $\beta_2$  (ref. 1). The oxygen binding characteristics of this new haemoglobin are of particular interest because haemoglobin J-Chesapeake, which has the substitution Arg  $\rightarrow$  Leu in the same position, has been shown to have an abnormally high affinity for oxygen<sup>2</sup>. Residue 92 of the  $\alpha$  chain occupies the position 4 in the non-helical sequence between the helical segments which have been denoted by F and G (ref. 3), and it is in this area that the  $\alpha$  and  $\beta$  chains are sliding past each other during oxygenation and deoxygenation of the haemoglobin molecule (personal communication from Perutz). A substitution of glutamine for arginine in this position can therefore be expected to influence the functional properties of the molecule and the haem-haem interaction.

Oxygen dissociation in haemolysates of blood containing haemoglobin A and haemoglobin J-Cape Town (35 per cent) is represented by Fig. 1. The slope  $n$  of the oxygen dissociation curve, which corresponds to the logarithmic form of Hill's empirical equation<sup>4</sup>, reflects haem-haem interaction. The figure also shows under the same experimental conditions data on fresh normal haemolysates, a normal control haemolysate from blood which was collected and transported from Cape Town to Cambridge at the same time as the A+J-Cape Town blood, and a fresh haemolysate of blood from a patient with haemoglobin A+haemoglobin J-Cambridge (50 per cent), the latter of which has its substitution in the periphery of the molecule<sup>5</sup>.

The oxygen dissociation curves were established on haemolysates of washed red cells diluted to a haemoglobin concentration of 200 mg per cent in 0.02 molar Sorensen phosphate buffer, pH 7.03, at 30° C. After washing with isotonic saline at 4° C, and lysing the erythrocytes with four volumes of chilled water, the red cell ghosts were removed by centrifugation at 22,000g for 45 min at 0° C. The oxygen dissociation curves were determined by the

tonometric procedure adapted from that used by Allen, Guthe and Wyman<sup>6</sup>; the measured volumes of moist air were admitted to the tonometer from a mercury meniscus pipette similar to that of Rossi-Fanelli and Antonini<sup>7</sup>. The tonometers used were all glass and designed to fit in the Unicam SP800 recording spectrophotometer, and had a 10 mm light path cuvette attached with a nominal capacity of 100 ml. The percentage saturation of the haemoglobin with oxygen was calculated by comparing the optical densities of fully oxygenated and fully deoxygenated with the partially oxygenated sample at a fixed wavelength near the maximum of the oxyhaemoglobin  $a$  absorption band (577 m $\mu$ ). The oxygen tension at each point on the curve was calculated from the volume and temperature of the air in the tonometer, and the volume, temperature, pressure and humidity of air admitted from the pipette (assuming partial pressure of oxygen in air to be 20.95 per cent). The amount of oxygen dissolved in the haemoglobin solution and that bound to haemoglobin was neglected in the calculations, for it was only about 0.1 per cent of the total present. Absorption spectra from 700–450 m $\mu$  were drawn of every specimen in the fully oxygenated state before and after each investigation to ensure that no methaemoglobin had formed and no other spectral change had occurred during measurement. The reproducibility of the method was within 1 per cent.

The control blood collected and transported with the A+J-Cape Town specimen showed, in the first place, no spectral or electrophoretic evidence of ageing and, when the oxygen dissociation curve was determined 2 days after the blood had been drawn, there was no significant difference between it and that of fresh haemolysates. The haemoglobin A+J-Cape Town haemolysate, however, which was examined only 1 day after it had been drawn, showed an increased oxygen affinity and the  $n$  value was 1.9 compared with 2.6 for normal haemolysates.

The change of oxygen affinity with pH—the Bohr effect—was found to be the same for the A+J-Cape Town haemolysate and normal haemolysate at 50 per cent saturation. For the mixed haemoglobin, however, the oxygen pressure was 0.13–0.14 mm of mercury less than in the normal haemolysate at each pH investigated (Table 1).

Table 1. BOHR EFFECT IN HAEMOGLOBIN J-CAPE TOWN, USING PHOSPHATE BUFFER 0.02 MOLAR AT 30° C

Haemolysate	pO <sub>2</sub> (mm of mercury) at 50 per cent saturation pH 6.41	pH 7.03	pH 7.80
Normal	1.31	0.95	0.52
A+J-Cape Town	1.18	0.81	0.39

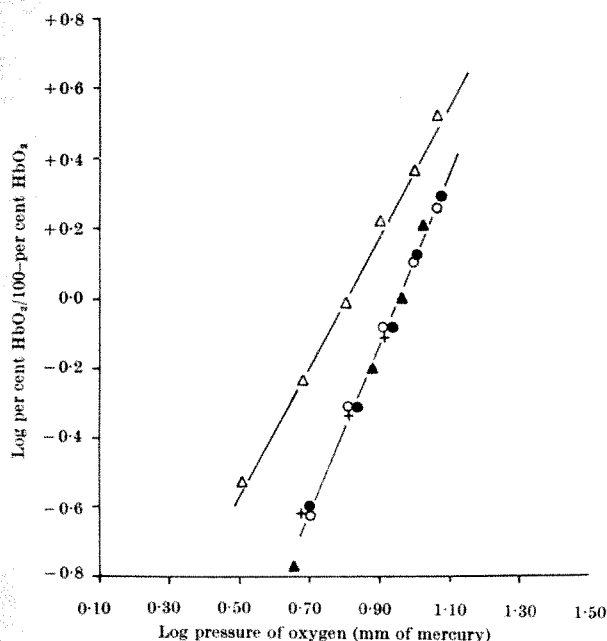


Fig. 1. Log plot of oxygen dissociation curves from J-Cape Town fresh whole haemolysate and controls.  $\Delta$ , J-Cape Town whole haemolysate;  $\blacktriangle$ , normal from Cape Town whole haemolysate, fresh;  $\circ$ , J-Cambridge whole haemolysate, fresh;  $+$  and  $+$ , normal whole haemolysates, fresh.

Charache *et al.*<sup>2</sup> have shown that the  $n$  value of the whole blood containing 30 per cent haemoglobin Chesapeake is 1.8 and mention that haemolysates also showed this smaller  $n$  value. The  $n$  value for whole blood haemolysate containing 35 per cent haemoglobin J-Cape Town was found to be similarly small, namely, 1.9 in our conditions. The altered oxygen binding by haemoglobin J-Cape Town ( $\alpha$  92 arginine  $\rightarrow$  glutamine) and presumably also that by haemoglobin Chesapeake ( $\alpha$  92 arginine  $\rightarrow$  leucine) are not merely the outcome of an additional negative charge in each half molecule. This can be concluded from the normal results with haemoglobin J-Cambridge which also has one negative charge more in each half molecule than haemoglobin A. It seems that the alteration in oxygen binding is related to the position of the amino-acid substitution in an area of the  $\alpha$  chain- $\beta$  chain contact within the haemoglobin molecule. The arginine residue of the normal  $\alpha$  chain which is replaced by a residue of leucine in haemoglobin Chesapeake and by one of glutamine in haemoglobin J-Cape Town is found in the  $\alpha$  chain of all mammalian haemoglobins so far investigated. It is not present in the haemoglobin of lamprey and in sperm whale myoglobin where there is no diversity of peptide chains and hence no interaction between unlike chains.



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### Effect of Catecholamines on Platelet Aggregation caused by Thrombin

THE addition of thrombin to human blood platelets results in their rapid aggregation, with release of 5-hydroxytryptamine and adenine nucleotides<sup>1</sup>. Whether thrombin-induced platelet aggregation is produced solely by adenosine diphosphate (ADP) released from the platelets or whether thrombin also has a direct aggregating effect is still not clear<sup>2,3</sup>. The aggregation by adrenaline or ADP of human platelets occurs in two phases, and it has been suggested that the second phase is brought about by ADP released from the platelets<sup>4</sup>. The second phase of platelet aggregation caused by ADP is inhibited by chlorpromazine and similar drugs, and potentiated by adrenaline<sup>5,6</sup>. Platelets are known to contain small amounts of catecholamines<sup>7,8</sup>. The evidence reported here suggests that sufficient catecholamines are released from the platelets during aggregation by thrombin to potentiate aggregation.

Blood was obtained from the antecubital vein of healthy volunteers. The blood was collected in plastic centrifuge tubes containing 10 per cent by volume of a 3.8 per cent solution of trisodium citrate, and centrifuged for 10 min at 220*g* at room temperature. The platelet-rich plasma was kept in plastic containers at room temperature and used within 3 h. Platelet aggregation was measured using the turbidimetric method of Born<sup>9</sup> as modified by Mills and Roberts<sup>5</sup>. A 1-ml. sample of platelet-rich plasma was pre-warmed to 37° C for 5 min in a transparent plastic tube and then placed in a constant temperature cell compartment in the modified EEL titrator. The plasma was stirred from below with a magnetic stirrer. Light transmission was recorded continuously on a 1-mV recorder at a chart speed of 2.5 cm/min. The recorder scale was calibrated by setting the titrator to 100 per cent transmission with each platelet-free plasma and adjusting the scales to give full-scale deflexion between 10 and 60 per cent. Reagents were added with microsyringes in volumes of up to 30  $\mu$ l.

Superimposed aggregation traces of platelet-rich plasma with varying doses of thrombin are shown in Fig. 1. A small increase in dose converted reversible aggregation into a second phase of aggregation which was irreversible. The critical concentration of thrombin at which the second phase of aggregation occurred varied from one donor to the next. With the concentrations of thrombin used, the plasma did not clot within the 4-min period of observation. Platelet aggregation could be separated into two distinct phases in seventeen of twenty-two plasma samples tested; in the remaining five samples, it was not possible to demonstrate this separation.

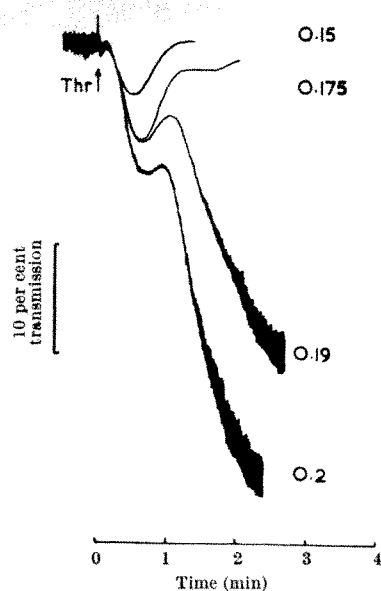


Fig. 1. Aggregation by thrombin of platelets in human citrated platelet-rich plasma. Thrombin was added at the arrow to give the final concentrations (N.I.H.  $\mu$ /ml.) shown by each curve.

The addition of low concentrations ( $10^{-8}$ – $5 \times 10^{-8}$  moles/l.) of adrenaline to platelet-rich plasma did not result in aggregation. Such concentrations of adrenaline, however, markedly potentiated the aggregation brought about by thrombin. As shown in Fig. 2,  $2 \times 10^{-8}$  moles/l. adrenaline pre-incubated for 1 min with platelet-rich plasma changed reversible first-phase aggregation into irreversible second-phase aggregation. While the potentiating effect of adrenaline was seen primarily in its ability to evoke the second phase, adrenaline also slightly increased the speed of the first phase. Aggregation by thrombin was also potentiated by noradrenaline, but its effectiveness was between two and three times less than that of adrenaline.

The effects of adrenergic blocking agents on thrombin-induced aggregation were then examined. With a dose of thrombin sufficient to evoke the second phase, the  $\alpha$ -blockers phentolamine and dihydroergotamine, added at 1–2  $\mu$ moles/l. 1 min before the thrombin, prevented development of the second phase (Fig. 3). The  $\beta$ -blocker

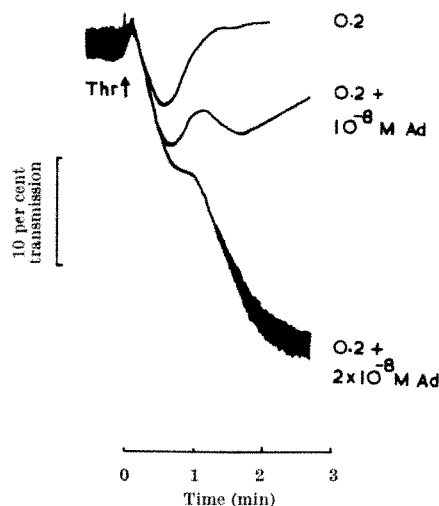


Fig. 2. Potentiation by adrenaline of platelet aggregation induced by thrombin. Thrombin was added at the arrow to give a final concentration of 0.2  $\mu$ /ml. Adrenaline (Ad) was added one minute before thrombin to give the final concentrations shown by each curve.

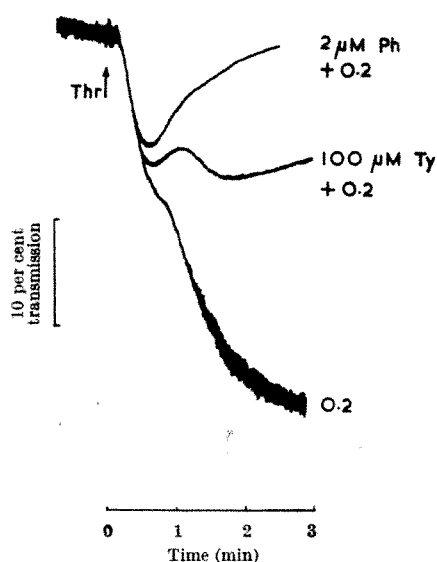


Fig. 3. Inhibition by phenolamine (Ph) and tyramine (Ty) of platelet aggregation induced by thrombin. Thrombin was added at the arrow to give a final concentration of 0.2 u/ml. Phenolamine (2  $\mu$ moles/l.) was added 1 min before thrombin, and tyramine (100  $\mu$ moles/l.) was incubated for 20 min at 37° C before adding thrombin.

propranolol had no effect on second phase aggregation at 2–5  $\mu$ moles/l. but inhibited it at 10  $\mu$ moles/l. It appeared therefore that propranolol could inhibit second phase aggregation, but less effectively than  $\alpha$ -blockers.

These observations suggested that thrombin was releasing sufficient catecholamines from the platelets to potentiate aggregation. Tyramine displaces catecholamines from various tissues and also 5-hydroxytryptamine from platelets<sup>10</sup>. The effect of pre-incubating platelets with tyramine was therefore examined. In fresh plasma, 100  $\mu$ moles/l. tyramine, added 20 min before thrombin, inhibited its ability to produce the second phase (Fig. 3). After 20 min pre-incubation the second phase was not blocked by tyramine at 20  $\mu$ moles/l. whereas 1 mmole/l. blocked after only 10 min.

Aggregation by ADP is potentiated by 5-HT<sup>11</sup>. Because thrombin releases 5-HT from platelets<sup>1</sup>, it is possible that the potentiating effects observed were due to released 5-HT rather than catecholamines. The specific 5-HT antagonist methysergide, at 2–5  $\mu$ moles/l., had no effect, however, on second phase aggregation caused by thrombin. In contrast, desmethylinipramine inhibits second phase aggregation by ADP without affecting the first phase<sup>5</sup>. It was found similarly that second-phase aggregation by thrombin could be inhibited by 20  $\mu$ moles desmethylinipramine, but first-phase aggregation could not.

The dose of thrombin required to demonstrate these effects was critical. On one hand, sufficient thrombin had to be added to induce the second phase of aggregation. On the other hand, too large a dose of thrombin produced rapid and irreversible aggregation, with subsequent clotting, and the effects of adrenergic blocking agents were obscured. The observations can be explained by assuming that thrombin releases catecholamines from platelets, which then either potentiate the aggregating action of thrombin itself or that of the ADP released by thrombin, or both. It has already been shown that exogenous catecholamines powerfully potentiate the aggregating action of ADP and thrombin<sup>6,11,12</sup>. Catecholamines are concentrated in platelets<sup>13</sup> although the amounts differ according to various reports. Pig platelets contained 0.2–2.6 ng adrenaline/10<sup>8</sup> platelets<sup>7</sup>. Human platelets contained 0.2–0.7 ng catecholamines/10<sup>8</sup> platelets<sup>14</sup>, or an average of 0.8 ng/10<sup>8</sup> platelets<sup>8</sup>. The number of platelets in 1 ml. of platelet-rich plasma (about 4  $\times$  10<sup>9</sup>)

therefore contain 1–3 ng catecholamines. Assuming that thrombin releases most of this from the platelets, the concentration of adrenaline in the plasma is similar to that which has to be added to potentiate aggregation (10<sup>-8</sup> moles/l. = 2 ng/ml.). It seems reasonable therefore to suggest that the aggregation of platelets can be potentiated by their own catecholamines, if released.

The release of catecholamines from platelets by degranulating agents such as thrombin and collagen may therefore play an important part in determining the extent of the reaction of platelets to such agents. It suggests that the contents of the platelets markedly affect their function in haemostasis and thrombosis.

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### Erythrocyte Glucose-6-phosphate Dehydrogenase Deficiency in Uganda

DEFICIENCY in erythrocyte glucose-6-phosphate dehydrogenase (G-6-PD) has been found to be widespread in the tropics and sub-tropics<sup>1-9</sup> as well as in the Negro population of America<sup>10</sup>. Surveys of G-6-PD deficiency in limited areas and populations in Uganda have been reported before<sup>11-13</sup>, but no country-wide screening for this deficiency in Uganda has been published. In the present investigation only African males were tested, by a screening test with the reduction of brilliant cresyl blue (National Aniline Division) as indicator<sup>14</sup>. The results, which are shown in Table 1, agree in general with findings in other countries, as well as with the 15 per cent prevalence in Kampala<sup>11</sup>. The low prevalence in Kabale, however, is striking and compares with that of 2 per cent found in South African male Bantu<sup>15</sup>. Kabale, at an altitude of 6,000 ft., is situated in the highlands of Kigezi in South-West Uganda near the Rwanda border, and the prevalence of malaria there has been very much lower than elsewhere in Uganda. Conversely, endemic malaria is known to be more prevalent in Bundebugyo than elsewhere<sup>16</sup>, and Lehmann and Raper<sup>16</sup> found there the exceptionally high sickling rate of 39 per cent. The prevalence of G-6-PD deficiency in Bundebugyo, which is slightly greater than average, was shown by tests made on a mixture of indigenous males and recent immigrants from the Fort Portal region. The prevalence among the people indigenous to Bundebugyo region may be considerably greater.

Table 1. PERCENTAGE OF MALES IN VARIOUS PARTS OF UGANDA WITH GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY OF THE RED CELLS

Town	No. tested	No. of abnormals	Percentage of abnormals
Fort Portal	100	11	11
Bundebugyo	65	12	18
Kabale	180	5	3
Mbarara	100	9	9
Mbale	100	14	14
Soroti	100	13	13
Moroto	60	8	13
Gulu	100	12	12
Aura	67	6	9

These findings support the theory of a connexion, direct or indirect, between G-6-PD deficiency and malaria<sup>17</sup>.

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### Protein Synthesis in Leucocytes of Diabetic and Normal Subjects

THERE are some indications that diabetes mellitus is a disease involving a disorder of carbohydrate, lipid and protein metabolism. Leucocytes possess a nucleus and mitochondria, and metabolize glucose, amino-acids and fatty acids, and therefore might be useful for the investigation of certain aspects of metabolism in human diabetes. Investigation of glucose uptake and oxidation, lipid metabolism and lactic acid formation in leucocytes has had varied results<sup>1-3</sup>. Protein synthesis has not been investigated in leucocytes, and we aimed to determine the differences in protein metabolism in leucocytes from control and diabetic patients.

Six diabetic patients who required insulin or oral anti-diabetic agents were examined. Their fasting blood sugar concentration was 100–180 mg/100 ml. After treatment with insulin for 24 h or an oral antidiabetic agent, heparinized blood was added to a tube containing dextran, to give a final concentration of dextran of 1 g/100 ml. This tube was refrigerated at 5°C for 45 min. After sedimentation of erythrocytes the supernatant was transferred into another tube and the suspension contained 10<sup>4</sup> leucocytes/ml.

To 0.2 ml. of leucocyte suspension was added radioactive glycine or cysteine in 0.5 ml. of buffer. The specific activity for cysteine was 9 mc./mmole and glycine 17 mc./mmole. The cysteine had an activity of 0.1 µc./ml. and the glycine of 0.5 µc./ml. The tubes were incubated for 120 min at 37°C. At the end of the incubation the leucocytes were washed twice with 10 per cent trichloroacetic acid. Radioactivity was determined with a gas flow counter in the case of radiocarbon, and with a Geiger-Müller thin-window tube in the case of cysteine.

The protein content was determined by the ninhydrin method. Samples of 1 ml. of blood from some patients was incubated with cysteine. In each experiment, both diabetic and control leucocytes were investigated. The chemicals used were pure.

The incorporation of glycine-1-<sup>14</sup>C and cysteine-<sup>35</sup>S by leucocytes from control and diabetic subjects is interesting. Comparison of the protein synthesis of control and diabetic leucocytes shows an increased protein synthesis in leucocytes from diabetic subjects (Table 1). The incorporation of labelled glycine into the protein fraction in the diabetic patients was three times that of normal controls. There was less incorporation of cysteine, but it was still very significant.

Table 1. INCORPORATION OF LABELLED GLYCINE AND CYSTEINE INTO THE PROTEIN OF LEUCOCYTES FROM NORMAL AND DIABETIC SUBJECTS

Test	Subject	Glycine-1- <sup>14</sup> C		Cysteine- <sup>35</sup> S	
		mg N	C.p.m.	C.p.m./mg N (specific activity)	C.p.m. × 10 <sup>3</sup> / mg N (specific activity)
Leucocyte suspension	Control	4.1	4,338	1,058	61.2
		4.0	1,478	369	68.9
		4.1	2,455	598	59.1
		3.9	1,704	437	110.9
	Mean value	4.0	2,493	615	75.3
Leucocyte suspension	Diabetic	4.6	6,495	1,412	120.8
		4.5	12,239	2,719	142.5
		4.6	5,050	1,097	103.9
		4.2	9,804	2,334	151.6
	Mean value	4.5	11,842	2,632	152.3
Whole blood	Control	1.5	—	—	43.2
Whole blood	Diabetic	1.5	—	—	54.9

When whole blood was used instead of leucocytes, there was more protein synthesis in the diabetics than in the controls, as indicated by the uptake of cysteine. Measurement of protein synthesis employing these two amino-acids gave varying results in the leucocytes from different patients, but the differences between normals and diabetics were still significant.

To find out about increased protein synthesis in leucocytes of diabetics we examined the effect of glucose in different concentrations. Leucocytes from controls were stored at increasing concentrations of glucose for 8 h and were incubated with cysteine-<sup>35</sup>S (Fig. 1). The results show that the protein synthesis of normal leucocytes depends on the concentration of glucose present.

These findings demonstrated that in every case the protein synthesis by leucocytes of diabetic subjects was significantly greater than the controls. In interpreting our results it might be supposed that the greater concentration of glucose caused increased protein synthesis in normal patients, for they suggest that the greater protein synthesis in diabetic leucocytes is also caused

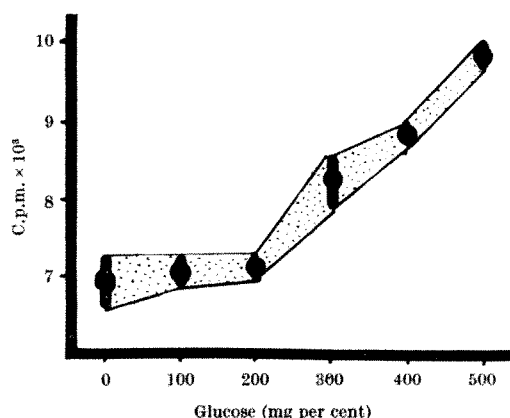


Fig. 1. Effect of glucose concentration on the cysteine incorporation into the protein fraction of leucocytes from normal subjects.

by the metabolic effect of glucose and not necessarily by the diabetic process itself.

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## PHYSIOLOGY

### Transmission of Receptor Potential in Dipteran Chemoreceptors

THE structure of the contact chemoreceptor sensilla on the labellum of certain muscid diptera has been studied by light<sup>1,2</sup> and electron<sup>3,4</sup> microscopy, but little attempt has hitherto been made to relate this structure to possible electrical phenomena within the receptor.

Morita and Tateda<sup>5</sup> and Wolbarsht and Hanson<sup>6</sup> have shown that the site of generation of action potentials is located in the region of the cell bodies of the receptor neurones proximal to the base of the receptor shaft and separated by as much as 500 $\mu$  from the distal tip of the sensilla to which the chemical stimulus is applied. There is no evidence for the generation of action potentials at the tip of the receptor shaft. Morita and Yamashita<sup>7</sup> showed that a slowly declining potential difference is established across the terminal membrane of the sensory dendrite when the tip is stimulated with water or aqueous solutions of sucrose or sodium chloride, such that the inside of the receptor membrane becomes positive relative to the outside. These distal potential changes must be transmitted to the site of generation of action potentials according to the cable properties of the sensillum.

The shaft of the sensillum contains two cuticular lumina, one large, thin walled and crescentic in the transverse section and one smaller, thick walled and circular in outline<sup>1,3</sup>. Larsen also showed that the distal dendrites of the receptor neurone fill the smaller lumen almost completely (Fig. 1a). The larger lumen communicates directly with the vacuole of the trichogen cell; this vacuole invests the proximal extensions of the smaller lumen wall (termed the scolopoid body by Stürckow<sup>2</sup> and Larsen<sup>3</sup>) which, in turn, surrounds the sensory dendrites just after they emerge from the cell body (Fig. 1b). Examination of Larsen's electron micrographs shows a transition from the thick wall of the small lumen (thickness 1.5 $\mu$ ) through the characteristically plicate section of the

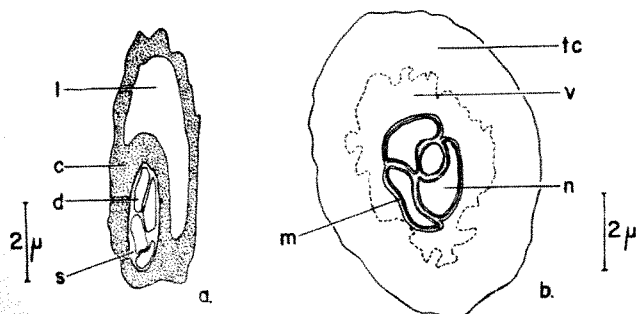


Fig. 1. (a) Diagrammatic transverse section through the chemoreceptor shaft of *Phormia* showing two lumina (*l*, large; *s*, small), sensory dendrites (*d*) and the cuticular architecture (*c*). (b) Diagrammatic transverse section through the trichogen cell of a labellar chemoreceptor complex of *Phormia* showing four sensory dendrites (*n*) passing through the vacuole of the trichogen cell (*v*) and separated from the vacuolar contents by a membrane (*m*). The plane of the section is parallel to the surface of the labellar cuticle. *tc*, trichogen cell cytoplasm. Both diagrams after Larsen (ref. 3).

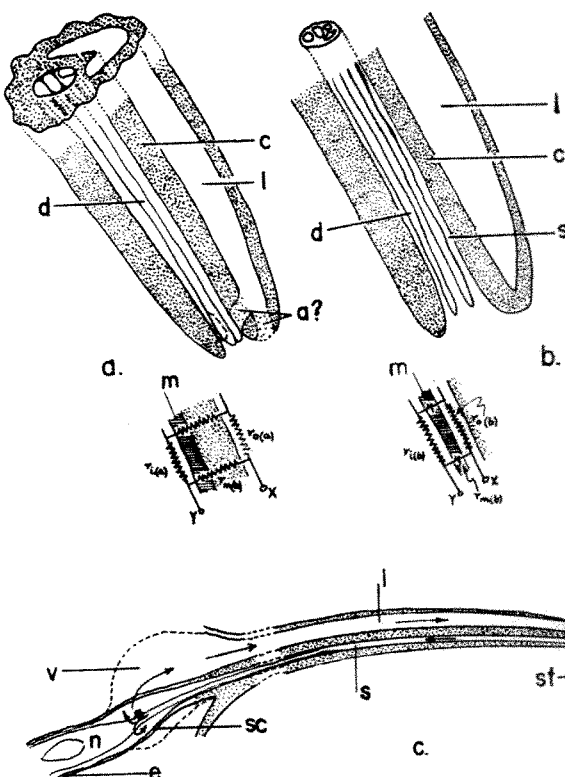


Fig. 2. (a, b) Diagrams of alternative structures for the tip region of the chemoreceptor shaft, with the associated resistive elements of two electrotonic transmission lines which these structures may possess. (a) Main longitudinal current flow by way of the dendrite axoplasm (*d*) and the large lumen space (*l*). (b) Current flow by way of the dendrite axoplasm (*d*) and the small lumen space (*s*). The cuticle (*c*) is shown stippled and the dendrite membrane (*m*) is shown hatched.  $r_o(a)$ ,  $r_o(b)$  Resistance per unit length of outer conductor (ohm/cm).  $r_i(a)$ ,  $r_i(b)$  Resistance per unit length of inner conductor (ohm/cm).  $r_m(a)$ ,  $r_m(b)$  Transverse resistance  $\times$  unit length of insulator (ohm cm). *a?* Conjectured alternative positions of a terminal aperture or low resistance pathway in the wall of the large lumen. *X*, *Y*, Points across which the receptor potential is developed. (c) Diagram to show the predicted pathway of the principal depolarizing current in the contact chemoreceptor of the labellum of muscid diptera. The pathway is shown by arrows. *l*, Large lumen; *s*, small lumen almost filled by receptor dendrites; *st*, stimulus solution; *sc*, scolopoid body; *e*, extension of scolopoid body; *n*, receptor neurone cell body; *v*, trichogen cell vacuole; \*, probable coincident location of the generator potential and the site of initiation of action potentials. Cuticle is shown stippled and the discontinuity in the receptor shaft (indicated by dashed connexions) is included for convenience in drawing. The separation of receptor tip and base may exceed 300 $\mu$ .

scolopoid body, with thinner walls, to separate tubular extensions formed by the closure of the lobes of the scolopoid body surrounding the sensory dendrites. In this latter region the walls have decreased in thickness to approximately 500 Å.

Longitudinal electrotonic transmission of the slow potential changes from the distal extremity to the basal regions of the sensillum cannot occur except in the presence of two conducting pathways separated by an insulator of relatively high resistance. Fig. 2 is a diagram of two alternative pathways of current flow which might account for the electrotonic transmission. In both cases one pathway must be the cytoplasm of the receptor dendrite; the return current might flow in the space between the dendrite and the wall of the small lumen (Fig. 2b) or through the contents of the large lumen (Fig. 2a). In *Phormia*, Larsen<sup>3</sup> has demonstrated a very restricted space between the receptor dendrite and the inner wall of the small lumen with a maximum thickness of 1500 Å. In *Stomoxys*, a closely related species of fly, Adams and Holbert<sup>4</sup> were unable to show such a space. There can be no doubt that the large lumen exists.

In the basal region of the receptor shaft maximal current flow between the dendrite cytoplasm and the



contents of the large lumen might be expected in any plane normal to the long axis of the receptor shaft at which the scolopoid body and its proximal extensions run through the trichogen cell vacuole. This vacuole has been shown to communicate directly with the contents of the large lumen (ref. 3 and Tominaga, Y., personal communication), and to be filled with material of similar appearance under the electron microscope. The generation site of action potentials is situated at about the same position as any of these planes of section<sup>5</sup>.

In electrotonic transmission, the relationship  $V_x = V_0 \cdot e^{-x/\lambda}$  may be used to describe the voltage attenuation—length characteristics of a bifilar conductor system, where  $V_0$  is the potential applied between the two conductors,  $V_x$  the potential measured between the two conductors in a plane normal to the longitudinal axis of the cable at a distance of  $x$  cm from the plane of application of  $V_0$  and where  $\lambda$  is the length constant of the cable.  $\lambda$  can be shown to equal  $(r_m/r_o + r_i)^{1/2}$ , where  $r_m$  is the transverse resistance  $\times$  unit length of the insulator between the two conductors measured in ohm cm, and  $r_o$  and  $r_i$  represent the resistances per unit length of the two conductors (in ohm cm). If  $r_m$  is increased,  $\lambda$  will increase with accompanying decrease in cable voltage attenuation, but if either  $r_o$  or  $r_i$  is increased,  $\lambda$  will decrease, resulting in greater voltage attenuation.

The diagrammatic structures shown in Figs. 2a and 2b may be discussed in relation to these properties. If the receptor dendrites are separated from the inner wall of the small lumen by a fluid layer forming the second longitudinal conductor,  $r_m$  becomes a parameter of the dendrite membrane alone (Fig. 2b). In Fig. 2a where the second longitudinal conductor is formed by the contents of the large lumen,  $r_m$  describes the series resistance of both dendrite membrane and a thickness of cuticle. Clearly,  $r_{m(a)} \gg r_{m(b)}$ . Further, the longitudinal resistance of a restricted fluid layer only 1,500 Å thick is likely to be much greater than that of the contents of the large lumen: ( $r_{o(a)} < r_{o(b)}$  in Fig. 2). Hence electrotonic transmission is attended by smaller cable losses in the network of Fig. 2a than that of Fig. 2b.

It is therefore suggested that the main depolarizing current of the site of generation of action potentials in dipteran chemoreceptors of this type may follow the pathway shown by arrows in Fig. 2c. In this system a Nernst potential set up across the membrane of the distal tip of the receptor dendrite drives a current through the dendrite cytoplasm, the dendrite membrane at the action potential initiation site, the attenuated wall of the proximal extension of the scolopoid body, the trichogen cell vacuole and the contents of the large lumen back to the membrane of the tip of the dendrite. The insulator allows little current to flow between the large and small lumina of the receptor except in the region where action potentials are initiated (the site of the generator potential). It is not yet known whether the application of the stimulating solution provides the final link in this current pathway, or, if this link is pre-existent, whether such an application merely establishes the receptor potential.

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## Sensitivity of L Cells in Exponential and Stationary Phase to 5-Fluorouracil

ANTICANCER agents have recently been classified according to their lethal effects on normal murine haematopoietic and lymphoma spleen colony-forming cells<sup>1</sup>. The members of one class, which includes 5-fluorouracil, reduce the survival *in vivo* of actively dividing lymphoma cells by a far greater degree than that of normal haematopoietic cells which are presumed to be in a resting state. When these two cell types were treated with 5-fluorouracil *in vitro* and assayed *in vivo*, a similar difference in survival was noted<sup>2</sup>. It was proposed that this difference in sensitivity is a consequence of the difference between the proliferative states of the two cell types<sup>1,2</sup>. In order to test this hypothesis and to establish a model system *in vitro*, the sensitivity of mouse L cells to 5-fluorouracil was determined by the colony formation method of Puck *et al.*<sup>3</sup>, either when the cells were in a rapidly proliferating state (exponential phase) or when they were in a non-proliferating state (stationary phase). Few reports of the survival of the colony forming ability of mammalian cells *in vitro* following exposure to chemotherapeutic agents have been published and in only one instance has the lethal effect of 5-fluorouracil been described in such a system<sup>4</sup>.

Mouse L cells (strain L60) were grown as suspension cultures in medium No. CMRL 1066<sup>5</sup>, from which nucleosides and coenzymes were omitted. The medium was supplemented with dialysed foetal calf serum at a concentration of 10 per cent. The serum was dialysed against phosphate buffered saline solution, volume ratio 1:20, for 24 h three consecutive times in order to remove nucleosides, which might affect the action of 5-fluorouracil from the serum. Cells from a stock bottle were diluted and 9.5 ml. of the cell suspension dispensed into each of a series of plastic test-tubes (Falcon Plastics, California) at a concentration of about  $4 \times 10^4$  cells/ml. The tubes were placed in a roller wheel and incubated at 37°. Fig. 1 shows a growth curve for cells grown under these conditions. Cells were treated with 5-fluorouracil on the first and second days after dilution into tubes (exponential

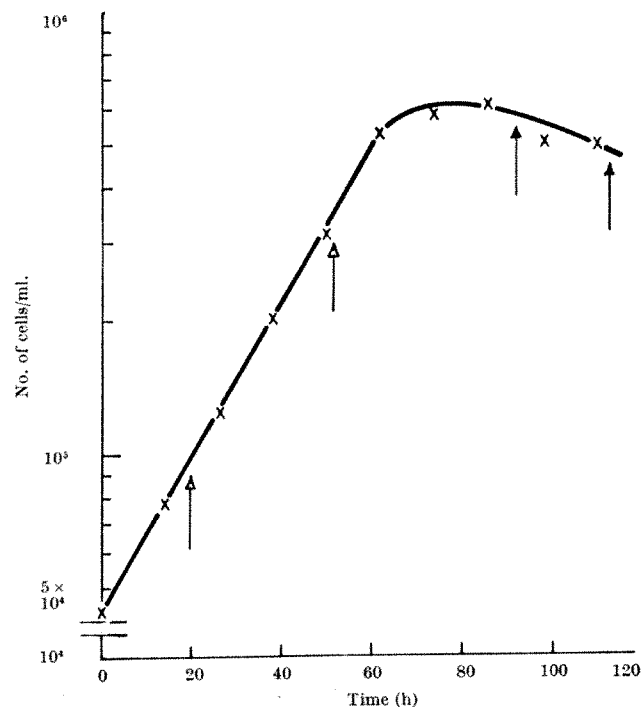


Fig. 1. Growth curve for mouse L cells in suspension culture. Arrows denote treatment times, see text and Fig. 2. Open arrows, exponential phase; closed arrows, stationary phase. The doubling time was 17 h.

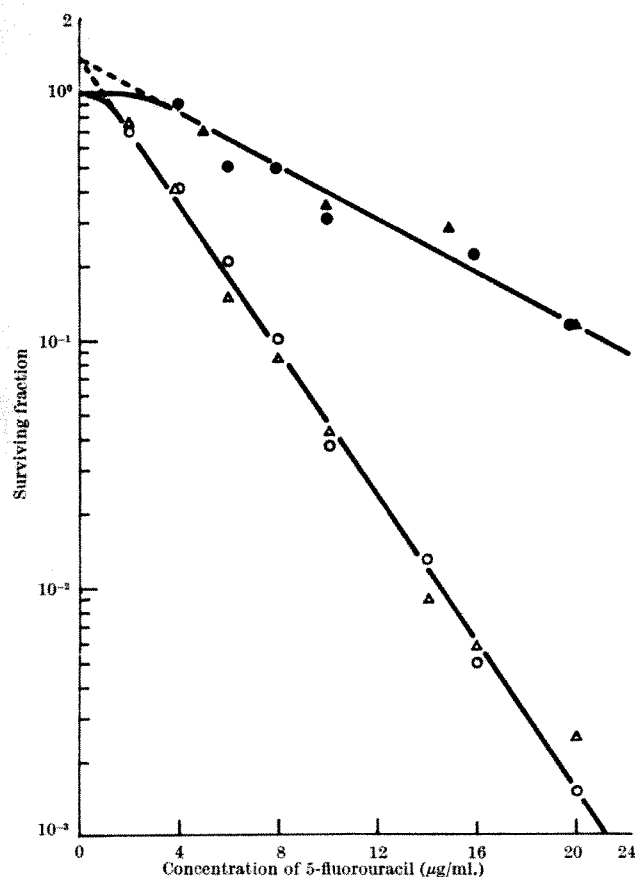


Fig. 2. Survival curves for mouse *L* cells exposed to 5-fluorouracil for 1 h *in vitro*. Open symbols, cells treated in the exponential (log) phase of the growth cycle (plating efficiency = 80 per cent;  $C_{10} = 6 \mu\text{g/ml.}$ ); closed symbols, cells treated in the stationary phase (plating efficiency = 50 per cent;  $C_{10} = 18 \mu\text{g/ml.}$ ). Triangles and circles represent treatment at different times within the given phase of the growth cycle, as denoted by the arrows in Fig. 1.

phase) and on the fourth and fifth days (stationary phase) as indicated by the arrows in Fig. 1. One half ml. of graded concentrations of the drug was added to the tubes, which were then returned to the incubator for 1 h. After this interval, the cells were spun down in a clinical centrifuge, the medium decanted and the cells resuspended in fresh medium. After appropriate dilutions, the cells were plated into Petri dishes, 5 per point, containing medium supplemented with undialysed serum. The number of cells added per dish was adjusted to give about 100 colonies per dish. After 14 days of incubation at  $37^\circ$  in a humidified atmosphere of 5 per cent carbon dioxide and 95 per cent air, the colonies were stained with methylene blue and counted. The survival curves thus determined are shown in Fig. 2.

The survival curves exhibit small shoulder regions and are similar to radiation survival curves. The  $C_{10}$ , that is the concentration of the drug required to reduce the survival to 10 per cent on the exponential region of the curve, was  $6 \mu\text{g/ml.}$  for exponential phase cells at both times examined. The  $C_{10}$  for stationary phase cells was  $18 \mu\text{g/ml.}$ , also at both times examined. It is clear that the rapidly dividing exponential phase cells were three times as sensitive to 5-fluorouracil, over the range of doses examined, as were the stationary phase cells.

To check that the stationary phase cells were not proceeding around the mitotic cycle, cells in either the exponential or stationary phase were exposed to  $^3\text{H}$ -thymidine for 1 h. Autoradiographic preparations showed that while 45 per cent of exponential phase cells were labelled, essentially none of the stationary phase cells was labelled. Thus the stationary phase cells did not proceed through the DNA synthetic phase of the mitotic cycle.

Because the cell concentration in the stationary phase was up to five times that in the exponential phase, the possibility that the drug was exhausted by the stationary phase cell population was examined. Cells in either phase were incubated with  $^3\text{H}$ -5-fluorouracil for 1 h and it was found that less than 0.01 per cent of the drug present in the medium was incorporated in either case.

The  $C_{10}$  values for mouse *L* cells may be compared with those for murine lymphoma cells and normal haematopoietic cells ( $C_{10} = 3.3$  and  $22.5 \mu\text{g/ml.}$  respectively), also exposed to 5-fluorouracil for 1 h *in vitro* (calculated from ref. 2). The difference in sensitivity to 5-fluorouracil between exponential and stationary phase *L* cells, although not as marked as that between murine lymphoma and normal haematopoietic cells, supports the hypothesis that the action of this drug depends on the proliferative state of a cell population. These results are in agreement with the observations of Bhuyan *et al.*, who reported that 5-fluorouracil had no detectable effect on resting human *KB* cells in tissue culture, as tested by a collagen plate assay, but did show a marked cytotoxicity to rapidly dividing *KB* cells tested by a broth dilution assay<sup>6</sup>. Our results also suggest that an *in vitro* colony-forming system may be utilized as a screening procedure for drugs which selectively destroy rapidly dividing cells.

Further interpretation of survival data for cells exposed to 5-fluorouracil *in vitro* is complicated by the fact that more recent work, to be described in a later publication, has shown that the survival of *L* cells exposed to 5-fluorouracil is affected by the presence of nucleosides in the medium, both before and after treatment. This effect may be evinced by the use of different batches of serum to supplement the medium, especially if the serum has not been dialysed.

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### Tritiated Water Space in Gastrocnemius of Rat

If a muscle is heated at  $105^\circ\text{C}$  until its weight is constant, the loss in weight (70–80 per cent of the original weight) represents the total amount of water in the muscle. More refined methods of estimating water contained in the tissue—normally applied to a whole animal *in vivo*—have been evolved; these methods depend on the equilibration of a marker molecule within the fluid spaces of the tissue or tissues under examination, followed by subsequent estimation of its concentration therein. The use of various markers, notably urea, thiourea and sulphanilamide, and their degree of correspondence with whole body water content as obtained by desiccation, has been comprehensively reviewed by Keys and Brozek<sup>1</sup>. Antipyrine spaces have been found to agree with tissue deuterium oxide spaces<sup>2</sup> and tritiated water spaces<sup>3</sup>. Antipyrine, however, is slowly metabolized<sup>4</sup>, and, like its *N*-acetyl-4-amino-derivative<sup>5</sup>, equilibrates relatively slowly with tissue fluids. Neither of these disadvantages is found in the case of deuterium oxide<sup>6</sup> or tritiated water<sup>3</sup>, although a small rapid exchange has been noted between the isotopic hydrogen atoms and the hydrogen atoms in the organic molecules of the tissues<sup>3,7</sup>.

Overton<sup>8</sup> considered that a fairly extensive proportion of the water in muscle is osmotically bound; that is, it is unable to participate in the normal osmotic exchanges of tissue fluid. Such water would not therefore be expected to take part in equilibration exchanges with the osmotically active markers referred to here, although it might be able to do so with deuterium oxide or tritiated water. Hill<sup>9</sup>, while confirming the experimental evidence on which Overton's deductions were based, suggested alternative explanations for them which are physiologically more reasonable, and doubted whether a significant proportion of muscle water was "bound". The present work was undertaken to assess not so much whether any muscle water is rigidly "bound", but whether there may be some proportion of it which might fail to equilibrate with marker molecules within a time sufficient for equilibration to occur between the marker and the majority of the tissue water. A non-osmotic marker, tritiated water, was used; this has the advantage of not being subject to any possible inequalities of distribution from Donnan effects. The investigation was confined to a single muscle, the gastrocnemius of the female rat.

The marker was introduced into the whole hind limbs of the rats by a perfusion technique already developed for other similar investigations<sup>10,11</sup>. It is felt that this method of perfusion permits thorough irrigation of the muscle vasculature, although because of employment of sub-physiological perfusion pressure, the attainment of equilibration is a more prolonged process than would be expected under completely physiological conditions.

Proteinized Ringer's solution<sup>10,12</sup> containing tritiated water (1 mc./100 ml.) was perfused through the hind limbs for predetermined periods which were varied from animal to animal. The perfusion pressure employed was sufficient to maintain the preparation in an isogravimetric state, that is, one in which the total fluid space of the perfused muscles neither decreased nor increased in size. After perfusion the two gastrocnemii were rapidly and carefully removed, blotted and weighed. They were then transferred to the microdistillation apparatus shown in Fig. 1, and there distilled at 105° C *in vacuo*. The distillate was trapped by freezing with acetone-solid carbon dioxide. In the final calculation allowance was made for small losses of distillate in the vacuum line. Weighed samples of distillate were analysed on a liquid scintillation counter. By reference to analysis of a similarly distilled aliquot of diluted perfusion fluid, the tritiated water space was back-calculated. In such calculations appropriate allowance was made for the loss referred to here; model

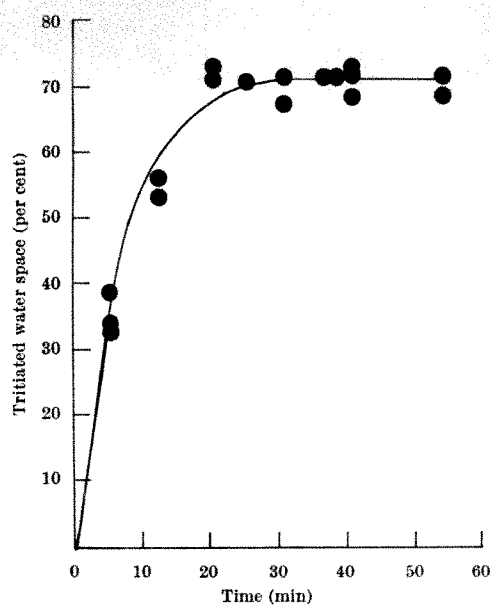


Fig. 2.

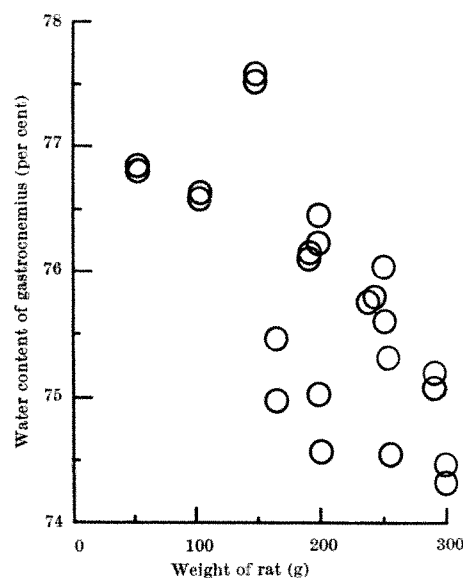


Fig. 3.

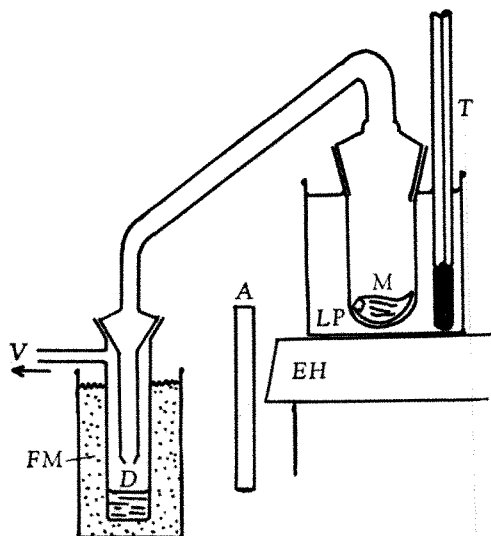


Fig. 1. Microdistillation apparatus. T, Thermometer; M, muscle mince; LP, liquid paraffin; EH, electric hotplate; A, asbestos shield; D, distillate; FM, freezing mixture; V, vacuum pump.

experiments showed that any minor inaccuracies incurred in the application of this correction would have an extremely small effect on the final figures. These are shown graphically in Fig. 2. All muscles analysed were taken from rats in the weight range 229–255 g. The equilibrium figure for the water spaces of these muscles was found to be  $70.2 \pm 1.4$  per cent.

For comparison, the percentage water content of another series of gastrocnemii was determined by desiccation at 105° C for 24 h (constant weight). These results are shown in Fig. 3. Rats with a wide range of body weights were used (55–300 g); but, confining attention to muscles taken from those rats the weights of which fell in the range 229–225 g, Fig. 3 shows that these should have had a water content of approximately 75 per cent. There is thus a discrepancy of 4–5 per cent between the two sets of values.

Although it is not possible from the present data to assess the parts played by various factors in bringing about this discrepancy, we can speculate on their nature, assuming completeness of perfusion. The possibility of a tritium-hydrogen exchange, which would give rise to retention of tritium in the dried muscle debris, and

therefore to spuriously low tritiated water spaces, has already been mentioned. Penetration of tritiated water into the extracellular regions within the tendinous parts of the muscle might well be so slow that whereas the entire fluid space of the muscle might appear to have equilibrated within 20–30 min, this small but specialized region of the extracellular space might well continue to equilibrate very slowly over a very much longer period.

Water in other regions of the extracellular space might behave in the same way. The hydration shells of proteins and of mucopolysaccharides are two such possible regions, corresponding, perhaps, to Overton's "bound" water. The extracellular space would therefore be the region to which the discrepancy between the desiccation and tritiated water spaces might most plausibly be ascribed.

Thus it is possible that the apparent equilibration plateau of the space/time curve in Fig. 2 might well approach a value of about 75 per cent over a long period, that is, it might correspond to the desiccation value. This suggestion would be consistent with the view that a small volume of the extracellular water in muscle is only very slowly exchangeable. Nor is this view necessarily incompatible with the finding of previous workers<sup>13</sup> that the whole body tritiated water and desiccation water spaces correspond, as the exclusive effect of protein and mucopolysaccharide hydration shells would be very much less significant in the whole body. Should this be the case there would be valid grounds for emphasizing that the differentiation of muscle water into "readily" and "non-readily" exchangeable moieties would have quantitative as well as qualitative significance.

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### Dorsal Light Receptors

THE dorsal ocelli of adult insects are present in some representatives of almost all orders except Collembola and Ephemeroptera<sup>1</sup>. Despite their wide occurrence among orders of insects, most hypotheses of their function have usually been *ad hoc* (for summaries of theories, see refs. 2–4). The physiological nature of the organs, their diversity throughout thousands of species, and their complex role in the integration of behaviour have not yet been thoroughly explored. Knowledge about these organs has begun to accumulate, and recent behavioural works on bees<sup>5,6</sup> and on wasps<sup>7</sup> give rise to the possibility that the ocelli may be associated more closely with visual perception than was previously thought. This communication is a preliminary report of the possibility that the ocelli act as visual sensitivity adjusters.

Much information has been accumulated about the ocelli, and the findings can be summarized as follows. (1) The ocelli do not form distinct images or, if they do, such images fall behind the retinal area and thus cannot

be perceived by insects<sup>2,4,8,9</sup>. (2) They react extremely rapidly to fluctuations in light<sup>10</sup>. (3) They usually occur in winged insects<sup>11</sup>. (4) Bees<sup>6</sup> and wasps<sup>7</sup> cease to forage at definite threshold intensities of light. The size of the ocelli appears to be related to the light intensity thresholds at which bees forage. Those with small ocelli are restricted to bright sunlight, while those with large ocelli (greater than 0.37 mm) can forage during day and night. (5) Blinding the ocelli decreases the visual sensitivity of bees<sup>6</sup> and of flies<sup>4</sup> during phototaxis. (6) Worker honeybees with blinded ocelli must forage at substantially higher light intensities than normal workers<sup>6</sup>.

**Function of the ocelli.** The general hypothesis of function which may fit all these behavioural and physiological findings is the following. The function of the ocelli can be thought of as analogous to that of the pupil of the human eye. Rather than being "stimulatory organs"<sup>4</sup>, they seem more likely to be moderators of the intensity of neural signals from the compound eyes, thus serving as extra-ocular adjusters of visual sensitivity. In particular, they allow a quickly flying bee or wasp to maintain its visual discrimination over a wide range of light intensity fluctuations, such as would be encountered in forests.

Light adjustment would seem to be a critical factor in visually oriented flight. Shielding pigment migrations in the compound eyes probably serve as long-term adjusters, because they are relatively slow<sup>12</sup>, while the ocelli would function centrally for quick adjustment. Hence large ocelli combined with sensitive, large compound eyes with pigment migrations would allow an insect to fly over a wide range of light intensities (both diurnal and nocturnal). Not all flying insects would need ocelli, but those without them would be limited in speed, direction and ability to continue flight under changing environmental light (for example, partly cloudy skies). A strongly diurnal flying insect (having reduced sensitivity of receptors, stationary shielding pigment, and few or no ocelli) could forage during daylight by avoiding shadows, but is unable to fly under reduced general environmental light (overcast sky). At the opposite extreme, ocelli and pigment migrations in the compound eyes may not be needed at all for a nocturnal flier which conceals itself well in the daytime and has no need to pass through strong light. For example, Johnson<sup>13</sup> has mentioned that most aphids (day fliers) will take flight if the light intensity is above 20 ft.-candles; whereas most noctuids, which are crepuscular or night fliers, will not usually take off when light is more than 20 ft.-candles in intensity. Such limits may not be vague behavioural entities, but may actually reflect restrictions on visual perception imposed by structural limitations.

The hypothesis that the ocellus serves as a sort of "pupil", that is, that it adjusts visual sensitivity, fits well with the pattern of occurrence of ocelli in bees. Bees (and, I strongly suspect, wasps) with different ocellar sizes tend to fly during different light intensities (Fig. 1). The larger the ocellus, the wider the range of light intensities under which they forage. The nocturnal bee, *Sphecodogastra texana*, has large ocelli in relation to the size of its head, and can forage not only under moonlight but also during the day. Whether the ocelli enlarge in order to maintain their function or whether their increased size enhances the ability to forage under low light remains to be determined by further testing.

**Control mechanisms.** For an indication of the necessity for quick and efficient visual adjustment mechanisms in accordance with increases in absolute speed, one needs only to look at birds. Unlike the slower mammals, including man, birds have not only photomechanical movements of receptors in the retina but also extremely fast pupillary movements often capable of great excursions<sup>14</sup>. The increase in visual sensitivity, along with their enhanced condition, is easily accepted as a prerequisite for dealing with visual perception at increased speeds. Movement necessitates good visual control not



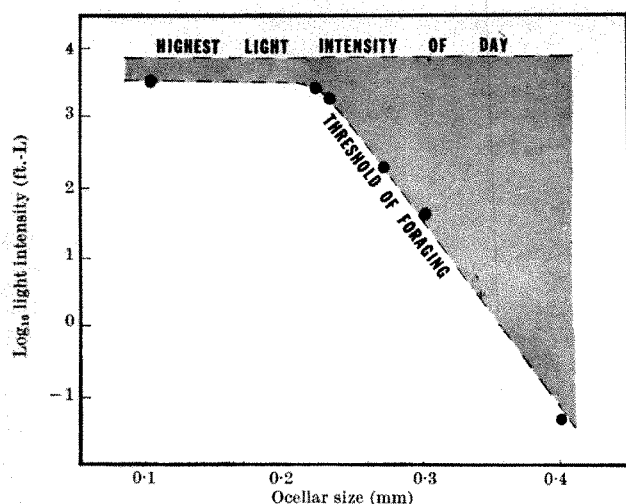


Fig. 1. The flight range of bees with different ocellar sizes. Left to right, the dots represent the light intensity thresholds of foraging for *Chilicola ashmeadii*, *Ezomalopsis (Ezomalopsis) sp.*, *Pseudogochloropsis nigerrima*, *Apis mellifera*, *Thygater analis*, and *Sphecodogastra texana*. The range of light intensities over which each of the species forages is the vertical distance from the highest light intensity of the day to the light intensity threshold of each bee.

only over fluctuations in background environmental lighting (day-night), but also over the increased rates of change of light intensity connected with passing through shadows. The rates of visual adaptation must be proportional to the absolute rates of light intensity change connected with velocity. If the visual adjustment mechanisms fail to keep pace with changes in light intensity, momentary loss of perception occurs which may result in collision and possible death.

As in birds, one would expect to find quick visual adjustment mechanisms in flying insects. This may

indeed occur. Interestingly, Kalmus<sup>11</sup> found that the possession of ocelli was correlated with the possession of wings. Likewise, neurophysiologists<sup>3,15</sup> have been impressed by the speed with which the ocelli respond to fluctuations in light intensity. Although visual pigment migrations occur<sup>12</sup>, they are rather slow, but they could certainly account for long-term adaptation to background light on a basis similar to the photomechanical movements of receptors in bird retinæ. Rather than having quick sensitivity adjustment and visual acuity tied up in the same structure, as in the human eyeball, these functions could be carried out in insects by two separate structures, the compound eyes and the ocelli.

In the human eye during quick fluctuations in light intensity, a "steady state" of incoming signals to the striate cortex is maintained by means of the pupillary reflex. This control mechanism can be imagined as a simplified feedback system which adjusts the magnitude of incoming signals (Fig. 2a). A drop in light intensity on the retina and on the macula lutea, in particular, causes a decrease in the magnitude of signals to the optic portion of the thalamus which then stimulates the iris to open, assuring a steady state of the signals coming into the decoding centre. (For a neurophysiological description of the synapses involved see ref. 16.)

In the insect, rather than a feedback system connected with the compound eye, a parallel, non-feedback system may have evolved. The ocelli monitor change in light intensity and adjust the magnitude of incoming signals (Fig. 2b). As the light intensity drops, decreasing the magnitude of signals from the compound eye, the ocellus increases its stimulation, probably increasing the sensitivity of secondary neurones to the lowered magnitude of signals, thus maintaining a steady state of signals into the decoding centre. Some caution is, however, needed here. This may only be the mode of operation in rather moderate background light. Although Ruck<sup>17</sup> found that during

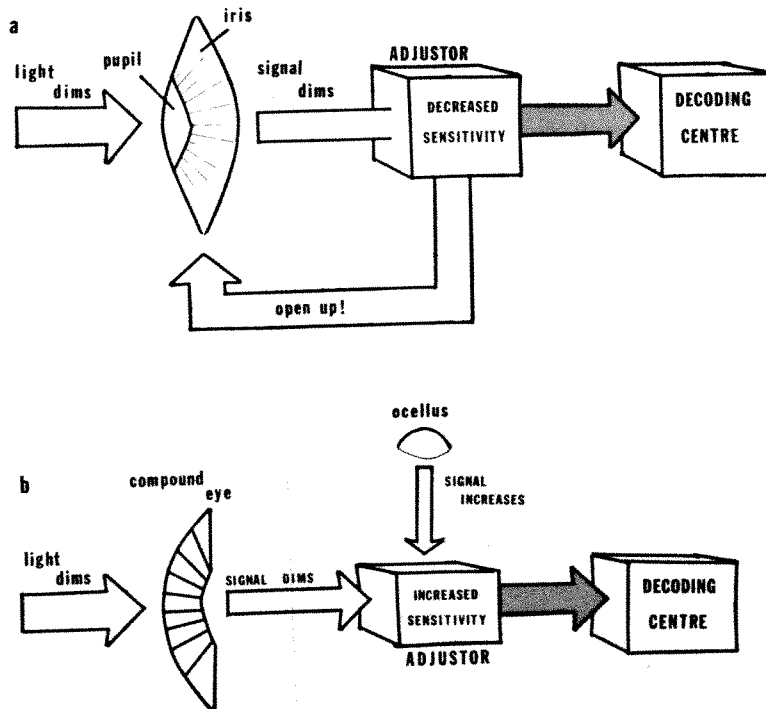


Fig. 2. a, Model of the pupillary reflex as a feedback system. Drop in light intensity decreases the magnitude of incoming signals to the optical portion of the thalamus (adjustor), increasing the signals of effector neurones to the iris. Opening the pupil increases the light intensity falling on the retina, thus maintaining a steady state of incoming signals (dark arrow) to the decoding centre. b, Model of ocellar adjustment as a simultaneous input. When the light level dims quickly, causing a decrease in the magnitude of signals coming into the optic lobe, simultaneously the ocellus reacts to the drop by increasing its signals. The ocellar stimulation presumably increases the sensitivity of neural ganglia to the lowered signals from the compound eyes, thereby maintaining a steady state of signals to the decoding centre.

moderate background light high light intensity tended to decrease or eliminate rapid spontaneous firings of the ocellar nerve stalk, very low background light also tended to create inhibition of spontaneous signals. If change of sensitivity would correspond to impulse rate, the latter finding of Ruck may explain the rise in the light intensity thresholds of honeybees with blinded ocelli<sup>5</sup>.

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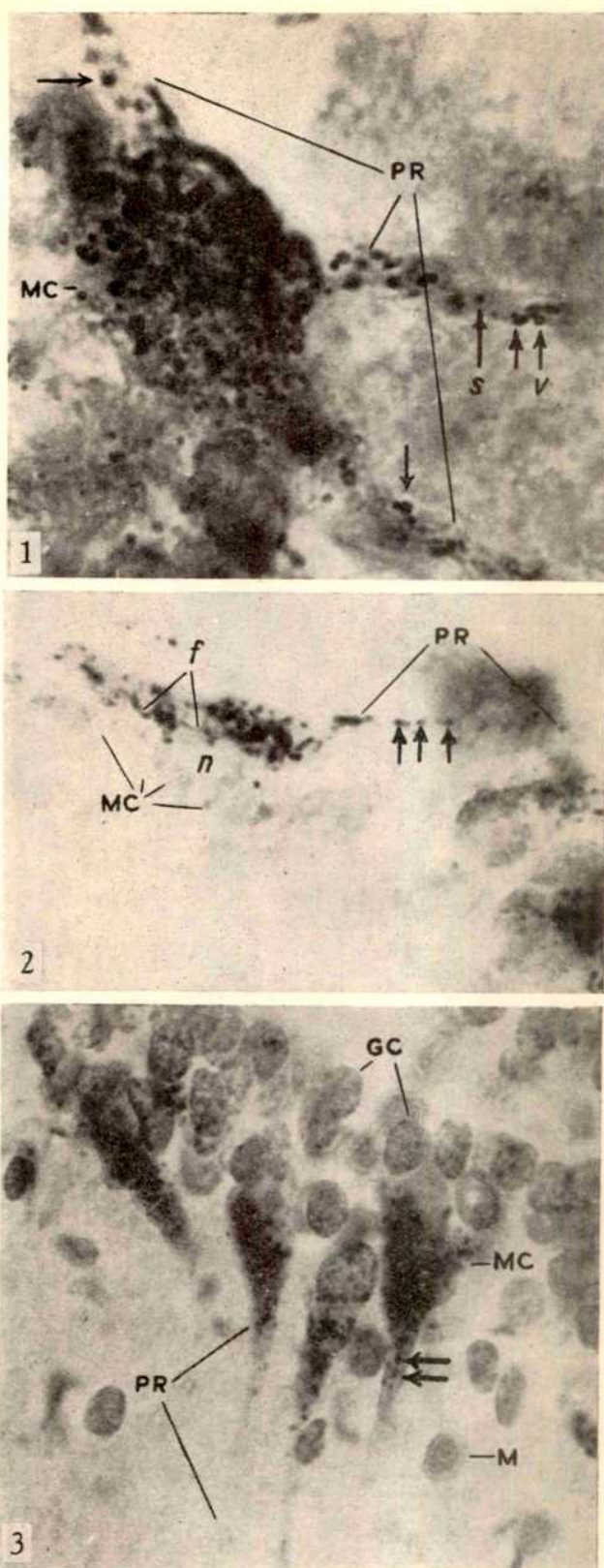
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### Lysosomes or Boutons?

ACID phosphatase has been localized by Gomori's technique in the form of vesicular bodies (*v*; Fig. 1) and solid spherules (*s*; Fig. 1) among the mitral and the tufted cells of the olfactory lobe of the rat and bat. Such inclusions vary in number and distribution in the mitral and tufted cells (Figs. 1-6). In some cells the granules are limited in number and are seen lying on one side of the nucleus (*n*; Fig. 2). There is polarization on the side from which the processes arise. Positive filamentous bodies occur in such cells (*f*; Fig. 2). It has been established that in neurons the acid phosphatase positive granules represent the lysosomes and they originate from the Golgi apparatus<sup>1</sup>; thus it is possible that the filamentous network may represent the part of the Golgi apparatus from where the lysosomes originate as often the lysosomes extend within the processes of the mitral cells (arrows *PR*; Figs. 1 and 2). In some of the processes, however, the acid phosphatase positive lysosomes are not seen (*PR*; Fig. 3) and in such cases the lysosomes are restricted to the main bodies. Figs. 4 and 5 show stages of the distribution of the lysosomes of the tufted cells of the bat in the main bodies (*TC*; Figs. 4 and 5), as well as along the entire lengths of their processes (arrows *PR*; Figs. 4 and 5). In Fig. 4 the two processes of the tufted cells seem to meet (within circle) and the acid phosphatase bodies are evenly distributed along the entire length of the two processes. In a low power microphotograph of a mitral cell of the rat the entire process (*PR*; Fig. 6) from the point of emergence at the mitral cell (*ML*; Fig. 6) to the point of fusion with the glomerulus (*GL*; Fig. 6) is seen studded with the acid phosphatase positive granules.

What is the exact nature and the physiological role of these acid phosphatase positive bodies? A comparison of



Figs. 1-5. All the figures represent acid phosphatase reaction (Gomori's technique) in fresh frozen sections.

Fig. 1 shows the vesicular and solid acid phosphatase positive bodies in the mitral cell as well as in its processes (*PR*), the solid type at *s* and vesicular type at *v*. ( $\times 1,600$ .)

Fig. 2 shows the concentration of positive filaments (*f*) and associated lysosomes near the nucleus (*n*), and the lysosomes in the process (*PR*) at the arrows in the mitral cell of rat. ( $\times 1,500$ .)

Fig. 3 shows the absence of the lysosomes in the process (*PR*) of the mitral cell of rat. Mitral cells are loaded with lysosomes. A molecular cell (*M*) and granule cell (*GC*) are also seen. ( $\times 500$ .)



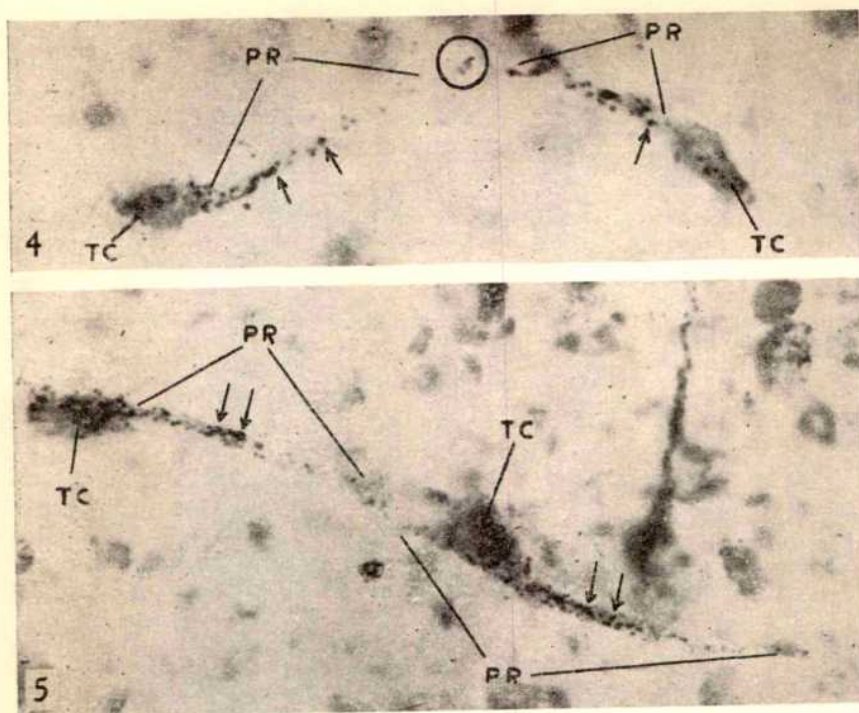


Fig. 4 shows two tufted cells (TC) of the bat with their processes (PR). Note the contact of the two processes within the circle and the lysosomes (arrows) contained in them. ( $\times 900$ .)

Fig. 5 shows two tufted cells of the bat (TC) with lysosomes (clear at arrows) distributed throughout the lengths of their processes (PR). ( $\times 800$ .)

our preparation (Fig. 1) with the photomicrograph of a freshly teased neurone stained by methylene blue by Hyden<sup>2</sup> shows exact resemblance as far as the distribution of the acid phosphatase positive bodies and the "synapses" in the neurones and the processes is concerned. The methylene positive bodies have been labelled as end feet (boutons) by Hyden<sup>2</sup> and the end feet or boutons have been considered as the synaptic sites located on the main bodies of the neurones and their processes. In this context it may be mentioned that two classes of glycolipids, namely, cerebroside and gangliosides, are present in the neurone system. It has also been suggested that the gangliosides are complexed with the proteins of the glycolipoprotein granules. The granules can be seen in neurones which have been stained by cationic (basic) dyes (such as neutral red and methylene blue) applied supravitaly or injected *intra vitam*<sup>3</sup>. It therefore seems possible that what Hyden has described as boutons after methylene blue staining may represent the glycolipoprotein granules. Further, as the glycolipoprotein granules have been proved to be homologous with the lysosomes<sup>4</sup>, the present results—which demonstrate that acid phosphatase positive lysosomes have the same appearance and topographical distribution as the boutons in Fig. 7—

add further evidence from the mitral cells that what has been regarded by Hyden as the synaptic sites may be simply the lysosomes distributed in the neurones and their processes. If such positive bodies are synaptic sites it is profitable to speculate about the role of the acid phosphatase, because recent evidence suggests that acid phosphatase is concerned with protein synthesis<sup>1,5-9</sup>. Ergastoplasm or ribosomal particles are found at the synapses<sup>9,10</sup>, and ion flux can produce changes in RNA during the passage of an impulse, resulting in the synthesis of qualitatively different protein for incorporation at the synaptic membrane (see ref. 10). Acid phosphatase may play a part in these processes. The acid phosphatase sites discussed here are thought to be lysosomes, however, and it is necessary to consider the role of these organelles at the dendritic sites.

Pappas and Purpura<sup>11</sup> have assigned an important role to the dendrites in the electrophysiological activity of brain, and Pope *et al.*<sup>14</sup> believe that certain enzymes play a significant part in the dendritic metabolism. The gangliosides (constituents of glycolipoprotein granules, that is, those of lysosomes)<sup>4</sup> have been reported to play a sig-

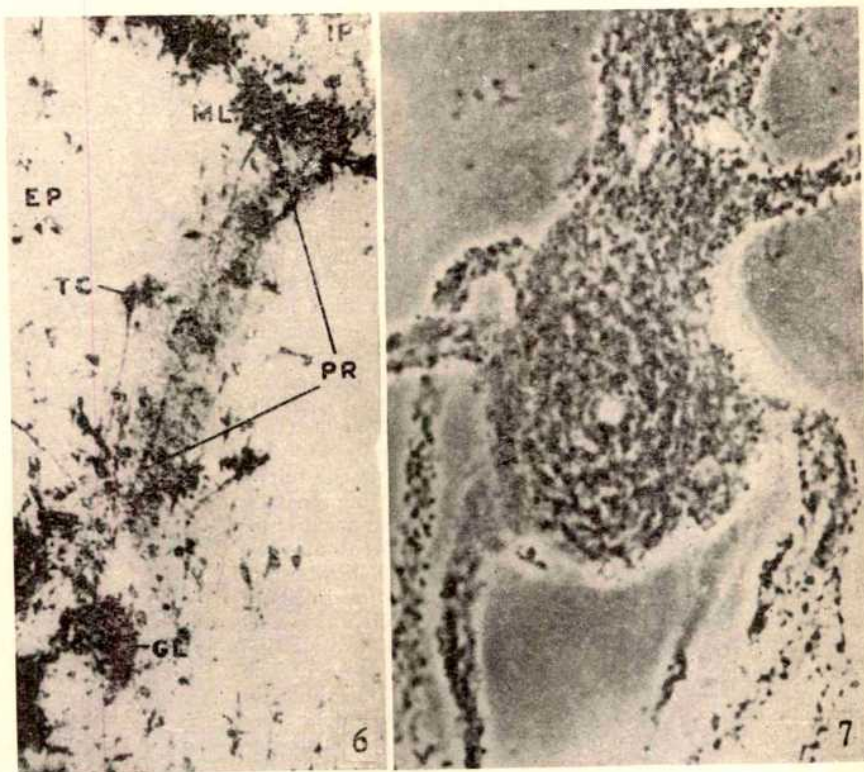


Fig. 6 is a low power photomicrograph which shows a mitral cell (ML) with its process (PR) positive along the entire length in the external plexiform layer (EP). Also note the positive glomerulus (GL) and the tufted cell (TC). The internal plexiform layer (IP) is also seen. ( $\times 170$ .)

Fig. 7 shows nerve cell from Deiters' nucleus. Dissected out by hand, photographed in phase contrast in isotonic sucrose solution slightly stained by methylene blue. This reveals the density of the synapses as small knobs on the surface. (Reproduced from Hyden<sup>2</sup>.)

nificant part in ion transport and other processes associated with membrane function<sup>12,13</sup>. Tewari and Bourne<sup>15</sup> have also suggested such a role of the acid phosphatase in the synapses of the cerebral cortex of rat. Thus it seems likely that the lysosomes, after originating from the Golgi apparatus in the perinuclear zone, migrate to dendrites to play their parts in ion transport and other phenomena like pinocytosis, with the acid phosphatase mediating the processes.

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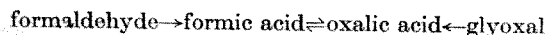
## RADIOBIOLOGY

### Cytotoxicity of Carbohydrates Heavily Irradiated in Solution

HEAVILY irradiated solutions of carbohydrates have been shown to influence the growth of cultures of bacteria<sup>1</sup>, and plant<sup>2</sup> and mammalian cells<sup>3</sup>, as well as producing chromosomal abnormalities in mammalian cells<sup>4</sup>. Steward *et al.*<sup>5</sup> have recently described the isolation from irradiated sucrose solution of a salt of formic acid which was characterized as sodium formate, and was shown to suppress the growth of carrot phloem explants.

In our earlier work on the effect of autoclaved and irradiated carbohydrate solutions on mammalian cells in tissue culture<sup>3,6</sup>, we concluded that the compound most likely to account for the persistent toxicity of irradiated solutions was the radiolytic product glyoxal. It was shown that the gross cytotoxicity of irradiated carbohydrate solutions was closely reproduced in our experimental system by a solution of glyoxal equivalent in concentration to the glyoxal present in the appropriate irradiated carbohydrate solution.

The presence of formaldehyde in irradiated carbohydrate solutions is known from the work of Phillips<sup>7</sup>, but it is unlikely that it is itself cytotoxic for the reasons discussed in our earlier paper<sup>3</sup>. The recent observation of Steward *et al.*<sup>5</sup> suggests that the oxidation of formaldehyde to formic acid, which might occur during irradiation or in the culture medium, results in the development of cytotoxicity. If this were so, glyoxal might similarly be converted to oxalic acid which must then be suspected as a potential cytotoxic agent. Because oxalic and formic acids are interconvertible, at least by chemical means, the gross effect of irradiated carbohydrate solutions might be accounted for by the system



This hypothesis was tested by the addition of formic and oxalic acids (as their sodium salts), glyoxal, or irradiated glucose solutions to the culture medium in which strain L mouse fibroblasts are grown. The culture

techniques have been described in detail in our earlier article<sup>3</sup>; the cells are grown attached to the glass surface of sealed bottles, collected by trypsinization, and the total growth after incubation at 37°C for 7 days counted electronically. The results are shown in Table 1, expressed as a percentage of growth in control cultures incubated simultaneously. Sodium formate exerts no cytotoxic effect, even at a concentration ten times higher than that shown by Steward *et al.*<sup>5</sup> to be present in solutions of irradiated carbohydrates. Sodium oxalate is also much less cytotoxic than glyoxal. It can be calculated that, even if all the glucose transformed during irradiation with 2.5 Mrads were to appear as oxalic acid, the amount of acid produced would still not account for the observed cytotoxicity of the irradiated glucose solution.

Table 1. GROWTH OF STRAIN L MOUSE FIBROBLASTS

Ordinary growth medium plus:	Percentage of control growth in 7 days	Ref.
Autoclaved distilled water (control)	100	3 and present experiments
1 per cent solution, dextrose, B.P., filter-sterilized	104	3
1 per cent solution, dextrose, B.P., irradiated, 2.5 Mrads	1.3	3
1 per cent solution, dextrose, B.P., irradiated, 2.5 Mrads, stored for two years	2.2	Present experiments
Glyoxal, 0.0073 mg/ml.	100	3
0.022 mg/ml.	71	3
0.073 mg/ml.	34	3
0.22 mg/ml.	1.4	3
0.73 mg/ml.	0.8	3
Sodium formate, reagent grade		
0.0017 mg/ml.	106	Present experiments
0.017 mg/ml.	102	" "
0.17 mg/ml.	109	" "
Sodium oxalate, 'AnalaR'		
0.022 mg/ml.	79	" "
0.22 mg/ml.	37	" "
2.2 mg/ml.	2.2	" "

These results indicate that the cytotoxicity of the formate salt which has been demonstrated by Steward *et al.*<sup>5</sup> is unlikely to be duplicated by the combined formic acid (which would appear as sodium formate in culture media for mammalian cells at pH 7.0-7.2) produced by radiolysis of carbohydrates in solution. This is also corroborated by the fact that labelled sodium formate is a common starting material used in the biological synthesis of a variety of compounds labelled with carbon-14. In these circumstances, oxidative processes involving the formation of formic and oxalic acids from formaldehyde and glyoxal cannot be expected to contribute to the cytotoxicity of heavily irradiated carbohydrate solutions.

Finally, the 1 per cent solution of glucose irradiated with 2.5 Mrads, which has been used in these studies, has been shown to retain its cytotoxicity for more than 2 years after irradiation.

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## Dependence of the Oxygen Enhancement Ratio on Neutron Energy

THE data reported by Barendsen and Broerse<sup>1</sup> for the oxygen enhancement ratios in human cell cultures subjected to neutron radiation have prompted us to report the values which we have obtained for a completely different biological system—that of induction of somatic mutation in the staminal hair cells of a diploid clone (02) derived from a variety of *Tradescantia occidentalis*. Briefly, the irradiation induces the appearance of homozygous recessive mutant cells of a red colour which are distinguishable from the normal heterozygous blue cells. The system has been used previously to obtain relative biological effectiveness (RBE) values for negative  $\mu$  mesons (ref. 2) and 650 keV neutrons (ref. 3).

The inflorescences of the plants were irradiated with 200 keV X-rays, cobalt-60  $\gamma$ -rays and mono-energetic neutrons with energies ranging from 0.1 MeV to 14.7 MeV. Contamination of the neutron radiation with X-rays is believed to be less than 5 per cent in terms of the absorbed dose. Both before and after irradiation the plants were kept in conditions of continuous light and constant temperature in order to obtain a fully asynchronous cell population. For the irradiation the plants were sealed in an aluminium container with their inflorescences held together in a small tissue equivalent plastic box for a total of 50 min in aerobic exposures; for anoxic exposures the container was evacuated for 13 min to a pressure of about 2 mm of mercury and was then filled and flushed with nitrogen for a further 17 min before the irradiation began; the total time in the container was 65 min. The oxygen content of the emerging nitrogen was measured with a Hersch cell<sup>4</sup> and never exceeded 300 volume parts per million at the beginning of an irradiation.

For both X-rays and cobalt-60  $\gamma$ -radiation the results are well represented by curves of the type

$$M = \alpha + \beta D + \gamma D^2$$

where  $M$  is the mutation frequency and  $D$  is the fluence. For neutron irradiations the results fit curves of the type

$$M = \alpha + \beta D$$

Depending on neutron energy, the dose rates varied between 0.02 rad/min and 0.4 rad/min, while for both X-radiation and  $\gamma$ -radiation dose rates of 1.5 rad/min were used. X-ray experiments indicate that the mutation frequency is not sensitive to dose rate in the range 0.15–1.5 rad/min. Death of the cells at the low doses used has been shown to be negligible<sup>5</sup>.

Because of some uncertainty in the exact absorbed doses we are unwilling to quote RBE values at the present time, although they seem consistent with the previous determination for 650 keV neutrons<sup>3</sup>. The oxygen enhancement ratios should, however, be independent of our uncertainty about the absorbed doses. The results, together with their standard errors, are summarized in Table 1. These oxygen enhancement ratios are the ratios of the coefficients of the linear terms of the above equations obtained by least square fits to the experimental data and correspond to the ratios of the initial slopes of the dose-effect curves. In the case of the quadratic response

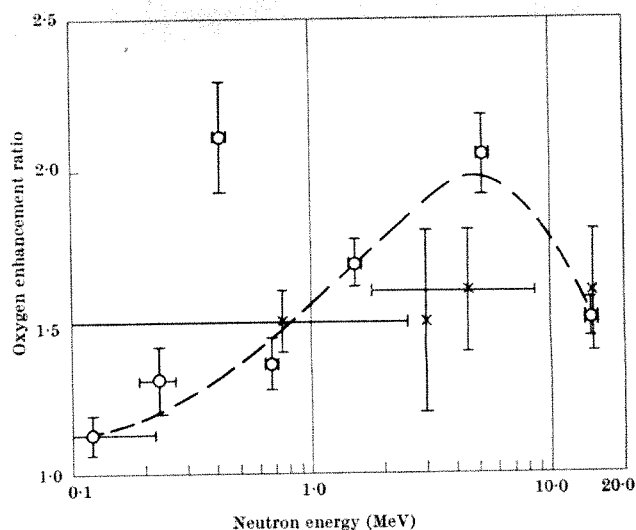


Fig. 1. Oxygen enhancement ratios as a function of neutron energy.  $\circ$ , Results from the induction of somatic mutations in *Tradescantia occidentalis*;  $\times$ , results from the survival of cultured cells of human origin<sup>1</sup>. ---, Hypothetical variation neglecting resonance scattering effects.

relationships given by the X-ray and  $\gamma$ -ray irradiations it may be demonstrated that for the oxygen enhancement ratio to be independent of the total dose the following relationship must hold

$$\text{oxygen enhancement ratio} = \frac{\beta_o}{\beta_N} = \left(\frac{\gamma_o}{\gamma_N}\right)^{\frac{1}{2}}$$

The suffixes  $O$  and  $N$  refer to the values obtained under aerobic and anoxic conditions, respectively. This is probably true in our case. The values for the cobalt-60 exposures are  $3.27 \pm 0.74$  for the ratio of the coefficients of the linear terms ( $\beta_o/\beta_N$ ) and  $2.38 \pm 0.71$  for the square root of the coefficients of the quadratic terms ( $\gamma_o/\gamma_N$ ); for the X-ray exposures the equivalent values are  $2.85 \pm 0.57$  and  $2.39 \pm 0.59$ , respectively. Within the standard errors these values are in agreement with the value of  $2.6 \pm 0.2$  found by Barendsen and Broerse<sup>1</sup> for 250 kVp X-rays despite the very different biological system.

It must be pointed out that the only neutron energy at which a true comparison between our results and those of Barendsen and Broerse is possible is at 14.7 MeV, when our result of  $1.51 \pm 0.06$  is in fair agreement with their result of  $1.6 \pm 0.2$ . For all other irradiations, either their energy differs markedly from ours, as with their 3 MeV value, or they used neutron radiation with a very broad energy spread while we have used substantially mono-energetic spectra. Both our data and those of Barendsen and Broerse are plotted in Fig. 1, showing the oxygen enhancement ratios at the peak intensities of the neutron spectra and giving the energy spread, where it is known, at half maximum intensity by means of the horizontal bars.

The high value for the oxygen enhancement ratio which we have found at 0.42 MeV requires some explanation. Apart from this energy, the ratios were obtained at energies chosen to avoid marked resonances in the scattering cross-sections of any of the elements from which the plant tissue is composed. In the region of 0.44 MeV, however, there is a marked resonance in the oxygen scattering cross-section. Chemical analysis (ref. 6 and unpublished results of Weiler and Strauss) has shown that the plant tissue contains about 80 per cent by weight of oxygen, so that the resonance was expected to produce a noticeable effect. The experiment demonstrates that heavy ion recoils must play an important part in the effect of neutron radiation on tissue, and that possibly range as well as linear energy transfer is a factor in the

Table 1. OXYGEN ENHANCEMENT RATIOS FOR THE INDUCTION OF SOMATIC MUTATIONS IN *Tradescantia occidentalis* BY MONO-ENERGETIC NEUTRON RADIATION, AND X-RADIATION AND  $\gamma$ -RADIATION

Radiation	Width of energy spectrum at half height	Oxygen enhancement ratio
<sup>60</sup> Co- $\gamma$ -ray	—	$3.27 \pm 0.74$
200 keV X-ray	80 keV	$2.85 \pm 0.57$
0.12 MeV neutrons	0.2 MeV	$1.131 \pm 0.066$
0.23 MeV neutrons	0.08 MeV	$1.31 \pm 0.11$
0.42 MeV neutrons	0.06 MeV	$2.11 \pm 0.18$
0.48 MeV neutrons	0.06 MeV	$1.365 \pm 0.083$
1.53 MeV neutrons	0.15 MeV	$1.695 \pm 0.077$
5.15 MeV neutrons	0.40 MeV	$2.05 \pm 0.13$
14.7 MeV neutrons	0.70 MeV	$1.514 \pm 0.063$

response. We hope to elucidate the relative importance of these factors in a quantitative way by an examination of the micro-dosimetry of our experiments.

We thank Dr D. R. Davies for the culture of the *Tradescantia occidentalis* and for comment in the preparation of this note. The experiments could not have been carried out without the help of the staff of the AERE Nuclear Physics Division's 5 MeV Van de Graaff and Cockcroft Walton accelerators.

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### Increased Resistance of Mice to X-irradiation after the Injection of Bee Venom

It is known that the response of animals to whole-body X-irradiation in the lethal range can be modified by certain changes in their physiological state induced before exposure. For example, the administration of oestrogens<sup>1</sup>, bacterial endotoxin<sup>2</sup>, colchicine<sup>3</sup>, or urethane<sup>4</sup> to mice 1 day or more before lethal irradiation results in a modest but definite increase in the number of animals surviving over a period of 30 days. The mechanism(s) by which these diverse agents enhance the radiation resistance of rodents is not clear; however, it can be stated that their protective effect must be mediated by way of modes of action different from those of the "classical" chemical radioprotectors such as cysteine, cysteamine or AET<sup>5</sup>, which are only effective when administered immediately before (about 30 min) irradiation.

It seemed to us possible that because of the chemical composition and pharmacological effects of the known components of bee venom it might be of value as a radio-protective agent. It was thought that the venom might produce a degree of physiological "stress" in animals, and thereby elicit a neuroendocrine response (pituitary-adrenal stimulation)—the so-called adaptation syndrome<sup>6,7</sup>—which would increase radiation resistance<sup>7</sup>.

Bee venom is a complex mixture of enzymes, toxins, and unidentified substances. The biologically active constituents in bee venom include the enzymes phospholipases A and B (ref. 8), hyaluronidase<sup>9</sup>, and the haemolytic and neurotoxic polypeptides mellitin<sup>10</sup> and apamine<sup>11</sup>. The last two substances constitute the largest fraction by weight of bee venom.

The experimental animals used were genetically homogeneous LAF<sub>1</sub> hybrid male mice, 12–16 weeks of age, from the NRDL colony. The estimated X-ray LD<sub>50</sub> for these mice is 740 r. The mice were placed in perforated 50-ml. 'Lusteroid' centrifuge tubes, radially positioned around a circular wooden turntable and rotated at 3.5 r.p.m. directly under the X-ray tube. After a single exposure to the radiation, the mice were housed in suspended galvanized metal cages, two per cage, and they were observed over a period of 1 month. 'Purina' chow pellet feed was available *ad libitum*. The drinking water contained added 'Neomycin' (100 mg per cent) and 'Polymyxin B' (840 u/ml.).

Two bee venoms (*Apis mellifera ligustica*) were used in this study: the venom used in the first experiment was collected on August 29, 1965, in Ithaca, New York, by Dr Roger A. Morse, Cornell University. The second venom

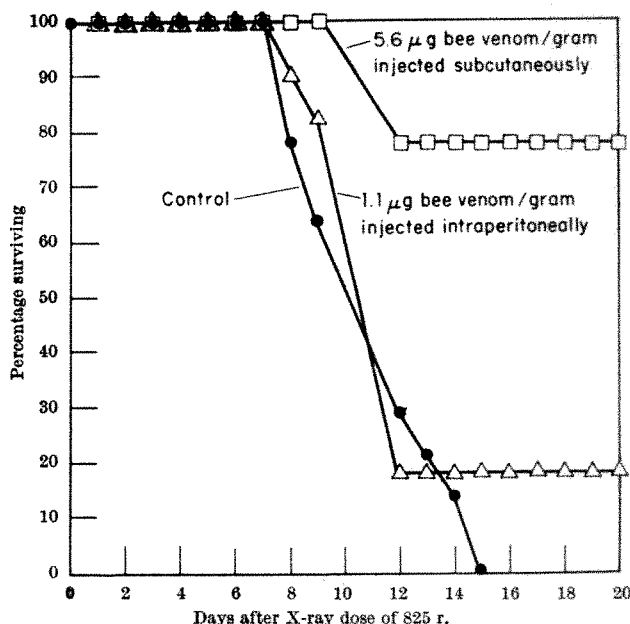


Fig. 1. Radioprotection by bee venom injected subcutaneously into mice 1 day before X-irradiation.

used was collected on April 2, 1966, by one of us (W. H. S.) in Santa Cruz, California. No differences could be detected in the toxicity for mice of these two venoms.

One group of eleven male mice (4 months old) received 1.24 µg of bee venom (dissolved in isotonic saline)/g of body weight and a control group of nine mice received the equivalent volume of saline only. The injections were delivered intraperitoneally 24 h before exposure to an X-ray dose of 800 r. At the end of the 30 day period of observation 64 per cent of the mice that received the bee venom were alive, while only 22 per cent of the control group had survived ( $P=0.005$ ).

In the next experiment each of a group of ten mice was injected subcutaneously with 5.6 µg of bee venom/g body weight and a second group received 1.1 µg/g intraperitoneally. A third group of fourteen mice was injected intraperitoneally with the same volume (0.2 ml.) of saline only. (A preliminary toxicity trial had revealed that these doses of venom were at the threshold of the toxic, that is, lethal, dose for mice, administered by each of these injection routes.) Twenty-four hours later, all three groups were exposed together to a dose of 825 r. As can be seen in Fig. 1, all members of the control group died, while about 20 per cent of the intraperitoneally injected group and 80 per cent of the subcutaneously injected groups were alive at 30 days. ( $P<0.001$  for subcutaneously injected mice versus saline controls.)

In two additional experiments, groups of mice were injected subcutaneously with doses of bee venom (4.3 µg/g, or 5 µg/g) 1 day before they were exposed to 850 r. of X-rays. In both these groups the 30-day survival was 70 per cent, while none of the controls receiving saline survived. At these dosages, however, the venom was not effective in increasing survival when it was administered 30 min before irradiation, nor when injected immediately after the exposure of mice to 850 r. The fact that the venom provides protection when it is administered 24 h before radiation exposure places it in a different category from cysteine, AET and related compounds, which if they are to be effective must be administered just before irradiation and which are thought to exert their action at a radiation-chemical level<sup>12</sup>.

We have carried out preliminary chemical fractionation studies in an attempt to identify the radioprotective constituent(s) of bee venom. A polypeptide fraction, the largest component of which was identified as mellitin, has

been separated and isolated by means of chromatography in columns of 'Sephadex' gel. In a single experiment, mice were injected subcutaneously with this fraction (5.4 µg/g) dissolved in isotonic saline containing 0.01 normal formic acid. One day later, the mice were exposed to an X-ray dose of 850 r. Thirty days after irradiation 57 per cent of the mice so treated were still alive, while all the irradiated control mice injected with saline-formic acid had died.

At least three mechanisms may be invoked to account for the radioprotective effect of bee venom reported here for mice: (1) that it has a stressor-like action, thereby eliciting an "adaptation syndrome", assumed to increase radioresistance; (2) that it produces changes in the haemopoietic system, for example analogous to the effect of urethane<sup>4</sup> or of certain bacterial endotoxins<sup>1</sup>; (3) that it has antibacterial properties<sup>12</sup>. These mechanisms are now being investigated and further experiments are being carried out to separate, purify and identify the biologically active constituents of bee venom.

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## MICROBIOLOGY

### Tetramethyldipicrylamine—a New Antibacterial Agent

Moore, Meyer and Hudson recently reported on a new analytical reagent, tetramethyldipicrylamine (3,3',5,5'-tetramethyl-2,2',4,4',6,6'-hexanitrodiphenylamine) and recommended it for further study in the analytical chemistry of the alkali metals<sup>1</sup>. A routine screening of this reagent by the National Cancer Institute, US National Institutes of Health, later showed its capacity to bring about a small but significant inhibition of tumour growth. A further check of its biological activity was then carried out, and is described here. The results indicate that this compound has antibacterial activity against Gram-positive organisms and several strains of sulphadiazine-resistant *Neisseria meningitidis*.

The sodium salt of tetramethyldipicrylamine was incorporated into trypticase soy broth and 1.5 per cent agar to give a final concentration of 10 and 50 µg/ml. medium. The following organisms were inhibited at the 10 µg/ml. concentration: *Staphylococcus aureus* (FDA-2098), *Staphylococcus epidermidis* (FDA-1200), *Streptococcus mitis*, *Streptococcus salivarius*, *Bacillus subtilis*, *Bacillus stercorophilus* (NIH-7953). The following organisms were not inhibited at 50 µg/ml. concentration: *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Proteus vulgaris*, *Salmonella heidelberg*, *Serratia marcescens* (ATCC-13880), *Escherichia coli*, *Salmonella montevideo*.

Fourteen strains of *Neisseria meningitidis*, including both sulphadiazine resistant and sensitive organisms of group B and C, were tested. The assay was carried out on Mueller-Hinton agar as described by Frank, Wilcox and Finland<sup>2</sup>. The minimal inhibitory concentration ranged from 0.06 to 0.5 µg of the compound/ml. of medium.

Using the serial dilution assay method, *Bacillus subtilis* was inhibited by the compound at 1 µg/ml. and *Diplococcus pneumoniae* at 2–5 µg/ml. (Muir, R. D., personal communication).

A Gram-negative plant pathogenic bacterium *Erwinia* species and the fungi *Candida albicans* and *Fusarium* species were tested by an agar diffusion method. The compound was only slightly active against *Erwinia* species and had no activity against the fungi in this test.

No inhibition was found in tests using the nematode *Turbatrix aceti* or the protozoan *Trichomonas foetus* (Muir, R. D., personal communication).

These results show high activity *in vitro* against Gram-positive organisms and little or no activity against most Gram-negative organisms, fungi, nematodes and protozoa. It is interesting, however, to note that the compound shows high activity against the Gram-negative sulphadiazine-resistant meningococcal strains.

The compound, which is acidic and of low solubility, forms well defined salts. The sodium salt is readily soluble and was used for all our tests. The free acid showed no antibacterial activity.

We thank Dr R. D. Muir of G. D. Searle and Co. for performing some of the microbiological tests.

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### Ultrastructural Localization of Coxsackie B<sub>4</sub> Virus in Mouse Myocardium

COXSACKIE viral infections are known to cause myocardial lesions in various animal species<sup>1</sup>. The identification and localization of the Coxsackie viral particles in the heart have not been adequately demonstrated because of the absence of morphological criteria which will differentiate virus particles from glycogen granules and ribosomes. This communication describes the ultrastructural localization of Coxsackie B<sub>4</sub> virus particles in the mouse heart by a ferritin antibody technique.

A randomly selected litter of 5 day old suckling mice of *HaM/ICR* strain was inoculated intraperitoneally with 0.1 ml. of fluid containing 10<sup>5</sup> TCID<sub>50</sub> Coxsackie virus B<sub>4</sub> in monkey kidney cells; this strain was originally recovered by Kibrick and Benirschke from a 10 day old infant who died of encephalohepatomyocarditis<sup>2</sup>. The mice were killed 6 days after inoculation. Multiple 0.5 mm cubic blocks of tissue were cut from the myocardial wall and fixed in 4 per cent buffered glutaraldehyde for 20 min. The tissue blocks were washed in phosphate buffered normal saline, immediately immersed in a ferritin conjugated anti-Coxsackie virus B<sub>4</sub> rabbit serum for 45 min,



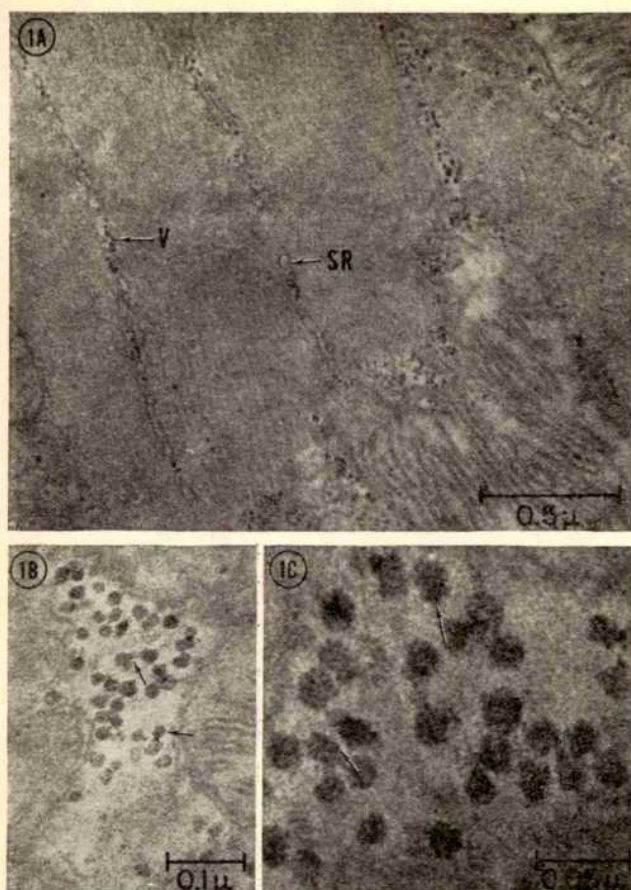


Fig. 1. An electron micrograph showing (A) the distribution of the Coxsackie B<sub>1</sub> virus particles (V) along the tubules of the sarcoplasmic reticulum (SR). (B) and (C) show viral particles at higher magnification, with the binding of ferritin molecules on the viral particle surface (arrows). Unstained.

washed in the buffer, fixed in osmium tetroxide, embedded in 'Maraglas' and examined with an electron microscope.

Conjugation of ferritin to previously prepared and purified hyperimmune anti-Coxsackie virus globulin (rabbit) was performed according to the method of Singer<sup>4</sup>. The ferritin acts as an electron dense "tag" which when coupled to antibody can be used to detect specific antigen-antibody reaction sites. Uninfected mice of the same age but from a different litter were used as controls. Control tissue was treated in the same way as that of experimental mice.

Virus particles were present only at certain foci within the infected hearts. In the infected muscle fibres the virus particles were oriented around tubules of the sarcoplasmic reticulum (Fig. 1A). No virus particles could be detected in the nuclei. The particles had a higher osmophilia than the surrounding matrix. Their average diameter was about 20 mμ. The viral particles were roughly hexagonal in profile and on their surface the small electron dense particles of ferritin could be easily identified (Fig. 1B, C). A few sparsely scattered free ferritin particles with no specific localization were also observed in the myocytes. In the control tissue, no such viral particles were noticed.

The early stages of Coxsackie infection produce few cytopathic features. Indeed, as with many other viral infections, synthesis of specific viral proteins apparently occurs simultaneously with normal cell protein and nucleic acid synthesis for varying periods of time<sup>5</sup>. The cells continue to function and show few alterations despite the presence of a latent infection. After 5-7 days definite pathological features appear in some cells and irreversible damage with necrosis becomes apparent<sup>1,2</sup>. The damage is focal, however, and some muscle cells appear entirely

normal. It seems likely from other studies that many cells are initially infected and that the latent phase may exist for weeks or even years without evidence of overt cellular damage<sup>6</sup>.

The distribution of the Coxsackie particles along the tubules of the sarcoplasmic reticulum seems significant. Since the inner membrane of the sarcolemma invaginates into the cell at the level of the Z-band and is in close approximation with the cavities of the sarcoplasmic reticulum, this may represent the primary route of virus entry into the sarcomere from the extracellular space.

The distribution of virus particles in the myocardium is evident from these observations. The ferritin labelling method is therefore a useful procedure for identification of viral infections of heart and other organs by routine electron microscopy.

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### Mutation towards Dextran Sulphate Resistance in Type 1 Poliovirus

THE use of type 1 poliovirus mutants resistant to dextran sulphate, as described by Takemoto and Liebhafner<sup>1</sup> and Takemoto and Kirschstein<sup>2</sup>, has proved extremely promising for genetic investigations. We have therefore carried out some additional investigations of these mutants with the view of using them in recombination experiments.

The present communication reports preliminary observations on some characteristics of the mutation towards dextran resistance and the relationship between the *ds* marker and other genetic characters either belonging to the group displaying covariation with neurovirulence or independent of the latter.

Our experiments were performed with clonal derivatives of the attenuated type 1 LSc2ab and of the neurovirulent type 2 MEF<sub>1</sub> poliovirus strains. The behaviour of the viral populations towards dextran sulphate 2,000 was tested by the plaque method with dextran sulphate incorporated in the agar overlay at a concentration of 100 μg/ml., as previously described<sup>3</sup>. In some cases, in which the inhibitor gradient method<sup>4</sup> was applied, the dextran sulphate concentration in the gradient plate ranged from 0 to 200 μg/ml. The other markers were tested according to techniques described in previous papers<sup>3,5</sup>. The hydrochloric acid salt of 2-(α-hydroxybenzyl)-benzimidazole (HBB) used in some of our experiments was synthesized and kindly supplied by V. A. Blaszek from the Institute of Medicine and Pharmacy in Tg. Mureș (Romania). The inhibitory action of HBB was assayed according to the same techniques as that for guanidine<sup>5</sup>.

When the parental L<sub>1</sub>S type 1 attenuated poliovirus was exposed to a dextran sulphate gradient, the large plaques formed by *ds*<sup>+</sup> particles were found to be scattered



Table 1. RELATIONSHIP BETWEEN THE *ds* CHARACTER AND OTHER GENETIC MARKERS OF POLIOVIRUS

Clonal strains		Genetic markers*						Genotype	
Origin	Selected in the presence of:	$ds \uparrow$ $\left(\frac{M}{DS\ 100\ \mu g}\right)$	Displaying covariation with neurovirulence			Not displaying covariation with neurovirulence			
			$MS \uparrow$ $\left(\frac{MK}{MS}\right)$	$t \S$ $\left(\frac{M}{50^\circ}\right)$	$mp \parallel$ $\left(\frac{nr.\ parallel}{nr.\ inoc.}\right)$	$g \uparrow$ $\left(\frac{M}{G\ 100\ \mu g}\right)$	$hbb \uparrow$ $\left(\frac{M}{HBB\ 400\ \mu moles}\right)$		
Derived from a neurovirulent wild type 2 population (MEF <sub>1</sub> )	— (Parental)	$r$ 0.25	+	+	+	$s$ 5.00		$S_2ds^rMS^+t^+mp^+g^s$	1
	$G\ 100\ \mu g$ (1 clone)	$r$ 0.00	+	+	+	$r$ 0.43		$S_2ds^rMS^+t^+mp^+g^r$	2
	$DS\ 100\ \mu g$ (10 clones)	$r$ 0.04	+	+	+	$s$ 4.48		$S_2ds^rMS^+t^+mp^+g^s$	3
Derived from an attenuated type 1 population (LSc2ab)	— (Parental)	$s$ $2.69 \pm 0.39 \P$	—	—	—	$s$ 5.46	$s$ 3.69	$S_1ds^sMS^+t^+mp^-g^shbb^s$	4
	$G\ 100\ \mu g$ (8 clones)	$s$ 2.95	—	—	—	$r$ 0.19		$S_1ds^sMS^+t^+mp^-g^r$	5
	$G\ 100\ \mu g\ (\times 2.3)$ (8 clones)	$s$ 3.07	—	—	—	$r$ 0.22	$s$ 5.35	$S_1ds^sMS^+t^+mp^-g^shbb^s$	6
	$DS\ 100\ \mu g$ (4 clones)	$r$ 0.01	—	—	—		$s$ 4.41	$S_1ds^sMS^+t^+hbb^s$	7
	$DS\ 100\ \mu g^{**}$ (2 clones induced with nitrous acid)	$r$ 0.20	—	—	—			$S_1ds^sMS^+t^-$	8
	$DS\ 100\ \mu g$ HBB 400 $\mu moles$ (4 clones)	$r$ -0.09	—	—	—	$s$ 3.69	$r$ 0.98	$S_1ds^rg^shbb^s$	9
	HBB 100–200 $\mu moles$ (3 clones)	$s$ 2.37	—	—	—			$S_1ds^shbb^s$	10

\* The figures represent either values obtained in single determinations or mean values recorded in partial or exhaustive testing of clones displaying similar phenotypes.

† The degree of inhibition. The values represent the virus titres (in log p.f.u./ml.) in the absence and in the presence of the inhibitor at the concentrations specified in the denominator of the fraction (*s* equals sensitive, *r* equals resistant).

‡ Difference between virus titres (in log dim/0.2 ml.) on MK and MS cells.

§ Difference between virus titres (in log dim/0.2 ml.) before and after heating of the virus at 50° C for 15 min.

|| *mp*, mouse pathogenicity, that is, number of mice paralysed/number of mice inoculated.

¶ Mean and standard deviation of individual values obtained in 11 determinations of the same strain.

\*\* Compare ref. 7.

†† Approximate indications resulting from the level at which plaques still appeared in inhibitor gradient (Grd) tests (in this case: 6 cm from the base of the plate, that is, about 200  $\mu moles/ml.$ , the gradient ranging from 0 to 400  $\mu moles/ml.$ ).

at random over the upper three-quarters of the gradient (ranging from about 50 to 200  $\mu g/ml.$ ) (Fig. 1). This distribution pattern strongly suggests that in contra-

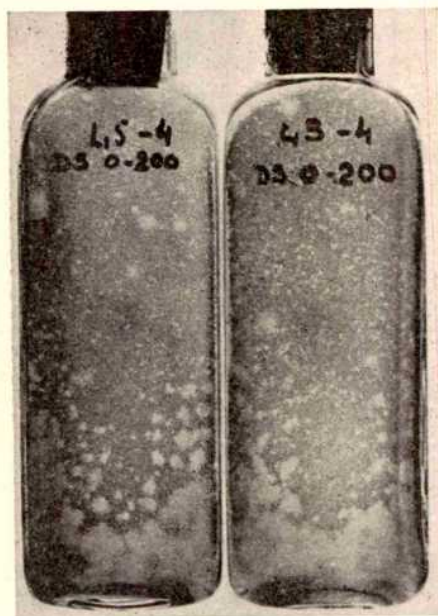


Fig. 1. Distribution pattern of normal sized plaques formed by the L<sub>1</sub>S type 1 poliovirus strain over a dextran sulphate gradient ranging from 0 to 200  $\mu g/ml.$  The dense crowding of normal sized plaques in the lower quarter of the flask is due to the fact that almost the whole viral population is insensitive to dextran sulphate at low concentration. This is in striking contrast to the upper three-quarters of the dextran sulphate gradient (ranging from about 50 to 200  $\mu g/ml.$ ) where a few normal sized plaques, formed by *ds<sup>r</sup>* mutants, are randomly scattered among inhibited plaques.

distinction to guanidine resistant mutants, which may be divided into several classes according to the concentration of the inhibitor to which they prove resistant, *ds<sup>r</sup>* mutants do not seem to be separable into distinct categories. If this could be confirmed, it would follow that dextran sulphate resistance is acquired by single step mutation. This inference is in agreement with Bengtsson's observations<sup>6</sup>. By plating LSc2ab strain under agar overlays which contained various concentrations of dextran sulphate Bengtsson found a sharp decrease in the number of normal sized plaques between concentrations of about 3 and 10  $\mu g/ml.$ , while larger amounts of dextran sulphate did not significantly enhance the degree of inhibition. This was considered to result from the presence of about 0.1 per cent dextran sulphate resistant mutants in the viral progeny.

In our experiments the mean proportion of *ds<sup>r</sup>* mutants in the parental L<sub>1</sub>S population for eleven determinations, expressed as a value of log<sub>10</sub>, was 2.69 (± 0.39). Thus it would appear that the *ds<sup>s</sup>* → *ds<sup>r</sup>* mutation involves only a very small region of the viral genome. This assumption was supported by further experiments<sup>7</sup> in which nitrous acid was shown to induce this mutation.

The results of our experiments concerning the relationship between the *ds<sup>r</sup>* character and several other poliovirus markers (*MS*, *t*, *mp*\*, behaviour towards guanidine and HBB) are shown in Table 1. The viral populations derived from the neurovirulent MEF<sub>1</sub> type 2 poliovirus strain (*MS<sup>+</sup> t<sup>+</sup> mp<sup>+</sup>*) were resistant to dextran (Table 1, rows 1–3), regardless of the circumstances in which cloning had been performed, that is, in the presence of dextran sulphate or of another selectant inhibitor (for example, guanidine). This tallies with the initial observation of Takemoto and Liebhafner<sup>1</sup> and Takemoto and

\* *mp* equals mouse pathogenicity.

Kirschstein<sup>2</sup> on the behaviour of the neurovirulent type 1 Mahoney strain.

The attenuated type 1 L<sub>1</sub>S parental strain and some of its clonal derivatives displayed the *ds*<sup>+</sup>, *MS*<sup>-</sup> and *t*<sup>-</sup> markers (Table 1). Both spontaneous mutants and those induced by nitrous acid, which maintained their *ds*<sup>+</sup> character after several passages, performed either in the presence or absence of the inhibitor, however, retained the *MS*<sup>-</sup> and *t*<sup>-</sup> markers of the parental strain (Table 1, rows 7 and 8). A similar pattern, that is the partial independence of the *ds*<sup>+</sup> → *ds*<sup>+</sup> mutation in relation to the *crt*/40° character, was recorded by Takemoto and Kirschstein<sup>2</sup>. Moreover, it will also be recalled that Boeyé<sup>6</sup> has described the occurrence of separate mutations as well as of covariation between the *d* and *E* markers. We think that the lack of covariation between the *ds* and *d* characters on one hand, and the so-called "group of markers covarying with neurovirulence" (compare Voss *et al.*<sup>9</sup>) on the other, emphasizes the necessity for an experimental re-appraisal of the actual relationships between these markers, as well as between each of them and neurovirulence.

Our experiments on the behaviour of all these strains towards guanidine unequivocally demonstrate (Table 1, rows 1-8) that this character is not affected by selecting for the *ds*<sup>+</sup> character and vice versa, thus indicating the mutual independence of the *ds*<sup>+</sup> → *ds*<sup>+</sup> and *g*<sup>+</sup> → *g*<sup>+</sup> mutations.

Preliminary findings concerning the relationship between the behaviour towards dextran sulphate and HBB indicated the lack of covariation of these two characters. The dextran resistant derivatives of the L<sub>1</sub>S *ds*<sup>+</sup> strain displayed the same susceptibility to HBB as the parental population. Conversely, no difference was observed between the *hbb*<sup>+</sup> character in a *ds*<sup>+</sup> population after selection in the presence of HBB (Table 1, row 9) and the *hbb*<sup>+</sup> character acquired by the L<sub>1</sub>S strain exposed directly to the drug. Moreover, no change was observed in the susceptibility of the latter strain towards dextran sulphate (Table 1, row 10) after mutation from HBB sensitivity to HBB resistance.

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## CYTOLOGY

### Genetic and Histochemical Studies on Mouse Spleen Black Spots

IN the course of immunological experiments involving the C57BL/Ka and C57BL/6 *Jax* strains of mice, black spots were noted on the spleens of some mice. These spots were usually found in the posterolateral aspect of the spleens, although a few cases were noted in which the spot was in the anterior portion of the spleen. Histological sections of these spots were characterized by a diffuse scattering

of pigmented cells, involving primarily the red pulp of the affected portion of the spleen. These spots occur in a number of (C57BL/Ka × Balb/C)*F*<sub>1</sub> hybrids, but not in Balb/c mice. They are present in affected strains in similar frequency from birth onwards. In order to test whether the appearance of this phenomenon is genetic, and perhaps indicative of some residual heterozygosity in our colony, we carried out laparotomies on a number of hybrid mice, then set up the following mating pairs: black spot × black spot, black spot × normal, normal by normal. The results are shown in Table 1.

Table 1. DISTRIBUTION OF BLACK SPOT OFFSPRING RESULTING FROM TEST MATINGS

Mating pair type	Black spot	Total	Percentage with black spot
Black spot × black spot	6	61	9.8
Black spot × normal	4	22	18.2
Normal × normal	12	75	16.0

The finding that two negative parents could have positive offspring rules out a dominant type of inheritance with complete penetrance. The finding that two positive parents did not have all positive offspring rules out a purely recessive type inheritance. In fact, the random distribution of positives, independent of parental "phenotype" raises some doubts about any genetic transmission of the phenomenon. We could not, however, rule out hypotheses involving the genetic transmission of a trait with variable penetrance.

The *F*<sub>2</sub> offspring were combined and assorted by skin and hair colour, and the results are shown in Table 2.

Table 2. DISTRIBUTION OF BLACK SPOT OFFSPRING IN *F*<sub>2</sub> OFFSPRING SORTED BY SKIN COLOUR

	Black spot	Total
Albino	0	40
Coloured	25	118

$$\chi^2 = 8.1, P < 0.005.$$

Thus the results demonstrate a genetic restriction on the phenomenon—the mouse must be capable of forming melanin pigment if it is to have a black spot on its spleen. The albinos were randomly distributed among the three classes of matings, and the removal of albinos from these groups did not change the proportion of black spot mice in any group.

To confirm the hypothesis that the black spot was indeed melanin, we found that the pigmented cells did not stain with Prussian blue (ruling out iron-containing pigments), did stain with DOPA oxidase, and could be bleached with hydrogen peroxide.

Because melanocytes are considered to originate from neural crest cells<sup>1</sup>, and the normal resting place of these cells is the skin (although reports of melanocytes in ovaries, thymus, and other organs have been published<sup>2</sup>), the finding of localized clusters of melanocytes in the spleen may be due to either a peculiar migration potential of these cells, a true metaplastic condition following localized prenatal trauma, or perhaps to the transport of melanin by leucocytes, as reported by Wasserman<sup>3</sup>.

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## BIOLOGY

### Electrophoretic Patterns of Larval Haemolymph-proteins in Autogenous and Anautogenous Forms of *Culex pipiens* L.

THE common house mosquito, *Culex pipiens*, has two different forms with respect to egg formation. Adult females of the so-called autogenous form can lay the first batch of eggs without taking a blood meal, whereas anautogenous females will not produce mature eggs until they have ingested blood. In spite of the many investigations of these two mosquito forms, very little is known about the physiological and biochemical basis of autogeny or anautogeny<sup>1-3</sup>. Egg formation in adult mosquitoes has been considered to depend on the nutritional reserve stored during larval development<sup>4,5</sup>; at emergence autogenous females contain more fat reserve than anautogenous females<sup>6,7</sup>. But, as Clements has pointed out<sup>8</sup>, the quantity of reserve materials cannot be the deciding factor for initiating ovarian development, because even in the anautogenous form there should be still a sufficient nutritional storage to produce a limited number of eggs. Investigation of the hormonal system revealed that the corpora allata of the autogenous females are active after emergence, whereas the anautogenous females do not secrete the gonadotrophic hormone until after they have fed on blood<sup>9-12</sup>. There is now no doubt that ovarian development in mosquitoes, as in all insects, is under hormonal control, but it is not known why the endocrine system is maintained in an active state even without feeding only in the autogenous form, while that in the anautogenous form requires a blood meal for activation.

There is no qualitative difference in the pattern of amino-acids and derivatives between autogenous and anautogenous mosquitoes<sup>13,14</sup>—paper electrophoresis showed only one protein fraction—and the total quantities of haemolymph proteins have been shown to be about the same in both forms<sup>2</sup>. We have separated the haemolymph proteins of the mosquito larvae by disc electrophoresis, which is superior to paper electrophoresis, mainly because of its high resolving power. As we report here, by means of this technique we separated more than ten fractions in the larval haemolymph and detected differences in protein patterns between autogenous and anautogenous forms.

The mosquito stocks used were *Culex pipiens* variety *molestus* (autogenous form) and *Culex pipiens* variety *fatigans* (anauto-genous form). The culturing method has been described before<sup>15,16</sup>. Larvae of both forms were grown at 25° C and fed daily with powdered dog biscuit. Haemolymph samples collected from individual larvae of desired developmental stages were centrifuged to remove haemocytes. The electrophoretic run was carried out according to the procedures used by Davis<sup>17</sup>. After separation the extruded gels were stained with amido black, destained, and preserved in 7 per cent acetic acid.

In agreement with our earlier findings<sup>2</sup> and as shown in Fig. 1, there is a rapid increase in the concentration of protein in the haemolymph during larval development. Furthermore, the protein pattern changes during larval life. In 10 and 12 day old larvae of *C.p.fatigans* there are three distinctly resolved protein bands of small mobility and large concentration; one of these remains very close to the origin (O) and has been identified as a lipoprotein. One moderately stained band moves very fast in the anodal direction and occupies a position only about 3 mm behind the tracking dye (TD). The middle part of the gel section is occupied by at least five to six faint bands which are difficult to resolve. The pattern is similar for *C.p.molestus* larvae aged 7 and 9 days except that the two bands ahead of the lipoprotein fraction have a relatively small concentration. The situation appears,

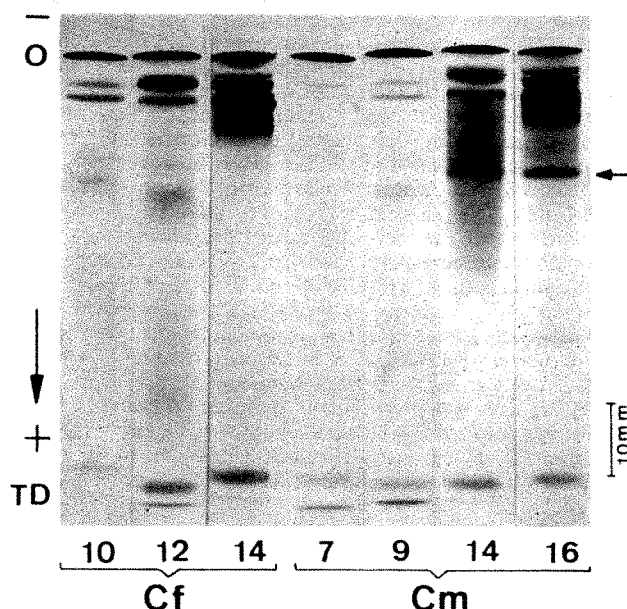


Fig. 1. Disc electrophoresis of haemolymph proteins in the larvae of *Culex pipiens* variety *fatigans* (Cf) and *C.p.* variety *molestus* (Cm). For each run 5  $\mu$ l. of haemolymph was used (120–270  $\mu$ g protein according to the stage of development). Numbers at the bottom of the electropherograms indicate larval ages in days after oviposition. O, Origin; TD, tracking dye.

however, entirely different for the fully grown larvae. In 14 day old *fatigans* larvae the gel region about 12 mm distant from the origin is occupied by five heavily stained bands, because of the increase in concentration of bands already present at earlier stages and the appearance of three new protein fractions. The concentration of the fastest migrating band also increases rapidly. The corresponding gel sections for 14 and 16 day old *molestus* larvae show likewise a much greater protein concentration compared with earlier stages, but the pattern differs from that in *C.p.fatigans*. For example, in the last electropherogram shown in Fig. 1 the first third of the distance from the origin is occupied by a single broad band. The appearance of a sharply demarcated band with a mobility of 18 mm from the origin is particularly interesting (indicated by arrow). This rather concentrated protein must be formed only towards the end of larval development, for it is absent at earlier stages. Analyses of larval tissues showed that it is present only in the haemolymph. In *fatigans* larvae of corresponding developmental ages we have found both haemolymph samples which contained no detectable amount of this protein (see Fig. 1) as well as samples which showed a moderately stained band. This variation is obviously a result of the genetic heterogeneity of the anautogenous stock.

The protein fraction already mentioned occurs regularly and is especially concentrated in the "autogenous" larvae, and so it would be interesting to know whether it is related to ovarian development in adult mosquitoes. Our preliminary examination of the protein composition in freshly laid eggs indicated that there are differences between autogenous and anautogenous forms, but no protein components with mobilities corresponding to those in the larval haemolymph could be found. This means that there is at least no direct utilization of this haemolymph protein for yolk formation. Its appearance shortly before pupation suggests that it is involved in metamorphosis. It is, of course, also possible that it is linked in a certain way to the endocrine system of adult mosquitoes.

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### Low Apparent Oxygen Requirements of Deep-water Fishes in Lake Tanganyika

THE lower limit of dissolved oxygen at the south of the very deep Lake Tanganyika (maximum depth 1,470 m) is normally around 200 m. Fish have been caught at about this depth by gill-nets on the bottom, and so I decided to make use of the unusual opportunity provided to investigate *in situ* the apparent tolerances of the fishes there to low oxygen concentration.

Gill-nets were set at intervals between about 150 m depth and the anoxic level, in the two southern arms of the lake. In each setting, one or two "fleets" of nets were set at about the same depth, that is, along the bottom contour. A fleet consisted of ten nets, each net 46 m long and each a different mesh size in the range 3.8–15.2 cm (stretched mesh) in 1.3 cm increments. Water was sampled for oxygen analyses (standard Winkler analysis) 1.5 m above the bottom near each fleet, and also at several levels above the nets at the times of fishing. A mean value from two or three titrations was taken for each sample. Bottom profiles and relative positions of the nets are shown in Fig. 1a and b, together with dissolved oxygen curves. Depths of settings and oxygen values near the bottom are listed in Table 1. Fish catches are detailed in Table 2.

At the lowest depth where fish were caught (212/215 m in the south-east arm), oxygen values varied from 19.2–

Table 1. LIST OF DEPTHS OF SETTINGS, LENGTHS OF GILL-NET SET, AND DISSOLVED OXYGEN AND TEMPERATURE VALUES CLOSE TO THE NETS AT THE TIMES OF FISHING

Depth of nets	Dissolved oxygen P.p.m.	Percentage saturation	Temperature (°C)	Total length of net set at each depth (m)
South-west arm				
150 m	3.06	40.8	23.80	460
160 m	3.39	45.1	23.70	460
170/174 m	2.54/2.29	38.8/30.5	23.70	460
181 m	1.78	23.7	23.70	460
189 m	1.44	19.1	23.65	460
206 m	0.76	10.1	23.60	460
South-east arm				
146/151 m	2.29/2.20	30.6/29.3	23.85/23.80	920
164/170 m	2.29/2.20	30.5/29.3	23.80	920
199/201 m	2.08/2.03	27.0	23.80	920
212/215 m	1.44/0.59	19.2/7.9	23.70	920
220 m	0.17	2.3	23.60	920
225/228 m	0.25/0.08	3.3/1.1	23.55	920
242 m	Nil	Nil	23.55	920

7.9 per cent saturation (1.44–0.59 p.p.m.). Of five species represented there, three species were caught along the nets to their lowest limit. No fish were caught nearby in 220 m in 2.3 per cent oxygen saturation (0.17 p.p.m.). Stomach contents indicated reduction in the variety of diet towards the deeper end of the distribution ranges. Most stages of maturity were represented with no significant preponderance of any stage, or in numbers of either sex. No marked size gradients were apparent near the oxygen limit.

Other information of relevance was obtained during bottom fishing in the south-east arm in July and August 1961, when deoxygenation of much of the bottom, caused apparently by shoreward surges of anoxic hypolimnetic water, was observed on two occasions, each of which lasted at least several days<sup>1</sup>. Gill-netting was carried out at the same time as the hydrological measurements, and species of fish caught when oxygen was absent or present in small concentrations are listed in Table 3.

It is important to establish whether the fish caught at the bottom normally live there. Some of the deepest dwelling species do undertake long diurnal migrations to near-surface levels<sup>2</sup>, and much shorter movements would be sufficient to reach well-oxygenated water; according to the curves in Fig. 1a and b such movements need not exceed 50 m from the bottom. Some evidence may be adduced to show that most benthic fish remain at the bottom. (a) In hundreds of purse-seine catches examined no benthic fish other than *Lates mariae* and *L. angustifrons*, species of *Bathybates* and *Dinotoplerus cunningtoni* were found. Furthermore, few *Lates* less than 40 cm long occur in purse-seine catches, yet a large part of the *L. mariae* population on the deep bottom is less than 40 cm<sup>3</sup>. The purse-seine often fishes within 20 m of the bottom, and more of the deep benthic species should appear in catches if they undertake vertical movements even of this

Table 2. CATCHES OF FISH SPECIES EXPRESSED AS NUMBERS CAUGHT BY EACH FLEET OF NETS, AND ALSO AS PERCENTAGE COMPOSITIONS OF THE CATCH, AT EACH DEPTH WHERE NETS WERE SET. NO FISH WERE CAUGHT DEEPER THAN 215 M

Fish species	Catch/fleet of gill-nets; south-west arm										Catch/fleet of gill-nets; south-east arm									
	150 m No.	%	160 m No.	%	170/174 m No.	%	181 m No.	%	189 m No.	%	205 m No.	%	146/151 m No.	%	164/170 m No.	%	199/201 m No.	%	212/215 m No.	%
<i>Limnochromis permaxillaris</i>																				
<i>L. abeelei</i>													1	0.5			0.5	0.5		
<i>Trematocara unimaculatum</i>													0.5	0.5			0.5	0.5		
<i>Hemibates stenosoma</i>	8	14.6	27	26.7	12	7.3	27	31.9	98	65.3	2	5	27	28.4	14	27.7	60.5	63.4	20	40.4
<i>Bathybates minor</i>													1	1.1			0.5	0.6		
<i>B. graueri</i>					3	1.8									1.5	3				
<i>B. vittatus</i>			3	3	2	1.2	1	1.2					1				0.5	0.5		
<i>B. leo</i>													2	2.1					0.5	1
<i>Haplotaxodon tricoti</i>							9	10.6					1	1.1	2	4				
<i>Plecodus paradoxus</i>													1	1.1			0.5	0.5		
<i>P. elaviae</i>							6	7.1							1	2	1.5	1.6	1	2
<i>P. multidentatus</i>	1	1.8																		
<i>Chrysichthys sianenna</i>	3	5.5											1	1.1			1	1.1		
<i>C. stappersii</i>	20	36.4	37	36.6	52	31.5	29	34.2	46	30.7	22	55	7.5	7.9	9.5	18.8	16	16.8	18	36.4
<i>C. graueri</i>	4	7.3											1							
<i>C. grandis</i>			1	1	1	0.6					1	2.5	0.5	0.5						
<i>Synodontis multipunctatus</i>			2	2																
<i>Dinotoplerus cunningtoni</i>	1	1.8					1	1.2	1	0.7			1	1.1						
<i>Lates angustifrons</i>			5	5	1	0.6	12	14.2	5	3.3					22.5	44.6	14	14.7	10	20.2
<i>L. mariae</i>	18	32.8	26	25.7	94	57					6	15	49.5	52.1						



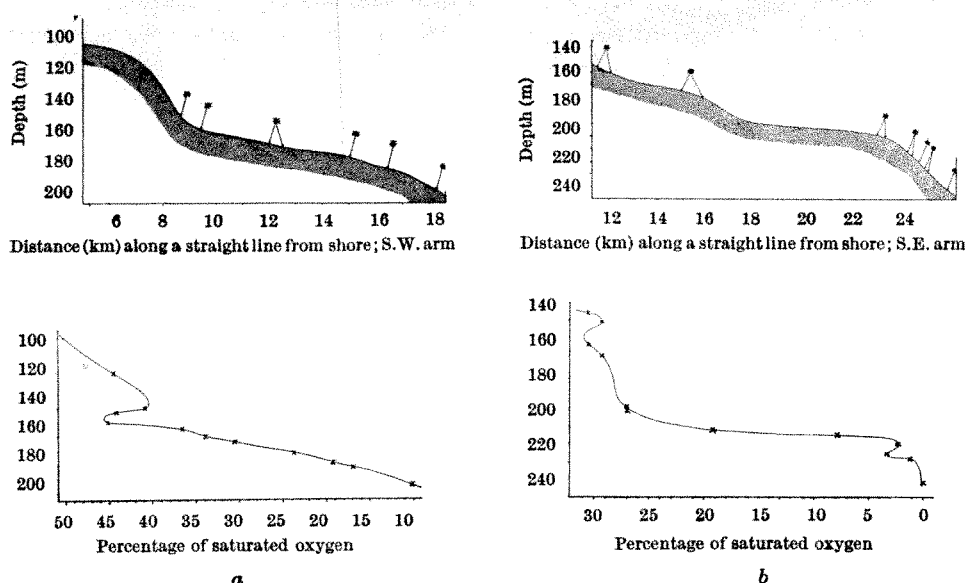


Fig. 1a and b. Gill-netting on the deep bottom in the south-west and south-east arms of the lake. Positions where nets were set are indicated on the bottom profiles by arrows. Dissolved oxygen values between 100 m and 205 m (south-west arm) and 146 m and 242 m (south-east arm) depth at the times of fishing are also shown.

order. (b) Several species appear to tolerate the temporary absence of oxygen (Table 3), which implies that they may be able to live normally in quite low concentrations (<2 p.p.m.).

In the extensive published data on minimum oxygen requirements of fishes determined in laboratory conditions at different temperatures and often with acclimated fish<sup>4-7</sup>, there are several instances where requirements are less than 1 p.p.m. On the other hand, the relatively few field observations<sup>8,9</sup> indicate that fish do not usually survive in nature where temperatures are between 15°–26° C in concentrations of less than 2.9 p.p.m. of oxygen. Discrepancies between thresholds observed in laboratory conditions and in natural habitats have been explained<sup>4</sup> in terms of the difference between survival in short-term adverse conditions, and in circumstances where feeding, growth, reproduction and competition demands must be met<sup>10,11</sup>.

The fish caught in Lake Tanganyika in concentrations of oxygen of almost 0.6 p.p.m. were presumably in an active metabolic condition. They must have been swimming actively to have become "gilled" in the net, and most stomachs not everted (because of rapid hauling to the surface) contained food. Provided that activity was sustained by respiration at the bottom, the oxygen concentrations there were remarkably low in relation to those believed to be necessary in general in natural environments elsewhere. Although some tropical Protozoa can live in anoxic freshwater<sup>12</sup>, no data concerning tropical fish are available for comparison except for *Tilapia nilotica*<sup>13</sup>. This species when forced to swim in water with 1.2 p.p.m. of oxygen at 21°–26° C survives at least 6 h.

In many fish species oxygen consumption increases sharply as temperature increases, but a relatively slow rate of consumption in tropical fish would have considerable adaptive advantages because of the inverse relationship between oxygen solubility and water temperature. If, however, rates of metabolism and energy requirements are similar in the same temperature range for tropical and temperate fish, the low oxygen tolerances cited here suggest that oxidative respiration may be supplemented by anaerobic respiration. (Work with trout<sup>14</sup> indicates that a fraction of its standard metabolism may be derived anaerobically.) Several of the common benthic fish in the south of Lake Tanganyika range from depths where oxygen concentrations should be great enough to satisfy all energy requirements to depths where concentrations are less than 1 p.p.m. and colonization of the deep bottom has perhaps been assisted by a capacity to change to anaerobic means of respiration. That this can occur without long acclimation is suggested by the survival of *Chrysichthys stappersii* and *Hemibates stenosoma* during the seasonal anoxic surges. Comparison of the haemoglobin content and tissue metabolism (in particular, the possible accumulation of lower acids or fat) of the same species taken at different depths may show that a build-up in anaerobic adaptation occurs towards the deeper end of their range.

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Table 3. FISH SPECIES PRESENT (IN ORDER OF ABUNDANCE) IN CATCHES MADE IN THE SOUTH-EAST ARM AT 120 M DEPTH WHEN OXYGEN WAS ABSENT OR PRESENT ONLY IN SMALL CONCENTRATIONS

3/7/1961 Nil oxygen	20/8/1961 Nil oxygen	28/8/1961 1% saturation (0.1 mg/l.) oxygen	4/9/1961 35% saturation (3.2 mg/l.) oxygen
<i>Hemibates stenosoma</i>	<i>Limnochromis permacellaris</i>	<i>Hemibates stenosoma</i>	<i>Trematocara nigrifrons</i>
<i>Bathybates fasciatus</i>	<i>Hemibates stenosoma</i>	<i>Chrysichthys stappersii</i>	<i>Hemibates stenosoma</i>
<i>Xenochromis hecqui</i>	<i>Chrysichthys stappersii</i>		<i>Bathybates fasciatus</i>
<i>Chrysichthys stappersii</i>			<i>B. minor</i>
<i>C. grandis</i>			<i>B. vittatus</i>
<i>Dinotopus cunningtoni</i>			<i>Chrysichthys stappersii</i>
<i>Lates mariae</i>			<i>C. graueri</i>
			<i>C. grandis</i>
			<i>Dinotopus cunningtoni</i>
			<i>Lates mariae</i>

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### Function of Combs in Ectoparasites

MANY ectoparasitic insects are characterized by one or more regular rows of closely set, large stout spines. These rows of spines are termed combs, or ctenidia, and have evolved independently in fleas (Siphonaptera), bat flies (Nycteribiidae), bat bugs (Polytenidae), and in the beaver beetle *Platypsyllus*. Hitherto there has been little evidence about their function. It has recently been shown<sup>1</sup> that the spacing of spines in the combs of fleas bears a close relationship to the diameter of the hairs of the appropriate host. The gap between the tips of the spines is usually about 1.75 times greater than the average diameter of the host's hairs, but the gap narrows almost to zero towards their base. The same relationship occurs between the combs of Nycteribiids and the fur of their Chiropteran hosts. The general applicability of this principle among ectoparasites was confirmed by measuring the comb of *Platypsyllus castoris* and the fur of the beaver. The results are summarized in Fig. 1.

The precise mode of action of the combs can be understood by considering the regularity of this quantitative correspondence and by examining the behaviour of the parasite on its host. Because the free edges of the combs always tend to point posteriorly, the hairs of the host do not become trapped between the ctenidial spines during normal forward locomotion. When a flea is dragged backwards, however, its combs catch in the host's fur. This process is assisted in two ways by the host's hair structure. First, hairs are not perfectly smooth, and in many mammalian species are characterized by deep serrations and by irregularities of cross section. Irregular projections, termed nodes, are also common on the downy barbules of birds. Second, the separate strands in fur or down do not lie parallel to one another. Most mammals show a differentiation of hairs into guard and contour hairs which are long and straight, and under-fur hairs which are much more abundant, and are finer, short and often wavy. The hairs of the under-fur often cross each other repeatedly. When an ectoparasite is moved backwards therefore it is not only the irregularities on individual hairs which become trapped between the spines, but there is also a hair tangling action analogous to that which occurs when a very fine comb is drawn through untidy human hair. The resistance to backward movement can be felt distinctly by dragging a flea through the fur of its host.

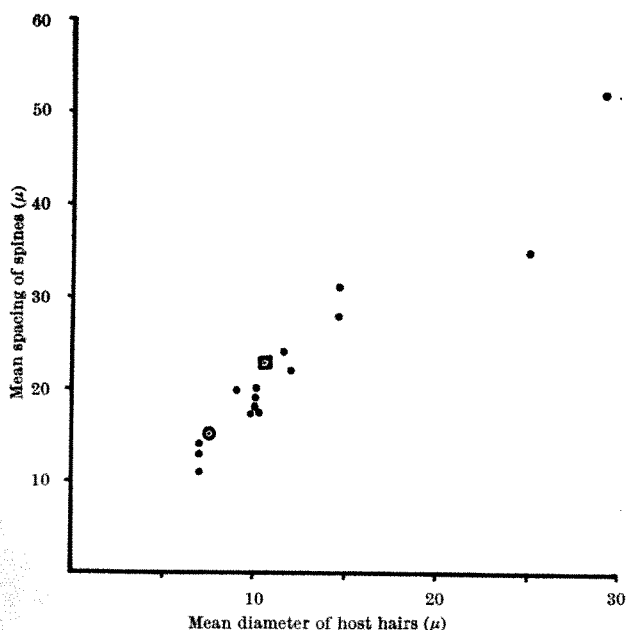


Fig. 1. Relationship between the diameter of host hairs and the spacing of ctenidial spine tips. ○, *Nycteribia biarticulata*; □, *Platypsyllus castoris*; ●, fifteen species of flea<sup>1</sup>.

The survival value of the action of the combs becomes clear when we consider the situations in which ectoparasites are likely to move backwards. Fleas, at least, never do so by their own activity. Backward movement is imposed by two factors: violent movements of the host which tend to dislodge the parasite, and gripping and pulling by the host's mouth or paws. When disturbed on the host, both fleas and Nycteribiids adopt a head-down posture in the under-fur near the skin surface. It is thus their hind parts which are likely to be seized by the host. In any event, it is only backward movement which can dislodge them, and this means that the under-fur will catch in the combs. There is abundant evidence<sup>2-4</sup> that the killing of ectoparasites by the host is a major factor limiting their populations. The mechanism of the combs can therefore be regarded as an adaptation resisting dislodgement or capture by the host. It is proposed that this is the selective advantage which underlies their convergent but independent evolution in such widely differing insect groups.

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### Leaf Status and Photoperiodic Control of Flower Initiation in a Late Variety of Pea

IN the absence of significant effects caused by partial defoliation in 'Greenfeast' peas, Sprent<sup>1</sup> has proposed that foliage leaves are involved in the long day flowering response of this pea variety in a qualitative rather than quantitative fashion. I agree with respect to the leaf area present at flower initiation<sup>2</sup>. I have investigated photoperiodic induction phenomena in 'Greenfeast', and suggest that the first formed foliage leaves do play an important though temporary, quantitative part in the flowering behaviour of this late variety.

I made a series of daily transfers to 8 h short days of plants germinated and grown in continuous light. The same light intensity (3,500 ft.-candles) was used for both photoperiods. Artificially lit controlled environment cabinets<sup>3</sup> allowed transfer from a cabinet giving continuous light to one giving an 8 h day without changes in light intensity or growing temperature. Fig. 1 shows that a stage of development was reached after 14 days in continuous light when the node of the first flower (NF) was predetermined and was independent of the later photoperiodic regime. Only 12 days of growth in continuous light was required for about 50 per cent of the plants to initiate the first flower at the same node as plants kept continuously in the light until flower primordia were present. This irreversible commitment to flower at a predetermined node suggests that photoperiodic induction has occurred in this quantitative long day plant. Induction was achieved at least 5 days or 3 plastochron intervals before leaf initiation at the flowering node (see N values). Also, a preliminary histochemical investigation has shown that the flower primordium initials are not formed until the subtending leaf is morphologically discernible by dissection. Thus the possibility that the commitment to flower was a result of flower primordia already present at completion of induction can be excluded.

The number of nodes bearing unfolded leaves (Fig. 1) before and at induction are particularly relevant to the role of the leaf in flowering. In general, the photoperiodic reactions involved in attainment of the induced

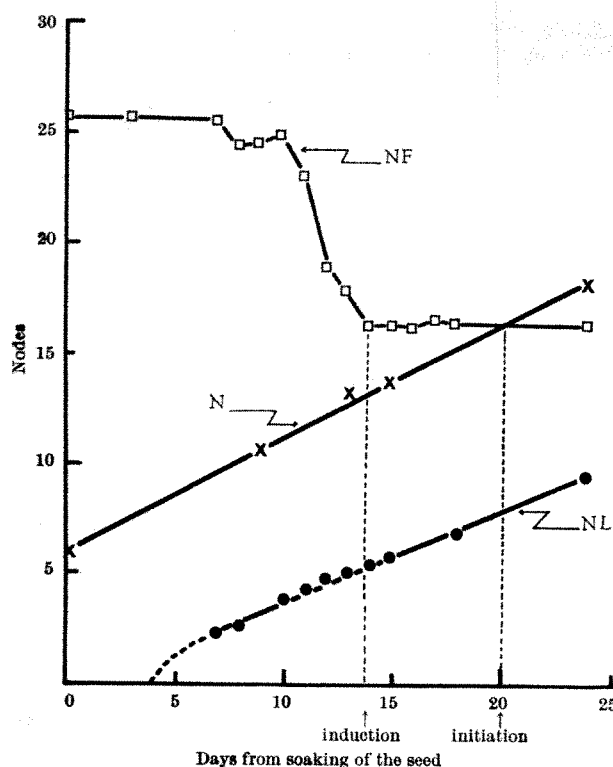


Fig. 1. The relationship between node of first flower (NF) and number of days in continuous light before transfer to 8 h day. Changes in leaf status for the same time scale are shown for nodes bearing unfolded leaves (NL) and total number of nodes formed (N). The former values refer to the number of nodes from the cotyledons (counted as 0) to the last unfolded leaf. Growing temperature was 20° C. Each point represents the mean of twenty to thirty plants (NF) or ten plants (NL and N). Leaf status values were unaffected by the day length and the values given are from the control kept in continuous light. Values for the standard error of the mean are too small to show graphically with the node scale used.

state were fully completed when the fifth leaf was unfolded. Growing temperatures affected this value to some extent and the leaf number at induction in several experiments at 20° C (5.3) was slightly but consistently larger than at 15° C (4.8) and 10° C (4.5).

The results of defoliation and masking experiments conducted at various times indicate that the foliage leaves at the third and fourth nodes are the most likely sites for the photoperiodic reactions concerned in induction in intact plants. The main results of one early experiment are typical (Table 1). Small but significant increases in NF values occurred only with complete removal of the third leaf (0.7 nodes) or the third and fourth leaves (1.6 nodes). The magnitude of these increases was reduced if the defoliation treatment was severe enough to cause a reduction in rate of leaf initiation. Partial defoliation at the third and fourth nodes is ineffective in similar conditions<sup>1</sup>; it seems likely that leaf area, particularly of the third leaf, is important. This has been tested by fractional defoliation treatments at different times before and after induction. Results so far obtained suggest a com-

Table 1. EFFECT OF COMPLETE DEFOLIATION AT DIFFERENT NODES ON NODE OF FIRST FLOWER AND TOTAL NUMBER OF NODES FORMED

Node position of leaves removed*	Node of first flower $\pm$ SE (days)	Total No. of nodes formed at day 41†
Intact control	15.7 $\pm$ 0.3	21.1
3	16.4 $\pm$ 0.2	21.1
3+4	17.3 $\pm$ 0.3	20.8
3+4+5	16.5 $\pm$ 0.5	18.5
3+all above	Dead	Dead
4+5	15.6 $\pm$ 0.2	21.0
4+all above	15.9 $\pm$ 0.3	19.2

This work was carried out at Hobart in June 1960 in a heated glasshouse in a 19 h day.

\* Leaflets and stipules removed before the leaf unfolded from the apical bud. Scale leaves at first and second nodes.

† All plants dissected 41 days after soaking. Note reduced rate of leaf initiation with removal of more than two foliage leaves.

plex relationship between critical area, node position and number, length, and light intensity of the inductive cycles. One clear result, however, was that post-induction defoliation did not affect NF values unless the rate of leaf initiation was also affected (compare Table 1). Evidently completion of photoperiodic processes in the leaf and passage of the inductive stimulus out of the leaf occurs at about the same time. Some of the hitherto puzzling features of flowering behaviour in peas and in particular the ineffectiveness of the type of defoliation treatments described by Sprent<sup>1</sup> may be explained on this basis.

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<sup>1</sup> Sprent, J. I., *Nature*, **209**, 1043 (1966).

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### Isolation of a Sex Hormone from the Water Mould *Achlya bisexualis*

*Achlya* is a genus of filamentous aquatic fungus inhabiting fresh-water lakes and ponds. Some years ago, Raper established that sexual reproduction is initiated, in certain members of this genus, by a diffusible substance secreted into the surrounding water by the female mycelium<sup>1</sup>. This substance was named hormone A. In response to the hormone, the male hyphae produce specialized branches<sup>2</sup> and at the tips of these branches antheridia are delimited<sup>3</sup>. An attempt to isolate the hormone from culture liquids of *A. bisexualis* gave a highly active though amorphous substance<sup>4</sup>. We have continued the investigation of hormone A and have isolated from strain T5 of *A. bisexualis* a crystalline compound which at high dilution induces branching and formation of antheridia in a number of male strains of *Achlya*. An account of the isolation of this hormone, which we wish to designate "antheridiol", is given here.

Antheridiol was produced in Fernbach flasks, each containing 360 ml. of sterile medium inoculated with 10 ml. of a suspension of zoospores from *A. bisexualis* T5. The composition of the culture medium was as follows: edamin (a hydrolysate of lactalbumin), 400 mg; dextrin, 2,400 mg; calcium glycerophosphate, 30 mg; calcium carbonate, 500 mg; calcium chloride (0.5 moles/l.), magnesium sulphate (0.5 moles/l.), KCl (1 mole/l.), 1 ml. each; distilled water, 1 l. The flasks were incubated in a stationary position for 3 days at 25° C. The mycelium was removed by filtration and the pH of the culture filtrate was adjusted to 4 by adding hydrochloric acid, which at the same time dissolved the fine suspension of carbonate. A weekly batch of 240 flasks gave 85 l. of culture liquid assaying about  $3 \times 10^6$  dilution units of hormone per litre<sup>5</sup>. More than 90 per cent of the active material was removed by two extractions with 13 l. of methylene chloride. Concentration of the extract *in vacuo* on a water bath (50° C) gave 800 mg of a brown gum which had an activity of about  $3 \times 10^6$  u/mg.

The gum (3 g) was chromatographed on silica gel (300 g) with ethyl acetate as solvent. The bulk of the material had low activity and was eluted in the early fractions (the volume of each fraction was 100 ml.). Next were fractions which gave about 50 mg of a crystalline amide,  $C_{11}H_{18}O_2N_2$ , melting point 158°–161° C, with negligible activity. The fractions of high activity moved as a narrow brown band on the column. They were collected, then subjected to preparative thin-layer chromatography on silica gel H (Merck) with chloroform-methanol (10:1) as solvent. This gave a pale yellow, highly active gum in

which crystals formed gradually. They were separated by adding a mixture of methanol and ether which dissolved the adhering gum. Approximately 2 mg of antheridiol was obtained as colourless crystals, melting point 240°–250° C, with an activity of about  $5 \times 10^7$  u/mg.

Because the recovery of the hormone was low and we suspected that it was being deactivated during chromatography, we turned to a method of isolation tried earlier, namely, countercurrent distribution, using a system of four solvents: water, methanol, ethyl acetate, petroleum ether, boiling point 60°–80° C. (1 : 1 : 1.25 : 1). About 6 g of gum was put through fifty transfers (volume of each tube, 150 ml.) and the most active tubes, 14–30, were combined and subjected to a second distribution of sixty transfers (volume of each tube, 20 ml.). The contents of tubes 22–34 were concentrated *in vacuo* below 50° C to remove the upper phase, and enough methanol was then added to give a clear solution which was seeded with antheridiol and set aside overnight. About 10 mg of crystals was obtained. Recrystallization from methanol removed a brown contaminant giving colourless crystals, melting at 250°–255° C, which assayed  $1.5 \times 10^{-8}$  u/mg (mean of six assays). A concentration of  $2 \times 10^{-8}$  mg/ml. is sufficient to induce branching in our assay strain, *A. ambisexualis* E87, but for the delimitation of antheridia a concentration of  $10^{-6}$  mg/ml. is required.

The ultra-violet spectrum,  $\lambda_{\text{max}}^{\text{soln}}$  220 m $\mu$  ( $\epsilon$  17,000), and infra-red spectrum,  $\nu_{\text{max}}^{\text{car}}$  3,390, 1,742, 1,672  $\text{cm}^{-1}$ , of antheridiol indicated the presence of hydroxyl and carbonyl functions. The high resolution mass spectrum determined by Drs G. Arsenault and K. Biemann, of the Massachusetts Institute of Technology, gave the formula  $\text{C}_{20}\text{H}_{42}\text{O}_5$  ( $M^+ = 470$ ). Treatment of antheridiol with acetic anhydride and pyridine yielded a diacetate ( $M^+ = 554$ ) which showed peaks due to loss of one and two moles of acetic acid,  $m/e$  494, 434, in the mass spectrum. Catalytic hydrogenation of antheridiol afforded a crystalline tetrahydro derivative in which the carbonyl peaks had shifted to 1,770 and 1,710  $\text{cm}^{-1}$ , indicating the presence of an  $\alpha$ - $\beta$  unsaturated  $\gamma$ -lactone and an  $\alpha$ - $\beta$  unsaturated ketone.

This work was supported by grants from the US National Institutes of Health. The countercurrent method and solvent system were recommended by Dr Marjorie Anchel, to whom A. W. B. is indebted for assistance in the initial attempts at isolating the hormone.

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<sup>5</sup> For method of assay, see Barksdale, A. W., *Mycologia*, **55**, 164 (1963).

## AGRICULTURE

### New Broad Spectrum Anthelmintic, Methyl 5(6)-Butyl-2-benzimidazolecarbamate

WE have found a series of anthelmintic compounds, one of the most potent of which is methyl 5(6)-butyl-2-benzimidazolecarbamate (II; SK and F 29044). Anthelmintic activity in this series of compounds was first found with thionocarbamate Ia. When a large number of compounds related to Ia were examined, it was found that 2-acylamino-benzimidazoles (Ib), 2-benzimidazolecarbamates (Ic) and 2-benzimidazolylureas (Id) also possess anthelmintic properties. In all cases, high activity was restricted to the lower members of the series in which the group R

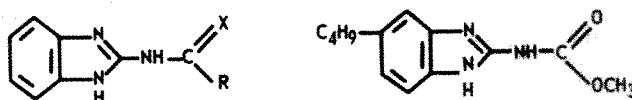


Fig. 1. Left hand side, anthelmintic benzimidazoles I (a) ( $X = S$ ,  $R = \text{OC}_2\text{H}_5$ ); (b) ( $X = O$ ,  $R = \text{alkyl}$ ); (c) ( $X = O$ ,  $R = \text{alkoxy}$ ); (d) ( $X = O$ ,  $R = \text{alkylamino}$ ). Right hand side, anthelmintic benzimidazole II (SK and F 29044).

contained between one and three carbon atoms. The methyl carbamate derivative (Ic,  $R = \text{OCH}_3$ ), which seemed to be the most promising, was selected for further modification, and this led to the discovery of SK and F 29044 (II). Compound II is conveniently prepared by the reaction of 4-butyl-*o*-phenylene-diamine with carbomethoxycyanamide in boiling 2-propanol. It is a white crystalline solid which melts at 224°–227° C with decomposition.

In our first efficacy trials with SK and F 29044 we used mice infected with *Syphacia obvelata*, *Nematospiroides dubius* or *Ascaris suum*. In mice naturally infected with *S. obvelata*, three oral or intraperitoneal doses at 10–12 mg/kg/day resulted in more than 90 per cent reduction in worm burdens. Single oral or intraperitoneal doses were less effective. Three intraperitoneal treatments at 25 mg/kg/day or treatment with 0.05 per cent in the diet for 5 days resulted in a marked reduction (88 per cent to 100 per cent) of adult *N. dubius* in mice infected 3 weeks previously with fifty larvae. Larval stages were virtually cleared (96 per cent) in mice fed diets containing 0.1 per cent of the drug for 2 days, starting on the day of infection. Mice infected orally with 50,000 embryonated ova of *A. suum*/day for 2 days and fed SK and F 29044 prophylactically for 12 days were protected against the lethal effects of the infection.

A single dose of 15 mg/kg administered orally or intraruminally to sheep naturally or artificially infected removed 93–100 per cent of *Haemonchus*, *Ostertagia*, *Trichostrongylus* in the abomasum; and *Strongyloides*, *Cooperia*, *Trichostrongylus*, *Nematodirus*, *Oesophagostomum* and *Chabertia* in the small and large intestine. Its activity against *Trichuris* was slight. In cattle a controlled trial was completed in four groups of three animals each. The compound was administered intraruminally at 10, 20 and 40 mg/kg, and the fourth group was untreated. The activity at 20 and 40 mg/kg was excellent, with 99 per cent to 100 per cent of *Haemonchus*, *Ostertagia*, *Trichostrongylus axei*, *Strongyloides*, *Cooperia* and *Oesophagostomum* being eliminated.

Excellent therapeutic activity in swine was obtained against natural infections with mature *Ascaris suum*, *Strongyloides ransomi*, species of *Oesophagostomum* and *Trichuris suis* at a single oral dose of 30 mg/kg. SK and F 29044, as 0.05 per cent of the diet, prevented the appearance of verminous pneumonia in pigs artificially infected with embryonated ova of *A. suum*.

Chickens fed a diet containing 0.05 per cent SK and F 29044 for 24 h were cleared of *Ascaridia galli* and *Heterakis gallinae*. Divided doses of SK and F 29044, when administered orally to rhesus monkeys or dogs at 50 mg/kg/day for 2–4 days, were effective in eliminating a variety of naturally occurring gastro-intestinal nematodes.

In sheep a peak concentration in the plasma was observed 6 h after oral administration of SK and F 29044 labelled in carbon 2. The compound and its metabolites were excreted in the urine, and 19 per cent of the administered radioactivity was recovered in the first 24 h. The parent compound and two important metabolites were excreted. Tissue residues in muscle, fat, skin, liver, kidney, heart, bile and plasma were less than 0.1 p.p.m. 16 days after administration of an oral dose of 45 mg/kg labelled with carbon-14.

Oral administration of labelled SK and F 29044 to swine resulted in a peak concentration in the plasma after 6 h.



In 24 h 11 per cent of the radioactivity was detected in the urine, which consisted of the parent compound and two important metabolites. Tissue residues were less than 0.1 p.p.m. 21 days after oral administration of 50 mg/kg of the compound labelled with carbon-14.

A single oral dose of 500 mg/kg was well tolerated by sheep and swine. The oral  $LD_{50}$  for rats and mice exceeds 4 g/kg.

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### Toxic Strain of *Fusarium culmorum* (W.G.Sm.) Sacc. from *Zea mays* L., associated with Sickness in Dairy Cattle

In 1928 there was an extensive North American outbreak of "scab" in barley, caused by the fungus *Gibberella zeae* (Schw.) Petch syn. *G. saubinetti* (conidial stage *Fusarium graminearum* Schw.). This was followed by the publication of a number of papers on the effect of feeding infected grain to domestic animals.

Bohstedt and Dickson<sup>1</sup> reported that ruminants showed no ill effects, but animals with simple stomachs, particularly pigs, were found to be very sensitive to "scab" infected grain. Mundkur<sup>2</sup> carried out feeding tests with hogs which demonstrated that barley containing 40 per cent scabby grain caused vomiting and slight intoxication but no diarrhoea or enteritis. Prentice *et al.*<sup>3</sup> extracted emetic material from culture filtrates of the following *Fusarium* species: *F. moniliforme* (one strain), *F. poae*, *F. culmorum* and *F. nivale*, when grown on synthetic liquid culture media. The only report of "fusariotoxiosis" in cattle seems to come from Russia, where oats infected with a very toxic strain of *F. sporotrichiella* produced poisoning symptoms when fed to yearling calves<sup>4</sup>.

The condition reported here occurred towards the end of autumn (May 1966), in south-eastern Victoria, at Berwick and Drouin, which are situated about 25 and 55 miles, respectively, from Melbourne. In both localities loss of appetite and decreased milk production were reported in dairy cattle after feeding on a crop of maize (*Zea mays* L.). At Berwick seven cows in a herd of forty-five showed loss of appetite and marked reduction in milk yield after feeding on maize; in addition some were scouring maize and two exhibited symptoms of staggering. Recovery and return to normal milk production occurred within a week of removing the cows from the maize crop.

When samples of the suspect maize crops were submitted to the Victorian Plant Research Institute for mycological examination, several species of *Fusarium* were isolated. Two were cultured from Drouin maize: *F. culmorum* (W.G.Sm.) Sacc. from the bract of a male inflorescence and *F. graminearum* Schwabe from the nodal region of the stem.

These species were also isolated from Berwick specimens of maize; *F. culmorum* was cultured from nodal stem tissue and *F. graminearum* from the bract of a cob. Also from the nodal stem tissue of Berwick maize plants the

following additional species were cultured: *F. acuminatum* Ell. and Ev., *F. moniliforme* Sheld., and an unidentified species, resembling but distinct from *F. dimerum*. All *Fusarium* species isolated were tested for dermal toxicity using the method described by Forgacs and Carll<sup>5</sup>. The fungal cultures to be tested were grown on sterilized maize grain for two weeks at 21°C and then extracted with ether in a cold Soxhlet apparatus. The dried ether extract was suspended in 2 ml. of olive oil and applied to a circular shaved patch of rabbit skin 2 in. in diameter. In each test, five applications of about 0.1 ml. of oily suspension were given during 2.5 days. The test was negative for all species except *F. culmorum*; however, isolants of this fungus from both Drouin and Berwick produced a very severe reaction. This species was tested on three rabbits. In each instance, the skin reddened on the third day, progressing in intensity and forming a typical haemorrhagic lesion, until finally death of the rabbit ensued on the fifth, sixth and seventh days, respectively.

We thank Dr C. Booth of the Commonwealth Mycological Institute, Kew, for identification of the *Fusarium* isolants.

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## ANTHROPOLOGY

### Hereditary Deficiency of Pseudocholinesterase in Eskimos

HIGH susceptibility to the neuromuscular blocking action of succinylcholine is usually associated with a defective form of pseudocholinesterase in blood plasma; the abnormal enzyme is distinguished by its resistance to inhibition by dibucaine<sup>1</sup>. The gene frequency of this abnormality in Caucasians is estimated to be 0.019; 3.8 per cent of the population are heterozygous; and 1 in 2,800 individuals is sensitive to succinylcholine<sup>2</sup>.

A much rarer form of sensitivity to succinylcholine results from the absence in serum of pseudocholinesterase<sup>3</sup>. Only ten cases in seven families have been reported<sup>4</sup>. The frequency of this "silent" gene has been estimated at about 0.003 in Caucasians, and one person in about 100,000 lacks pseudocholinesterase<sup>5</sup>.

Two Eskimo children were apnoeic for 3-4 h after administration of a single dose of succinylcholine amounting to 1 mg/lb. of body weight. The children came from the same geographical area but were unrelated. When their sera were tested no pseudocholinesterase activity could be demonstrated. While testing random samples of serum to determine the reliability and reproducibility of the enzyme method, the thirteenth sample proved to be from an Eskimo who also lacked pseudocholinesterase.

A survey was then made of 708 samples of serum from hospital patients. Four unrelated persons among 354 southern Eskimos were found with pseudocholinesterase deficiency. No abnormal sera were found in 122 northern Eskimos, 141 Athabaskan Indians, fifty-eight Aleuts or thirty-three persons of other ethnic groups. None of the samples was resistant to inhibition by dibucaine, except

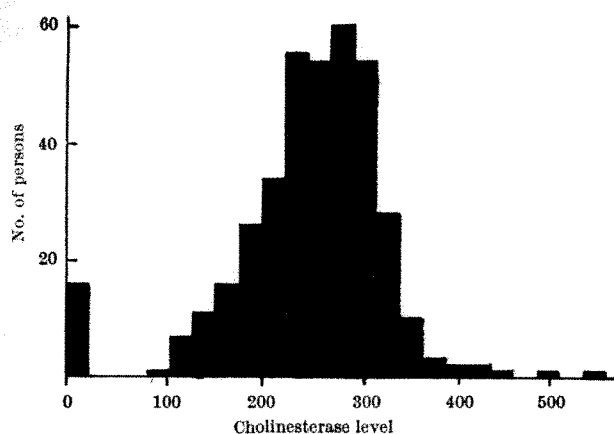


Fig. 1. Distribution of pseudocholinesterase concentrations in serum of 379 Eskimos. Units are μmoles of acetylcholine hydrolysed by 1 ml. of serum in 1 h at 37° C.

for one sample from an Aleut, and all showed normal inhibition by fluoride.

All six of the deficient individuals came from an area in Alaska extending from Hooper Bay to Unalakleet and centred on the lower Yukon River. Three came from near Mountain Village, and a general survey of all persons, 6 yr old or more, was made of the inhabitants of the villages of St. Mary's, Mountain Village and Pilot Station. Fifty-seven people examined were close relatives of known cases, while 322 were unrelated to them. As a result, nineteen cases of pseudocholinesterase deficiency are now known in eleven Eskimo families in Alaska. Only two of these families are related; and in only three families have all members been examined.

The distribution of concentrations of pseudocholinesterase in this investigation is shown in Fig. 1. The mean concentration of pseudocholinesterase in fourteen obligatory heterozygotes was 175 U with a standard deviation of 19 U. Assuming that all individuals with less than 175 U were heterozygotes, and that this was half the total, the gene frequency in 322 random samples was calculated to be 0.115. The calculated and observed distributions of genotypes are shown in Table 1.

Table 1. CALCULATED AND OBSERVED DISTRIBUTIONS OF GENOTYPES OF AN ESKIMO POPULATION

	322 random Eskimos		379 Eskimos	
	Calculated*	Found	Calculated†	Found
Normal homozygotes	252	252	292	295
Heterozygotes	66	66‡	81	76‡
Deficient homozygotes	4	4	6	8

\* Gene frequency was taken as 0.115.

† Gene frequency was assumed to be 0.121.

‡ Twice the number of persons found with concentrations of less than 175 U.

This gene frequency is too small, because index cases and their relatives were excluded. If they are included, the gene frequency is 0.121 in 379 persons. Calculated from the number of enzyme deficient persons only, the gene frequency was 0.111 in 322 random persons, and 0.145 in 379 individuals. The most probable value for gene frequency in this locality is thus about 0.12. About 1.5 per cent of the population can be expected to be sensitive to succinylcholine.

The method used in these investigations was adapted so that more serum could be used, and concentrations as small as 1 U of pseudocholinesterase could be measured. Eight deficient persons in five families had detectable activity (2-8 U) while nine in four families had none whatever. In one of the latter families, a father and five of his nine children were deficient in the enzyme.

The problem of the frequent occurrence of a benign, ordinarily rare polymorphism in a specific ethnic group has previously been encountered in southern Eskimos<sup>6</sup>.

The only explanation that has thus far been proposed is that any mutation that does occur may become quite common in a small population group that has been relatively isolated for many generations.

We thank J. Crispin Smith, Department of Anaesthesia, Montefiore Hospital, New York, for demonstrating pseudocholinesterase deficiency in the first two cases.

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### Olduvai Hominid 10: a Multivariate Analysis

In a recent article<sup>1</sup> a hominid terminal toe phalanx was described (Olduvai Hominid 10). The bone was recovered from site FLK North (FLK N1), Upper Bed I, Olduvai Gorge, Tanzania. In that article morphological evidence was put forward to support the view that the bone belonged to an upright hominid with a plantigrade propulsive or striding gait. Further studies on the toe bone, and comparative series of toe bones, have included calculation of the  $D^2$  generalized distance coefficient of Mahalanobis and canonical variate analysis. The results entirely confirm the previous morphological assessment.

Nine functionally significant characters were selected for analysis; these included the maximum length, the length/breadth index, the mean mid-shaft thickness, the index of robusticity, the breadth of the head, the convergence angle (a measure of the relationship between the head breadth and the base breadth), the angle of deviation and the angle of axial torsion of the shaft. Measurements were taken from three populations of modern man; fifty specimens were obtained from London dissecting rooms, twelve specimens from the series of Bushmen skeletons held by the British Museum (Natural History) and thirty-seven specimens from a series of East African skeletons held by the University of Makerere, Uganda. Measurements were also taken from eleven gorilla and twelve chimpanzee toe bones.

The statistical analysis<sup>2</sup> of the data was based on the average covariance matrix, although there was some evidence of heterogeneity of variance between the groups. The Mahalanobis  $D^2$  test (Fig. 1) showed that the distances obtained between any two groups gave  $F$ -values which were significant at the 0.1 per cent level of probability, but the three groups of men were never separated by more than 8.5  $D^2$  units and the two groups of apes by more than 9.9  $D^2$  units. The human and ape groups were never less than 21.9  $D^2$  units apart, however, and on occasion were more than 36  $D^2$  units apart. Calculation of the single value of  $D^2$  for the Olduvai toe bone showed that its distance from the ape groups was about twice its distance from the human groups (Fig. 2). At the 0.1 per cent level of probability the Olduvai toe bone was different from those of the apes but not significantly different from those of the men.

Canonical analysis on the same data showed a clear separation between the human and ape groups when Variates I and II (corresponding to the two largest eigen-

values) were plotted (Fig. 3). Four variates were achieved but further plots produced no further discrimination. In the canonical diagram the circles are four standard deviations in diameter and thus should encompass 95.4 per cent of the observations in a group. Calculation and plotting of the variates for the single case of Hominid 10 showed that it fell clearly among the human groups.

In interpreting these results it would seem wise to bear in mind that any conclusions which are drawn must relate to the parameters which were selected for analysis. In this case the selected measurements were chosen on the grounds that they each reflect an aspect of the functional morphology of the terminal phalanx. A multivariate technique, by taking account of the correlations between individual parameters in each bone, can present the functional morphology of that bone as a unified concept and assign it a position in relation to others; clustering of points will produce a grouping of bones with similar functional affinities. It is

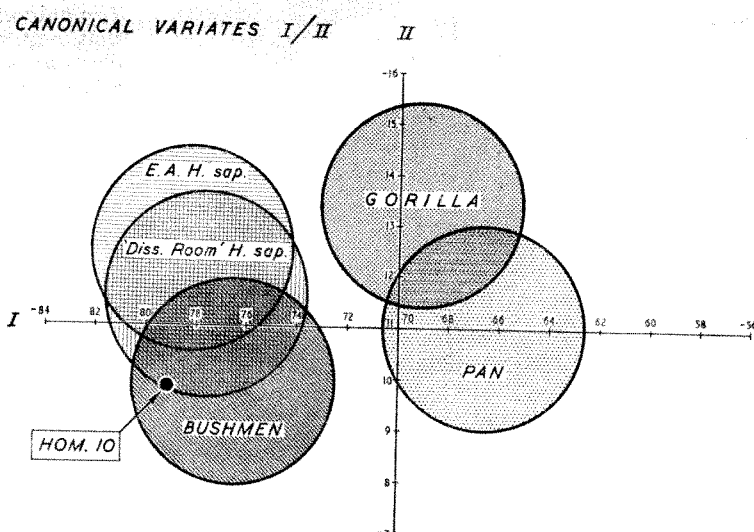


Fig. 3. Canonical diagram for Variates I and II showing the separation of hominid and pongid groups and the position of Olduvai Hominid 10.

#### TERMINAL PHALANGES

Groups		1	2	3	4	5
Modern Man (50 European)	1	-	6.2	21.9	31.4	2.9
Modern Man (12 Bushmen)	2	6.2	-	27.8	27.3	8.5
Gorilla (12)	3	21.9	27.8	-	9.9	23.3
Pan (11)	4	31.4	27.3	9.9	-	36.4
Modern Man (37 E.African)	5	2.9	8.5	23.3	36.4	-

#### D<sup>2</sup> GENERALISED DISTANCE MATRIX

Fig. 1. Matrix showing the Mahalanobis  $D^2$  generalized distances between the comparative groups of hominid and pongid great toe terminal phalanges.

#### THE TERMINAL PHALANX FROM OLDUVAI (Hominid 10)

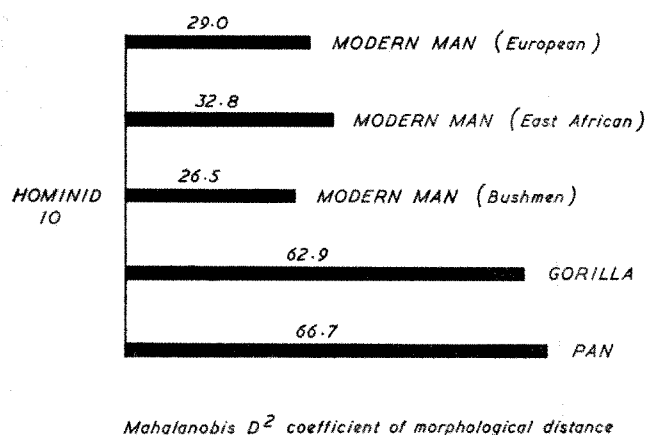


Fig. 2. A linear representation of the Mahalanobis  $D^2$  generalized distances between Olduvai Hominid 10 and comparative hominid and pongid groups.

likely that these groups will represent populations of a Linnean species, but this is not inevitable because Linnean taxonomy is usually based on a wider selection of parameters than those which can be realized by examining a single bone.

From this analysis it is clear that, by anatomical and statistical examination of the terminal phalanx of the great toe, it is possible to discriminate functionally between pronograde quadrupedal apes and plantigrade bipedal men. It is particularly interesting that the same group of parameters taken from a fossil hominid toe bone, recovered from the Lower Pleistocene of East Africa, should so clearly demonstrate the functional affinity of this bone with those of plantigrade men. The palaeo-anthropological implication of this finding is that propulsive bipedalism, of the human type, was the form of gait of at least one group of early hominids evolving in East Africa at this time.

I thank Dr L. S. B. Leakey for allowing me to examine and report on this bone, Alan Walker for data from the osteological collection of the University of Makerere, Brian Newman for mathematical advice and the Institute of Computer Science, University of London, for the use of the Atlas computer.

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## PSYCHOLOGY

### Centre of Gravity Movement in the Standing Human Body

THE position of the centre of gravity of the human body has been measured in a wide range of conditions, but in many cases a high degree of accuracy was not possible because of the crude nature of the equipment. Much less work has been carried out on the movement of the centre of gravity in quasi-static conditions, but similar remarks apply to the accuracy of the results. For the erect human body the swaying motion associated with long durations of standing is readily observed, but measurements of this movement are rare. Hellebrandt<sup>1</sup> recorded a series of positions of the centre of gravity to determine an average

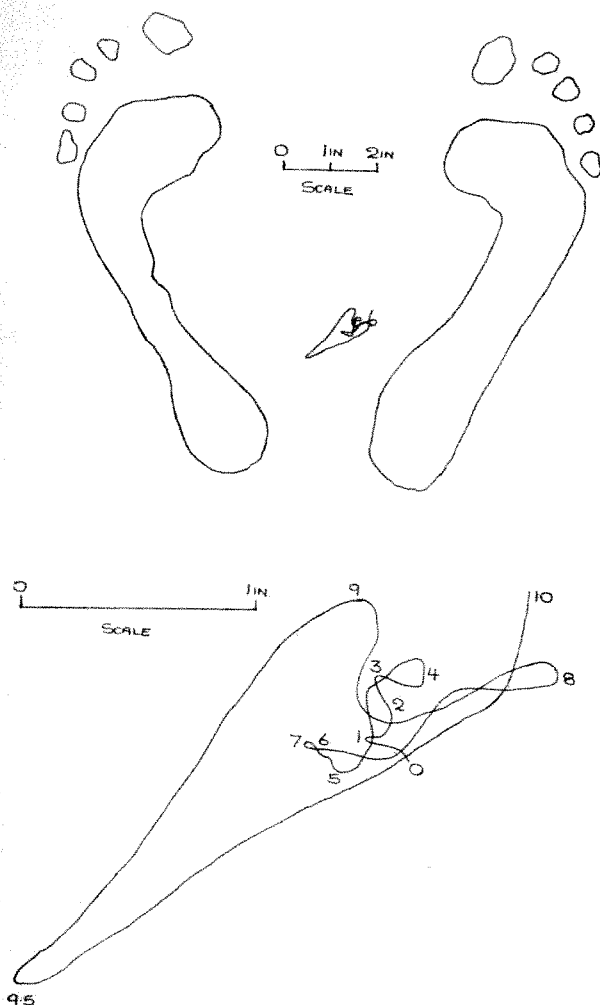


Fig. 1. (a) Base of support and path of centre of gravity. (b) Path of centre of gravity drawn to a larger scale than (a). 0-5, Eyes open; 5-10, eyes closed. This path was plotted at intervals of 0.25 sec for 10 sec.

location, while Contini *et al.*<sup>2</sup> describe apparatus by which such measurements may be made but do not seem to have produced any continuous record of the movement of the centre of gravity.

Preliminary measurements of this continuous movement of the centre of gravity have been made. The subject stands on a horizontal circular platform which is supported by three cantilevers at equally spaced positions around the periphery of the platform. Each cantilever has strain gauges attached to it, giving a continuous reading of the load on each support. Analysis of these readings allows the continuous movement of the centre of gravity of the body to be determined.

Fig. 1 shows a typical curve of the movement of the centre of gravity. The movement relative to the position of the feet is shown, together with an enlargement of the path of the centre of gravity. The numbers along the path represent the elapsed time in seconds. The figure indicates a common feature of these preliminary tests, in that each subject tends to be biased towards one foot and the movement of the centre of gravity tends to follow a line parallel to the axis of the biased foot. The greater movement of the centre of gravity with the eyes shut is as one might expect; even greater movement is found with a subject placed on the apparatus at the edge of a high flat roof, indicating a possible measurement of "dizziness".

It is proposed to develop the equipment to investigate the effect of posture and stance on stability and to provide quantitative data for the analysis of the balance mechanism

of the body; further suggestions regarding the use of the equipment would be welcomed.

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## APPLIED SCIENCE

### Physical Adsorption on Porous Glass Beads

A PREVIOUS study<sup>1</sup> has indicated that a soda-lime-silica glass may be attacked by liquid water to form a porous layer on the surface. The present investigation was to show the effect of atmospheric water vapour on the surface structure of a lead glass.

Lead glass beads of 0.87-0.91 mm diameter and composition by weight 58.6 per cent silica, 24.2 per cent lead mono-oxide, 0.7 per cent barium oxide, 1.9 per cent calcium oxide, 4.5 per cent sodium oxide, 9.4 per cent potassium oxide, and 0.6 per cent alumina and ferric oxide, varied in appearance from opaque to clear. Microscopic examination of the opaque beads showed them to have rough surfaces which were characteristic of weathered glass. Atmospheric weathering processes in glass occur generally only over prolonged periods of time. The beads under investigation had never been in contact with liquid water, and therefore any deterioration of the glass could be attributed to vapour phase weathering processes. The fact that opaque beads which were given a light etch in hydrofluoric acid became clear demonstrated that the opacity was a surface property. Some of the etched beads reverted to their original appearances after treatment with water vapour at elevated temperatures, indicating that this surface effect was the result of a weathering process.

The glass beads were separated mechanically into arbitrary clear and opaque fractions. Benzene adsorption isotherms were measured at 45° C by frontal and elution gas chromatography<sup>1,2</sup> on the clear and opaque fractions and also on the original mixture of beads. All three fractions had been degassed previously under a flow of dry nitrogen for 4 h at 200° C.

Fig. 1 shows that the extent of adsorption for the various fractions of glass beads was the same. Application

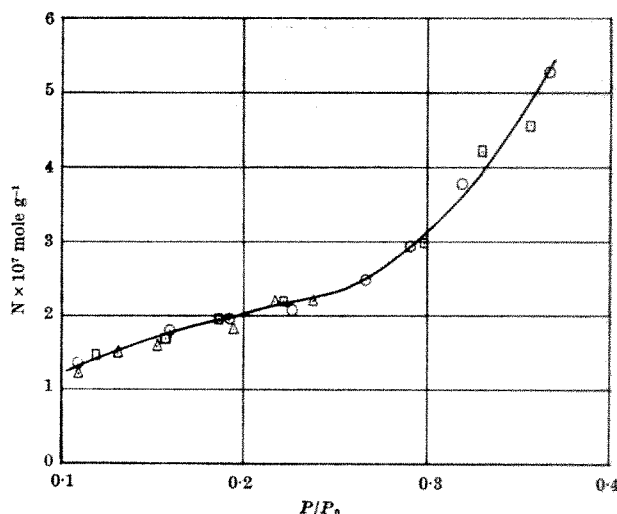


Fig. 1. Adsorption of benzene on lead glass beads at 45° C. ○, Mixed beads; △, clear beads; □, opaque beads.



of the Brunauer-Emmett-Teller equation to the isotherms gave a monolayer capacity ( $N_m$ ) of  $2.35 \times 10^{-7}$  mole  $g^{-1}$  and a Brunauer-Emmett-Teller constant ( $c$ ) of 8.2. Using a cross-sectional area of  $43.7 \text{ \AA}^2$  for the adsorbed benzene molecule<sup>1</sup>, the calculated specific surface area of the beads was twenty-seven times the geometric area. The magnitude of the  $c$  value indicated that the pores were greater than  $35 \text{ \AA}$  in diameter<sup>2</sup>. Benzene adsorption on non-porous glass gave a specific surface area in agreement with the geometric area<sup>1</sup>.

If it is assumed that the extent of porosity gives a measure of the degree to which the glass had been attacked (because all the beads had been subjected to the same weathering conditions), then all the beads had been attacked to the same extent. The appearance of the beads, therefore, was not related to the extent of attack. Because all the beads had been attacked to the same extent, it was expected that the clear and opaque fractions would have the same bulk chemical compositions, and this proved to be the case. Differences in appearances of the beads were therefore probably due to variations in the surface properties of the glass.

Ross and Roberts<sup>4</sup> have shown that devitrification is a source of high specific surface areas in glass. The more opaque glass beads gave an electron diffraction pattern which was typical of saponite [ $(Mg, Al, Fe)_3 (Al, Si)_4 O_{10} (OH)_2$ ]. This crystalline material, however, was only present in trace amounts, and its state of hydroxylation indicated that it was a product of weathering. The agreement between the isotherms suggested that the opaque products of weathering (which may have been partly crystalline) did not contribute significantly to the surface areas of these porous systems.

In conclusion, atmospheric water vapour may attack glass to form a porous surface layer. The appearance of the attacked glass is not necessarily related directly to the degree of attack, but may be correlated with a discrete surface property of the glass which may initiate or inhibit the formation of opaque products of weathering. Part of the products of weathering may be crystalline.

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<sup>1</sup> Sewell, P. A., *Physics and Chemistry of Glasses* (in the press).

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<sup>3</sup> Kiselev, A. V., *The Structure and Properties of Porous Materials, Colston Papers* (edit. by Everett, D. H., and Stone, F. S.) (Butterworths, London, 1958).

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## GENERAL

### Numerical Taxonomy and Textual Criticism of a Classical Latin Author

CRUDE forms of numerical tabulation were used as far back as the 1890s in efforts to determine genuineness or relative chronology of ancient writings, for example, by Lutoslawski<sup>1</sup> for the dialogues of Plato. The results were not without value when used in combination with other criteria, but an author's stylistic habits cannot be assumed to be constant throughout his period of literary activity, and much also turns on the investigator's subjective choice of *differentiae*.

Classification of manuscripts, an essential preliminary to any systematic textual criticism, is perhaps a more promising field for the more precise techniques now available. A manuscript tradition may be "pure bred", with successive generations of manuscripts directly copied from earlier exemplars; clerical errors and other indicative features will then be regularly distributed over the whole group of manuscripts. The interrelations of such a

monothetic (monotypic) array can be traced by routine methods and presented cladistically in the form of a family tree or *stemma*. Thus if half-a-dozen manuscripts all exhibit an identical dislocation (for example, the misplacing of the same block of lines by removal to the same place in the text), they are faithfully reproducing a feature of their common parent, and no particular problems arise.

When, however, readers or scholars in late antiquity (that is, before about A.D. 600) or in the Middle Ages (from 800 to about 1500) began to insert variant readings, either in the margins or between the lines with the desire to ease obscurities or otherwise to smooth the reader's progress, a complex situation arose. In these cases extant manuscripts show confused affiliations, which criss-cross in an apparently random fashion. Current doctrine in textual criticism treats these cases of "contaminated" or "horizontally interpolated" traditions as unamenable to systematic classification<sup>2</sup>. Editors of such texts have therefore had to fall back on considerations of idiom and style when choosing between readings in doubtful places, rather than on a critical assessment of the credentials of the manuscripts offering the variants.

This may be a premature confession of defeat, however. Inspection shows that these patterns of manuscript interrelations have the characteristics of a polythetic (polytypic) array, where *differentiae* are no longer common to the whole group under investigation. In other disciplines taxonomic methods aided by computers have enabled research workers to take such problems in their stride. In the case of Juvenal, the Latin writer of verse satire (about A.D. 100), whose manuscripts have been collated in sufficient detail for the present purpose, the number of passages which exhibit significant variants is of the order of 1,500, too few to justify investigation using a computer programme. The number of manuscripts (the operational taxonomic units in this problem) varies between fourteen and sixteen, and warrants the use of computer methods although the actual calculations are laborious.

An analogy between the behaviour of the manuscript variants and that of mutant genes suggests itself: thus manuscript variants sometimes skip one or more generations, and something akin to chiasmatic arrangement appears on occasion. Although this resemblance should not be pressed, it offers a strategy of analysis for textual criticism. Coincidence of readings in blocks of more than 300 lines in two or more manuscripts can be plotted. Each block yields not less than 40-50 points of significant variation. Thus, using similarity matrices, I was able to establish a sequence of near-neighbour relations. This is not an order of merit; it is meaningless to speak of a "best" manuscript. It is, however, helpful to further investigation to know which manuscripts can be grouped together, especially where, as in the case of the Juvenal tradition, obvious but superficial differences obscure any underlying affinities. The groupings of manuscripts for successive stretches of the satires have been tested by Spearman's rank correlation coefficient and values of the order of  $R = +0.8$  obtained, except in some instances where isolated manuscripts, which are suspect for other reasons, affect the calculation. With thirteen or fourteen degrees of freedom, such values of  $R$  yield a figure for Student's  $t$  greater than 0.1 per cent probability, that is, the odds against such consistent near-neighbour groupings being caused by mere chance are higher than a thousand to one.

Whether this taxonomic approach succeeds in penetrating to fundamental "genetic" affinities of the extant manuscripts of Juvenal is now open to debate, but I hope shortly to publish the data and reasoning in full.

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<sup>1</sup> Lutoslawski, W., *Origin and Growth of Plato's Logic* (London, 1897).

<sup>2</sup> Maas, P., *Textkritik* (English translation), 7 (Oxford, 1958).

## BOOK REVIEWS

### ORIGIN OF THE ROYAL SOCIETY

#### The Royal Society

Concept and Creation. By Margery Purver. With an introduction by H. R. Trevor-Roper. Pp. xvii+246+12 plates. (London: Routledge and Kegan Paul, Ltd., 1967.) 35s. net.

It seems strange that there should be so much obscurity about the origin of the Royal Society, and that there should be so much difference of opinion among historians who have written on the subject.

On one point, however, there seems to have been general agreement—that the movement which led to the foundation of this new scientific society drew its inspiration from the writings of Francis Bacon; and yet that this should be so is no less strange: Francis Bacon, Lord Verulam, statesman and philosopher, was no experimental scientist. Strongly as he advocated a departure from the teaching of the medieval schoolmen, based as it was on the works of Aristotle, and the cultivation of a new scientific approach to the problems of nature, his ideas of the methods by which this should be achieved were widely different from those of the actual founders of the Royal Society, and indeed of its fellows for the past three centuries. His conception, it might be said, was of an organized attack on the problems of science, not of experimentation by individuals and deduction from their discoveries. It is doubtful if he had any belief in the idea of individual experimental research.

It is more probable that the true origin of the Royal Society is to be sought in the character and ideas of the men constituting the group or groups which met in Oxford and London in the first half of the seventeenth century, in the years following the Civil War and immediately before the restoration of King Charles II.

It is this period and this enquiry which is the subject of Miss Purver's book. Her endeavour has been to clear up some of the obscurities and to indicate, more clearly than has been done hitherto, which of the groups of learned men, among several competitors for the honour, were the true founders of the Royal Society. It should be said at once that, although there is still room for difference of opinion on certain points, she has been highly successful in her research and her book is most illuminating.

The first historian of the Royal Society was Thomas Sprat, whose book appeared in 1667, only five years after the society received its Royal Charter. Sprat was in no doubt that the Royal Society originated in the meetings at Oxford of the group which centred around the personality of John Wilkins, the Warden of Wadham College. Later writers have tended to disregard Sprat, thinking that his view of the happenings was personal to himself, and it is one of the most valuable features of Miss Purver's book that she is able to show conclusively that his history was authorized and approved by the council of the young society.

The "experimental science club" at Oxford included among others, and besides John Wilkins, Seth Ward, Robert Boyle, John Wallis, William Petty and Christopher Wren. They met for about ten years, from 1648 to 1658. At the end of that period the members dispersed, for various reasons, the greater number going to London. There the meetings were continued, at Gresham College

and elsewhere, and the group was joined by others sympathetic to their ideas, among them Lord Brouncker, John Evelyn and Dr George Ent.

Other meetings of a similar or a comparable character had been held in London as early as 1645. Dr John Wallis, writing in 1678, and disregarding Sprat's version, considered these to have been the origin of the Royal Society. There was certainly some overlapping, for some of the members at these earlier London meetings appeared afterwards at the Oxford club. Wallis gave an interesting list of subjects discussed at his meetings, including the work of William Harvey on the circulation of the blood. Another notable figure at these earlier London meetings was Theodore Haak, a German who had settled in Great Britain, and to him Wallis was inclined to attribute the origin of this London club and therefore of the Royal Society.

Miss Purver has also investigated the activities of Samuel Hartlib and his collaborators, Comenius and others, with their ideas, put forward about 1646, of a "Universal College" in England. With these Robert Boyle and his "Invisible College" were involved, and all these early discussions and proposals clearly have their bearing on the origins of the Royal Society.

Much of the confusion, the conflict of evidence, which surrounds these activities is undoubtedly due to the period in which they occurred, for the times were out of joint. The Civil War had ended in 1646. King Charles II was recalled from exile in 1660. The intervening period was one of divided loyalties, and men would have been cautious in expressing their opinions and speaking of their associates. A little later, in 1665, when Sprat was writing his history, there was the disturbance caused by the plague and the fire of London.

There is the remarkable case, for example, of John Wilkins, whose position and influence were such that he might well have been the first president of the new society, and he would have seemed the obvious person to solicit the favour of the King and the grant of a Royal Charter. But Wilkins was married to Cromwell's sister; and the choice of a person to carry this responsibility fell on Sir Robert Moray, a Royalist who had been in exile with the King in Holland. The charter was obtained and the Royal Society was established with Lord Brouncker as its first president.

T. MARTIN

### ASTRONOMY'S HEROIC AGE

#### Quasars

Their Importance in Astronomy and Physics. By F. D. Kahn and H. P. Palmer. Pp. 112. (Manchester: Manchester University Press, 1967.) 30s. net.

THE race to publish the first book on quasars has been won by Professor Kahn and Dr Palmer of the University of Manchester. Their book is addressed to the general reader, and so the three questions we should ask first are, in descending order of importance, is it intelligible, is it accurate, and is it unbiased? The quasars have such astonishing properties, and our knowledge of them is developing so rapidly, that it is hard to be any of these things. The book is certainly accurate, as we should expect from the authors, who are distinguished for their work in theoretical astrophysics and observational radio astronomy—a happy combination. It is also reasonably unbiased, which in view of the controversies now raging is a remarkable achievement. I was, however, surprised to find that the marks they allot to the various theories of the origin of quasars add up to exactly 100 per cent. In view of the calculated sobriety with which they write about these astonishing objects, it seems inconsistent of them to be sure that the correct explanation has already been given!

The book is lucidly written, so that any difficulties it may present would come from the ideas described and not from their expression. The early chapters which are devoted to the discovery of quasars and their main observational properties are fairly straightforward, and should be understood by the general reader with scientific and astronomical interests for whom the book, according to the blurb, has been written. The theoretical chapters discussing the physical basis of current interpretations are inevitably more difficult. A brief account is given of radiation processes, the acceleration of fast particles, magnetohydrodynamics, relativity and cosmology. This is a formidable list, but I believe that the general reader will glean from this part of the book the type of idea which each of these subjects is called on to provide.

Separate mention should be made of the chapter entitled "Stars", which is the most difficult but most rewarding in the book. It deals with the stability of large masses in the manner made famous by Eddington, that is, by using simple physical considerations to derive their main properties. I would like to direct attention especially to the extension of Eddington's method to the case when general relativistic effects are important. Chandrasekhar has found by some difficult analysis exactly how the stability criterion is altered by these effects. Here a relatively simple analysis is used to obtain a reasonable approximation to this new criterion. This section will be read with gratitude not only by the general reader but also by astronomers who are not experts in relativity. I think Eddington would have enjoyed this extension of his method, although perhaps he would have added whimsically that the authors give a rather low mark (10 per cent) to this relativistic instability as the possible origin of quasars.

One day we will understand the origin of quasars, and then this book will have to be rewritten. Even now, however, we can claim that we are living in the heroic age of astronomy (and it is no disrespect to the great astronomers of the past to recognize this). The reader who masters a fair fraction of Kahn and Palmer can share in the excitement of this age, and will be prepared to understand in good part the great discoveries that are still to come.

D. W. SCIAMA

## OPTIMAL CONTROL

### Optimization of Stochastic Systems

Topics in Discrete-Time Systems. By Masanao Aoki. (A Series of Monographs and Text-books in Mathematics in Science and Engineering, Vol. 32.) Pp. xv + 354. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1967.) 116s.

This is the thirty-second text in an excellent series of books on mathematics with practical application which is being edited by R. Bellman. The present author, M. Aoki, is an eminent research worker in the field of control system optimization and fifteen of his 143 references are to important publications of his own.

As indicated by the word "stochastic" in the title, the problem of optimization is studied for cases in which the system is subject to randomly varying inputs. These inputs can be either additive, as with noise or random signals to be followed, or multiplicative, as when gains and time constants vary randomly. The whole study is performed using discrete-time so that systems are always described by difference equations. Thus the development of statistical methods is more straightforward than with a continuous-time study which may be ultimately necessary in many cases. The resulting unified treatment, however, is a commendable feature of the book.

Much of the book is concerned with the derivation of what the author calls "optimal Bayesian control". This involves the manipulation by the chain and Bayes's rules

of conditional probability densities which are used in recurrence relations for expected future cost. Then the process of deriving optimal control corresponds to the method of dynamic programming. The optimal control policies are applied firstly when noise and plant parameters are known constants, then when some of the parameters are unknown but assumed constant, and finally when such parameters are themselves stochastic.

A chapter is devoted to the problem of how to operate on noisy measurements in order to make the best estimate of the quantities being measured. Three methods are studied: least-squares, maximum likelihood and optimal Bayesian estimation, and their inter-relation is examined. The Kalman filter is derived as an example of the latter method.

The convergence of Bayesian optimization is considered together with Kalman's concept of controllability and observability applied to stochastic systems. The question of the stability of systems is also studied using stochastic Lyapunov functions. There is a long chapter concerned with approximations which may be made to simplify the formulation and implementation of optimal control policies. More work may be needed in this direction before the methods are applied in practice. The certainty equivalence principle, pseudoinverse matrices and sufficient statistics and martingales are interesting features which enter naturally into the work and are given some explanation.

The basic requirement of the reader is a working knowledge of matrices, vector spaces and conditional probabilities. Throughout the book, the author tries to be gentle to the uninitiated by the use of illustrative examples given in ascending order of difficulty. Although no exercises are set for the reader, it will form a good textbook for students of advanced control theory and it will be an extremely valuable reference book for researchers and teachers of control of stochastic systems.

A. P. ROBERTS

## THIS IS TRIBOLOGY

### Lubrication and Lubricants

Edited by Eric R. Braithwaite. Pp. xiv + 568 + 191 illustrations and 46 tables. (London, Amsterdam and New York: Elsevier Publishing Company Ltd., 1967.) 170s.

PRESENTATION of a subject which has its origin in traditional practice but wherein modern development has occurred in a number of scientific disciplines poses many problems. Not least of these is the probability that the reader may be expert in one facet while having only a superficial knowledge of the remainder. Thus, as in this book, which deals with chemistry, metallurgy and several aspects of engineering, matter which is descriptive of present or past practice must be blended with rigorous treatment of current scientific contributions. Collaborators in this volume have overcome these difficulties quite skilfully, presenting good reviews of current research in each field against a background of historical or other introductory matter sufficient to facilitate a multi-disciplinary approach. Perhaps, because the planning of the volume anticipated the Jost Report, the word tribology is not used although the volume is itself a good example of the multi-disciplinary approach denoted by this term.

In an introductory survey, Rowe departs from the conventional concern with the contact of asperities and introduces the subject of boundary lubrication by analogy with the lubrication by a soft solid of flat metal plates loaded in compression. The resistance to lateral sliding is proportional to the yield strength in shear of the soft solid, and the maximum compressive stress which can be withstood by the metal plates is, of course, their yield strength in compression. The yield strength in shear is related to yield strength in compression by a ratio of 1/2,

therefore coefficient of friction = yield strength of soft solid /  $2 \times$  yield strength of plate material. Evidence is provided from measurements of friction in forging titanium with copper or aluminium used as a lubricant which reveals close qualitative agreement with the above prediction. Reviewing recent developments of frictional theory, Rowe concludes that "welding" is not an essential feature of boundary friction but that most of the energy can be dissipated in shearing the lubricant itself. The theory of hydrodynamic lubrication is introduced in general terms without making excessive demands on the mathematical skill of the reader and without the detailed treatment which characterizes most texts on the subject. Greater emphasis is given to a descriptive treatment of the causes and prevention of wear.

Peace summarizes recent work on solid lubricants. Additives for lubricating oils present a confusing array of chemical types which must provide commercial solutions to special problems posed by engines or other machinery requiring lubrication. The final product cannot, however, be characterized in chemical terms alone and Molyneux describes some of the basic chemical formulations available, but he is mainly concerned with functional tests as prescribed in the various specifications. Although the text is quite correct some difficulty appears in connexion with the captions of some of the illustrations. Thus, Fig. 3/4*b* illustrates "ridging" failure rather than "rippling" and these are stated to occur in high-speed, low-torque conditions rather than the low-torque, high-speed conditions. The quality of reproduction of Fig. 3/4*a* is insufficiently good to determine whether or not it illustrates "scuffing" as claimed in the caption. This type of failure is characteristic of the high-speed, low-torque condition as correctly stated in the text and not the high-torque, low-speed case referred to in the caption.

The increasing use of gas turbine engines in aircraft has produced demand for improved performance of lubricating oil which can no longer be met by natural lubricants and which can only be met by synthetic fluids, notably the diester types. Goddard reviews the chemical nature and performance characteristics of synthetic lubricants generally.

For less exacting duties lubricating greases are used, and Harris shows that these are no longer based on tradition but have been the subject of much scientific study. Electron micrographs of the structures of several types of grease reveal the important part played by the network of soap fibrils or the fine structure built up from mineral based solid phases.

In a review of lubrication in metal cutting Rowe introduces the theory of Ernst and Merchant which relates the coefficient of friction at the tool face with the rake angle. This theory envisages that all the shearing takes place on a single plane of negligible thickness. To accommodate a finite relative displacement, however small, an infinite shear strain would be required which could only be associated with complete rupture. A micro-section of the cutting zone in beryllium copper suggests that shear occurred successively on a number of planes but, in the general case of a continuous chip, rupture is excluded. The authors rectify this concept by introducing what they call the "minimum energy" theory of machining. Although shearing of metal would be initiated on one plane, the material in its vicinity would become strain hardened so that subsequent shearing would take place on an adjacent plane. This leads to the prediction of a wedge-shaped shearing area, which is confirmed by photomicrographs of sections of machined areas of annealed mild steel. We are thus concerned with shearing zones rather than the shear planes postulated by Ernst and Merchant. Recent work<sup>1</sup> shows that the formation of a continuous chip is the result of plastic deformation in two zones, one embracing the shear plane of Ernst and Merchant and the other adjacent to the face of the tool. These are shown to be attributable to the variation of

strength with shear rate and the postulation of strain-hardening may be unnecessary.

Many books on lubrication concentrate on the hydrodynamics or chemistry of the lubricant to the exclusion of the nature and contribution of the surfaces being lubricated. This book avoids this criticism, for fully illustrated chapters by Morris and Pratt deal with metallic and plastic based bearings respectively.

During the discussion of any lecture on lubrication some member of the audience invariably asks, "What do I do about my car?" Such questions are answered by Greene, who deals fully with lubricants for reciprocating engines, gas turbines, rotary engines and gears. No doubt for the enlightenment of non-engineers, diagrams are provided illustrating the common types of rolling bearing. Unfortunately the captions have been transposed and a ball thrust bearing is described as a double row angular contact bearing. The illustration of the latter type is captioned as a self aligning bearing. The self aligning ball bearing is described as a roller bearing and the roller bearing is described as a ball thrust bearing. A final chapter by Cockcroft on lubrication in metal working adequately describes a wide range of processes.

Notwithstanding minor blemishes, this book is probably unique in the range of information included and will serve equally well as an introduction for the newcomer into the field or as a work of reference for the established practitioner.

F. T. BARWELL

<sup>1</sup> *J. Mech. Eng. Sci.*, 8, 266 (1966).

## X-RAYS FOR METALLURGISTS

### A Handbook of Lattice Spacings and Structures of Metals and Alloys

Vol. 2. By W. B. Pearson. (International Series of Monographs in Metal Physics and Physical Metallurgy, Vol. 8.) Pp. viii + 1,446. (Oxford, London and New York: Pergamon Press, Ltd., 1967.) 500s. net.

THE second volume of this important reference book follows closely the pattern of its well known predecessor published in 1958. The tabulated data assembled in the first volume are brought up to date, and the enormous literature in the intervening years (1955-1964) is reviewed. This volume is substantially larger than the first, but the overall presentation, limited to six chapters, is more concise.

A new classification of the known structure types of metals and alloys is introduced in the first chapter, based not on the usual Strukturbericht symbols but on a new notation employing two characters to represent each of the fourteen Bravais lattices. This new notation will present no problem to the reader, but its adoption here will no doubt cause debate.

The bulk of the book can be divided broadly into two sections. The first of these, three chapters and 400 pages, takes the form of a vast table, compiled in alphabetical order, which summarizes the structural data and lattice parameters of many thousands of compositions of intermediate phases, metallic borides, carbides, hydrides, nitrides and binary oxides—a truly monumental compilation of most useful data. It must be noted, however, that should the references corresponding to the tabulated data be required, they lie partly in the review sections of the first volume.

The second and larger section of the book (the fifth and sixth chapters) is devoted to a meticulous critical review of X-ray structure data published between the writing of the first and second volumes. The accounts of earlier work in the corresponding chapters of the first volume are not repeated in this section, but are widely referred to, so that in this respect the two volumes are complementary. The coverage is very wide indeed and the condensed results,



detailed and amply illustrated, are presented in a most accessible and useful form.

It is difficult to find fault with this book; it would be unreasonable to do so on the narrow basis of a few omissions and inadvertent misrepresentations. It is a pleasure, therefore, to say that the second volume lives up to the standard of its predecessor to constitute a magnificent reference work on X-ray structure data, which will be greatly valued by research metallurgists and physicists.

I. WILLIAMS

## EASTERN RADIATION CHEMISTRY

### Proceedings of the Second Tihany Symposium on Radiation Chemistry

Edited by J. Dobo and P. Hedvig. Pp. xvi + 813. (Budapest: Akademiai Kiado, Publishing House of the Hungarian Academy of Sciences, 1967.) 200s.

To those who had the pleasure of participating in the first Tihany Conference on Radiation Chemistry in 1962, the announcement of a second conference promised a further useful interchange of the latest information from a wide range of sources. Subsequent reports show that this promise was more than fulfilled and this is confirmed by the many excellent contributions reported in this volume. As may be expected, east European countries were strongly represented, and the meeting presented an excellent opportunity of reviewing the present status of research which, because of language difficulties, cannot always be readily followed from published literature.

The proceedings are divided into four main parts: general (eight papers), aqueous solutions (eighteen papers), organic compounds (thirty papers), polymers (forty-two papers). This division, however, does not reveal the wide scope of the papers. These include several papers concerned with radiation mechanisms and with radiobiology, some valuable contributions on the radiation chemistry of water and related problems and a range of papers dealing with intermediates in simple organic compounds, using recently developed techniques. An unusual feature is the large number of contributions concerned with various aspects of polymer research: polymerization, graft and block polymers, solid state reactions, crosslinking and thermoluminescence. One of the most useful features is that many of the articles are commendably short, summarize the conclusions reached as a result of a comprehensive research programme and give references to the original papers in which fuller details can be found. Thus, a selection of important papers not yet available in English can be made easily. This approach is to be highly recommended; only too often papers presented at conferences are too detailed and present one aspect of a lengthy programme, with which the author but not the audience is familiar; such results are more appropriately published in the usual scientific journals.

One feature of these conferences is the opportunity it offers for informal discussions in very pleasant conditions on work in progress. I have a vivid recollection after the first conference of trying to follow an informal scientific discussion in a Budapest restaurant, while a violinist of the nationalized Zigeuner Orchestra played highly romantic music at a range of six inches alternately from left and right ear. For radiation chemists who have participated in this meeting and enjoyed excellent Hungarian hospitality, and for those who have not had that opportunity, this volume is most highly recommended as a source book. On the present showing we may expect future Tihany conferences to become an important feature in the calendar of international conferences on radiation chemistry, and their published proceedings a valuable collection of up to date short review articles.

A. CHARLESBY

## CENTURY OF STUDY

### Mongolism

Edited by G. E. W. Wolstenholme and Ruth Porter. (Ciba Foundation Study Group, No. 25.) Pp. ix + 99. (London: J. and A. Churchill, Ltd., 1967.) 18s.

In 1866 Dr John Langdon Haydon Down published a paper "Observations on an Ethnic Classification of Idiots", in the London Hospital reports, in which he drew attention to the group of children now classed as showing the features of mongolism or Down's anomaly. Any suggestion implied in Down's paper of an ethnic link between these children and the members of the Mongolian race has long since been refuted, but the term mongolism remains in use despite attempts to supplant it by Down's anomaly. The centenary of Down's paper was commemorated by the meeting of a study group at the Ciba Foundation in London on May 10, 1966, under the chairmanship of the late Lord Brain.

In numerical terms mongolism is important within the context of the more general problem of congenital abnormality. Children with this syndrome are born with a frequency of about 1 in 800 of all live births, and apart from the characteristic physical stigmata they eventually show a severe degree of mental sub-normality. Mongols form an important component of the children in any hospital for the mentally sub-normal, and mongolism is possibly the example of gross congenital abnormality with which the general public is most familiar, a familiarity enhanced in recent years through the medium of television.

Seven papers were given at the Ciba meeting and six of these had roots in various advances already made in our understanding of mongolism. Dr E. Matsunaga discussed Japanese data on parental age and birth order in mongolism, while Drs H. Forssman and H. O. Akesson discussed Swedish work on consanguinity and mongolism. Both Professor L. S. Penrose and Dr G. F. Smith, of the Kennedy-Galton Centre at Harperbury Hospital, gave papers on dermatoglyphics, while Dr Ursula Mittwoch of the Galton Laboratory dealt with the DNA content of mongol and normal cells as measured by a microdensitometer on Feulgen-stained fibroblasts. Dr M. Fraccaro and collaborators from Pavia and Stockholm discussed their data on the timing of DNA replication in *G* group autosomes in cultures of lymphocytes from mongols.

The past thirty to forty years have seen important advances in our knowledge of mongolism, and the highlights have been an appreciation of the significance of maternal age in relation to the risk of conceiving a mongol, the specificity of the dermatoglyphic findings, and the understanding that mongolism may be the phenotypic expression of trisomy of one of the *G* group autosomes, conventionally regarded as No. 21 in the Denver classification. A further important advance has been the linking of familial mongolism with the transmission of a translocation chromosome of the *D/D* or *G/G* centric fusion type, while a perplexing piece of information is the markedly increased risk of the development of leukaemia incurred by mongol children. It is perhaps a pity that on the centenary of Dr Down's paper there was no review of the advances that had been made and of the hopes for the future. In the study of mongolism we seem to have come up to one of those barriers to the acquisition of knowledge, the successful negotiation of which opens up wide and new fields of understanding. Much of the burden of future concern in research into mongolism must surely be towards knowledge of the mechanisms linking gene action and phenotypic expression. The last paper of the Ciba meeting, by Dr W. J. Mellman and collaborators from Pennsylvania, begins to deal with just such questions and is concerned with whether increased enzyme activities observed in the polymorphonuclear leucocytes of mongols can be accounted for by the localization of

specific gene determinants to chromosome 21. Perhaps the nature of gene action and phenotypic expression in mongolism will be understood long before the bicentenary of Dr Down's paper comes round.

W. M. COURT BROWN

## HUMAN INHERITANCE

### Genetics of Man

By Paul Amos Moody. Pp. viii + 444. (New York: W. W. Norton and Company, Inc., 1967.) \$7.50.

THIS book is an introduction to the principles of genetics and to inheritance in man. Based on a course of elementary lectures, it is suitable for medical students and those who want to know more about man. Each chapter has retained the characteristics of a lecture, offering problems for further study and a list of references to original works. The book remains a collection of lectures, and the references are not collected at the back and the names of the authors do not occur in the index.

The introduction to theories of inheritance is more successful than the outline of human cytogenetics. It is doubtful whether a reader would gain an adequate knowledge of the structure and behaviour of chromosomes to appreciate the applied science that is referred to as genetic engineering. He should find, however, the gradual introduction to theories of probability and methods of calculating inheritance an excellent groundwork for the study of human pedigrees.

A prospective reader need not be put off by the figures and formulae he may see as he turns the pages, for Professor Moody has succeeded in his aim of avoiding the intricacies of mathematics.

There is a simple but adequate exposition of twin studies. This leads to well balanced appreciations of the inheritance of intelligence, natural selection in human populations, the genetics of race and, finally, to a chapter on genetic counselling. Genetic counselling is becoming the fashion and those who think it has reached an advanced stage might read the last chapter for a straightforward factual explanation of its difficulties and limitations.

WILMA GEORGE

## OUR ABNORMAL CHROMOSOMES

### Human Population Cytogenetics

By W. M. Court Brown. (North-Holland Research Monographs: Frontiers of Biology, Vol. 5.) Pp. xii + 107 (31 tables, 25 plates). (Amsterdam: North-Holland Publishing Company, 1967.) 40s.

DR W. M. COURT BROWN and his colleagues at the Medical Research Council Clinical Effects of Radiation Unit have played a unique part in the history of human cytogenetics. Dr Court Brown was active in the subject from the start of its recent efflorescence (1956), and the unit which he heads is now the largest one for the mass study of human chromosomes in the world. Moreover, the geographical position of the unit is such that it is able to survey, if it wishes, the whole population in the sea-limited Edinburgh-Glasgow axis. It not only has a comprehensive filing system for all karyotypes studied, but also a team of social workers trained to follow up any case in which the subsequent history, or familial studies, are likely to be of interest. For these reasons the unit has been able to collect a unique mass of information on the incidence of chromosomal variations and abnormalities in a large population.

This book gathers together in one volume several topics on which reports have hitherto been scattered in various journals. It does not claim to be comprehensive; rather it reflects the problems which have been of particu-

lar interest to the author and his colleagues in the past few years. The topics fall under three main headings: the incidence of clearly visible abnormalities (and variants) in the mitotic chromosomes in a normal population; the problem of the chromatin-positive male; and the role and nature of the Y chromosome.

The findings are such as might be expected from a large-scale study of those morphological peculiarities of chromosomes which can clearly be seen with the aid of the microscope. The incidence of the various anomalies found is clearly listed, and the clinical manifestations of the resultant phenotypes are defined briefly; it is also shown how chromosomal alterations may be inherited in families.

In the first chapter the increase of aneuploidy with age is clearly dealt with; then the author considers the controversial subject of chromatid aberrations (these are so dependent on cultural conditions that he can only say that the whole status of chromatid abnormalities in preparations from population surveys remains to be evaluated). The conclusions about chromosome aberrations and their relation to environmental chromosome damaging agents are not much more definite, although the extensive work (of Buckton and others) on the long-term effects of radiation on the behaviour of lymphocytes has given us much basic information about the consequences of chromosomal damage. Finally, in this chapter, the author considers inherited variants in autosomes found in phenotypically normal individuals; these are found especially in the acrocentric chromosomes of groups D and G and in pair 16. It is surprising that no specific mention is made of the chromosomal satellites, for these exhibit morphological variations and it has been shown that abnormally long ones can on occasion be inherited. Perhaps the subject has been generally disregarded because of early erroneous ideas of others on the use of the satellites in chromosomal classification (now abandoned) and the relationship of long satellites to certain diseases which had been proposed on rather slender evidence.

The second chapter contains a most valuable contribution to the literature on chromatin-positive males (who do not necessarily, as Dr Court Brown points out, present with Klinefelter's syndrome), and a study of the ways in which they present for diagnosis and of their ultimate fate.

The third chapter deals with the Y chromosome; two findings of particular interest are the evidence that there may be a sex-determining locus on the short arm of the Y (this is based on the phenotypes of individuals found to have abnormal Y chromosomes) and the well known recent discovery of the high incidence of tall XYY males in institutions reserved for unusually violent and aggressive individuals.

In the last chapter the author considers chromosomal rearrangements in the population and relates them to the classical theories of Muller and others relating to radiation-induced rearrangements. Here again the main types of rearrangements found are catalogued together with their incidence, which totals (on the basis of mitotic studies) about 0.5 per cent in the normal population. The author indicates, however, that the recent meiotic studies of McIlree and others in the Clinical Effects of Radiation Unit show clearly that this figure is likely to be an underestimate, for they have brought to light probable heterozygosity in the chromosomes not visible in mitotic cells.

Altogether this is a monograph which will be essential in the library of any human geneticist or cytogeneticist. It gives the basic data on the incidence and genetic behaviour of such gross alterations in the autosomes as can be seen by microscopic inspection. Abnormalities of the sex chromosomes are not dealt with as a whole, because these were thoroughly documented in an earlier monograph from this unit. The layout of this work is particularly clear and easy for reference. Cytologists

may regret that the author has not gone more deeply into the biological significance of the chromosomal abnormalities described; into little-understood things such as heterochromatin or chromosomal coiling, or the whole problem of why the acrocentric chromosomes are so much more often involved than the others in rearrangements; but these matters were perhaps outside his terms of reference. The fundamental significance of this book is the sheer volume of research on which it is based. It is to be hoped that the possible computerization of chromosome analysis may enable the unit to undertake even more extensive studies in the future.

E. H. R. FORD

## QUALITY OF MILK

### Nutrition and the Composition of Milk

By Manfred Kirchgessner, Henning Friesecke and Gunter Kock. Translated from the German by Catherine T. M. Herriott. (International Monographs: Aspects of Animal and Human Nutrition.) Pp. xiv + 273. (London: Crosby Lockwood and Son, Ltd., 1967.) 55s. net.

THE factors controlling the composition of cow's milk are partly of genetic and partly of environmental origin. This book is primarily concerned with one of the most important of the environmental factors, the effect on the quality of milk of different feeds and methods of feeding. In the book, which is a translation of the German edition published in 1965, the authors outline the chemistry of the major constituents of milk and present comprehensive reviews of the influence of the diet on the physical properties of milk and on the content in milk of fat, protein, minerals and vitamins. The enzymes of milk and certain anomalous constituents, including antibiotics, pesticides and radionuclides, are discussed in the final chapters. A feature of the presentation is the large number of tables of compositional data.

The several thousand references cited in the text, mostly to work published within the past twenty years, bear witness to the considerable interest in these topics. Whereas some of this work was purely observational, much of it was carried out with the important immediate practical objective of maintaining and, if possible, improving the quality and thus the nutritive value of milk, particularly through increasing the content of non-fatty solids. At the same time advances in knowledge of the influence of the diet on microbial processes within the rumen and of the physiological and biochemical processes of milk secretion have facilitated a clearer understanding of the results of feeding experiments. The authors of this book have succeeded in drawing together the threads of knowledge in discussing the reasons for the effects of diet on the composition of milk.

With its very complete bibliography this book will prove a most useful work of reference and it is a worthwhile addition to this series of monographs.

J. W. G. PORTER

## JOURNAL FOR TAXONOMISTS

### *Journal of Natural History*

An International Journal of Taxonomic and General Biology. Vol. 1, Number 1 (January-March 1967). (Formerly the *Annals and Magazine of Natural History*.) Pp. 152. Price per part 52s. 6d. (\$7.50) plus postage. Subscription per volume (4 parts) £10 (\$28.60) post free, payable in advance. (London: Taylor and Francis, Ltd., 1967.)

It was in January 1952 that the *Annals and Magazine of Natural History* first appeared without the traditional pictorial design on its blue cover and so abandoned its dedication to lovers of nature. Now, as announced to

subscribers in a letter which was enclosed with the issue of last September, it has modernized itself in the manner of several other journals, even to the extent of changing its name. There will no doubt be mixed feelings. Some taxonomists will welcome this sign that their subject merits a journal with the same general appearance as those devoted to branches of the so-called "modern" biology. Others may be sad at the passing of an old friend. Librarians, always practical people, may curse the need to find a slightly taller shelf, but will be glad that the subscription price has remained unchanged, and that the numbers will now appear quarterly by intent as well as in practice.

The old journal was "conducted" by a distinguished list of experts. The new journal is edited by only two, with one assistant and an international panel of specialist consultants. It is a pity that we are not told who these are. The editor, in a note, says that it will now be possible to accept longer papers, and that contributions are invited on more general topics as well as the shorter papers on taxonomy and morphology that were a characteristic feature of the *Annals*. Like its predecessor, the *Journal of Natural History* remains the property of the publisher and is independent of any society or institution. The typographical format has been changed, the page size enlarged and the slightly tinted, loaded paper enables illustrations to be included in the body of the text. These changes should certainly lead to economies in production and to an improvement in the reproduction of the line drawings that are the most frequent form of illustration to this sort of communication. The practice does not, however, suit half-tone plates and the author may well be dissatisfied with the one example that appears in this first number.

The contents are of a high scientific standard, and include an important addition to the series of papers by Dr Sidnie Manton on the origin of the Arthropoda. The wide range of taxonomy is indicated by the fact that the ten other authors are drawn from university departments of botany, geology and zoology and from institutes of entomology, freshwater and marine biology as well as from the traditional museums. There is no doubt that knowledge of the range and form of animals and plants remains at the root of biological science, and can be ignored by biologists only at great loss both to themselves and the subject. It is to be hoped that all true natural historians will welcome this new journal and will see to it that it is never short of material for publication.

J. W. S. PRINGLE

## ANTIBIOTICS IN THE MAKING

### Biosynthesis of Antibiotics

Vol. 1. Edited by J. F. Snell. Pp. x + 234. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1966.) 80s.

THE first volume in this series provides a survey of the literature pertaining to the biogenesis of selected groups of antibiotics up to early 1966. The book is divided into six chapters, each covering a clearly defined topic, and each written by different authors. As a result, a different emphasis within each topic is readily apparent. Somewhat surprisingly the chemically more simple antibiotics such as D-cycloserine, chloramphenicol, griseofulvin, sarkomycin and L-azaserine are not dealt with as might have been expected in a first volume, especially because some of these compounds illustrate simpler versions of biogenetic pathways which are covered in the work.

The first chapter gives a rather superficial treatment of microbial processes which have been applied to the preparation of labelled antibiotics. The chapter is partly redeemed, however, by the excellent table of selected specific examples (where a strict adherence to alphabetical order would have been an improvement) and by the

comprehensive bibliography which provides ready access to more detailed material.

The second chapter, which certainly could have been better presented, deals with the biogenesis of the penicillins and cephalosporins and includes useful sections on penicillin acylases and the "semisynthetic" penicillins and cephalosporins. Particularly poor features, so far as students are concerned, are the complete lack of stereoformulae and the portrayal, in Figs. 2, 3, 5 and 7, of sequences of biochemical conversions solely by use of the names of the compounds involved. The presentation of formulae with individual asymmetric centres labelled D or L is no substitute for proper stereoformulae in the portrayal of absolute configuration, and structural formulae are essential for instant appreciation of chemical changes. Closer attention could have been given to analogy in organic chemistry of the reactions involved in the biosynthesis of the penicillins and cephalosporins, and the statement that cephalosporin P appears to be of steroidal nature in terms of a 1957 reference reveals a lack of breadth in literature coverage.

A better presentation is evident in the third chapter which covers the biogenesis of the tetracycline group. Here, as in the fourth chapter which deals with the biosynthesis of streptomycin, with very little mention of antibiotics chemically similar to streptomycin, stereoformulae are given, and due attention is paid to possible reaction mechanisms in the biogenetic pathways.

The fifth chapter seems completely out of place in this work, for it is concerned with a broad review of polyacetylene biogenesis with no apparent relevance to any specific antibiotics. Moreover, the introductory text which states that polyacetylenes have two main taxonomic loci in higher plants, of which only one is mentioned by name, is poorly married to Table 1 which shows four major loci, with the result that only an expert botanist could gain much from the discussion of the distribution of acetylenic compounds in Nature.

The sixth chapter, which deals with the macrolide antibiotics, is perhaps the best chapter in the book, with a good balance between biochemistry and reaction mechanism.

In summary, the book is rather uneven, and while it will serve as a valuable source of references in the library to workers in various biological fields it does not appear to be a book to inspire private purchase by graduate students or university staff members.

M. MARTIN-SMITH

## OBITUARIES

### Dr S. B. Challen

STEPHEN BENJAMIN CHALLENGER died at his home in Havant, Hampshire, at the age of 42. Educated at Queen Elizabeth's School, Blackburn, and the Blackburn Technical College, he served his pharmacy apprenticeship during the Second World War, and graduated from the University of London School of Pharmacy in 1947. After two years national service with the Royal Air Force he was appointed assistant lecturer at the School of Pharmacy and subsequently became a lecturer in 1951. He spent a year as visiting professor of pharmacognosy at the University of Saskatchewan and was appointed head of the School of Pharmacy at Portsmouth College of Technology on April 1, 1964.

Dr Challen was a pioneer in pharmacy education and was secretary, president and organizing secretary of the British Pharmaceutical Students' Association, and associated with the formation of the International Pharmaceutical Students' Federation. He had gained in 1952 a special degree in botany by part-time study; and his researches

on the chemical constituents of plants and the retention of pesticides on leaf surfaces reflected his interest in botany. His achievement in revitalizing the Portsmouth school and developing Council for National Academic Awards degrees and strong research groups represents his major work, and the future of the school based on the firm foundation which he laid will provide his best memorial. His death is a sad loss to pharmacy and to pharmaceutical education in particular.

W. DAVEY

### Professor Richard S. Schweet

RICHARD S. SCHWEET was killed in an air crash on April 3, 1967, at the age of 48.

He graduated BS at City College, New York, in 1938, and MS at Iowa State College in 1941. After military service during the Second World War he continued his research studies and graduated PhD in 1950. For the next two years he was a research fellow with D. E. Green at Wisconsin where he studied the properties and functions of pyruvic oxidase. He then moved to California Institute of Technology where he worked in the laboratory of H. Borsook, holding a senior fellowship from 1953 until 1958. During this period he initiated his studies on protein biosynthesis which he actively pursued until his premature death. In this field, which has moved forward extremely rapidly during the past decade, Schweet made many major contributions and was consistently at the forefront of ideas.

In 1958 he reported studies in which the soluble system for incorporation of amino-acids into RNA was resolved into an activating enzyme fraction and an RNA acceptor. His experiments supported the earlier proposal that there is a specific activating enzyme for each amino-acid and provided evidence that there is a specific RNA acceptor for each amino-acid. This was an important point in establishing that soluble RNA fulfilled the role of an adaptor molecule.

Later in the same year Schweet (with H. Lamfrom and E. Allen) reported the synthesis of haemoglobin in a cell-free system prepared from rabbit reticulocytes. This system provided the first example of the cell-free synthesis of a known soluble protein and its use has facilitated the elucidation of many aspects of protein synthesis. Studies, in collaboration with J. Bishop and J. Leahy (in 1960), using the reticulocyte cell-free system led to the conclusion that the peptide chain is synthesized sequentially from the amino terminus.

In 1960 Schweet went to the University of Kentucky Medical Center as full professor in the new department of biochemistry headed by Dr G. W. Schwert. There, with a series of collaborators, he studied many aspects of protein biosynthesis, including the role of transfer RNA, the steps involved in the transfer of amino-acids from amino-acyl RNA into protein, the functioning of polyribosomes and the action of various inhibitors of protein synthesis.

In 1964 Schweet published (with R. Arlinghaus and J. Shaeffer) important evidence showing that peptide chain growth occurs by the alternate action of two transfer enzyme fractions. The first fraction, requiring guanosine triphosphate, appears to function in the binding of amino-acyl RNA to the ribosome and the second (peptide synthetase) catalyses the formation of the peptide bonds as each amino-acid is added sequentially to the chain.

During 1965 Schweet left the department of biochemistry to become chairman of a new department of cell biology, and much of his time during the past two years was devoted to that venture. He served on grant reviewing committees for the National Institutes of Health and it was in this role that he undertook the fatal flight to Colorado. In the scientific world he will be especially missed by those who had the pleasure of collaborating with him and learning from him, and who thereby shared his warm friendship.

A. R. WILLIAMSON



## University News:

### Birmingham

THE following appointments have been made: Professor J. G. Hawkes, to the Mason chair and headship of the Department of Botany; Professor P. G. H. Gell, to the chair of experimental pathology and headship of the Department of Experimental Pathology; Dr D. B. Brewer, to the chair of morbid anatomy in the Department of Pathology. Mr P. M. Woodward, of the Royal Radar Establishment, Malvern, has been appointed to the honorary professorship in the Department of Electronic and Electrical Engineering.

### Leeds

DR E. ALLIBONE, chief scientist of the Central Electricity Generating Board, has been appointed external professor to the Department of Electrical and Electronic Engineering.

### Southampton

PROFESSOR T. E. H. WILLIAMS, professor of civil and transport engineering in the University of Newcastle upon Tyne, has been appointed to a chair of civil engineering; Dr E. W. Lee, reader in physics in the University of Sheffield, has been appointed to a chair of physics. The title of honorary professor in the Department of Mathematics has been conferred on Dr K. W. Mangler, deputy chief scientific officer in the Aerodynamics Department of the Royal Aircraft Establishment, Farnborough.

## Appointments

THE Minister of Technology has appointed Air Commodore C. S. Betts to be director of guided weapons research and development (air) in succession to Air Commodore R. R. Holder.

THE Minister of Technology has appointed Air Commodore H. A. C. Bird-Wilson to be director of flying (research and development) in succession to Air Commodore T. R. Pierce.

## Announcements

DR F. G. WALTON SMITH, director of the Institute of Marine Sciences at the University of Miami, has been elected chairman of the Executive Committee of the Joint Oceanographic Institutions Deep Earth Sampling Program.

THE administrators of the Sir George Beilby Memorial Fund, representing the Royal Institute of Chemistry, the Society of Chemical Industry and the Institute of Metals, have made an award from the fund of a gold medal and 100 guineas to Dr Anthony Kelly in recognition of his work in chemical engineering.

THE US National Academy of Sciences-National Research Council is accepting, on behalf of the James Picker Foundation, applications for post-doctoral awards of fellowships and grants in the field of radiology and nuclear medicine for the year 1968-69. The awards are made in the following four categories: Advanced Fellowships in Academic Radiology; Research Fellowships; Grants for Scholars; Research Grants. Further information can be obtained from the Committee on Radiology, National Academy of Sciences-National Research Council, 2101 Constitution Avenue, N.W., Washington, D.C.

## Meetings

PSYCHOTHERAPY—Prevention and Rehabilitation, August 21-26, Wiesbaden (Mrs Fay Pannell, Conference Services, Ltd., 11 Whitehall Court, London, S.W.1).

MARINE Biology, August 24-28, Norwegian College of Economics and Business Administration (Symposium Office, Biological Station, Espeyrend, Blomsterdalen, Norway).

INTERACTIVE Systems for Experimental Applied Mathematics, August 26-28, Washington (Juris Reinfelds, Computer Center, The University of Georgia, Athens, Georgia).

REACTIONS of Oxygen with Organic Compounds, August 28-September 1, San Francisco (Dr T. Mill, Stanford Research Institute, Menlo Park, California).

REPLICATION and Recombination of Genetic Material, August 28-September 1, Canberra (Dr W. J. Peacock, P.O. Box 109, Canberra City, A.C.T.).

SUMMER School on Quantitative Methods in Reflected-light Microscopy, September 19-23, Department of Mineralogy and Petrology, University of Cambridge (Mr G. A. Kingston, Department of Geology, Imperial College, London, S.W.7).

ERRATUM. In the note "Ice and Snow" in *Nature*, 214, 1073 (1967), it was stated that G. Ostrem and V. Schytt had spoken as chairmen of two working groups. In fact, they were reporting work carried out in their own countries—Norway and Sweden, respectively.

## THE NIGHT SKY IN AUGUST

All times are in Universal Time

		MOON		CONJUNCTIONS WITH THE MOON			
		New Moon	6d 03h	Venus	8d 01h, 10° S.		
		Full Moon	20d 02h	Mars	12d 08h, 0.4° S.		
				Jupiter	—		
				Saturn	23d 20h, 1° S.		
PLANETS		Times of rising ( <i>R</i> ) and setting ( <i>S</i> ) during the month					
Name	<i>R/S</i>	Beginning	Middle	End	Mag.	<i>D</i> <sub>9</sub> (10° miles)	Zodiacal position
Mercury	<i>R</i>	2h 45m	3h 35m	Unfavourable	− 1.2	116	Cancer
Venus	<i>S</i>	20h 40m	Unfavourable for observation		− 3.8	30	Sextans
Mars	<i>S</i>	22h 25m	21h 40m	21h 00m	+ 0.6	111	Libra
Jupiter	<i>R</i>	Unfavourable for observation		3h 30m	− 1.3	589	Cancer
Saturn	<i>R</i>	22h 00m	21h 00m	19h 55m	+ 0.7	817	Cetus
<i>D</i> <sub>9</sub> is the distance of planet from the Earth on the 15th of the month.							
OCULTATIONS OF STARS BRIGHTER THAN MAGNITUDE +6 AT GREENWICH							
Star		<i>R/D</i>		Time	Mag.		
32 Tau		<i>R</i>		27d 23h 22.6m	+ 5.8		
(D, disappearance; R, reappearance.)							
METEORS							
Name		Active period	Date of maximum	Radiant	Remarks		
Perseids		July 27–Aug. 17	12d	46° R.A., +58° Dec.	Favourable		

### OTHER PHENOMENA

5d 04h, Mercury 7° S. of Pollux.  
12d 08h, Mars occulted by the Moon, visible Asia, N. Australia.  
23d 20h, Saturn occulted by the Moon, visible E. Europe, N.W. Asia.

## FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Tuesday, July 18

ROYAL SOCIETY OF MEDICINE (at 1 Wimpole Street, London, W.1), at 5.30 p.m.—Annual Meeting.

Tuesday, July 18—Thursday, July 20

INSTITUTION OF ELECTRICAL ENGINEERS and the INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS (at the University of Manchester Institute of Science and Technology, Manchester, 1)—Conference on "Computer Technology".

Tuesday, July 18—Saturday, July 22

MEDICAL RESEARCH COUNCIL, MICROBIAL SYSTEMATICS RESEARCH UNIT (in conjunction with the COSPAR-IQSY Assembly, at Imperial College, London, S.W.7)—Symposium on "Sterilization Techniques for Instruments and Materials as Applied to Space Research".

Friday, July 21—Saturday, July 22

BRITISH ACADEMY OF FORENSIC SCIENCES (at the Institute of Psychiatry, The Maudsley Hospital, Denmark Hill, London, S.E.5)—Meeting on "The Hazards of Modern Life".

## APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

ASSISTANT LECTURER IN PHYSICAL CHEMISTRY—The Registrar, University of Strathclyde, George Street, Glasgow, C.1, quoting Ref. 39/67 (July 19).

ASSISTANT LECTURER (with research interests in theoretical mechanics or pure mathematics) IN MATHEMATICS; and an ASSISTANT LECTURER IN STATISTICS—The Registrar, University of Strathclyde, George Street, Glasgow, C.1 (July 19).

ASSISTANT LECTURER IN HUMAN GEOGRAPHY—The Registrar, University College of Wales, Aberystwyth (July 20).

DEMONSTRATORS (3) (with a higher degree or equivalent postgraduate experience, and preferably experience in (a) mineralogy and petrology; (b) palaeontology; or (c) sedimentology and stratigraphy) IN THE DEPARTMENT OF GEOLOGY—The Registrar, The University, Newcastle upon Tyne, 2 (July 21).

LECTURER (with academic or industrial research experience in organic chemistry and/or experience in the application of physical instrumentation to the elucidation of problems of organic structure, and preferably a pharmaceutical qualification or experience of drug research) IN MEDICINAL CHEMISTRY—The Staff Officer (577/6), University of Aston in Birmingham, Gosta Green, Birmingham, 4 (July 22).

ASSISTANT LECTURER or LECTURER IN CROP AGRONOMY IN THE DEPARTMENT OF AGRICULTURE—The Registrar, The University, Nottingham (July 24).

DEMONSTRATOR/SENIOR DEMONSTRATOR (with a higher degree or equivalent postgraduate research experience, and preferably experienced in experimental entomology) IN THE DEPARTMENT OF ZOOLOGY—The Registrar, The University, Newcastle upon Tyne, 2 (July 24).

PROFESSOR (with interests in any branch of pure mathematics) to take charge of the division of PURE MATHEMATICS; and a PROFESSOR (with interests in any branch of applied mathematics) to take charge of the division of APPLIED MATHEMATICS, La Trobe University, Melbourne, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, July 24).

JUNIOR RESEARCH FELLOW (with a good honours degree in physics and research interests in solid state physics) IN PHYSICS—The Registrar, The University, Sheffield (July 25).

CHAIR OF AFRICAN ANTHROPOLOGY at the School of Oriental and African Studies—The Academic Registrar, University of London, Senate House, London, W.C.1 (July 28).

SCIENTIFIC OFFICER (Bacteriologist) (with a degree in bacteriology or equivalent, and preferably practical experience of water treatment and of freshwater algae and fungi) to investigate bacterial diseases of fish—The Chief Pollution and Fisheries Officer, Trent River Authority, Meadow Lane, Nottingham (July 28).

LECTURER or ASSISTANT LECTURER (chartered civil engineer with a good honours degree) IN CONCRETE MATERIALS TECHNOLOGY—The Academic Registrar (LFG), University of Surrey, Battersea Park Road, London, S.W.11 (July 31).

MARINE ECOLOGIST (graduate with a research degree or equivalent experience) to join a research group studying present-day marine carbonate sedimentation processes off the west coast of Ireland—Dr A. Lees, Sedimentology Research Laboratory, University of Reading, Whiteknights, Reading, Berkshire (August 1).

CHAIR OF INORGANIC CHEMISTRY—The Registrar, University of Strathclyde, George Street, Glasgow, C.1, quoting Ref. 51/67 (August 5).

UNIVERSITY DEMONSTRATOR (with an honours degree in agriculture or agricultural science and preferably postgraduate experience of teaching or research in crop production) IN AGRICULTURE to demonstrate to classes on the University Farm, lecture and participate in research on crops—The Secretary, School of Agriculture, University of Cambridge, Downing Street, Cambridge (August 5).

RESEARCH FELLOW (with a degree in physics or engineering with Ph.D. or other postgraduate experience, and preferably an interest in aerodynamics or heat transfer, and computing) IN ENVIRONMENTAL PHYSICS for a group working on transfer of energy and matter in forest canopies using micrometeorological techniques—The Secretary, The University, Aberdeen, Scotland (August 6).

ENTOMOLOGIST (with a degree in agricultural science or equivalent and prepared to work in any part of New South Wales) IN THE DIVISION OF SCIENCE SERVICES, DEPARTMENT OF AGRICULTURE, New South Wales, to carry out research on insect pests—The Recruitment Section, New South Wales Government Offices, 56 Strand, London, W.C.2 (August 9).

ANIMAL TECHNICIAN (with appropriate training and/or experience) in the DEPARTMENT OF BIOLOGICAL SCIENCES, to be responsible for the day-to-day running of the animal house—The Principal, Lanchester College of Technology, Priory Street, Coventry (August 10).

LECTURER IN MATHEMATICAL STATISTICS at the University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office),

Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 11).

LECTURER and an ASSISTANT LECTURER in the DEPARTMENT OF PSYCHOLOGY—The Registrar, The University, Reading, Berkshire (August 12).

RESEARCH FELLOW (or in certain circumstances a Staff Fellow) in CHEMISTRY (Inorganic, Organic or Physical)—The Master, Selwyn College, Cambridge (August 15).

TEMPORARY LECTURER (preferably with interests in one or more of the following: urban, applied or social geography) IN GEOGRAPHY—The Registrar, The University, Newcastle upon Tyne, 2 (August 15).

DIRECTOR (with research experience in a field dealing with the production, processing and marketing of meat and meat products) of the M. C. FRANKLIN LABORATORY and WILLIAM MCILRATH FELLOW IN ANIMAL HUSBANDRY at the University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 18).

PROFESSOR OF PHYSICS at the University of Natal, Pietermaritzburg—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, August 18).

LECTURER IN HAEMATOLOGY and BLOOD TRANSFUSION in the DEPARTMENT OF PATHOLOGY, University of Ibadan, Nigeria—The Inter-University Council, 33 Bedford Place, London, W.C.1 (August 21).

RESEARCH ASSISTANT (preferably with an interest or training in electrophysiology, statistical methods or biochemical techniques) in the DEPARTMENT OF ZOOLOGY, to work with Dr D. M. Guthrie on the physiology of invertebrate nervous systems—The Secretary, The University, Aberdeen (August 26).

RESEARCH FELLOW (with medical qualifications) in the DEPARTMENT OF BIOCHEMISTRY, University of Ibadan, Nigeria—The Inter-University Council, 33 Bedford Place, London, W.C.1 (August 26).

LECTURER IN SOCIOLOGY at the Australian National University—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 28).

SENIOR LECTURER/LECTURER IN ELECTRICAL AND ELECTRONIC ENGINEERING—The Registrar, University of Bradford, Bradford, 7, quoting Ref. EE/L/2/E (August 31).

LECTURER/SENIOR LECTURER IN MATERIALS SCIENCE at Monash University, Melbourne—The Academic Registrar, Monash University, Wellington Road, Clayton, Victoria, Australia; or the Secretary-General, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (September 1).

SENIOR LECTURER in the DEPARTMENT OF ANATOMY at the University of Western Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 2).

SENIOR LECTURER IN METALLURGICAL ENGINEERING in the DEPARTMENT OF METALLURGY, University of Melbourne, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 30).

CHAIR OF EDUCATION—The Secretary and Registrar, The University, Southampton (October 9).

ENTOMOLOGISTS (2) (with an honours degree in biology with emphasis on entomology and practical postgraduate experience, preferably in biocontrol) for field work on biocontrol projects—The Secretary, Commonwealth Agricultural Bureaux, Farnham House, Farnham Royal, Bucks (October 15).

FORBES CHAIR OF ORGANIC CHEMISTRY—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh (October 31).

OFFICIAL FELLOW AND TUTOR IN PHILOSOPHY to teach ancient philosophy, and to take part in (or make arrangements for) the teaching of philosophy for the School of Philosophy, Politics and Economics and other honours schools—The Vice-Provost, Oriel College, Oxford (October 31).

ASSISTANT EXPERIMENTAL OFFICER or EXPERIMENTAL OFFICER (with a pass degree, H.N.C. or equivalent in an appropriate subject, and a general background of organic and physical-organic chemistry) to operate a combined gas chromatograph-mass spectrometer instrument in the new Meat Research Institute under construction at Langford, near Bristol—The Secretary, Meat Research Institute, Agricultural Research Council, Low Temperature Research Station, Downing Street, Cambridge, quoting Ref. CM 13.

CHAIR OF GEOGRAPHY—The Registrar (Room 39, O.R.B.), The University, Reading, Berkshire.

DEMONSTRATORS (preferably with an interest in the field of inorganic solvents, including liquid metals) IN INORGANIC CHEMISTRY—The Registrar, The University, Nottingham.

GAME MANAGEMENT OFFICER (normally national of the United Kingdom or the Republic of Ireland, with a B.Sc. degree in zoology and wildlife or equivalent, and preferably experience in wildlife management in East Africa and/or research into wildlife) IN TANZANIA, for duties which will include the planning, development and implementation of wildlife projects, and perhaps also the carrying out of scientific research into wildlife—The Appointments Officer, Ministry of Overseas Development, Room 301, Eland House, Stag Place, London, S.W.1, quoting Ref. RC 275/267/01.

POSTDOCTORAL RESEARCH FELLOW at the Victoria University of Wellington, New Zealand, for work in the applications of Mössbauer effect to solid state reactions involving the development of high potentials—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1.

RESEARCH ASSISTANT to Professor J. E. Gibson in the DEPARTMENT OF CIVIL ENGINEERING to work in structural research sponsored by the Science Research Council. The research covers computer analysis of bridge, slab and shell structures and model and photoelastic experiments in this field—The Assistant Academic Registrar, The City University, St. John Street, London, E.C.1, quoting Ref. No. 8/CL.

RESEARCH ASSISTANT (with a degree or equivalent qualification) IN BIOCHEMISTRY to work on cation transport and the involvement of cations in metabolism—The Secretary, University College Hospital Medical School, University Street, London, W.C.1; quoting Ref. Ex.P.1.

RESEARCH ASSISTANT (with an honours degree in mechanical engineering or physics) in the DEPARTMENT OF MECHANICAL ENGINEERING to work on exciting new developments in the prediction of the fatigue life of metals—The Secretary, University College London, Gower Street, London, W.C.1.

RESEARCH FELLOW (with, or expecting to obtain shortly, a Ph.D. degree) IN SOLID STATE PHYSICS to work on the conducting and dielectric properties of thin films with special reference to switching phenomena—Prof. A. K. Jonscher, Department of Physics, Chelsea College of Science and Technology, Manresa Road, London, S.W.3.

STUDENT/JUNIOR TECHNICIAN (preferably with experience of histology) in a UNIT OF PHARMACOLOGY which has a special interest in the central nervous system—The Secretary, Institute of Psychiatry, De Crespigny Park, Denmark Hill, London, S.E.5.

TECHNICIAN (young man or woman, preferably over 21 and suitably qualified) to assist in a programme sponsored by the Medical Research Council,

directed at understanding and improving the behaviour of materials used for surgical implants in the human body—The Staff Officer, University of Surrey, Battersea Park Road, London, S.W.11.

## REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

### Great Britain and Ireland

- Building Research Station Digest 82 (Second Series): Improving Room Acoustics. Pp. 8. (London: H.M. Stationery Office, 1967.) 4d. [185]  
Desalination and Its Role in Water Supply. Prepared by the Central Office of Information, in conjunction with the Ministry of Technology, the United Kingdom Atomic Energy Authority, the Board of Trade and the Foreign Office. Pp. 103. (London: Ministry of Technology, 1967.) [195]  
Ministry of Technology. Second Report of the Committee on Common Standards for Electronic Parts. Pp. vii+36. (London: H.M. Stationery Office, 1967.) 6s. 6d. net. [195]  
Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences. No. 776, Vol. 252 (1 June 1967): Studies on Early Tetrapods. 1: The Lower Carboniferous Microsaur. 2: *Microbrachis*, the Type Microsaur. 3: The Genus *Gephyrostegus*. By Margaret C. Brough and J. Brough. Pp. 107-165+plate 13. (London: The Royal Society, 1967.) 27s.; \$4.05. [195]  
British Museum (Natural History). The John Murray Expedition 1933-34. Scientific Reports, Vol. 11, No. 3: The Deep-Sea Bivalvia. By J. Knudsen. (Publication No. 657.) Pp. 237-243+3 plates. (London: British Museum (Natural History), 1967.) 57s. 6d. [195]  
The School of Pharmacy, University of London. Library Guide with some Notes for Preparing Dissertations. Compiled by Magda Pasztor and Jenny Hopkins. Second impression, revised. Pp. 31. (London: The School of Pharmacy, University of London, 1967.) [225]  
Science and the Soviet Economy. By Prof. R. W. Davies. (An Inaugural Lecture delivered in the University of Birmingham on 18th January 1967.) Pp. 21. (Birmingham: The University, 1967.) 2s. 6d. [225]  
The Society for Analytical Chemistry. Report of the Analytical Methods Committee 1966. Pp. 29. (London: The Society for Analytical Chemistry, 1967.) [225]  
Bryanston School, Blandford. Report of the Natural History Society for 1966. Pp. 36. (Blandford: Bryanston School, 1967.) [225]  
Department of Agriculture and Fisheries for Scotland. Freshwater and Salmon Fisheries Research Report No. 38: The Food, Growth and Population Structure of Salmon and Trout in Two Streams in the Scottish Highlands. By Dr. Henry J. Eglishaw. Pp. 32. (Edinburgh and London: H.M. Stationery Office, 1967.) 11s. 6d. net. [235]  
Central Electricity Generating Board. Electricity and Nuclear Power. By Donald Clark. Pp. 20. (London: Central Electricity Generating Board, 1967.) [235]  
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### Other Countries

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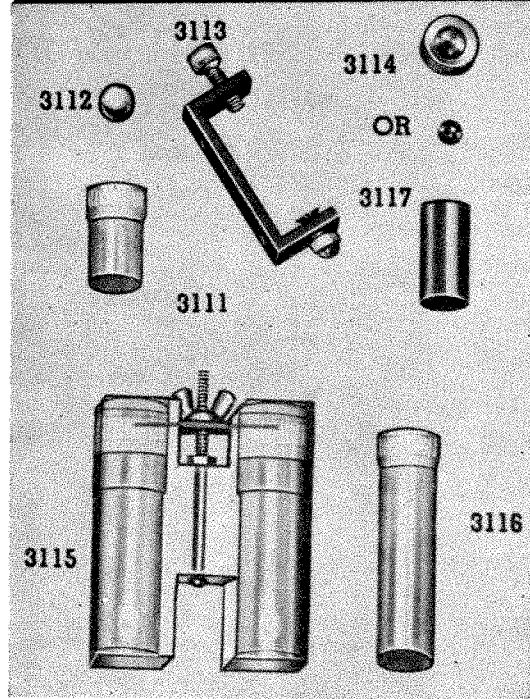
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### APPOINTMENTS VACANT

#### UNIVERSITY OF MALAYA DEPARTMENT OF GEOGRAPHY

Applications are invited for the following posts:

**SENIOR LECTURER** from population and/or settlement geographers with additional experience of research and teaching on population movements, settlement patterns and urban growth in south-east Asia.

**LECTURER/ASSISTANT LECTURER** in Surveying. A good degree in surveying is essential and the experience of research and teaching in photogrammetry would be advantageous.

**LECTURERS / ASSISTANT LECTURERS** (two) from urban geographers with additional experience in economics or from economists and demographers with interests in urban regional studies. The ability to apply quantitative techniques in the analysis of locational and distributional problems would be an advantage.

Salary scale (approximate sterling equivalent): Senior Lecturer, £1,890 by £70 to £2,310 per annum; Lecturers, £1,288 by £56 to £1,624/£1,680 by £63 to £1,995 per annum; Assistant Lecturers, £1,120 by £56 to £1,232 per annum. In addition the following allowances are payable: Variable allowance—35% of basic salary subject to certain maxima depending on marital status; minimum £210 per annum, maximum £560 per annum. Inducement allowance (if awarded) varies—(a) for Senior Lecturers, up to £560 per annum; (b) for Lecturers, up to £420 per annum; (c) for Assistant Lecturers, up to £280 per annum. Housing will be provided, if available, for which a rent of £140 to £175 per annum will be charged.

Further particulars and application forms are obtainable from the Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1. The closing date for the receipt of applications in Kuala Lumpur and London is August 7, 1967.

(146)

## Meat Research

An important and challenging research post on meat packaging is available for a **CHEMIST, BIOCHEMIST** or **MICRO-BIOLOGIST** in the Animal Sciences Division of An Foras Taluntais (The Agricultural Institute). The person appointed will be required to carry out research on the problems associated with extending the shelf life of packaged fresh meat. Starting salary up to £2,465 depending on qualifications and experience. Marriage and children's allowances apply in addition. Pension scheme is non-contributory (special arrangements can be made in the case of an F.S.S.U. member) and working conditions are excellent.

**ESSENTIAL:** Honours University Degree or equivalent.

**APPLICATIONS ALSO CONSIDERED FROM 1967 FINAL DEGREE CANDIDATES.**

Further particulars and application forms from the Director, An Foras Taluntais, 33 Merrion Road, Dublin, 4, Ireland. Latest date for receipt of completed forms—August 21, 1967. (250)



## Scientific Civil Service

### MINISTRY OF TECHNOLOGY

#### NATIONAL PHYSICAL LABORATORY

##### DIVISION OF RADIATION SCIENCE

**PRINCIPAL SCIENTIFIC OFFICER** post for man or woman, preferably aged between 30 and 50, at the National Physical Laboratory, Teddington, Middlesex, involving responsibility for the current 2 MV exposure calibration service for radiotherapy and other purposes together with its future extension to protection-level exposure rates; also the development of absorbed dose and intercomparison services at higher energies, and collaboration on the installation of a linear accelerator facility.

**QUALIFICATIONS:** Normally 1st or 2nd class honours degree or an equivalent or higher qualification in an appropriate science subject. Knowledge of the work of hospital and health physicists and post-graduate experience in radiation dosimetry desirable.

**SALARY** (Outer London): £2,315 to £3,172. Entry may be above minimum for a candidate with qualifications and/or experience of special value. Promotion prospects to grades with national salary scales £3,500 to £4,000 and £4,175 to £4,625.

(Reference: S/6720/67.) Closing date August 9, 1967.

### MINISTRY OF AGRICULTURE,

#### FISHERIES AND FOOD

##### FISHERIES LABORATORY, Lowestoft

**MATHEMATICIAN** (graded E.O./A.E.O.; for man only) with background of biology or physical oceanography to join a computer systems team responsible for the transfer to an I.C.T. 1907 computer for most of the computational work called for by the laboratory's research programme; and the development of ship-borne computer systems aboard fisheries research vessels. Much of the work is in the field of fish population dynamics, physical oceanography and quantitative marine ecology. Once most of the transfer to the computer has been achieved the successful candidate will be switched into the general stream of the laboratory's research work. Participation in research vessel cruises involved. Previous experience in computer system analysis and/or programming desirable.

**QUALIFICATIONS:** Degree, H.N.C., or equivalent in appropriate subject. Under 22, minimum qualification is G.C.E. in five subjects; including two Scientific/Mathematical subjects at 'A' level or equivalent level.

**SALARY:** E.O. (minimum age 26) £1,365 to £1,734; A.E.O. £568 (at 18) to £803 (at 22) to £1,017 (at 26 or over) to £1,243.

Promotion prospects.

(Reference: S/579-580/MAFF.) Early application advised.

The above posts are pensionable and **APPLICATION FORMS** are obtainable from the Secretary, Civil Service Commission, Savile Row, London W.1. Please quote appropriate reference. (211)

#### UNITED NEWCASTLE UPON TYNE HOSPITALS

**PROBATIONER BIOCHEMIST** required for duties in the Department of Clinical Biochemistry. This is a joint University/Teaching Hospital department and a considerable amount of research work is undertaken. Candidates, who must possess an appropriate science degree will serve a probationary period before appointment to basic grade. Salary scale £855 to £1,210 per annum; after probationary period £1,040 to £1,658 per annum. Whitley Council conditions of service applicable.

Applications, giving full details of age, education, qualifications and experience (if any), with names of two referees to the House Governor and Secretary, Royal Victoria Infirmary, Newcastle upon Tyne 1. (223)

#### EAST ANGLIAN REGIONAL HOSPITAL BOARD

Cambridge Chest Clinic in conjunction with the Botany School, University of Cambridge

**RESEARCH ASSISTANT** for investigation of the daily and seasonal variation in the pollen and spore content of the atmosphere. Applicants should preferably hold an Honours degree in botany or equivalent technical qualifications and be willing to undertake a substantial amount of microscopy. Salary scale £850 to £1,150 per annum according to experience. Tenable for three years from October 1, 1967.

Further details from, and applications to, Dr. R. G. West, Botany School, Cambridge, by August 19, 1967. (215)

# Return to Research in Britain

Are you a British Scientist in North America thinking of returning to work in Britain? You may find what you are looking for, without having to go home first, in the Civil Service, the United Kingdom Atomic Energy Authority, or the Central Electricity Generating Board.

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There are openings in most branches of science. The order in which they are most numerous is (i) physics, mathematics, engineering, and materials science; (ii) chemistry; (iii) biochemistry. The research ranges from the most fundamental to the most applied. Payment of fares (including family) to the United Kingdom will be considered.

### A Selection Board of scientists will be visiting centres as follows:-

WASHINGTON	-	8th to 22nd November, 1967
NEW YORK	-	27th November to 8th December, 1967
OTTAWA	-	16th January to 2nd February, 1968
CHICAGO	-	19th March to 5th April, 1968
SAN FRANCISCO	-	11th April to 8th May, 1968

If you would like to see them would you please write to one of the following at least six weeks, if possible, before the Board's arrival at the Centre nearest to you.

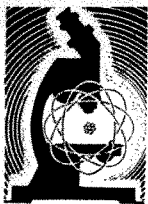
#### For Candidates in the U.S.A.

Mr. R.G. Voysey, Director,  
United Kingdom Scientific Mission,  
British Embassy,  
Washington 20008, D.C.

#### For Candidates in Canada

Dr. A. Huggard, Senior UKAEA  
Representative in Canada,  
P.O. Box No. 1245, Deep River,  
Ontario.

Issued jointly by the Civil Service Commission, the  
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# MATERIALS ANALYSIS

There are vacancies for Experimental Officers or Assistant Experimental Officers in the Materials Analysis Group of the Analytical Sciences Division, A.E.R.E., Harwell.

The Division has new laboratories equipped with a comprehensive range of modern instruments, and staff are given frequent opportunities to improve and widen their professional knowledge and are encouraged to publish original work. There are vacancies in two sections.

In the Spectrographic Section successful candidates will use the techniques of emission and atomic absorption spectroscopy to investigate the analytical problems arising from the study of a wide range of materials. They will also be required to assist scientists in the design and development of new spectroscopic instruments and methods. Some experience in analytical spectroscopy is desirable.

In the Physical Methods Section the successful candidate would work with a small group on instrumentation for chemical analysis. The work includes the development of new instruments and the elaboration of systems for the recording and reduction of data, and for automatic control and programming. Typical systems involve the use of operational amplifiers and logic circuits.

Training and/or experience in electronics is required, and the ideal applicant would also have subsidiary interests in computing and chemistry.

Applicants for Experimental Officer or Assistant Experimental Officer posts should preferably have a degree (or equivalent) but the minimum qualifications are G.C.E. in 5 subjects (including English Language at 'O' level and two scientific or mathematical subjects at 'A' level). The starting salary will be in the range £735-£1,335 (for an Assistant Experimental Officer) or £1,465-£1,860 (for an Experimental Officer), and will be related to age and experience.

Married officers living beyond daily travelling distance may in certain cases be eligible for Authority housing.

Please write for further details and application forms to:

**Personnel Department 'A' (519634),  
Atomic Energy Research Establishment,  
Harwell, Didcot, Berks.**

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## UNIVERSITY OF NOTTINGHAM

### DEPARTMENT OF AGRICULTURE

Applications are invited for an ASSISTANT LECTURESHIP or LECTURESHIP in Crop Agronomy in this Department. The appointment will be effective from November 1, 1967, or as soon as possible thereafter. Salary will be within the range £1,105 to £1,340 (Assistant Lecturer) or £1,470 to £2,630 (Lecturer).

Forms of application and further particulars, returnable not later than July 24, 1967, from the Registrar. (208)

## ST. MARY'S HOSPITAL MEDICAL SCHOOL (UNIVERSITY OF LONDON) PADDINGTON, W.2

Applications are invited for the appointment of

### LECTURER IN PHYSICS

for a period of three years, or TEMPORARY LECTURER for one year from September 1 or October 1, 1967. Consideration might be given to a part-time applicant. Commencing salary within the scale £1,470 to £2,270 to £2,630 plus London Allowance.

Applications (two copies), together with the names of two referees, should be sent by July 29, 1967 to the Secretary, from whom further particulars should first be obtained. (219)

## UNIVERSITY OF WESTERN AUSTRALIA

### SENIOR LECTURESHIP IN ANATOMY

Applications are invited for appointment to a Senior Lectureship in the Department of Anatomy. The salary range for the post is \$A6,500 to \$A7,600 per annum, plus superannuation similar to F.S.S.U.

The Department provides courses for undergraduate and postgraduate students in the Faculties of Science, Medicine and Dental Science. The position would best be filled by an Anatomist having training and an interest in research in the fields of Human Biology and Physical Anthropology. The appointee will be required to develop courses in Human Biology for third-year degree students in the Faculty of Science and would, as the department develops, be responsible for training postgraduate research students in association with the International Biological Programme (Human Adaptability Section). He will be expected to assist in the teaching of Gross Anatomy to Medical students.

The Department is currently undergoing expansion and will be moving into new buildings within two years. Existing research facilities include a laboratory for the study of ultrastructure equipped with a Philips E.M.300 high resolution electron microscope and necessary ancillary equipment. Further information concerning the Department may be obtained from the Head (Professor David Allbrook).

Intending applicants are requested to obtain details of the procedure to be followed in applying for the post and a copy of the appropriate conditions of appointment before submitting their applications. This information is available from the Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1. Applications close in Australia and London on September 2, 1967. (205)

## UNIVERSITY OF GLASGOW RESEARCH ASSISTANTSHIP IN DERMATOLOGY

Applications are invited from science graduates, either in chemistry or in biochemistry, for the post of Research Assistant. The successful applicant would be required to undertake work on the histochemistry of the skin with special reference to steroid synthesis. Salary scale: £1,105 to £1,340 per annum. Initial salary according to experience and qualifications. F.S.S.U.

Applications (three copies) should be lodged, not later than August 11, 1967, with the undersigned, from whom further particulars may be obtained.

ROBT. T. HUTCHESON,  
Secretary of the University Court. (261)

# Parasite Immunity

The Parasitology Research Department of the Therapeutics Research Division of Pfizer Limited is presently engaged in research programmes in the field of Parasite Immunity. Teams of scientists are working on basic and applied aspects of the problems related to animal and human diseases.

Two additional scientists are now needed to complete the teams.

## Immunologist

This appointment calls for a biological or chemical science graduate who has already gained some postgraduate experience in immunology, preferably in the areas of helminth and/or protozoal diseases.

## Parasitologist

This position involves fundamental work on the in vitro culture of helminths. Postgraduate experience is not absolutely necessary and new graduates will be considered together with those already possessing experience in this field or other branches of parasitology.

Both appointments offer the successful applicants excellent long-term career prospects. The starting salaries will be competitive and the fringe benefits generous.

Applications, which will be treated in the strictest confidence, should be addressed to:

**The Personnel Manager,  
Research Division, Pfizer Limited,  
Sandwich, Kent.**

(214)

## FIGHTING THE WRONG BATTLE

THE big guns of Imperial College have not chosen the happiest of moments for their protest at the difficulty of equipping their new laboratories (see page 341), which is not to say that they have nothing to complain about. To be sure, it is a modest misfortune that they now find themselves having to pore over their books of account in search of economies or extended credit much as if they were housewives overcommitted at the grocer's. It is also plain that the University Grants Committee has managed things badly, for the academic community as a whole has been badly informed about the change from one system of providing equipment to another. One problem is that too much protest about present difficulties may obscure the plain truth that the proposed change is in the best interests of the universities as a whole. Whether academic protests will strengthen the hand of the Minister of Education in his fight with the Treasury about allocations for the quinquennium which begins a year from now or, alternatively, persuade him to spend more of his energies on some other part of the educational system is another matter, but there is plenty of evidence that Mr Crosland will not be indefinitely sympathetic to the universities.

On the virtues of the new system which the UGC is seeking to introduce, there is very little argument. Hitherto it has been the practice for universities being endowed with new buildings to be given a capital grant to cover the cost of equipment as well. Traditionally, the UGC has operated this system generously—for several years in the recent past, the capital grant for furniture and equipment has been roughly a third of the cost of new buildings. In practice this has often meant that the newest university laboratories have also been the best equipped. The replacement of equipment in existing buildings has, by contrast, been a charge on the recurrent grant to the universities, and proposals for the replacement of equipment have often been at a disadvantage in the competition with other ways of spending academic money. Everybody will be better off if there is now a more orderly system for the replacement of old equipment and furniture, and if universities are provided each year with a sum of money related to the scale of their academic operations and not to the historical accidents of the building programme. This, indeed, was the view pressed on the UGC by a broadly based committee and endorsed by the Committee of Vice-Chancellors a year ago. Not the least of the benefits of the new system will be the sense of autonomy that universities should derive from it.

Why, then, is there so much irritation in the universities? One of the troubles is that it is a long time to

August 1968, when the new system will begin to operate. But it is also plain that the UGC has failed to explain as fully as it should have done how the new system will affect the individuals most directly concerned—the heads of university departments blessed with new buildings in the past two or three years. Probably the committee has put too much faith in the processes of communication within universities. Telling a vice-chancellor of a proposed change in regulations may seem to the committee to be the same as telling the university, but there is no way of making sure that the message will filter through. It would do no harm if the UGC took the fuss about equipment grants as a proof that better channels of communication within the academic system are an urgent need. It is also possible that the UGC has underestimated the extent to which the new system will alter the balance of power within universities between vice-chancellors and university departments. The old equipment grant was a kind of dowry for the head of a department moving to a new building. The new one will tend to be much more the gift of a vice-chancellor and his committees. Until there is some first-hand experience of how this system will operate, the chances are that heads of departments will be apprehensive.

Universities as a whole are likely to remain uneasy about their relations with the Government at least until the scale of UGC support for the period beginning in 1968 has been defined. The plan is that subventions for the next quinquennium should be defined by the autumn of this year, although it is plain from what the Secretary of State for Education and Science has been saying in public that he will be hard pressed to wring from the Treasury all the funds which he may consider to be necessary. In this connexion, it may be important that the academic year about to start is the first in which the immediate target of the Robbins Report—197,000 university places of all kinds—will be attained. Although there will be some growth of the university system in succeeding years, not least of all because the new universities are half-way through their natural growth, the Government will not be publicly committed to forced expansion after 1968. Left to itself, and with all the pressures of demand from other fields, nobody should be surprised if the British Government seeks to keep university spending on a tighter rein after 1968.

In the circumstances, what academics should really be worrying about is the character of university teaching in the decade ahead. Those who signed the letter to *The Times* are probably right in identifying the cold hand of Government restriction behind their present discontents about equipment, but they have probably



picked too small an issue on which to fight. It is a much more serious threat that the sense of parsimony now settling on the universities may put a stop to developments in university teaching which are essential if British universities are to remain respectable in the years ahead. It is particularly important that the need of more postgraduate teaching, recognized by Robbins, should now be translated into fact. There are plenty of opportunities, expensive in the immediate future but economical in the long run, for collaboration between groups of universities well placed geographically. There is a great and universal need to make experiments with new kinds of teaching equipment. It is vital that universities, or those universities which feel so inclined, should be free to make experiments with undergraduate courses lasting four years coupled with less stringent requirements of students on entry. The most immediate danger is that the coincidence of renewed financial pressure from the Treasury and the attainment of the Robbins goal will bring in a period of stagnation in British universities. To demonstrate that such an outcome would be disastrous is more important than paying bills on time. After all, it is always possible for universities to borrow from the banks.

## BURN MORE COAL

THE Minister of Power is not planning to produce a comprehensive statement of British fuel policy before the autumn, but its broad outlines were plainly to be seen in the long and repetitive debate on the coal industry which spanned Tuesday and Wednesday in the Commons this week. Briefly, Mr Richard Marsh has gone further than most of his predecessors to acknowledge that the decline of the British coal industry, which has been continuous for the past decade, will persist for several decades to come. But, like his predecessors, Mr Marsh has chosen to take the edge off some of the problems which need solving by providing further measures of protection for the coal industry. Against their better economic judgment, the electricity and gas industries are to be asked to burn an extra six million tons of coal a year between now and 1971, and the British Government is to subsidize them for doing so. The fact that the minister is planning to do more than his predecessors to tackle directly the social problems of a declining coal industry will not entirely make palatable this dubious decision.

A plain recognition of what is happening to coal is nevertheless a considerable public asset, even though the trends have been plain ever since the mid-fifties, when the potential economic advantages of nuclear power first became apparent. Then the annual production of coal from British mines amounted to more than 200 million tons in each of several consecutive years, and there were plenty of people willing to burn the coal. Although electricity generating stations were the largest single customer, their collective consumption was hardly more than 12 or 15 per cent of all the

coal produced in Britain. Since then, however, events have moved rapidly to the disadvantage of the coal industry. The most serious and persistent influence has been the high cost of mining coal from British coal seams. The immediate result has been enormously to stimulate the growth of petroleum consumption in Britain. In spite of various measures of discrimination against petroleum which are equivalent in aggregate to a tax of something like 40 per cent, it is an open secret now openly acknowledged by the minister that the electricity generating industry would have preferred to substitute oil for coal at several modern power stations. But coal has also in recent years been able to shelter behind a ban on imports of cheaper coal from overseas and a ready supply of capital from public funds. It is no wonder that Lord Robens has been exuding sweetness and light in the past few weeks, for even he cannot have been entirely sure that yet another brake on the decline of his industry would be provided. The result—or at least the hope—is that by 1971 the annual consumption of coal in Britain will amount to 155 million tons, and that the nationalized secondary fuel industries—electricity and gas—will consume well over half of everything produced.

Protection as such, of course, is not entirely outrageous, and governments are frequently justified in shielding new and potentially important industries from competition which may be too fierce for survival. But the protection of declining industries is more often a snare than an economic benefit—the attempts to keep the British cotton industry on its feet in the decades since the war are a vivid illustration of that. With coal, the evil consequence of too much protection is likely to be an inflated price structure throughout the fuel economy. Ironically, Mr Marsh will probably find this out for himself when he is required in the next few weeks to adjudicate on the price to be paid by the Gas Council for North Sea gas. His freedom to fight hard for a low price will be compromised, to say the least of it, by his readiness to support the domestic market at too high a level. At the same time he may find that he has artificially unbalanced the development of nuclear reactors in the critical years immediately ahead. The British nuclear power industry—which does not deserve protection even though the economic case for it is stronger than with coal—now needs a steady flow of orders on which to cut its teeth and firm incentives to keep costs low enough to be able to compete successfully abroad. By asking the power stations to burn more coal than they want to, the Government has probably done more harm than good.

It is entirely possible, of course, that further measures of protection are not a kindness for the communities which depend on coal, but merely a means of providing them with a false sense of security. For what is to happen after 1970? Will the British Government then be prepared to maintain an artificial level of demand for coal? And if not, will there be a sudden contraction of the industry? In the early seventies

it is entirely possible that the natural demand for coal could be more like 100 million tons a year than the 155 million tons now planned for. The traditional customers are melting away. In a few years there will be a demand for 70 million tons or so of coal each year from power stations now working or being built, a demand for 20 million tons or so of coking coal each year for making steel, and the rump of that traditional but anachronistic market for coal to be burnt on open hearths in people's houses. But who else will want to burn coal? And who else will be able to afford to do so? In all the circumstances, it might have been safer and in the long run kinder to the men concerned if the Government had sought to anticipate the changes which are now well under way and not to delay them. It would have made more sense to aim at a contraction from the present rate of production of 164 million tons a year to something more like 140 million tons a year or even less by the end of the decade, and then to have done whatever may be necessary to see that people and communities robbed of work are given other things to do.

## UNLUCKY STRIKE

COLUMBIA UNIVERSITY seems to have made a serious error of judgment in its decision to undertake the management of a new cigarette filter. Even if the filter turns out to be as effective in removing tar and other condensates from cigarette smoke as its inventor, Dr Robert Strickman, claims, the university will have some tedious explanation to provide. It is therefore important to be quite clear that nobody objects—or should object—to the university making money. The independent universities in the United States are respected as well as envied by similar institutions elsewhere. The way in which they are able to live by their own laws must naturally seem a great opportunity to others less favourably placed. The fact that they have chosen to devote so much of their resources to creative scholarship is a striking proof of their high-mindedness. In the last resort, their freedom is based on their financial independence. Yet there are some ways in which universities, however great, cannot live by the simple rules which govern commercial enterprises. There are some kinds of money which they should not make, and there are some ways of making money which they should avoid. Some of this seems to have been forgotten in all the ballyhoo attending the launching of the new filter on July 13.

In the first place, it is entirely mystifying that the university should have taken such trouble to make a great occasion out of its public announcement that Dr Strickman had been generous enough to transfer to the university most (though not all) of his rights in the new filter. Columbia knows enough about the ways in which newspapers work to know that it is almost impossible to hint that an important announcement on smoking and health is on the way without whipping up excitement. In the event, trading in

tobacco shares on the New York Stock Exchange was stopped between midday and the point later in the afternoon at which stockbrokers could inform themselves about the news which Columbia had to broadcast.

And that, of course, was a bitter disappointment. As yet, there is only the most sketchy information about what the filter may accomplish. At Columbia on July 13, both the President, Dr Houston Kirk, and Dr Strickman steadfastly declined to say what the new filter is made of, or how its supposed effectiveness has been demonstrated. With so little to say, it is unlikely that an ordinary commercial company would have been able to create quite such a sensation. Indeed, announcements of improved filters are frequently to be heard. When the New York Stock Exchange stopped trading in tobacco, that was as much a measure of respect for the university as a proof of the statement that the new filter "is 70 per cent more effective" in removing tar from tobacco than other filters now on the market. And in any case, of course, whatever tests there may have been in Dr Strickman's laboratory, it is exceedingly improbable that anybody has had time to carry out clinical tests of the extent, if any, to which the new filter can diminish the incidence of lung cancer.

But should a great university rely on income from tobacco smoking? This is one of the questions now to be asked. Perhaps the first, though the most trivial, thing to say is that the Chancellor of the Exchequer in Britain would immediately be lost if he were deprived of the revenues from tobacco taxes. There will also be echoes of the way in which the Church of England was frequently criticized, before and after the war, for collecting ground rents on houses in London which were known to be used as brothels. The truth is that in a competitive economy it is exceedingly difficult to demand that respected institutions should exercise restraint about investment which others are entitled to ignore, and for practical purposes, of course, few people would object if Columbia were now seeking to make a fortune out of speculation in tobacco stocks. It has exposed itself to criticism only because it has set up what could easily become an intolerable conflict of interest. People will now be tempted to suppose that Columbia in its wisdom has decided that the probable connexion between smoking and lung cancer is illusory. At the press conference last week, Dr Kirk seems to have acknowledged, ineffectually perhaps, that his announcement was not to be taken as a pronouncement that smoking, after all, is safe. He and his colleagues will have to go much further than this if they are to remove the false impression which will have been created by the link between Columbia and the filter—and if they are to conform with the tough new spirit of the regulations of the Federal Trade Commission on tobacco advertising. The trouble, of course, is that if Columbia now embarks, as it should, on a campaign to inform tobacco smokers that there is no evidence that filters bring immunity from lung cancer, it will be undermining the chances of commercial success for its new enterprise.

## NEWS AND VIEWS

### One Step Up

At the European Space Conference last week in Rome, it was unanimously agreed by the 18 countries present that the conference should become a permanent body with national representation at ministerial level and that it should meet "at least once a year". In future it is to have executive power as well as an advisory and co-ordinating function—its unanimous decisions will be binding on all member nations. Any nation belonging to one or all of the three institutions ELDO, ESRO and CETS (Conference for European Telecommunications by Satellite) becomes a member of the conference. It was accepted at Rome that it is "necessary and urgent" for Europe to be given a co-ordinated space policy but that "amalgamation of existing space bodies was not yet practicable". Instead, there is to be an advisory committee on programmes, under a French chairman, to "frame proposals for the establishment of a European space policy" with special reference to scientific and technical research projects and "useful" space activities, and consideration of the launchers required to carry them out. The committee will be chiefly concerned with communications satellites and "a meaningful scientific programme concentrated on activities few in number but opening up new prospects in the research area"—a form of words intended to mean projects which can stand comparison with American experiments in one way or another.

To balance the possible enthusiasm of the scientists and technologists, there is to be a second sub-committee to assess the viability of all proposals for the European economy as a whole. This committee has been asked to bear in mind that Europe has limited resources not only of money but also of trained manpower, and that a balance must be struck between the demands of a space programme and those of other more immediately profitable technologies, and that programmes should accommodate the real interests to consumers (particularly relevant for the communications satellite proposals).

The British delegation, led by Mr John Stonehouse, Minister of State at the Ministry of Technology, considered this machinery for economic screening of great value, but its immediate effect has been to postpone for perhaps nearly a year any decision on the European communications satellite project which has been the subject of a design study by ESRO on behalf of CETS. The project has now been hanging about for two years. ESRO has been voted a further £1 million to keep the communications satellite study team together until the end of the year. (The original contract placed last autumn was worth about £100,000.)

Another key project which has been waiting for a decision and must now undergo further screening is the Large Astronomical Satellite for which the Culham Laboratory was awarded the contract for the scientific equipment last August. Apart from administrative decisions, the project has been caught in the general

budgetary freeze which has afflicted ESRO this year because of the failure of member countries to agree to the organization's budget for its second three year period (1967–70). Last week's meeting has got ESRO temporarily moving again by releasing on British initiative £10 million of money underspent against the first budget for immediate use.

### Space Jamboree

IF all scientific conferences were as over-subscribed as the joint meeting between IQSY (International Quiet Sun Year) and COSPAR (Committee for Space Research) which is going on in London this week, the hotel business would have little to complain about. Instead of the expected 650 delegates, 1,000 turned up, forcing the organizers to change the venue from the Department of Mechanical Engineering at Imperial College to the Royal Geographical Society. The Royal Society, which is organizing the meeting, seems to have been left rather breathless by the task of catering for so many, but the slight air of disorganization at least adds more excitement and urgency to the meeting.

At the opening ceremony, there were plaudits for international scientific co-operation from Professor P. M. S. Blackett, Mr Anthony Crosland and (in a message read by Dr A. H. Abdel Ghani) the Secretary-General of the United Nations, U. Thant. Only Professor W. J. G. Beynon, President of the IQSY Committee, gave any hint how such co-operation might be prolonged now that the IGY and the IQSY are things of the past. There was, he said, a closely knit fraternity of geophysicists, who had been able to forget disciplinary and international differences, and at the very least they should try to maintain the central administrative machinery which had been developed. Perhaps, he suggested, this could be done through the new Commission for Solar Terrestrial Physics.

The meeting is really two meetings. The first, the IQSY meeting, from July 15 to 22, was concerned with an initial broad assessment of the IQSY. The second meeting, from July 24 to 29, the COSPAR meeting, will cover an enormous range of space research, including a symposium on sterilization techniques, and sessions on tracking, telemetry and dynamics of satellites, collection and detection of interplanetary dust, reviews of upper atmosphere structure and variations, and a seminar on stratospheric circulation.

### Sponsors Wanted

At least three large radio-astronomy projects are at present exercising the judicial capacities of the National Science Foundation in the United States. Each has asked the NSF for support, and it is most unlikely that there will be enough money to support all three.

Thirteen universities in New England and New York State have grouped together to form the Northeast Radio Observatory Corporation, a non-profit organization. They want to build a very large dish, 440 ft. in diameter, covered by a space-frame radome. The radome would need to be 550 to 600 ft. in diameter, and it is designed to be built of fibreglass sheets 40 thousands of an inch thick inside a steel framework. The blockage of the radio signal which the framework

will introduce will be equivalent to a reduction in the size of the dish from 440 to 400 ft., but it will offer a new freedom in design. The dish inside will be free from the effect of the wind, and therefore will need to be designed to withstand only gravitational forces. It can be built of very light alloy, and the distortion of the structure in different positions can be taken up by hydraulic jacks. It should be possible to operate the telescope at wavelengths down to 5 cm. The instrument will be mounted on a turntable, and carried on a horizontal beam with a row of bearings. The cost of the project—called Camroc—will be about \$25 million, and Dr Jerome Wiesner, Provost of MIT, has been elected chairman of the corporation which hopes to see it built.

In the West, there is another proposal for a large dish, this time 328 ft. in diameter. The project is supported by the University of Michigan, California Institute of Technology, Stanford University, and the University of California. Caltech has responsibility for the design, construction and ultimate operation of the instrument, which would be built at the Owen's Valley Observatory. The cost would be \$17.8 million, and again the NSF has been asked for support.

Meanwhile at the National Radio Astronomy Observatory at Greenbank there is a proposal to build a large array consisting of a series of smaller dishes. This project would be the most expensive, about \$50 million, but would cost a good deal less than an ambitious plan for a very large dish which was in the air at Greenbank some years ago.

## Fuss about Equipment

THE fuss over equipment grants for British universities has broken out again. When the row first surfaced in May this year, bursars complained that the transition to a new system for equipment grants had left their science departments so short of money that students would have to be turned away. At the time, the University Grants Committee explained (*Nature*, 214, 756; 1967) that the difficulty had been caused by the transition to the new system, and would soon resolve itself. Some university departments are now beginning to see much more discreditable motives behind the decisions. In a letter to *The Times* on July 17, Sir Cyril Hinshelwood and some of his colleagues from Imperial College claimed that the UGC had dishonoured firm pledges and gone back on financial commitments made in the past. Sir Cyril also saw a conscious decision to limit expenditure on equipment grants during 1967.

Ironically, there seems to be no doubt that the UGC has in fact provided more money for equipment this year than in 1966-67. The figures are £22.5 million for 1967-68, against £21.5 million in 1966-67 and £17 million in 1965-66. In any normal year, this would have been enough, but this year is the last before the new quinquennium. Grants awarded at the beginning of the quinquennium for equipment have in many cases not been entirely taken up by the universities; in the past, the UGC has found that it often takes universities 6 or 7 years to draw the whole of the grant. On April 1, 1967, this unspent balance held by the UGC amounted to £38 million, to which universities felt they were entitled. To this extent, the £22.5 million which the UGC coaxed from the Treasury does represent a

cut, of £15.5 million. The UGC says that this money will not be lost, but will go into the kitty when the new system starts to operate. The universities, on the other hand, may fear that if they do not get it out soon they will not get it at all.

The UGC claims to have foreseen the problem early in 1966 and to have discussed it with the Vice-Chancellors. The problem was again explained to the Vice-Chancellors in September 1966, and was the subject of a letter from Sir John Wolfenden to the universities in December 1966. In this letter, Sir John warned that it might be necessary to control the rate of drawings. In order to control the situation, the UGC decided to distribute the £22.5 million in two stages; £20 million in the first stage, leaving £2.5 million in reserve for universities which felt deprived. Universities were asked to make supplementary claims for this £2.5 million by June 31; when the claims came in, the UGC was alarmed to see that they amounted not to £2.5 million, but to £21.5 million. Clearly the reserve could not hope to keep the universities happy.

## Look, No Hands

IN the engineering industry it is estimated that about 40 per cent of the production time consists of putting together finished components to produce the final article for sale. In the motor industry, the figure is even higher—60 per cent on assembly, and only 40 per cent on metal working. Faced with these figures, it is perhaps surprising that there has so far been no coherent attempt to automate the assembly process, cutting costs and releasing men for other jobs.

The Ministry of Technology is now trying to catch up with lost time. The minister, Mr Wedgwood Benn, announced on July 18 three projects for the development of automatic assembly. The first is a development contract with Staveley Industries to which the ministry will lend £200,000 over three years for the design and development of units which can be used as a basis for producing automatic assembly machines. Staveley will not be attempting to produce complete machines—the intention is to develop modular units which can be fitted together in different ways to do different jobs. A simple machine might be made up of a bowl feeder which selects one part from storage, an escapement mechanism which offers the part up in correct alignment with other parts, and a screw driving head which connects the two parts together. No human intervention will be necessary.

The ministry is also thinking of providing financial support for the Institution of Production Engineers, which is to produce what are called production data memoranda. These will supply engineers with the critical data needed to make use of automatic assembly, and will also include the availability of equipment and the economics of application. The ministry intends to support work in universities, starting with the Department of Production Engineering at the University of Nottingham. Professor Heginbotham and his team will be investigating the methods used for packaging parts, and teachable machines. These are machines which can be taught to carry out assembly functions by being first taken through the job by an operator and storing the information in a memory.



They promise to be particularly flexible and thus suitable for small batch production.

## Brain Drain, Both Ways

THE Meteorological Office announced with considerable pleasure this week that Professor Raymond Hide, professor of geophysics and physics at the Massachusetts Institute of Technology, will be joining it in September of this year. Professor Hide graduated from the University of Manchester in 1950, took his doctorate at Cambridge in 1953, and became a lecturer at the University of Newcastle. He was appointed a full professor at MIT in 1961 at the early age of 32, and is well known for his work on fluid motions in the atmosphere, the Earth's core and the atmosphere of Jupiter. A special senior post has been established in the Meteorological Office for Professor Hide, who will be in charge of a new laboratory of geophysical fluid dynamics.

Dr John Napier of the University of London is going the other way across the Atlantic. He has been appointed by the Smithsonian Institution in Washington to investigate the possibility of establishing an International Center for the Study of Primate Animals. Dr Napier, a leading authority on primate biology, will be trying to plan programmes for the best training of primate biologists on both sides of the Atlantic, and will also be studying primate classification, morphology, anatomy, genetics, palaeontology, ecology and behaviour.

## Who Does What

THE Department of Education and Science and the British Council have produced their guide to *Scientific Research in British Universities and Colleges* (HMSO, Vol. I, Physical Sciences, £2; Vol. II, Biological Sciences, £2; Vol. III, Social Sciences, £1 12s. 6d.) at the end of the academic year to which it relates. Although the great value of this publication may be somewhat tarnished by the fact that roughly 10 per cent of those listed in it may have moved to universities other than those to which they are said to be affiliated, or may have changed their research projects, it remains a considerable achievement that this compendium should be published at all. This year, the volumes are somewhat more free from error than is usually the case. The subject index remains wayward, with some coarsely defined categories and some defined in great detail. The character of descriptions of research projects varies enormously from one university to another—some spell out what individual members of a department do and some still include them all under one great umbrella.

The social sciences volume breaks new ground by including among the institutions with which research projects are identified organizations which are not strictly universities at all—the London Transport Board, for example, the Central Office of Information and the Shirley Institute. Apparently the publishers would be prepared to include in this volume details of work carried out in industrial establishments. The future of the other compilations will depend on a survey being carried out by the Office of Scientific and Technical Information. Potential users in universities and government departments have been asked to

specify the kinds of enquiries they would like to see made. One possibility is that a magnetic tape index to the volumes will be produced, and that the work of government establishments may be lumped in with that of universities. There seems as yet no prospect that industrial research in the full-blooded sense will be included.

## More Plans for Food

ANOTHER attempt to provide policies for feeding the world population has been made, this time by a committee which advises the Economic and Social Council of the United Nations. The Advisory Committee on the Application of Science and Technology to Development, under whose aegis the report is published, has been advised by a series of sub-committees, expert panels, and working parties, but it seems that responsibility for most of the recommendations rests with a small group of three: Dr W. F. J. Cuthbertson (UK), Dr D. S. Bhatia (India) and Dr A. A. Pokrovsky (USSR).

The committee has concentrated on one aspect of the problem, increasing the production and use of edible protein. Unfortunately it has come up with some rather well worn suggestions. Protein from conventional sources should be increased, using modern technology, and should be supported by better use of marine and inland fisheries. The committee also sees a role for unconventional sources of protein; soya, peanuts, and textured vegetable products are all given a favourable mention. Fish protein concentrate (fish meal) can also contribute, the committee feels, and it recommends greatly intensified research on single cell protein sources, which can be produced without using agricultural land. There are also recommendations about research, training and marketing.

The report will be debated by the Economic and Social Council at its meeting this month, which has in the past been a signal for similar reports to disappear without trace. This time the committee has made a worthwhile attempt to cost its proposals, and some of them are surprisingly modest—\$5 million annually to prevent waste, for example, or \$5 to \$8 million a year over the next five years to set up regional centres for research and training in agricultural technology and food science. One problem which the report ignores—in common with almost all reports of this kind—is the effect of the price structure on food production. Until producers, distributors and retailers can go ahead with full confidence of making profits from their work, nothing is likely to come of the plans. This, perhaps, was what President Johnson's Science Advisory Committee was hinting at in its report on food production (*Nature*, 215, 234; 1967) when it said that few countries were making full use of the power of the market economy.

## Rockefeller against Hunger

THE Rockefeller Foundation remains dedicated to the problems of food and population in developing countries. According to Mr J. G. Harrar, the president, in his review of 1966, out of a total spending of \$31.9 million on the foundation's five principal areas of interest \$8.6 million was spent on the programme called 'Towards the Conquest of Hunger' and \$3.8

million on 'Problems of Population'. The foundation also spent \$7.8 million on university development, \$3.3 million on 'cultural development' and \$4.9 million on 'equal opportunity for all'.

Mr Harrar points out in his review that all efforts to provide food and other material requirements will fail unless the rate of world population increase can be significantly reduced. The Rockefeller Foundation adopted the 'Conquest of Hunger' as one of its principal objectives in 1963. Important grants in this area during 1966 included one of \$533,000 for five years to the University of Nebraska Foundation for research on the physiology of sorghum yield and sorghum management, and another of \$100,000 for a three year period to the US National Academy of Sciences, for a multidisciplinary research and training programme, administered by the Africa Science Board's Subcommittee on the Development of Water Resources.

Much of the foundation's effort in the problems of population has gone into demography. Among the grants awarded were \$350,000 for a two year period to the Population Council, New York, for an international study of the effectiveness of family planning measures undertaken in the postpartum period, and \$400,000 for a three year period to the University of North Carolina for the preparation of educational material on population and the development of a computerized information retrieval service.

## Teaching Abroad

AN exhibition to introduce teachers in developing countries to recent advances in British science teaching has been organized by the British Council under the title of "New Approaches to Science Teaching". The emphasis of the exhibition is on teaching pupils to discover for themselves and thus to learn by understanding. With such an emphasis it is hardly surprising that the displays lean heavily on the Nuffield Science Teaching Project and the Scottish Education Department for their material. The exhibition covers the teaching of science in junior and secondary schools, and includes records and films. The organizers hope that the exhibition will encourage teachers in developing countries to evolve their own projects using local materials; from this point of view it may be a handicap that the contents of many of the displays are so western in character, from the examination question about tube trains to the projects on English insects. Nevertheless this exhibition, which goes first to Nigeria, will doubtless do a lot to advance science teaching in underdeveloped countries.

## Museum for Stargazers

THE £84,000 spent by the Ministry of Public Building and Works on the restoration of the Meridian Building at the Old Royal Observatory, Greenwich, is money well spent. The building has been restored to all its former glory, with the rooms and instruments reinstated, as far as possible, as they were used. The building, part of the National Maritime Museum, was opened to the public last Wednesday (July 19) by Sir Richard Woolley, the Astronomer Royal. Its history of nearly 300 years in the forefront of astronomical and navigational discovery is well presented for all types of visitor, from the package-tour rusher to the

serious student. It contains Airy's transit circle room from which the Prime Meridian is defined. Some visitors will be a little startled to find that there are, in fact, three meridians: Flamsteed's of 1681, Bradley's of 1750 and Airy's of 1850. Most of the instruments shown are the original ones used by the various Astronomers Royal. They include Bradley's 8 ft. Mural Quadrants, Airy's Transit Circle, Halley's Transit and many others, all tributes to the design and workmanship which made accurate observations possible.

## Progress in Welding

THE annual report of the British Welding Research Association for 1966 makes confident reading. The past year, as well as showing a significant growth in membership and research facilities, has brought better grant conditions from the Ministry of Technology. The ministry is now prepared to contribute 150 per cent on subscription income instead of the standard 100 per cent, up to a maximum of £350,000. Income last year was £644,487, an increase of nearly £80,000 over 1965. Expenditure increased by £105,000, to £614,363, and BWRA employs about 150 technical staff, almost half of them graduates.

A large part of the association's income is spent on advisory and liaison services. This includes visits by members of staff to member firms to advise on problems, and the information service run by the association. The most interesting problem studied by the information service during the year was the brittle failure of a pressure vessel while still under test at the manufacturer's works. Another investigation was one carried out for Samuel Fox and Co., Ltd., which showed that a new series of austenitic stainless steels (Hi-proof 316) developed by the company are suitable for pressure vessels, and offer considerable savings in cost and weight.

Much of the work was naturally concerned with welding processes. One of the largest friction welding machines in the world was designed and built during the year. In friction welding, a rotating piece is brought into contact with a stationary piece under pressure. The heat generated causes the two to bond, and the association reports that the technique is now being used industrially for welding turbine wheels to shafts and valve heads to shafts. In the metallurgical laboratories, work has gone on on burning and hot tearing in structural steels, and the avoidance of embrittlement and stress corrosion in welds in high strength aluminium alloys. A considerable amount of work on brittle failure seems to have been carried out, chiefly because brittle fractures are almost invariably initiated from weld defects.

## Preservation by Co-operation

IN an effort to educate gamekeepers and anyone else who goes to the country to shoot predatory animals the Council for Nature, in association with the British Field Sports Society, the Fauna Preservation Society and the Game Research Association, has produced a booklet *Predatory Mammals in Britain*, a code of practice for their management (The Council for Nature, price 5s.). The booklet was prepared by a working party—set up at a conference on mammalian predators in Britain in 1965—whose members represented con-

servationists, sportsmen and gamekeepers. Professor H. R. Hewer, the chairman of the Council for Nature, has described this co-operation as "a triumph of common sense", for the naturalists have agreed that predators must sometimes be killed, and the sportsmen have accepted that they can protect their game without eradicating all the predators.

The booklet contains illustrated notes on recognition, distribution, behaviour and control of twelve British predatory mammals. The need for control is clearly stated—the mink, for example, is potentially very destructive and should be eradicated; the hedgehog can cause serious losses of nests on a partridge beat, but elsewhere there is no need for control; the pine marten is virtually harmless, and although the otter can damage fishing interests it is doubtful whether it does much harm in Great Britain as a whole. The appropriate methods of control are given for each animal; the mole, which is an agricultural pest, proves particularly difficult to exterminate, and the working party has had to recommend poisoning with strychnine hydrochloride, although it had hoped to eliminate this poison from the countryside. Chapters on the law explain the methods of snaring, trapping, gassing and poisoning which can be used, and also the most humane ways of carrying these out. In a chapter prepared by the Universities Federation for Animal Welfare the best ways of killing trapped animals are described.

The working party is hoping to see *Predatory Mammals in Britain* in the pockets of all gamekeepers and sportsmen, perhaps an unduly optimistic target. In any case, these people may prove difficult to persuade. Promotion of the booklet in schools, which is planned, may help to produce a new, educated generation of gamekeepers and sportsmen.

## Radiological Committee

THE British Committee on Radiological Units has been reconstituted under the title of the British Committee on Radiation Units and Measurements by the Radioactive Substances Advisory Committee. It will be chaired by Professor F. W. Spiers, professor of medical physics at the University of Leeds and honorary director of the MRC Environmental Radiation Research Unit, and based at the National Physical Laboratory.

The committee consists of experts, serving in a personal capacity, who are actively interested in the quantitative use of radiation and radioactive substances in industry, science and medicine. It will act in an advisory capacity to government departments and other bodies, interpret ICRU recommendations, make recommendations to the ICRU and other bodies, and also set up forums and working parties for specific purposes.

## Design for Astronomy

THE Optical Group of the Institute of Physics and the Physical Society and the Royal Astronomical Society picked the right time to hold a conference on Astronomical Optics, at Imperial College last week. With astronomical telescopes promised at Kitt Peak in Arizona, Cerro Tololo in Chile, and in Australia, the next few years are bound to be full of interest in telescope design. The conference covered a wide field, some of it directly concerned with the new instruments.

One preoccupation is the possibility of replacing photographic recording by photoelectric methods. Among advantages this would confer are a higher quantum efficiency, a wider range of wavelengths covered and a linear response, as Professor J. D. McGee from the Department of Physics at Imperial College explained. The snag is that the equipment is heavier and more complex than the simple photographic plate. Professor McGee suggested four possible systems: television cameras, solid state detectors, electronographic tubes and image intensifier tubes, and went on to discuss his own speciality, cascade tubes with thin mica windows. Professor M. A. Lallemand from the Paris Observatory was hopeful that the electronic camera could achieve sensitivities ten to a hundred times greater than photographic emulsion, and offered more reliable and precise measurement. Dr M. J. Smyth from the Royal Observatory at Edinburgh described the working of the twin 16 inch photoelectric telescopes at the observatory. One telescope continuously monitors light from a reference star, while the second measures actual stars of interest within a  $10^\circ$  diameter field. In this way the reference beam can be used to compensate for changes of atmospheric transparency, an inherent limitation for ground based observations.

For the new large telescope projects, the Richey-Chretien optical system which gives an aplanatic secondary focus is being adopted in preference to the Newton-Cassegrain layout. Dr C. G. Wynne of Imperial College discussed what the change involves and described his own development of extended field systems which will be adopted on the new telescopes. Dr E. H. Richardson of the Dominion Astrophysical Observatory in Canada described the design of spectrographs for the Queen Elizabeth II 156 inch instrument proposed in Mount Kobau, British Columbia. Dr E. H. Linfoot of the observatories at the University of Cambridge discussed coma tolerances for photographic star images. Coma is an aberration produced when oblique rays falling on the same zone of the mirror are not brought to coincidence in the focal plane; the two mirror Richey-Chretien combination avoids comatic aberration, but suffers from astigmatism. Because of the non-linearity of photographic response, coma tends to shift the mean centre of the image of stars by a distance depending on the stellar magnitude, giving rise to systematic errors in the determination of star positions. Dr Linfoot, with Professor R. O. Redman, has attacked the problem of tolerances with a computer, and explained how his results affect telescope design.

There were several contributions concerned with astronomy from rockets or satellites. Mr R. H. Christie of AWRE, Aldermaston, has designed a system for an all-reflecting satellite telescope in which stability of image is maintained by rotation of the secondary mirror, controlled by a signal derived by a sensor placed near one image point. The advantage is that only a small mass need be moved to maintain alignment, instead of the entire spacecraft, and the telescope can be made independent of the guidance electronics of the spacecraft. Several speakers described spectrographs for rocket-borne studies, and Mr C. T. Farr from RAE, Farnborough, described a small photometer to be carried in a rocket. On the last day, Dr R. Q. Twiss of the National Physical Laboratory gave an

account of the stellar intensity interferometer at Narrabris, Australia, with which the angular diameter, and hence the radiation temperature of hot bright stars can be measured. Dr P. J. Trianor from the Vatican Observatory at Castel Gondolfo showed how he had measured the angle of polarization of stars. His method enables the statistical distribution of angles of polarization to be measured from one exposure.

## Four Classes of Microtubules

from our Correspondent in Cell Biology

ONE of the problems that continually faces electron microscopists is deciding whether organelles with the same fine structure have identical chemical composition and cellular function. Usually, the more complex the structure of the organelles, the easier it becomes to make this decision. Few would question, for example, that mitochondria and chloroplasts seen in different cells perform the same cellular functions. But does this apply to structurally simple organelles such as microtubules? Are they identical? According to Behnke and Forer (*J. Cell Sci.*, **2**, 169; 1967) there are at least four classes of microtubules.

Microtubules, simple cylindrical structures a few hundred angstroms in diameter and up to several microns long, are thought to be a virtually ubiquitous cell organelle. They occur in specifically arranged aggregates in cilia, flagella, sperm tails and the mitotic spindle, but also occur free in the cytoplasm without any obvious pattern of arrangement. The latter are known simply as cytoplasmic microtubules. Their function remains a mystery although various pieces of circumstantial evidence have led to a host of more or less likely suggestions: involvement in cell motility, cytoplasmic streaming, skeletal support, cellular plumbing, and the like. Surprisingly, however, despite all these proposed functions, it has generally been held that all microtubules are the same.

Now Behnke and Forer report that treatment of crane fly spermatids, rat sperm and rat tracheal cells with pepsin, colchicine, or storage at 0°C or 50°C clearly differentiates four classes of microtubules. In crane fly spermatids the cytoplasmic microtubules and those in the 9+2 arrangement, the central pair and each tubule of the nine outer doublets of their sperm tails respond differently to these treatments. Behnke and Forer conclude that these four classes have intrinsic differences in their chemical and/or physical composition. Furthermore the same four types occur in rat sperm and rat tracheal cells.

When examined after negative staining these four types of tubules have identical substructure. It appears that despite other differences, microtubules are in general constructed to a basically similar pattern, and Behnke and Zelder (*J. Ultrastruc. Res.*, **19**, 147; 1967) present more evidence of this. They have examined the substructure of the microtubules in mammalian blood platelets, which, like those in many other cell types, are labile to osmium tetroxide fixation but are preserved by glutaraldehyde fixation. They find the structure of these G-tubules is very similar to that of O-tubules, microtubules fixed by both osmium and glutaraldehyde. The wall of both types is constructed of about twelve fine filaments each about 35–40 Å in diameter and arranged in a cylinder with a 60 Å centre to centre distance.

## Parliament in Britain

### University Computers

THE Secretary of State for Education and Science, Mr A. Crosland, stated that purchase of computers for universities to the total value of £6.5 million had been approved so far against the programme outlined by the Flowers Committee, which was being kept under review by the Computer Board. Further major provision was under consideration by the board. It was still expected that expenditure in the first three years would average about £3 million a year when account was taken of building and operating costs. Expenditure by the research councils on "off line" computers totalled nearly £2 million in 1966–67 and was expected to be £1.5 million in 1967–68. (Written answer, July 12.)

### Teachers

REPLYING for the Government in a short debate in the House of Commons on July 10 on regulations for the training of teachers, the Minister of State, Department of Education and Science, Mr G. Roberts, said that full-time staff in the colleges now numbered 7,900. He agreed on the need to attract more graduate teachers in mathematics and science. The target was now 110,000 teacher training places, outside the universities, in England and Wales in 1973–74 and 100,000 by the end of this decade, the latter figure corresponding with an annual intake of 35,000 at a cost of £68 million in 1966–67. Since 1962–63 the number of new entrants had increased from about 5,000 to 9,500 but was still only about 28 per cent of all entrants, instead of the desired 35 per cent. The number of entrants aged 25 and more trebled between 1962–63 and 1966–67, when it reached 6,100. The proportion of those trained who left teaching was probably well below 3 per cent. On consultative machinery a wide range of views had been expressed and it would not be easy to reach a conclusion which would be universally acceptable. The Weaver report had been generally welcomed, and colleges of education had been invited to prepare schemes of government and provided with models from which they could work. Schemes were coming forward, but it was not intended to seek to impress any rigid uniformity in the articles of government. A modern scheme of government for voluntary colleges had also been agreed with the voluntary bodies and circulated to all colleges, and they were now submitting their proposals for new schemes of government. Mr Roberts hoped both types of college would emerge with the fullest possible academic and student autonomy appropriate to their nature. (House of Commons, July 10.)

### Steelworks

MR ARTHUR BOTTOMLEY, Minister of Overseas Development, gave details of the aid provided by the British Government for the development of the Durgapur Steelworks in India. Loans totalling £68 million had been offered since 1957, he said, and £60 million of this had so far been taken up. The expansion of the steelworks from 1.6 to 3.4 million tons had been postponed because the project was too expensive for the Indian Government, and the growth of the steel market in India had been slower than anticipated. Before expanding, the Indian Government wanted to attain full production at the existing capacity, he added. (Written answer, July 12.)



# Organochlorine Pesticides in Antarctica

by

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The first reports of organochlorine pesticides in wildlife in the vicinity of the US Antarctic base at McMurdo Sound suggested that local human activity might be responsible. Measurements of samples from the British Antarctic Survey Base at Signy Island in the South Orkneys now suggest that there is a world-wide pattern of distribution by means of the oceans and the atmosphere.

CONSIDERABLE interest was aroused in 1965 by news from the United States that DDT had been discovered in Antarctic wildlife. Details of this finding were given later in two papers. The first of these by Sladen *et al.*<sup>1</sup> described the detection of DDT and two of its toxic metabolites, DDE and TDE, in six Adèle penguins (*Pygoscelis adeliae*) and one crab-eater seal (*Lobodon carcinophagus*). All these specimens were taken at Cape Crozier on Ross Island (Fig. 1). The second paper by George and Frear<sup>2</sup> described a more ambitious study of samples taken on Ross Island and in the nearby McMurdo area. DDT only was detected in four out of sixteen Weddell seals (*Leptonychotes weddelli*) and four out of sixteen Adèle penguins. Of sixteen skuas (*Catharacta skua maccormicki*) examined, nearly all contained DDT and DDE. No pesticides were detected in water and snow samples, nor in samples of four phyla of marine invertebrates and an Emperor penguin (*Aptenodytes forsteri*). Of three species of fish taken in the Ross Sea, only one specimen of *Rhigophila dearborni* contained DDT but no DDE.

These discoveries suggested that the contamination of the environment by the new persistent organochlorine pesticides had spread to what is usually regarded as the most remote and isolated part of the Earth. The nearest land mass to Antarctica is Cape Horn in South America (Fig. 1), about 1,000 km distant, and most other land masses are at least 2,000 km away. The character of Antarctica is such that the only human residents on the continent are explorers and research teams, but the traffic of men and supplies brings visiting airmen and sailors to the region. Although the number of men on the continent has grown in recent years<sup>3</sup> and at times there may be as many as 4,000, the population is still minute relative to the land mass of about 13 million sq. km. There are no insect pests in the Antarctic and therefore no need for

insecticides. Moreover, there is a general international agreement, under the Antarctic Treaty of 1959, that Antarctica shall remain a faunal and floral preserve and that the existing ecosystems shall not be disturbed by, for example, the introduction of alien species or such powerful influences as pesticides. The detection of DDT in Antarctic wildlife therefore raised immediately the problem of its origin.

Both of the above reports referred to samples taken in the area served by the large United States base at McMurdo and it is perhaps significant that only DDT, and its first two metabolites DDE and TDE, were detected. It is now well known that the air and coastal waters of, for example, the United States and the British Isles contain traces of organochlorine pesticides<sup>4-9</sup> so that pesticides could have reached Antarctica by way of these two media. Air, rain and coastal waters, however, usually show traces not only of DDT and its metabolites but also of other organochlorine pesticides in common use, such as BHC and dieldrin. None of these other pesticides was detected in the samples from the McMurdo area. Therefore, there was always the possibility that no matter how vigilant the authorities concerned may have been, DDT had reached McMurdo simply because man himself had transported it there in his ships, food, clothing or stores.

If this latter supposition were correct, then other areas of Antarctica outside the environment of McMurdo might still be free from contamination. It was considered that this could be investigated by sampling in the area of the British Antarctic Survey Base at Signy Island in the South Orkney Islands (Fig. 1) some 4,500 km from the American base at McMurdo. If no DDT were detected there, then it would suggest that the contamination in the McMurdo area was directly due to local human activities. If DDT were found on Signy Island then again it might be

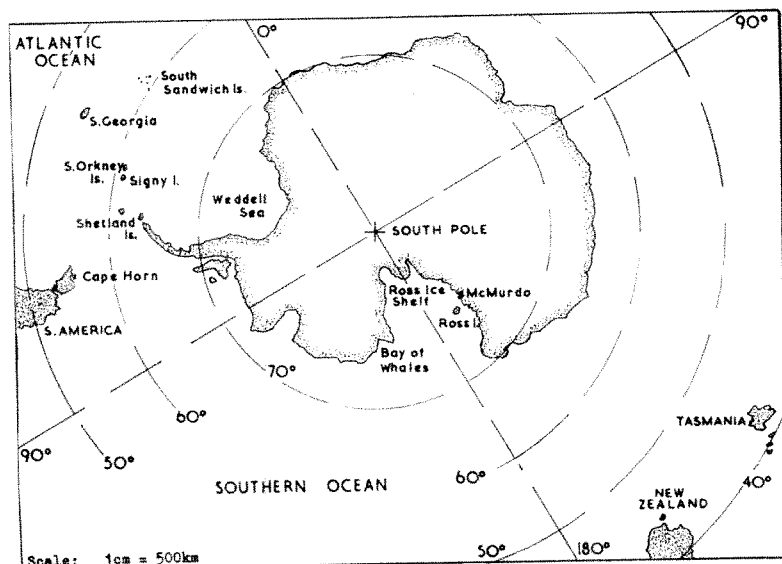


Fig. 1. Antarctica.

Table 1. ORGANOCHLORINE PESTICIDE RESIDUES IN ANTARCTIC WILDLIFE (p.p.m.)

	No. of samples	Alpha-BHC	Beta-BHC	Gamma-BHC	Heptachlor epoxide	Dieldrin	<i>pp'</i> -DDE	<i>pp'</i> -TDE	<i>pp'</i> -DDT
Penguin liver	11	0.000-0.002 Mean 0.001	0.002-0.008 Mean 0.005	0.000-0.003 Mean 0.002	0.000-0.006 Mean 0.002	0.001-0.006 Mean 0.002	0.001-0.018 Mean 0.006	ND	0.001-0.010 Mean 0.005
Penguin blubber	10	0.001-0.002 Mean 0.002	0.000-0.006 Mean 0.003	0.001-0.002 Mean 0.001	0.001-0.003 Mean 0.002	0.004-0.010 Mean 0.007	0.013-0.048 Mean 0.032	0.000-0.006 Mean 0.003	0.005-0.012 Mean 0.008
Penguin abdominal fat	5	0.001-0.002 Mean 0.001	0.000-0.004 Mean 0.001	0.000-0.002 Mean 0.001	0.001-0.002 Mean 0.002	0.006-0.010 Mean 0.008	0.029-0.048 Mean 0.039	0.000-0.004 Mean 0.001	0.006-0.011 Mean 0.008
Penguin stomach contents	2	0.001, 0.001	0.005, ND	0.001, 0.001	ND, ND	0.001, 0.001	0.001, 0.001	ND, ND	0.001, 0.001
Penguin eggs	3	0.003-0.005 Mean 0.004	ND	0.003-0.005 Mean 0.004	ND	0.003-0.008 Mean 0.005	0.014-0.032 Mean 0.021	0.003-0.005 Mean 0.004	0.005-0.012 Mean 0.008
Notothenia (fish)	4	0.001-0.007 Mean 0.003	0.002-0.008 Mean 0.005	0.001-0.004 Mean 0.003	0.002-0.004 Mean 0.003	0.001-0.009 Mean 0.003	0.002-0.013 Mean 0.007	0.000-0.018 Mean 0.007	0.006-0.020 Mean 0.011
Brown skua liver	2	ND	ND	ND	0.100, 0.035	ND	4.00, 0.890	ND	0.33, 0.23
Brown skua fat	2	0.004, ND	0.040, ND	0.006, ND	0.120, 0.730	ND	5.80, 26.0	ND	0.890, 2.50
Blue-eyed shag liver	2	0.001, 0.002	0.006, 0.003	0.001, 0.003	0.002, ND	0.002, 0.001	0.011, 0.015	ND	0.003, 0.009
Blue-eyed shag fat	2	ND	0.009, 0.010	ND, 0.002	ND	0.004, 0.006	0.051, 0.140	0.009, 0.018	0.012, 0.023

ND, not detected.

attributed to human activities but it would also reinforce the argument that there was general contamination of all Antarctica whether sea-borne or air-borne or both. The British Antarctic Survey generously agreed to co-operate in obtaining suitable samples from the Signy Island area and to transport them to England in a permanently frozen condition.

The samples included the livers, blubbers, abdominal fat and stomach contents of a number of chinstrap penguins (*Pygoscelis antarctica*). Most of these were adult birds, 3-8 years old, at the end of the breeding season when they were moulting and very fat; three of the birds were described as immature but at least 1 year old. The samples also included three penguin eggs, probably chinstrap, the fat and livers of two brown skua and two blue-eyed shags (*Phalacrocorax atriceps*), and the livers of four fish (*Notothenia neglecta*) about 3 years old. All these specimens, except for the fish, were taken on Signy Island, as far removed as possible from the Survey's base. The fish are the most abundant species in local waters and were taken in Borge Bay on the east side of the island. They are bottom feeders and are preyed on by shags and seals. The birds were killed by pithing or with a blunt instrument, except for the shags which were shot with a rifle. Dissecting instruments were washed in acetone which was also sampled as a precaution and later found to be completely free from pesticide residues. The items for analysis were placed in glass containers with caps lined with metal foil. These containers had been specially cleaned and prepared in this Laboratory. The samples were then maintained in a continuously frozen state during the journey to London where they were stored at  $-25^{\circ}\text{C}$  pending analysis.

The samples were all in an excellent state of preservation when examined and showed no signs of decomposition. They were extracted with hexane or an acetone-hexane mixture, and these extracts cleaned up by the method of de Faubert Maunier *et al.*<sup>10</sup> which includes a dimethylformamide-hexane partition followed by passage through a column of prepared alumina. The solutions which resulted were then examined by gas-liquid chromatography using silicone, Apiezon and cyanosilicone columns, with electron-capture detection<sup>11,12</sup>. The solutions were further examined by a thin-layer chromatographic method<sup>13,14</sup>, by which the solutions were spotted on to silica gel plates and developed with a 1 per cent solution of acetone in hexane. After development, the appropriate areas of the thin-layer, corresponding to concurrently developed standard solutions of pesticides, were removed and extracted with hexane. These extracts were examined by gas-liquid chromatography and the identity and proportions of each of the compounds detected by the initial examination confirmed. The presence of *pp'*-DDT in the samples was further confirmed qualitatively and quantitatively by warming the appropriate thin-layer extract with ethanolic sodium hydroxide. The *pp'*-DDE formed by hydrolysis of the *pp'*-DDT was then determined by gas-liquid chromatography as already described. The presence of *pp'*-TDE was confirmed in a

similar manner by hydrolysis to *pp'*-DEM (1-chloro-2,2-di-4-chlorophenylethylene).

The results of the analyses are set out in Table 1. They give clear indication that all the penguins examined contained small amounts of BHC isomers, dieldrin, *pp'*-DDT and *pp'*-DDE in their liver, blubber and fat. Most of these samples also contained small amounts of heptachlor epoxide and *pp'*-TDE. Not shown in the table, but also detected in some of the samples, were small amounts, generally less than 0.005 p.p.m., of *pp'*-DME, a further breakdown product of DDT and generally regarded as non-toxic. Most of the pesticide residues detected were of the order of 0.001-0.010 p.p.m. but some of the samples contained nearly 0.050 p.p.m. of *pp'*-DDE in the blubber and fat. There was no apparent relation between the age of the birds and the distribution of results. The three penguin eggs, each weighing about 100 g, also contained BHC isomers, dieldrin, *pp'*-DDT and *pp'*-DDE in similar proportions to those found in the birds.

The stomach contents of the birds consisted almost wholly of krill (*Euphausia* sp.). The Antarctic seas are very rich in krill which form the main food of these penguins. Analysis of these krill samples indicated that here at least was one source of the pesticides found in the birds. Precisely the same compounds, but in lower proportions, were detected in the krill as in the birds, except that heptachlor epoxide was not detected in the krill, probably because the concentrations were less than those capable of being detected, allowing for the size of the samples and the methods used.

All the fish livers showed BHC isomers, heptachlor epoxide, dieldrin, DDT and its metabolites, in small amounts similar to those found in the penguin livers.

The skua is known to nest in Antarctica and the specimens taken for this study came from nests on Signy Island. Outside the breeding season, the birds range widely and often appear in the tropics. They are scavengers and predators and such birds have often been shown to contain large amounts of pesticide residues<sup>17,18</sup>. Nevertheless, 0.73 p.p.m. of heptachlor epoxide, 26 p.p.m. of *pp'*-DDE and 2.5 p.p.m. of *pp'*-DDT are, by any standard, very high concentrations to find in the fat of a wild bird.

Blue-eyed shags nest in rookeries and are not wanderers. The specimens examined here were from a shag rookery on Signy Island and had probably never left the local waters. As might be expected, therefore, both the identity and proportion of the pesticides found in these birds are the same as those found in the penguins except that the *pp'*-DDE and *pp'*-DDT contents appear to be a little higher.

Shortly after receiving and analysing the samples described above, we also received the livers of three sheathbills (*Chionis alba*). These had been taken on a separate occasion by the British Antarctic Survey on Signy Island following the sudden deaths of a number of these birds from unknown causes. All these livers contained *pp'*-DDE (0.030, 0.100 and 0.014 p.p.m.) and two of them contained dieldrin (0.015 and 0.012 p.p.m.) but no other pesticide residues.

The picture revealed by this present investigation shows that all the wildlife sampled on Signy Island and in its surrounding waters was contaminated by the persistent organochlorine pesticides. The great majority of the samples contained small amounts of four of the most commonly used of these compounds or their toxic metabolites—BHC isomers, heptachlor epoxide (a very toxic metabolite of heptachlor), dieldrin, *pp'*-DDT, and two of its toxic metabolites, *pp'*-DDE and *pp'*-TDE. The pesticides themselves have now been in large scale agricultural and veterinary use for about 15–20 years. In this time, substantial amounts would have found their way down rivers to the oceans and have been carried away by deep sea currents. Large amounts would also have been lost to the atmosphere by volatilization and some of this would have returned to some other part of the land or sea by precipitation<sup>8</sup>. It is clear from the analysis of the krill in the stomachs of the penguins and of the fish livers that the Antarctic waters contain not only traces of DDT but also traces of other organochlorine pesticides. The analysis of the penguins and their eggs and of the blue-eyed shags shows that this contamination is also present in the avian wildlife of the region. It thus seems possible that the DDT found in the early studies may, in fact, have been the first signs that contamination had reached Antarctica through the media of the seas and air.

The penguins of Antarctica have the auk family (*Alcidae*) as their ecological equivalent in the northern hemisphere. Some measure of the degree of contamination of Antarctic waters might be gained from a comparison of the results from the present study and those from studies already made of the auk family. Around the shores of the United Kingdom this family is represented chiefly by razorbills (*Alca torda*), puffins (*Fratercula*

*arctica*), guillemots (*Uria aalge*) and black guillemots (*Cepphus grylle*). The pesticide residues occurring in the eggs of the first three of these species have been studied closely for the past 4 years so that the general concentrations of these compounds in their eggs are well established<sup>5,15,16</sup>. The concentrations are of the order of fifty times higher than were contained in the penguin eggs studied here which would suggest that the waters of Antarctica are still far less contaminated than those around the British Isles.

We thank the British Antarctic Survey for their co-operation in this enterprise and, in particular, Dr J. R. Brotherhood, of the Survey, who took all the samples and arranged their dispatch to England.

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## The Alaska Earthquake of 1964

by

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Now that the dust has settled it is possible to assess the long term effects of the Alaskan earthquake on March 27, both on the activities of man and on the wild life of the State.

THE greatest earthquake to strike North America in this century rocked southern Alaska on March 27, 1964, at 5.36 p.m. local time<sup>1</sup>. The generative area, however, was mostly uninhabited, and although the long period vibrations that reached distant inhabited areas (such as Anchorage) heavily damaged many large or tall buildings, they spared most of the small wood-frame buildings. Thus, despite its great size, this earthquake ranks far below many other natural disasters in terms of property damage and loss of life. Damage due to vibration was significant chiefly at Anchorage and Whittier. At Anchorage, all buildings of ten stories or more sustained moderate to heavy damage, four structures of four to nine stories collapsed or were damaged beyond repair, and several low buildings were totally destroyed<sup>11</sup>. But many small dwellings, stores and commercial buildings were hardly damaged, if at all. Nevertheless, thousands were left homeless.

The timing of the earthquake—during a school holiday and at a time when many people were returning from work—also contributed to the low casualty rate, which was 130 killed, 16 of whom died by drowning outside Alaska. In fact, drowning in sea waves caused more deaths than all other factors combined. The earthquake disrupted the economy of the entire State, and total property damage to Alaska exceeded 311 million dollars.

Earthquake damage to the cities, towns and villages of southern Alaska was caused by direct seismic vibration, ground breakage, mud or sand emission from cracks, ground lurching, subaerial and submarine landslides, fires, sea waves, and land-level changes (Table 1). Not all these factors caused damage in every community. Some communities were devastated by only one. The village of Chenega in Prince William Sound, for example, was destroyed by a sea wave.

Overall, landslides probably caused the most damage to man-made structures and property, but sea waves took the most lives. Many Alaska communities can be reached only by water or air, water transportation being the base of the State's fishing industry and one of her vital links with the outside world. Severe damage to port and harbour facilities, therefore, was a great blow to the economy. In addition, destruction of railroad terminal and port facilities at Seward and Whittier, coupled with destruction of the highway port of Valdez, deprived Alaska of any ice-free, all-weather ship terminals. The submarine sliding at Seward, Valdez and Whittier generated large local sea waves that completed the destruction of docks, piers, and small-boat harbours. At Valdez, more than forty boats were smashed. At Seward, tugs, fishing boats and a tanker were washed ashore, and burning petroleum swept into the bay by submarine sliding

Table 1. SUMMARY OF EARTHQUAKE DAMAGES TO ALASKAN COMMUNITIES

Place	Popu- lation* 1960	Deaths (total, 114)	Subsi- dence	Uplift	Principal causes of damage					Fire	Type of structures damaged							
					Landslides Land	Sub- marine	Ground cracks	Vibra- tion	Waves		Homes	Business and public	Mili- tary	Har- bour	Water supply	Other utilities	High- ways	Air- ports
Afognak	190	0	x	—	—	—	—	—	x	—	x	x	—	x	x	0	x	—
Anchorage	44,237†	9	—	—	x	—	—	x	x	—	x	x	x	x	x	x	x	x
Cape St. Elias	4	1	—	—	x	—	—	—	x	—	—	—	—	—	—	—	—	—
Chenega	80	23	—	x	—	—	—	—	x	—	x	—	—	—	—	—	—	—
Chugiak	51	0	—	—	—	—	—	—	x	—	—	—	—	—	x	—	—	—
Cordova	1,128	0	—	x	—	—	—	—	x	—	x	x	—	x	x	x	—	—
Cordova FAA airport	40	0	—	—	—	—	—	x	x	—	x	x	—	—	x	x	x	x
Eagle River	130	0	—	—	—	—	—	—	—	—	—	—	—	—	x	—	—	—
Elleumar	1	0	—	x	—	—	—	—	—	—	—	x	—	—	—	—	—	—
Girdwood	63	0	x	—	—	—	x	—	—	—	x	x	—	—	—	—	—	—
Homer	1,247	0	x	—	—	x	—	x	—	—	—	x	—	x	—	—	x	—
Hope	44	0	x	—	—	—	—	—	—	—	x	—	—	—	x	—	—	—
Kodiak Fisheries Cannery	2	—	x	—	—	—	—	x	x	—	x	x	—	x	x	x	—	—
Kaguyak	36	3	—	—	—	—	—	—	x	—	x	x	—	—	—	—	—	—
Kodiak	2,628‡	15	x	—	—	—	—	—	x	—	x	x	x	x	x	x	x	x
McCord	8	—	—	—	—	—	—	x	x	—	x	x	—	—	—	—	—	—
Old Harbor	193	0	—	—	—	—	—	—	x	—	x	x	—	x	x	0	—	—
Ouzinkie	214	0	x	—	—	—	—	—	x	—	x	x	—	—	—	—	—	—
Point Nowell	1	1	—	—	—	—	—	—	x	—	x	—	—	—	—	—	—	—
Point Whittshed	—	1	—	x	—	—	—	—	x	—	x	—	—	—	—	—	—	—
Portage	71	0	x	—	—	—	x	—	—	—	x	x	—	—	0	—	x	—
Port Ashton	—	1	—	—	—	—	—	—	x	—	—	—	—	—	—	—	—	—
Port Nellie Juan	3	3	—	—	—	—	—	—	x	—	—	—	x	x	—	—	—	—
Seldovia	460	0	x	—	—	—	—	—	—	—	x	x	—	—	—	—	—	x
Seward	1,891	13	x	—	x	x	—	—	x	x	x	x	x	x	x	x	x	x
Tatitlek	—	—	—	x	—	—	—	—	—	—	—	—	—	x	—	—	—	—
Valdez	1,000	31	x	—	x	x	—	—	x	x	x	x	—	x	x	x	x	x
Whittier	70	13	x	—	x	—	—	x	x	x	x	x	x	x	x	x	x	x

\* Alaska Depart. Health and Welfare (1964). † 82,833 including military personnel. ‡ 4,788 including personnel at Kodiak Naval Station.

was carried back across the waterfront by the returning surge of water. At Kodiak, damage was caused mostly by a succession of huge seismic sea waves, compounded by tectonic subsidence of about 2 m.

### Geophysical Observations

The earthquake had a magnitude variously computed at 8.3–8.75 on the Richter scale. The epicentre of the main shock was near the north margin of Prince William Sound, about 130 km east-south-east of Anchorage at lat. 61.1° N., long. 147.7° W.<sup>2</sup> Its hypocentre was about 21 km deep. The thousands of aftershocks were dispersed chiefly along the continental margin of the Aleutian Trench between Prince William Sound and the Trinity Islands south of Kodiak. This area, about 100 km across and 700 km long, coincided with a zone of tectonic uplift and was the probable source of the seismic sea waves that washed the shores of the Pacific Ocean as far south as Antarctica<sup>3</sup>.

Ten aftershocks stronger than Richter magnitude 6 followed within 24 h of the first shock. Within a week seventy-five aftershocks with magnitudes greater than 4 had been recorded<sup>2</sup>, including one of magnitude 6.7 on March 29 (March 30, 02:18:05.6 G.M.T.). About 12,000 aftershocks with magnitudes equal to or greater than 3.5 probably occurred in the 69 days after the main shock<sup>4</sup>, and several thousand more were recorded in the next year and a half<sup>5</sup>.

The immediate effect of the earthquake was increased by the duration of strong ground motion. Although strong motion instruments were not at the time emplaced in the affected area, so that the duration of the motion had to be surmised from the best estimates of eye-witnesses, timings by wrist or pocket watch ranged from 1.5 to 7 min or more. Most estimates were grouped within the range of 3–4 min.

The main shock was felt throughout most of Alaska, including points as far as 1,200 km from the epicentre. It caused significant damage to ground and structures within an area of about 120,000 km<sup>2</sup> (ref. 6). Marked fluctuations were recorded in water wells as far away as Georgia, Florida and Puerto Rico<sup>7</sup>.

Although the earthquake was felt throughout Alaska, the intensity diminished appreciably northwards from mountainous southern Alaska to the intermontane plateau of the interior. Damage extended generally throughout an arcuate area within about 250 km of Prince William

Sound roughly coincidental with a zone of tectonic land-level change. In this zone, crustal deformation due to land-level change was more marked than any deformation known to be related to any previous earthquake<sup>8</sup>.

Probably 200,000 km<sup>2</sup>, possibly much more, an area larger perhaps than the British Isles, was tectonically elevated or depressed (Fig. 1). The line of maximum subsidence coincided with the mountain axis of the Kenai Peninsula and Kodiak Island, where downwarping exceeded 2 m. Uplift over wide areas of Prince William Sound averaged about 2 m, and in an area of surface rupture on Montague Island on the south side of the sound uplift locally reached 10 m (ref. 3); south-west from Montague Island the sea bottom was uplifted locally more than 15 m (ref. 9), and uplift of the sea bottom in the Gulf of Alaska generated the tsunamis that spread across the Pacific Ocean<sup>10</sup>. Triangulation by the Coast and Geodetic Survey also indicated large-scale lateral translations of the Prince William Sound area, probably accompanied by crustal extension. In general, Prince William Sound and the contiguous Chugach Mountains moved southward as much as 3 m relative to adjacent areas west and north. On the other hand, crustal shortening was greater than 3 m in a north-south direction between Montague Island and Latouche Island, which are only about 10 km apart. Landslides, sometimes submarine, caused spectacular damage in Anchorage, Seward and Valdez. Four large slides in built-up parts of Anchorage were caused by failures along bluff lines in soft clay the water content of which at critical depths exceeded its liquid limit. At Valdez and Seward, spontaneous liquefaction of granular deltaic materials initiated submarine slumping which carried away the waterfronts of both towns. The seaward slopes of the deltas, moreover, were less stable after the quake than before.

Probably few earthquakes have so strongly affected the fauna and flora of a region as that in Alaska. The total biological effects will not be known for a long time to come. Wholesale extermination of marine organisms followed crustal changes which have completely altered the ecologic setting of the shore. Broad expanses of shore and sea bottom were raised above tide water in Prince William Sound, and in the subsided areas of the Kenai Peninsula and Kodiak Island, which had previously provided winter forage for moose and nesting grounds for migratory birds, coastal marshlands and forest were inundated by salt water.



Sea waves caused enormous direct destruction of organisms. In addition, salt water invasion of many coastal lakes destroyed the freshwater habitat, at least temporarily. Many fish were destroyed when streams and lakes temporarily lost water into ground cracks, or when streams were dammed by landslides. Subsidence in some areas, however, opened new spawning habitats by inundating previously impassable falls and velocity barriers in coastal streams. Some spawning beds for salmon were destroyed by siltation on river deltas, and direct kills of eggs and fry were caused by disturbance of the gravel beds of streams<sup>14</sup>.

The salmon fishery is one of Alaska's foremost resources, and the full impact of the earthquake on this fishery will not be felt until the matured hatch of 1964 returns from the sea to spawn. Spawning areas for pink and chum salmon, which are intertidal spawners, received major damage in nearly all coastal sections affected by sea waves, uplift, or subsidence. On Kodiak and Afognak Islands, moreover, waves struck at a critical time when pink salmon fry were just moving from the spawning beds to the estuaries. Sport fisheries, including salmon, trout, char and smelt, were all damaged by environmental disturbances, especially in spawning areas.

Vast numbers of red snapper (red rock cod) were killed in Port Valdez, Port Wells, and in other parts of Prince William Sound, perhaps by turbulence or sudden upwelling associated with submarine slumping. Countless thousands of these fish—normally bottom dwellers—were left floating dead at the surface<sup>8,9</sup>. Dead dungeness crabs were found in the Copper River Delta area after the earthquake, but the commercial catch was not affected. Much of the commercial clam habitat in Prince William Sound and in the Copper River Delta was damaged or destroyed. There was a high mortality of razor clams, cockles and duck clams. Duck clams, moreover, are an important food for sea ducks, divers and other birds, as well as the starry flounder. Recovery of clams as a commercial resource is expected to be slow. Many of the new beach areas are unsuited for clam habitat. In suitable areas, moreover, reseeded clams will require 8–12 yr of growth before reaching commercial size<sup>14</sup>.

The effects of the earthquake on wildlife on land are mixed, and some short term effects have even been beneficial. Some mountain goats were said to have been killed by avalanches, as probably were some mountain sheep, deer and moose. Uplift favourably altered some nesting habitats of ducks, geese and trumpeter swans by

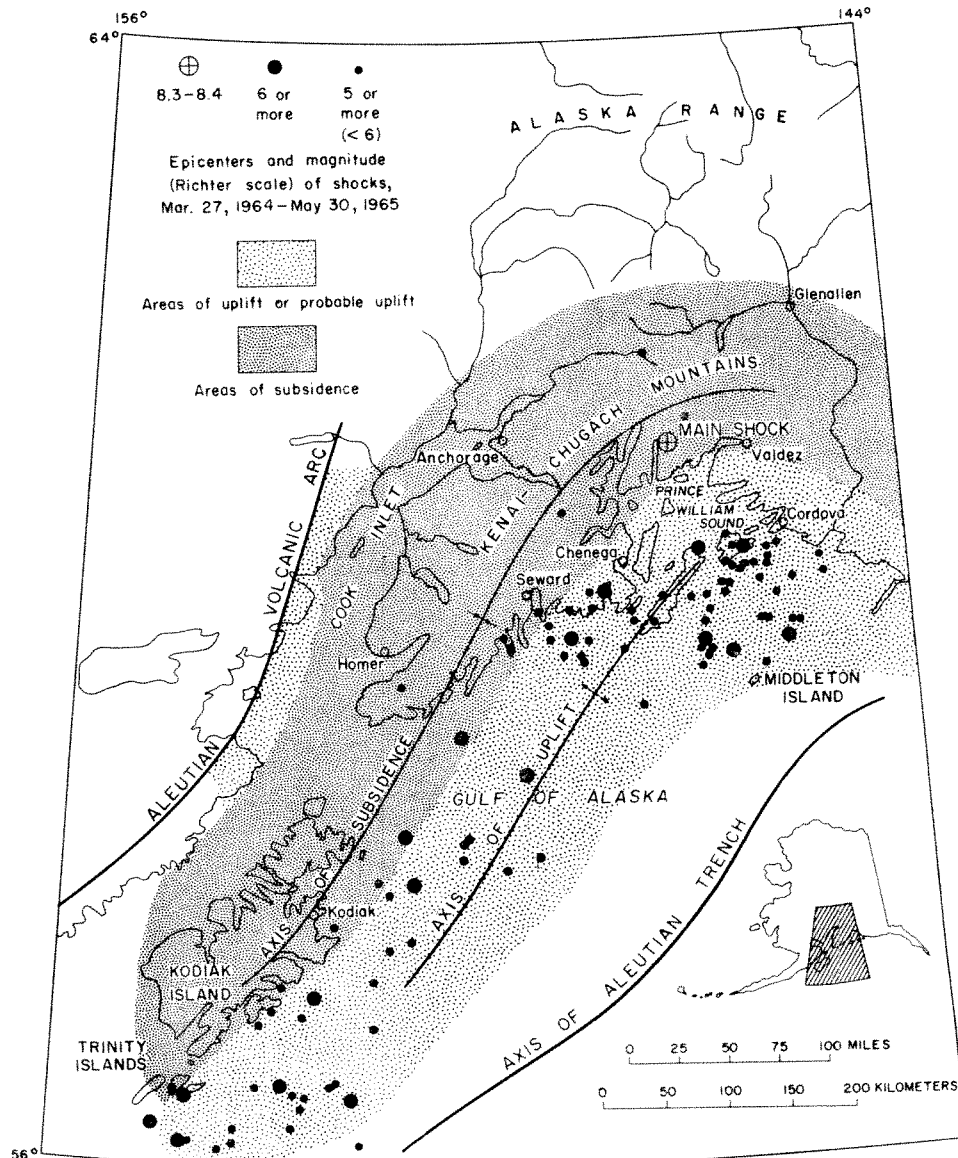


Fig. 1. Map of south central Alaska showing epicentre, major aftershocks, and areas of tectonic changes in land level accompanying the earthquake of March 27, 1964. Data chiefly from refs. 2, 3, 5, 6.

eliminating flood dangers, but the long term ecology may be less favourable—a new balance will be established as brush gradually invades upland areas and vegetation spreads over former mud flats; nesting places will shift accordingly (J. W. Brooks, personal communication). In tectonically subsided areas, where extensive freshwater marshlands and meadows were invaded by salt water, populations of moose will have to readjust downward to the new restricted food supply.

Secondary damage effects of the earthquake reached far beyond Alaska as seismic sea waves spread rapidly across the Pacific Ocean to Hawaii, Japan and Antarctica<sup>10</sup>. Van Dorn concluded that the waves were caused by the sudden displacement of water in the Gulf of Alaska accompanying the uplift of thousands of square kilometres of sea floor. A maximum wave height of more than a metre was reported from the Antarctic Peninsula (Palmer Peninsula) but heights in Japan were only 0.3 m or so. Hilo, Hawaii, had a 2 m wave, but only minor damage. Apparently the source was directional, the waves radiating preferentially south-eastward along the west coast of North America.

Atmospheric effects also reached far beyond Alaska. A pressure wave was recorded by microbarographs at Scripps Institute of Oceanography at La Jolla, California, more than 3,200 km from the epicentre, and also at the University of California at Berkeley<sup>10,17</sup>. This wave, which resembled pressure waves generated by nuclear explosions, travelled at acoustical velocity, reaching La Jolla 3 h 19 min after the onset of the quake. It was an atmospheric counterpart of the seismic sea waves generated at sea, and must also have been caused by the rapid tectonic uplift of the sea floor and the overlying water column<sup>3</sup>.

Atmospheric waves coupled to surface seismic waves were also recorded at Berkeley. These waves began at

Berkeley about 14 min after the onset of the quake and lasted about 4 h (ref. 17).

The earthquake also generated ordinary sound waves of very low sub-audible frequencies in the atmosphere<sup>18</sup>. These sound waves were radiated by the earthquake at the epicentre and by seismic waves passing through the Earth remote from the epicentre, exciting the atmosphere with their passage. Thus, the Rocky Mountains and the Mississippi Delta were local sources of sound as they vibrated with the passage of the shock. In addition Rayleigh waves crossing the continent displaced the ground surface about 5 cm in the conterminous United States and produced strong sub-audible sound waves; they then travelled vertically upward to the ionosphere, amplifying greatly as they ascended. The ionosphere, in turn, oscillated up and down at a rate of several hundred m/sec in motions that were detected by means of reflected radio waves broadcast from one ground station to another.

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<sup>3</sup> Plafker, G., *Science*, **148**, 1675 (1965).

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<sup>6</sup> Grantz, A., et al., *US Geol. Survey Circ.* 491 (1964).

<sup>7</sup> Waller, R. M., et al., *Amer. Water Works Assoc. J.*, **57**, 123 (1965).

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<sup>17</sup> Christensen, M. N., and Bolt, B. A., *Science*, **145**, 1207 (1964).

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## Human Red Cell Peptidases

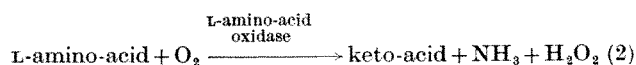
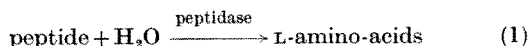
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A new method for characterizing peptidases in terms of electrophoretic behaviour in starch gel and of substrate specificity is described. A survey of red cells from a large number of people has revealed genetically determined variants of two of these enzymes. Separate loci appear to be involved.

PEPTIDASES with different specificities are known to occur in erythrocytes<sup>1-6</sup>. Our procedure for characterizing several of these enzymes depends on the detection of peptidase activity after electrophoresis by the following sequence of reactions



The free amino-acid liberated by peptide hydrolysis (reaction (1)) is subjected to oxidative deamination by L-amino-acid oxidase (reaction (2)). The complete reaction sequence results in the appearance of a dark brown zone (oxidized dianisidine) at the site of peptidase activity

(reaction (3)). In the present work we have mainly used rattlesnake (*Crotalus adamanteus*) venom as the source of L-amino-acid oxidase. This has been found to be particularly active with leucine, isoleucine, phenylalanine, tyrosine, methionine and tryptophan; weakly active with arginine, valine and histidine; and inactive with aspartic acid, asparagine, cysteine, glutamic acid, glutamine, glycine, alanine, serine, lysine, threonine and proline. Only peptides from which one or another of the amino-acids in the most active group may be released have so far been tested as substrates with this staining procedure.

The procedure adopted is as follows. Haemolysates prepared by mixing one volume of washed packed red cells with two volumes of water or by sonication are subjected to starch gel electrophoresis at pH 7.4 for 18 h at 5 V/cm (bridge buffer, 0.1 molar *tris*/maleate; gel buffer, 0.005 molar *tris*/maleate). The samples are inserted into the gel on filter paper (Whatman No. 3 MM). The gels are kept cold during the run by metal cooling

plates through which water at 8° C is circulated. The gels are sliced in the usual manner, and the reaction mixture is poured on to the cut surface of the gel. The reaction mixture is prepared immediately before use by mixing 25 ml. of 2 per cent aqueous agar at 55° C with a solution containing 25 ml. of 0.2 molar phosphate/hydrochloric acid buffer, pH 7.5, 20 mg of peptide, 5 mg of crude *Crotalus adamanteus* venom (Sigma), 10 mg of horseradish peroxidase POD II (Boehringer Corp., Ltd.), 5 mg of *o*-dianisidine hydrochloride and 0.5 ml. of 0.1 molar manganese chloride. The starch gel with its agar overlay is then incubated for 1 h at 37° C.

Using the twelve dipeptides and the four tripeptides listed in Table 1, it has been possible to distinguish five sorts of peptidase present in erythrocytes in terms of their electrophoretic properties and of substrate specificity. They will be referred to as peptidases *A*, *B*, *C*, *D* and *E*. The electrophoretic components seen in each of these are shown in Fig. 1, and their relative activities with the different substrates are indicated in Table 1. Certain peptides, for example Phe-Tyr and Phe-Leu, appear to be readily hydrolysed by several of these peptidases, so that when they are used as substrates a complex pattern of electrophoretic components is seen. With other peptides simpler patterns are observed because they are mainly hydrolysed by only one of the peptidases present. Thus activity against Val-Leu appears to be restricted to peptidase *A*, activity against Leu-Gly-Gly to peptidase *B*, and activity against Leu-Pro to peptidase *D*. Fig. 2 illustrates the electrophoretic patterns observed with the same haemolysate when different substrates are used.

Preliminary studies on extracts from a variety of tissues indicate that the peptidases found in the red cell are probably widely distributed. But other peptidases not seen in the red cells also occur, and their characterization by the methods described here is in progress.

Peptidase *A* hydrolyses all the dipeptides (except Leu-Pro) listed in Table 1, although the activity varies somewhat from substrate to substrate. No hydrolysis of any of the tripeptides has been observed. At least three electrophoretically distinct zones of activity can be regularly detected and all appear to show the same substrate specificity. The slowest of these zones is always very much more intense than the others and would appear to account for most of the activity of peptidase *A* present in red cells.

Peptidase *B* is very active against the tripeptide Leu-Gly-Gly and also the dipeptides Phe-Tyr and Phe-Leu. It shows weaker activity against the tripeptides Leu-Gly-Phe, Tyr-Tyr-Tyr and Leu-Leu-Leu and also against the dipeptides Pro-Phe and Lys-Leu. This enzyme is probably the same as the erythrocyte tripeptidase described by Adams, Davis and Smith<sup>4</sup>. If so the present findings indicate that this enzyme has a broader specificity than previously thought, because certain dipeptides are also hydrolysed quite readily. Depending on the total level of activity in the haemolysate and the substrate

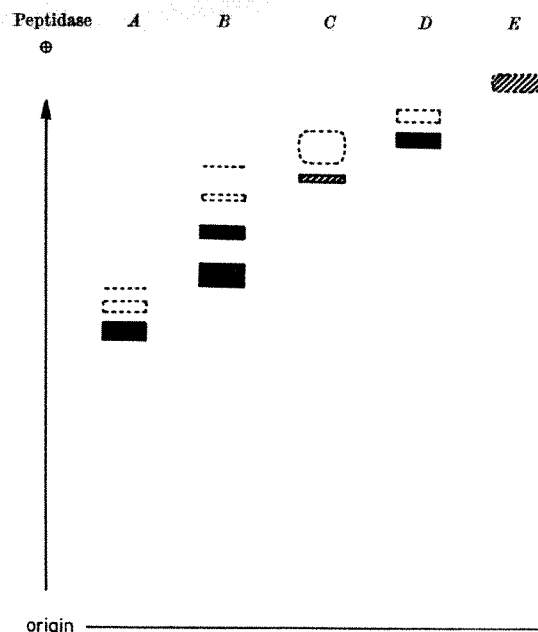


Fig. 1. Diagram showing electrophoretic components in five different red cell peptidases.

used, four or more distinct electrophoretic components with peptidase *B* activity can be seen. The slowest is the most active and the others show diminishing activity with increasing electrophoretic mobility.

Peptidase *C* shows activity against a number of the dipeptides listed in Table 1, but not with the tripeptides. Its substrate specificity, however, is very different from that of peptidase *A* which also appears to be primarily a dipeptidase. Thus peptidase *C* shows much more activity against Lys-Leu than peptidase *A*, and also relatively more activity against Phe-Tyr. In contrast, Gly-Leu and Gly-Phe are much less readily hydrolysed by peptidase *C* than by peptidase *A*. In freshly prepared red cell sonicates most of the peptidase *C* activity is present in a single zone as indicated in Fig. 1. A weaker and more diffuse area of activity migrating more rapidly also occurs, however, and this is sometimes seen to be differentiated into two distinct zones. If the sonicate is kept for a few days at +4° C and then re-examined the main zone originally seen is weaker, but the faster zones are more intense. The changes seem to be progressive with time. As the slowest of the three zones diminishes in activity the middle and subsequently the fastest become more active. If mercaptoethanol is added to the older sonicates to give a concentration of 20 mmolar and they are incubated for 60 min at 37° C before electrophoresis, the pattern reverts to that originally observed with the fresh sample. On the other hand, if a fresh sonicate is preincubated with oxidized glutathione (25 mmolar, 60 min at 37° C), a pattern similar to that seen in the older samples is obtained, and the fastest of the three zones is the most intense. These findings suggest that the electrophoretic changes with ageing of the sample may possibly be explained in terms of reactions between free -SH groups on the enzyme molecule and oxidized glutathione present in the red cell sonicate. The formation of a disulphide bridge linking a half molecule of oxidized glutathione to the enzyme would increase the net negative charge by 1 unit for each -SH group engaging in this reaction. It is noteworthy that similar changes are not seen in the electrophoretic patterns of peptidases *A* or *B* in the older sonicates, nor are these patterns modified by preincubation with oxidized glutathione.

Peptidase *D* has so far only been found to show significant activity with Leu-Pro. It probably therefore,

Table 1. RELATIVE ACTIVITY OF THE FIVE RED CELL PEPTIDASES WITH TWELVE DIPEPTIDES, FOUR TRIPEPTIDES AND LEUCYL- $\beta$ -NAPHTHYLAMIDE (LEU- $\beta$ NA)

Peptide	Peptidase				
	<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>	<i>E</i>
Val-Leu	+++	-	-	-	-
Gly-Leu	+++	-	+	-	-
Leu-Gly	+++	-	+	-	-
Gly-Phe	++	-	+	-	-
Leu-Ala	++	-	+++	-	-
Leu-Leu	++	-	++	-	+
Gly-Trp	++	-	++	-	-
Lys-Leu	+	+	+++	-	+
Pro-Phe	++	+	++	-	-
Phe-Leu	++	++	+++	-	+
Phe-Tyr	++	++	+++	-	+
Leu-Pro	-	-	-	++	-
Leu-Gly-Gly	-	+++	-	-	-
Leu-Gly-Phe	-	+	-	-	-
Tyr-Tyr-Tyr	-	+	-	-	-
Leu-Leu-Leu	-	+	-	-	-
Leu- $\beta$ NA	-	-	-	-	+



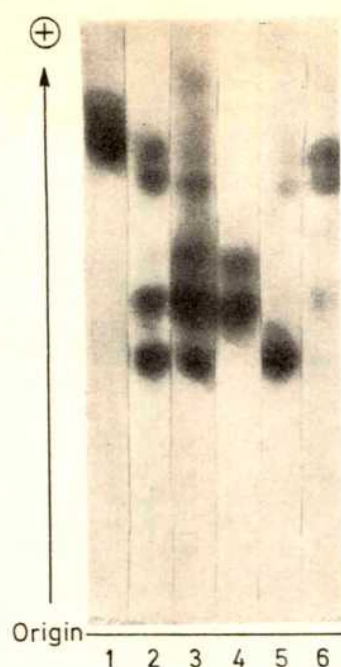


Fig. 2. Peptidase patterns seen after starch gel electrophoresis of a single haemolysate and development with six different substrates. (1) Leu-Pro; (2) Pro-Phe; (3) Phe-Tyr; (4) Leu-Gly-Gly; (5) Leu-Gly; (6) Lys-Leu.

corresponds to the enzyme "prolidase" described by Adams, McFadden and Smith<sup>2</sup>. As indicated in Fig. 1, there is one main zone of activity with a weaker and more diffuse zone moving ahead. Changes in the electrophoretic pattern occur in red cell sonicates which have been kept for a few days at 4° C and resemble those seen with peptidase C. The patterns can be modified in a similar way by treatment with mercaptoethanol, and apparently can be induced in fresh sonicates by incubation with oxidized glutathione.

Peptidase E has been detected with Leu-Leu, Lys-Leu, Phe-Leu and Phe-Tyr, but its activity in each case is relatively weak. Older sonicates show electrophoretic changes resembling those seen with peptidase C and D, and analogous changes are also brought about by treatment with mercaptoethanol and oxidized glutathione. It is interesting that peptidase E appears to be the only red cell peptidase which shows significant hydrolysis of leucyl-β-naphthylamide. This substrate has been widely used both histochemically and after electrophoresis for the detection of what has been referred to as "peptidase" or "naphthylamidase" activity in various tissues.

The electrophoretic patterns shown in Figs. 1 and 2 are those most commonly seen. During quite extensive population and family studies, however, several genetically determined variants of peptidase A and of peptidase B have been identified. A systematic search for variants of peptidase C, D or E, however, has not yet been carried out. The variants of peptidase A and B have been found to be independent of each other and are not associated with any variation of the pattern of peptidases C, D or E, thus confirming the conclusion based on the substrate specificity investigations that several quite different peptidases are demonstrable in these conditions.

We will refer to the usual form of peptidase A as Pep A 1 and the variant types so far identified as Pep A 2-1, Pep A 2, Pep A 3-1 and Pep A 4-1. Similarly the usual form of peptidase B will be called Pep B 1 and the variant types Pep B 2-1, Pep B 3-1 and Pep B 4-1. The frequencies with which these different types have been observed in a survey of more than 2,000 unrelated people of European origin and in more than 500 people of African origin are

Table 2. INCIDENCE OF PEPTIDASE PHENOTYPES IN DIFFERENT POPULATION SAMPLES

	Europeans	Resident in UK	Negroes Yoruba (Nigeria)	Bantu (S. Africa)
Peptidase A 1	2,279	254	127	87
2-1	—	36	25	11
2	—	3	3	2
3-1	1	1	—	—
4-1	3	—	—	—
Total tested	2,283	294	155	100
Peptidase B 1	2,188	294	155	100
2-1	6	—	—	—
3-1	2	—	—	—
4-1	1	—	—	—
Total tested	2,197	294	155	100

given in Table 2. The Negroes which were investigated were from three different population groups; 155 were Yoruba from Nigeria, 100 were Bantu from South Africa and 294 were Negroes of various origins resident in England, largely recent immigrants from the West Indies. Pep A 2-1 has not been seen in the European population, but it is relatively common in Negroes, occurring in 11 per cent to 16 per cent of people in the three population groups examined. Similarly Pep A 2 is absent in the European sample, but has an appreciable frequency among the Negroes (0.5 per cent to 2 per cent). Pep A 3-1 has been found once in the unrelated European sample and once among the Negroes. Peptidase A 4-1 is also relatively rare. Three examples were observed among the Europeans and none among the Negroes. Of the peptidase B variants, Pep B 2-1 is the commonest and appears to occur in about one in 350 Europeans. It has not been seen in the Negro groups. In a series of 104 "Cape Malay" individuals (not listed in Table 2) from South Africa, however, two examples of Pep B 2-1 were observed. It is thus possible that this type may have a relatively higher incidence among people of oriental origin.

Pep A 2-1 and Pep A 2 have the electrophoretic patterns shown in Fig. 3. Segregation data in a series of families in which at least one parent was either Pep A 2-1 or Pep A 2 are given in Table 3. These data, as well as the segregation patterns observed in several pedigrees

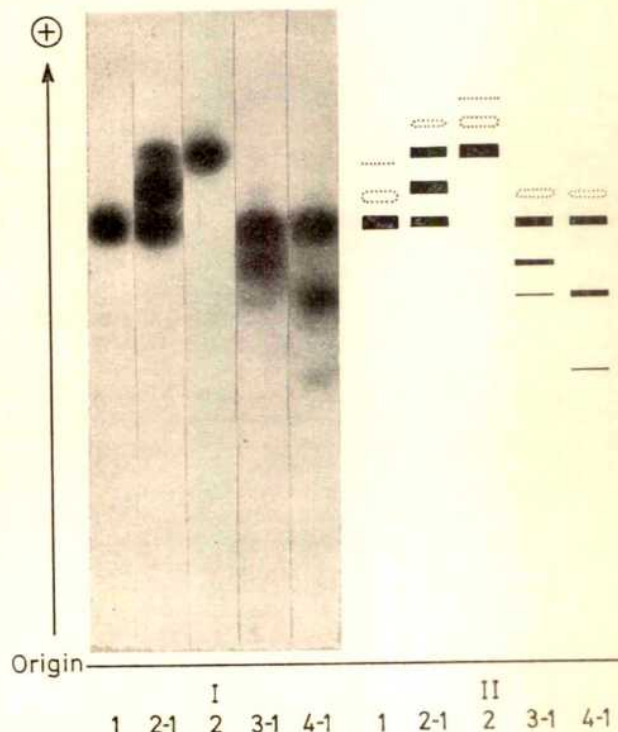


Fig. 3. I, Electrophoretic patterns of five different peptidase A phenotypes. Development with Leu-Gly as substrate. II, Diagram of the principal and minor bands in the same five phenotypes.



Table 3. SEGREGATION OF PEPTIDASE A PHENOTYPES IN THIRTY MATINGS IN WHICH AT LEAST ONE PARENT IS PEP A 2-1 OR PEP A 2

Parents	No. of matings	Children			Totals
		Pep A 1	Pep A 2-1	Pep A 2	
1 × 2-1	26	25	34 (7)	—	59
2-1 × 2-1	3	1	2 (1)	1	4
1 × 2	1	—	2 (1)	—	2
Totals	30	26	38 (9)	1	65

Figures in parentheses indicate the number of families selected through a child who was Pep A 2-1. In the other families the propositus was a parent who was Pep A 2-1.

involving three generations, is consistent with the simple hypothesis that Pep A 2-1 individuals are heterozygous for an autosomal allele which in homozygotes produces type Pep A 2.

The presumed heterozygote Pep A 2-1 shows three main components, two of which correspond in mobilities to the main components of the presumed homozygotes Pep A 1 and Pep A 2, while the third has an exactly intermediate mobility. This characteristic triple-banded pattern in heterozygotes is seen in a number of other enzymes and is usually thought to arise in situations where the enzyme in the homozygote contains two identical polypeptide chains or sub-units, whose primary structure is determined by a single allele. In the present case we suppose that the common allele determines a polypeptide  $\alpha^1$ , and that the allele which in homozygotes gives Pep A 2 determines a polypeptide  $\alpha^2$  which differs in structure from  $\alpha^1$  possibly by a single amino-acid substitution. If so, the main enzyme component in Pep A 1 would have the structure  $\alpha^1\alpha^1$ , the main component in Pep A 2 would have the structure  $\alpha^2\alpha^2$ , and the three main components seen in Pep A 2-1 would have the structures  $\alpha^1\alpha^1$ ,  $\alpha^1\alpha^2$  and  $\alpha^2\alpha^2$ .

Pep A 3-1 and Pep A 4-1 (Fig. 3), like Pep A 2-1, each show two new components as well as a component with the same mobility as the principal component in Pep A 1. In both these types, however, the new components migrate more slowly than the Pep A 1 component. In an investigation of the families of two individuals with Pep A 3-1, seven further examples of this phenotype were found. In the case of the individuals with Pep A 4-1 six further examples of the same phenotype were found among the immediate relatives. The pedigrees indicate that these phenotypes occur in individuals who are heterozygotes. If the sub-unit hypothesis of peptidase A outlined earlier is correct, then it is likely that types Pep A 3-1 and Pep A 4-1 are heterozygous for rare alleles which determine variant sub-units which may be designated  $\alpha^3$  and  $\alpha^4$  respectively. If so, the three components seen in Pep A 3-1 will be expected to have the structures  $\alpha^1\alpha^1$ ,  $\alpha^1\alpha^3$  and  $\alpha^3\alpha^3$ ; and the three components in Pep A 4-1, the structures  $\alpha^1\alpha^1$ ,  $\alpha^1\alpha^4$  and  $\alpha^4\alpha^4$ . The slowest bands in both Pep A 3-1 and Pep A 4-1 are somewhat weaker than the others. This could arise if sub-units  $\alpha^3$  and  $\alpha^4$  were catalytically less efficient than  $\alpha^1$ , or if they were present in smaller amounts because they were less stable or were synthesized at a slower rate than  $\alpha^1$ .

Minor components similar to those seen ahead of the main component in Pep A 1 may also be seen ahead of the main component in Pep A 2 and of its homologue in Pep A 2-1. They are also seen ahead of the fast principal component in Pep A 3-1 and Pep A 4-1. It is possible that similar minor components may also occur in association with the other principal components present in Pep A 2-1, Pep A 3-1 and Pep A 4-1 but are obscured by the more intense components in the patterns.

The pattern of components in the phenotype Pep B 2-1 is shown in Fig. 4. There is a series of zones with mobilities similar to those in Pep B 1, but there is also an extra slower moving zone. Among the offspring of eleven matings where one parent was Pep B 1 and the other Pep B 2-1, sixteen children were found to be Pep B 1 and sixteen Pep B 2-1 (excluding propositi). These findings, as well as the overall segregation pattern in the

pedigrees, indicate that the type occurs in individuals heterozygous for a rare autosomal gene.

The electrophoretic patterns observed in Pep B 2-1 can be explained most simply by supposing that the rare allele for which these individuals appear to be heterozygous alters the mobility of each of the series of Pep B isoenzymes in a regular manner. The effect of this allele would be to slow each of the isoenzymes by an amount equivalent to the normal difference in mobility between any consecutive pair of them. In an autosomal heterozygote the products of both alleles can be expected to be equally represented. Thus the pattern in Pep B 2-1 would be made up of the series of isoenzymes present in Pep B 1 and the series determined by the rare allele. These series are presumed to overlap, and so the distinctive feature of the Pep B 2-1 pattern would be the appearance of an extra slow component.

In the phenotype Pep B 3-1 (Fig. 4) the zones seen match those of Pep B 1 in electrophoretic mobility, but their relative intensities are different. The slowest is relatively weaker while each of the faster ones is relatively more intense than the corresponding zone in Pep B 1. Thus while in Pep B 1 the slowest zone is the most intense of the series, in Pep B 3-1 the next zone is the most intense. Two families in which the propositus was of type Pep B 3-1 have been investigated and four further examples of this type were found. The pedigrees suggest that individuals with this unusual phenotype are also heterozygous for a rare allele.

We can account for the electrophoretic pattern seen in Pep B 3-1 by supposing that the rare allele concerned causes an increase in the mobility of each of the usual series of components seen in Pep B 1 by an amount equivalent to the difference in mobility between any pair of them. The pattern in Pep B 3-1 would represent a mixture of the usual series of components determined by the common allele and the more rapidly moving series of components determined by the rare allele. In the usual series the slowest component is the most active, and this would presumably also be the case in the new series. The net effect in the mixture would be for the slowest zone to have approximately half the activity it has in Pep B 1, but the next zone, which represents a mixture of the second component in Pep B 1 and the slowest component in the new isoenzyme series, would be distinctly more intense.

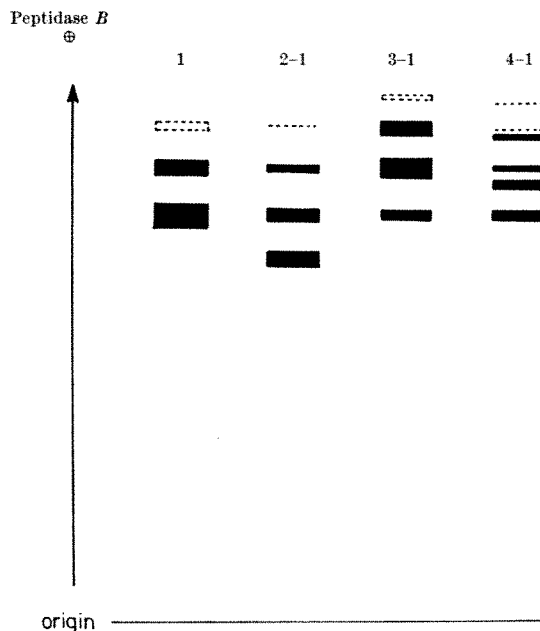


Fig. 4. Diagram of electrophoretic patterns of four different peptidase B phenotypes. Development with Leu-Gly-Gly as substrate.

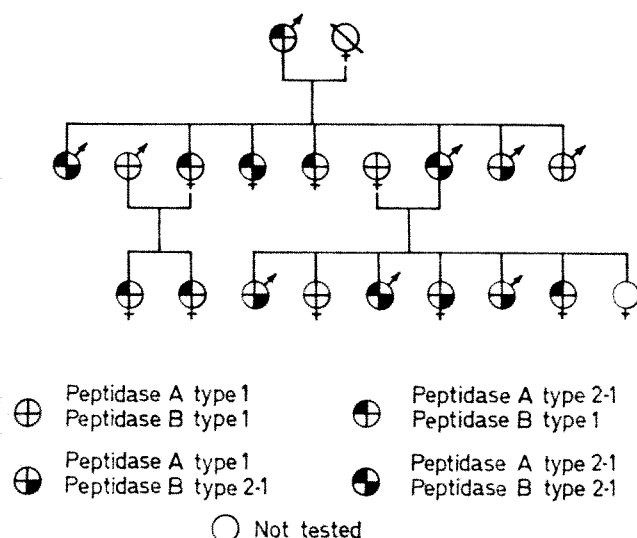


Fig. 5. Pedigree of a family in which peptidase A 2-1 and peptidase B 2-1 are segregating.

Similarly, each of the faster zones would appear to be relatively more intense than the zones with the same mobilities in Pep B 1.

In the phenotype Pep B 4-1 (Fig. 4) each of the components characteristic of Pep B 1, showing about half their usual activity, are seen and in addition there is a new series of components each of which has a greater mobility than the apparently corresponding component in Pep B 1. The investigation of the family gave results

consistent with the view that individuals showing this phenotype are heterozygous. We suppose that the rare allele involved determines the new series of components seen, and that the common allele present determines the usual series of peptidase B components which occur in this phenotype.

In one of the families investigated both the phenotypes Pep A 2-1 and Pep B 2-1 occurred. The pedigree is shown in Fig. 5. There is one mating between an individual who has the phenotype Pep A 2-1/Pep B 2-1 and an individual who is Pep A 1/Pep B 1. Among the six children tested one is Pep A 1/Pep B 1, one is Pep A 2-1/Pep B 1, three are Pep A 1/Pep B 2-1, and one is Pep A 2-1/Pep B 2-1. Each of the four possible combinations occurs, and so recombination must have taken place. We conclude that the loci determining peptidase A and peptidase B are not closely linked. They are either well separated on the same chromosome or on different chromosomes.

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## Non-randomness of Amino-acid Changes in the Evolution of Homologous Proteins

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A calculation of the extent of the differences between homologous proteins shows that the amino-acid "mutations" are not as random as might have been thought. In fact, natural selection seems to act to favour those amino-acid substitutions that tend to maintain the original conformation of the protein.

Two principal methods have been used to assess the degree to which homologous proteins are related. One relies on estimating the number of amino-acids in the sequences that are identical<sup>1</sup>, and the second involves the determination of the smallest number of mutations necessary to convert one sequence into another<sup>2-4</sup>. Unfortunately, neither method provides information about the nature of the differences in the sequences or about the forces that have influenced these differences. To approach these questions, amino-acid exchanges (otherwise referred to as substitutions, replacements, or "mutations") have been analysed with regard to the degree to which the properties of the residue side-chains have altered<sup>5,6</sup>. In a previous study, exchanges were divided into three groups on the basis of the polarities of the amino-acids<sup>6</sup>. Group I exchanges were those in which polarity was not altered; group II exchanges were between polar amino-acids and glycine or alanine; and group III exchanges involved an alteration in polarity. The reasons for these assignments have been discussed pre-

viously<sup>6,7</sup> but will be briefly re-stated here. Experimental and theoretical considerations indicate that the polarity of a residue is very important in determining its effect on protein conformation. The interiors of globular proteins consist primarily of hydrophobic residues, while polar (or hydrophilic) residues are found on the surface and are accessible to the solvent. Glycine and alanine residues can be found in either place<sup>8</sup>. All other considerations apart, therefore, type I exchanges would be expected to produce much smaller alterations of protein conformation than would type III exchanges; the effects of type II exchanges would be large or small depending on the actual localization of the residues in the molecule. It was predicted therefore that analysis of amino-acid exchanges between homologous proteins with similar conformations would reveal principally type I exchanges and that type III exchanges would be few<sup>6</sup>. This was, in fact, found, and the results further suggested that the differences in sequence were less random than could be attributed to simple mutations even if the organization



Table 3. COMPARISON OF HOMOLOGOUS AND NON-HOMOLOGOUS AMINO-ACID SEQUENCES

Proteins (refs.)	Total	Residues different	$\Delta p$	All exchanges		Interior exchanges		'Surface' exchanges	
				$\Delta m$	Base changes	$\Delta m$	Base changes	$\Delta m$	Base changes
<b>Homologous</b>									
Haemoglobin: human $\beta$ and $\gamma$ chains (17, 18)	146	37	0.07	0.29	1.27	0.11	1.13	0.34	1.31
Direction known		18		0.28	1.22				
Haemoglobin: human $\alpha$ and $\beta$ chains (17, 19)	141	73	0.15	0.29	1.32	0.19	1.25	0.32	1.32
Haemoglobin: human and horse $\alpha$ chains (19, 20)	141	17	0.04	0.35	1.30				
Myoglobin (whale) and haemoglobin $\beta$ chain (horse) (9)	144	110	0.31	0.40	1.45	0.18	1.20	0.46	1.49
Tobacco mosaic virus: vulgaris and Dahlemensis (21)	158	30	0.07	0.32	1.20			0.43	1.47
Subtilisin: BPN' and Carlsberg (22)	274	82	0.09	0.31	1.27				
Ribonuclease: bovine and rat (23)	124	40	0.10	0.30	1.25				
Cytochrome c: yeast and horse heart (5)	102	39	0.14	0.36	1.44				
Trypsinogen and chymotrypsinogen (24)	216	115	0.19	0.36	1.50				
<b>Non-homologous</b>									
Myoglobin and hen egg lysozyme (9, 25)	129	124	0.50	0.52	1.64				
Ribonuclease (bovine) and lysozyme (23, 25)	124	118	0.45	0.47	1.56				

be determined<sup>2</sup>, all indices were calculated using the coefficients  $\Delta_{AB}$ . Because a significant number of residues in the homologous sequences are identical,  $\Delta p$  is always less than  $\Delta m$ . Both sets of values, but particularly the latter, are considerably less than the values observed with the non-homologous proteins (Table 3) and the calculated random values of 0.49 (for  $\Delta p_{\text{expected}}$ ) and 0.52 (for  $\Delta m_{\text{expected}}$ ). Except for the comparison of myoglobin and the haemoglobin  $\beta$ -chain, all values of  $\Delta m$  were 0.36 or less. Furthermore, the actual distribu-

tions of the coefficients of the individual amino-acid exchanges show a higher proportion of low values than do the distributions of the exchanges between non-homologous proteins (Fig. 1). The paucity of values of  $\Delta_{AB}$  greater than 0.75 is particularly striking in the subtilisin (BPN' and Carlsberg), ribonuclease (bovine and rat), and the various haemoglobin comparisons.

Because a knowledge of the types of amino-acid interchanges found among the chains of haemoglobin and myoglobin<sup>8</sup> has influenced the assumptions underlying the derivation of the coefficients of difference, it would be expected that the values of  $\Delta m$  and the distributions of  $\Delta_{AB}$  for the haemoglobin comparisons should be non-random, and this is what was found. On the other hand, the finding of non-randomness for the other comparisons indicates that the rules for haemoglobin are not unique and are applicable to other groups of proteins. We can therefore be more confident in accepting the non-random values for  $\Delta m$  as indicating the correctness of the hypothesis that the preservation of the "conformational" attributes of amino-acids in a protein sequence is important in the evolution of homologous proteins.

It has repeatedly been pointed out that the amino-acid or genetic code is non-random in its organization and that single base changes will often result in the substitution, if not of the same amino-acid, of a similar or related amino-acid<sup>7,11,14,15</sup>. This is readily apparent from an examination of the code<sup>15</sup> as depicted in Fig. 2. To obtain an estimate of the degree to which the structure of the code influences the nature of the substitutions, the coefficients of difference for all the amino-acid substitutions resulting from single base changes have been averaged for each codon, and the values for all codons for the same amino-acid have then been averaged. The resulting values (defined as  $\Delta_A$ ) which represent the expected coefficients of difference for random single base changes in amino-acid codons (the specific codon not being known) are shown in Table 4. These coefficients have been expressed in two ways: as coefficients for all possible mutations and as coefficients for observable mutations. The former values provide a quantitative estimate of the "conservative" nature of the amino-acid code and indicate the degree of non-randomness that the amino-acid code imposes on the results of random single-base mutations. Because comparisons of homologous amino-acid sequences cannot detect "isosemantic" mutations—mutations which do not result in a change of amino-acid—the  $\Delta_A$  for observable mutations must be used for comparative purposes. Its values range from 0.19 to 0.58, with a mean of 0.42 over all codons and of 0.38–0.39 if averaged over the compositions of haemoglobin (human  $\beta$ -chain) or ribonuclease (bovine). The latter thus represent the expected values for  $\Delta m$  if only single base changes can occur. It has been conjectured that transitions (mutations which substitute one pyrimidine for another or one purine for another) may occur more frequently than transversions<sup>16</sup> and may be more "conservative" in nature<sup>14</sup>, and values for  $\Delta_A$  and for expected  $\Delta m$  have therefore been calculated.

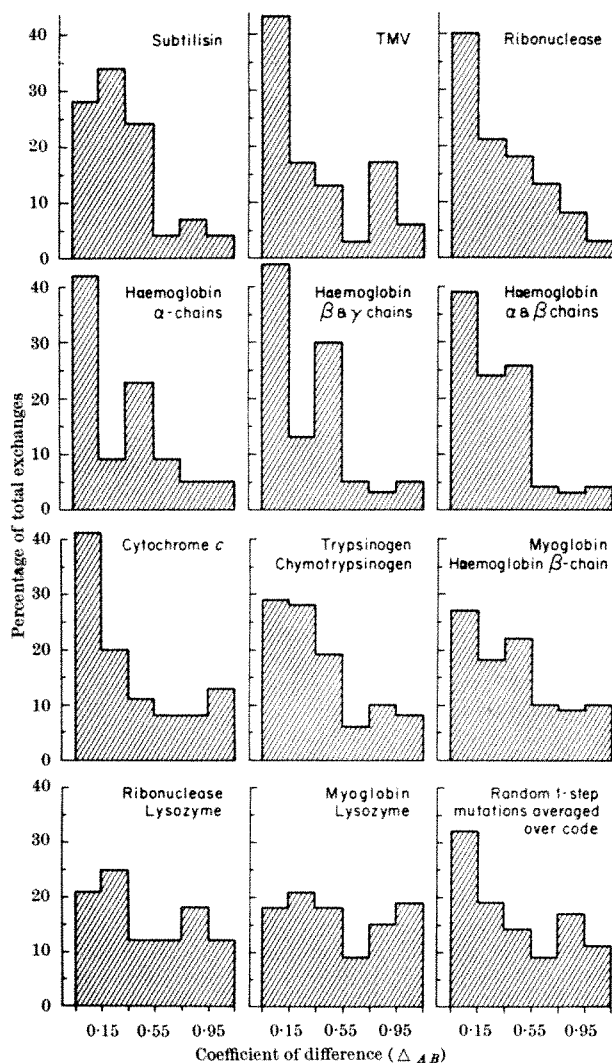


Fig. 1. Distributions of coefficients of difference for amino-acid exchanges between homologous proteins. For reference, distributions for comparisons of non-homologous proteins and for observable substitutions produced by randomly occurring single base changes are also shown.



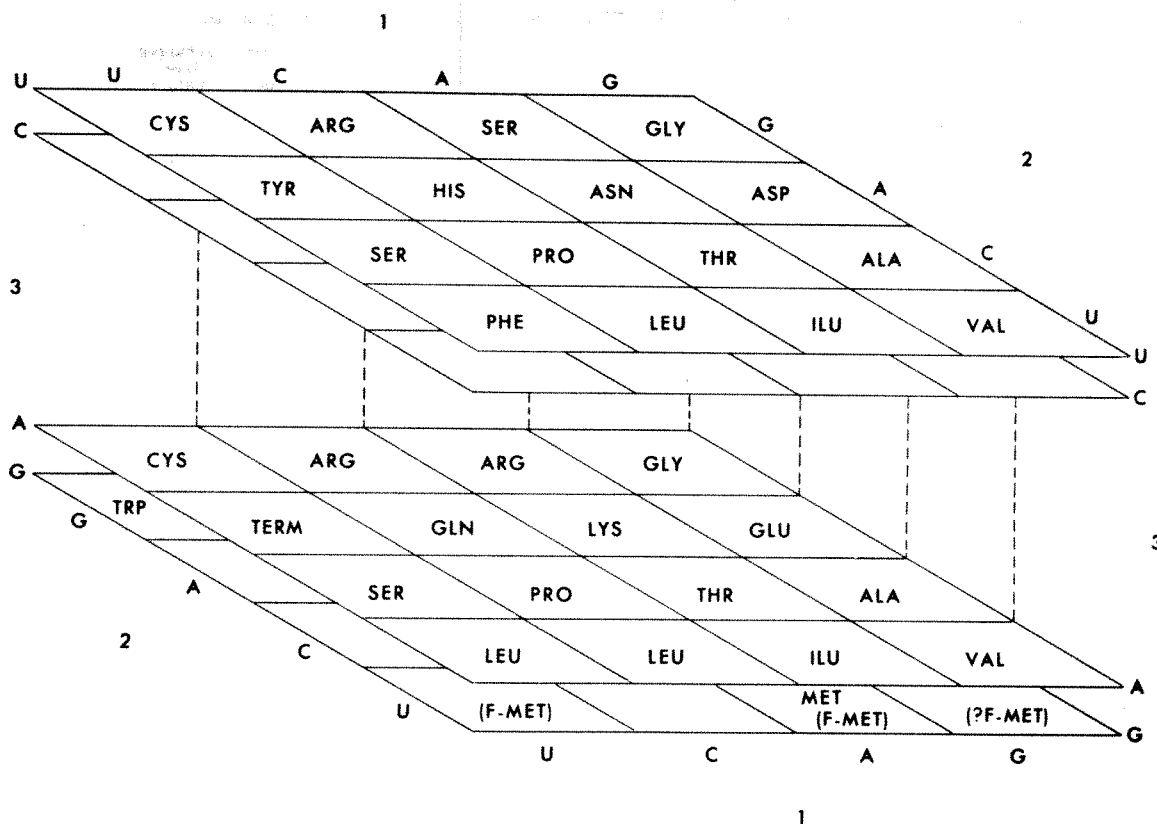


Fig. 2. Organization of the amino-acid code<sup>15</sup> represented as a three-dimensional matrix to emphasize the relationships among the codons of the various amino-acids. The numbers, 1, 2, and 3, refer to the position of the base in the codon. The close spacings of the planes for U and C and for A and G in the third position indicate that, unless otherwise marked, the codons are degenerate for pyrimidine or purine bases in this position.

assuming each codon can undergo six (rather than three) transitions for each six transversions<sup>7</sup>. The expected  $\Delta m$ s fall slightly to about 0.37 (Table 4).

Calculations have also been carried out for double mutations in which a single codon is subjected to two successive base changes at either the same or in different positions. The values for  $\Delta_A$  (Table 4) are greater than were obtained for single base changes, although still not

as great as for random substitutions. If double base changes are examined from the point of view of how many base changes appear to be necessary in each codon, rather than how many actually were necessary, the number is considerably less than 2.0 and ranges from 1.17 to 1.52 (Table 4); the mean is 1.30. In other words, if each codon in a sequence were subjected to two successive but independent mutations, the resulting amino-acid sequence would appear to differ from the original one by only 1.3 mutations in each codon. If amino-acids are paired at random, the value for the apparent number of base substitutions is in the range 1.45–1.61 (ref. 2).

With the results presented here, it is possible to consider the following question. Are observed amino-acid substitutions non-random merely because they are of the type favoured by the organization of the amino-acid code, or are they even less random than the code would ensure? As was already noted, a series of one-base changes with transitions twice as frequent as would be expected for purely random mutations gives an expected  $\Delta m$  of 0.37. How different is this value from those for observed  $\Delta m$  (Table 3) which range from 0.28 to 0.40? Unfortunately, the two numbers are not directly comparable because observed amino-acid substitutions cannot be explained solely on the basis of single base changes within codons. In fact, the minimal number of base changes that must be postulated is of the order of 1.20–1.50 per substitution (Table 3). An estimate of the value of expected  $\Delta m$  for a series of random mutations giving a similar apparent number of base changes for each amino-acid substitution is therefore required as a standard of reference. Such a value has already been obtained for the random two-base changes: an expected  $\Delta m$  of 0.45 with 1.30 apparent base changes for each substitution. To facilitate the comparison of the observed values of  $\Delta m$  with random expectations, the appropriate results have been displayed graphic-

Table 4. PREDICTED COEFFICIENTS OF DIFFERENCE ( $\Delta_A$ )

Amino-acid	One-base changes					Two-base changes				
	Possible		Observable		Trans. $\times 2$ + transv.	Possible		Observable		Apparent number of base changes
	All	Trans.	All	Trans.						
Phe	0.18	0.30	0.21	0.45	0.26	0.32	0.37	0.42	0.46	1.24
Met	0.32	0.28	0.32	0.28	0.31	0.42	0.46	0.33	0.34	1.37
Leu	0.21	0.11	0.33	0.31	0.32	0.33	0.34	0.40	0.48	1.19
Ilu	0.31	0.28	0.40	0.38	0.40	0.40	0.48	0.36	0.44	1.29
Val	0.24	0.16	0.36	0.23	0.33	0.36	0.44	0.50	0.62	1.22
Pro	0.44	0.28	0.67	0.43	0.61	0.44	0.49	0.42	0.47	1.38
Tyr	0.45	0.24	0.53	0.37	0.49	0.44	0.49	0.40	0.49	1.24
Trp	0.43	0.49	0.43	0.49	0.44	0.42	0.47	0.34	0.42	1.26
Cys	0.37	0.43	0.45	0.49	0.49	0.40	0.49	0.37	0.45	1.26
Ala	0.26	0.27	0.39	0.41	0.40	0.34	0.42	0.35	0.45	1.17
Gly	0.29	0.34	0.45	0.51	0.46	0.37	0.45	0.34	0.42	1.22
Ser	0.33	0.42	0.47	0.62	0.51	0.35	0.45	0.38	0.44	1.38
Thr	0.26	0.40	0.40	0.60	0.45	0.34	0.42	0.41	0.47	1.42
His	0.36	0.27	0.40	0.40	0.40	0.38	0.44	0.41	0.45	1.41
Glu	0.27	0.17	0.31	0.26	0.30	0.41	0.47	0.42	0.49	1.39
Gln	0.31	0.03	0.35	0.05	0.25	0.41	0.45	0.35	0.41	1.45
Asp	0.36	0.17	0.41	0.26	0.37	0.42	0.49	0.34	0.40	1.46
Asn	0.28	0.07	0.31	0.11	0.27	0.35	0.41	0.45	0.59	1.17
Lys	0.17	0.02	0.19	0.03	0.15	0.34	0.40			
Arg	0.38	0.27	0.58	0.41	0.55	0.45	0.59			
Mean:										
Over codons	0.31	0.26	0.42	0.38	0.41	0.38	0.47			1.28
Over haemo-globin $\beta$ -chain	0.28	0.23	0.38	0.35	0.37			0.45		1.30
Over bovine ribonuclease	0.30	0.24	0.39	0.35	0.37					

ally in Fig. 3. When arranged in this form, all the observed exchanges lie considerably below the line representing codon-determined random substitutions, and the organization of the amino-acid code, conservative as it is, is not sufficient to explain the "conformational" non-randomness of amino-acid substitutions. It is inferred therefore that natural selection has operated to reduce the amount of variability in amino-acid residues to less than that which would result from a series of random mutations.

Within the limits imposed by the numerical system that has been used in these calculations selective forces do not appear to have eliminated all substitutions with large coefficients of difference. Otherwise it would be expected that the observed values of  $\Delta m$  would be closer to 0.1–0.2 than to 0.3. Part of the explanation for this situation may lie in the initial assumptions and in the arbitrary assignments of values to certain amino-acid substitutions. This is at present unavoidable. Another factor may be that when at least 25 per cent of the residues remain unchanged all amino-acids in a sequence are not subject to the same intensity of selection. Thus as has been

pointed out by Perutz *et al.*<sup>8</sup>, the residues on the surface of globin may be more variable with respect to polarity than those in the interior. To determine whether this could affect the values observed for  $\Delta m$ , the residues listed by Perutz *et al.*<sup>8</sup> as interior residues (based primarily on the analysis of the structure of myoglobin) were removed from consideration, and  $\Delta m$  was calculated separately for the interior residues and the surface residues of the  $\alpha$  and  $\beta$  and the  $\beta$  and  $\gamma$  chains of haemoglobin, and of the  $\beta$ -chain of haemoglobin and myoglobin (Table 3, Fig. 3). In all cases,  $\Delta m$  for the interior residues was less than 0.2, indicating that selection is indeed more rigorous for the interior. In the first two cases,  $\Delta m$  for the surface residues became slightly but not significantly higher, but for the myoglobin- $\beta$ -chain it rose to a value not very different from that corresponding to the random arrangement. In the latter instance it must be concluded that either selection of the type envisioned is not operating on the surface residues or that the discrepancy is in some way connected with the fact that myoglobin does not form tetramers while the  $\beta$ -chain of haemoglobin does. To test the latter possibility, all  $\beta$ -chain residues implicated in the formation of inter sub-unit contacts<sup>9</sup> were removed and  $\Delta m$  recalculated. This results in a fall of  $\Delta m$  from 0.45 to 0.43, the  $\Delta m$  of the group of inter-sub-unit contact residues being 0.58. The new value of  $\Delta m$  for the surface residues, while lower than before, is still too close to random to allow the absence of tetramerization to be invoked as the sole explanation for its relatively high value.

To sum up, a series of numerical values have been derived to express the conformational differences between amino-acids. These values, or coefficients of difference, have been used to calculate indices of difference for amino-acid exchanges between homologous proteins. All comparisons give indices of difference considerably lower than could be accounted for by random mutations, even if the organization of the amino-acid code is taken into account. We infer therefore that natural selection has acted in the evolution of homologous proteins to favour amino-acid substitutions that would be compatible with the retention of the original conformation of the protein, and conversely to eliminate those that would not.

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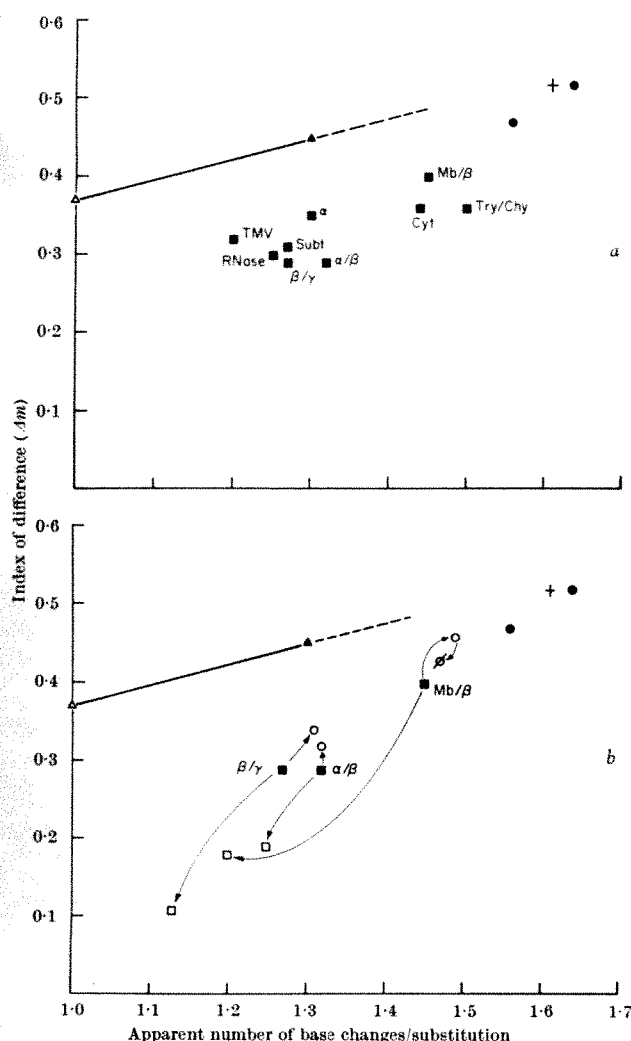


Fig. 3. *a*, Relationship of  $\Delta m$  to apparent number of base changes per codon for comparisons of homologous (■) and non-homologous (●) proteins. All points for the former fall below the line representing results expected for unselected random mutations. *b*, Separation of  $\Delta m$  for the globin comparisons into  $\Delta m$ -surface (○) and  $\Delta m$ -internal (□). For the myoglobin/haemoglobin  $\beta$ -chain comparison,  $\Delta m$ -non-inter-sub-unit-surface (○) is also shown. Except for  $\Delta m$ -surface for myoglobin/haemoglobin  $\beta$ , all points, particularly those for the internal residues, again fall below the line for unselected random mutations. TMV, Tobacco mosaic virus; RNase, ribonuclease; Subt, subtilisin; Cyt, cytochrome *c*; Try/Chy, trypsinogen and chymotrypsinogen; Mb/β, myoglobin and haemoglobin  $\beta$ -chain;  $\alpha$ ,  $\alpha/\beta$ ,  $\beta/\gamma$ , haemoglobin  $\alpha$ ,  $\alpha$  and  $\beta$ , and  $\beta$  and  $\gamma$  chains, respectively.

# Characterization of Macromolecules by Constant Velocity Sedimentation

by

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To exploit the analytical power of zone velocity centrifugation, a novel method has been developed using sucrose gradients calculated to give constant particle velocity.

ZONE velocity centrifugation is an indispensable tool for the fractionation and characterization of macromolecules<sup>1,2</sup> (ref. 1 has a collection of articles on this subject), but in practice the analytical power of the method has rarely been exploited either with respect to resolution or quantitative determination of the rates of sedimentation. Determination of sedimentation constants in sucrose gradients is complicated because, with the gradients and rotors normally used, the molecules slow down as they move away from the top layer, for the viscous drag of the medium increases more rapidly than the centrifugal force. The viscosity and density of the medium change in a complicated fashion along the sedimentation path, and so the mathematical computation of sedimentation coefficients<sup>3,4</sup> becomes too cumbersome for routine analyses. This difficulty can be overcome in part by the use of tables prepared by a computer programme<sup>5</sup>. Without standardization of the gradients, however, this is not practical, because each type of rotor, gradient and particle density requires a different set of solutions. For the special case of protein molecules of a certain density range Martin and Ames showed that a particular rotor combined with a specific gradient produced nearly constant sedimentation rates<sup>4</sup>. The sedimentation coefficient of the unknown components could then be determined from the ratio of mobilities relative to an internal standard. Application of this linear extrapolation to different gradients, rotors and particles is normally not justified and would lead to grossly erroneous *S* values. A general method that gives fairly accurate values and is applicable to all particles of equal density and osmotic behaviour, and to any given rotor type and gradient, makes use of an experimentally determined, usually non-linear calibration curve<sup>6-8</sup>. Although this method requires a valid internal standard of known sedimentation coefficient, it is simpler and more rapid than mathematical integration by computer. Furthermore, unlike the mathematical methods, it is not dependent on the validity of whatever assumptions are made about the hydrodynamic behaviour of the molecules which are being investigated.

In addition to causing these analytical and computational difficulties, the decrease in rates of sedimentation observed in the commonly used gradients also seriously limits the resolving power of the method, for the particles tend to pile up towards the bottom of the tube. In most gradients the separation between two components fails to improve with sedimentation beyond a third to a half of the entire length of the tube. Thus the potential resolution offered by the available length of path remains largely unexploited.

A very different approach expected to eliminate most of these difficulties and shortcomings would be the use of gradients that are specially designed to keep the sedimentation rate of particles of a given density constant throughout the length of the tube. This would be accomplished by constructing gradients in which the linear increase in the driving force acting on a particle with increasing distance from the centre of the rotor is exactly compensated by an equivalent increase in the opposing forces of viscous drag and buoyancy.

In this communication I derive the theory of constant velocity sedimentation in sucrose gradients and document its validity by experimental measurements with ribosomes and RNA. An extremely simple method for preparing isokinetic gradients will be described which makes it possible to determine sedimentation coefficients in the preparative ultracentrifuge with ease, speed and accuracy.

To derive the shape of the isokinetic sucrose gradient, I use the same basic equations used before<sup>3-5</sup> to describe the sedimentation behaviour of particles in arbitrary sucrose gradients. These equations have been derived with some restrictive assumptions: the particles should be (a) spherical and (b) unaffected by changes in the suspending medium with respect to shape and density. The first condition, derived from Stoke's law for spherical particles, implies that the frictional resistance is proportional to the viscosity of the medium. This condition is probably not very stringent, for non-spherical particles would not be expected to deviate much from this relationship. The second condition could be more critical; while it is certainly not fulfilled by osmotic particles, it is difficult to predict to what extent changing sucrose concentrations might influence the overall shape of various non-osmotic macromolecules. By comparison with spherical latex particles which are unaffected by the medium, however, this question can be put to a rigorous test.

At a given temperature, the rate at which a particle of density  $D_p$  sediments through a medium of density  $D_m$  and viscosity  $\eta_m$  in a centrifugation field  $\omega^2 r$  is

$$dr/dt = S_{20,w}(\omega^2/A)r(D_p - D_m)/\eta_m \quad (1)$$

The term  $A = (D_p - D_{20,w})/\eta_{20,w}$  has been introduced to normalize measurements with respect to temperature and viscosity of water at 20° C; it is constant for any given value of  $D_p$ . In a sucrose gradient both  $\eta_m$  and  $D_m$  are functions of the concentration of the sucrose and thus of the distance  $r$  of the medium from the centre of the rotor; within the concentration range being considered, they are approximated by the expressions<sup>9,10</sup>

$$\eta_m(r) = \exp\left(\alpha \frac{C}{100 - C} + \beta C^2 + \gamma\right) \quad (2)$$

in which  $\alpha$  and  $\beta$  are temperature dependent constants,  $C$  the sucrose concentration in per cent (w/v) and  $\gamma$  the natural logarithm of the viscosity of water at  $T^\circ\text{C}$ ; and

$$D_m(r) = \mu(C - C_t) + D_t \quad (3)$$

where  $\mu$  is a constant.

The initial conditions are defined by the distance  $r_t$  of the meniscus from the centre of rotation, by the choice of the sucrose concentration  $C_t$  at the top of the gradient and the corresponding values of  $D_t$ ,  $\eta_t$ .

If we now demand that the particles move at constant velocity, the second term in equation (1) must remain constant with increasing  $r$ , that is, as the particles increase their distance from the top layer

$$dr/dt = r(D_p - D_m)/\eta_m = \text{constant}$$

or

$$\eta_m(r)/\eta_t = r(D_p - D_m(r))/r_t(D_p - D_t) \quad (4)$$

Inserting into (4) the viscosity and density functions (2) and (3) we obtain the desired constant velocity sedimentation gradient

$$\exp\left(\alpha \frac{C}{100 - C} + \beta C^2 + \gamma\right) = m r [D_p - \mu(C - C_t) - D_t] \quad (5)$$

in which

$$m = \eta_t/r_t(D_p - D_t)$$

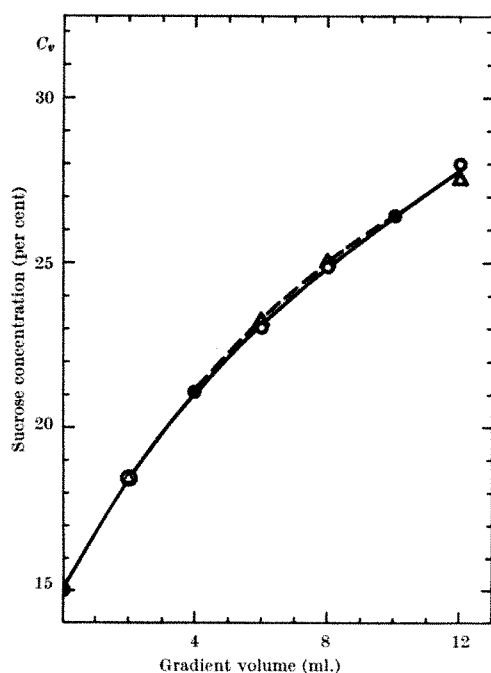


Fig. 1. Shape of the isokinetic gradient. Solid line represents theoretical curve, broken line indicates approximation with mixing device in Fig. 2. To find the parameters ( $V_m$ ,  $C_R$ ) giving satisfactory approximation, three points were selected on the theoretical curve corresponding to the top concentration  $C_t$ , an intermediate concentration  $C_i$  and the bottom concentration  $C_b$ . The values of  $V_m$  and  $C_R$  of that curve of equation (6) which is fixed by these three points were then found. This produced the transcendental equation

$$(C_v - C_t) e^{-V/V_m} (1 - e^{-V/V_m})^{-1} = (C_b - C_t) e^{-V_b/V_m} (1 - e^{-V_b/V_m})^{-1}$$

which is solved for  $V_m$  by graphical methods or computer approximation.

$\triangle$  ---  $\triangle$ ,  $C_v = C_R - (C_R - C_t) e^{-V/V_m}$ ;  $\circ$  ---  $\circ$ ,  $C = f(r)$  from equation (5).

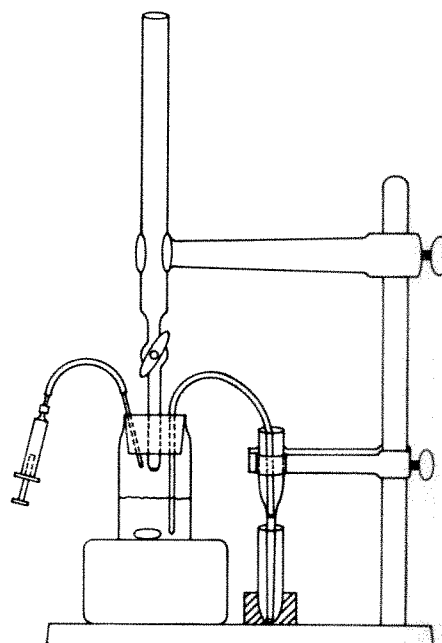


Fig. 2. Preparation of the isokinetic gradients. Convex exponential gradients according to equation (6) are conveniently prepared with a burette and mixing chamber<sup>11</sup>. The burette contains a sucrose solution of a concentration  $C_R$  and is connected through an air-tight rubber stopper to a mixing flask with magnetic stirring bar. A thin piece of polyvinyl tubing connects the mixing flask with the gradient tube. A small syringe is attached to the mixing chamber through a short piece of tubing to adjust in a reproducible way the amount of pressure at the beginning. The mixing flask is now filled with sucrose solution of a concentration  $C_t$  equal to that desired at the top of the gradient. The mixing volume  $V_m$  is determined by the type of isokinetic gradient to be chosen (Table 1) and remains constant. The procedure is started by increasing the air pressure in the mixing chamber with the syringe until the solution has risen to a fixed point in the ascending portion of the outlet tubing. The tubing between the burette and mixing flask is then clamped off. The stopcock of the burette is opened to add the heavy sucrose solution with rapid stirring at a rate of about 1.0 ml/min. As the volume  $V$  added from the burette increases, a corresponding volume of the mixture is displaced into the gradient tube. The total volume added to the centrifuge tubes of the IEC rotor 283 used in these experiments was 12.0 ml. To prevent turbulence in the gradient tube, the end of the outlet tubing from the mixing chamber is inserted into a capillary pipette whose tip must touch the bottom of the gradient tube, so that the light sucrose introduced first is allowed to float to the top, as solution of increasing density is fed in. At the end of the procedure the capillary pipette is carefully pulled out through the gradient. The gradients, prepared at room temperature, were precooled to  $2^\circ\text{C}$  by storing in the cold room before use.

This equation is transcendental, and cannot be solved explicitly for  $C$ . The function  $C=f(r)$  may be evaluated by computer or graphical methods. The graphically determined solutions of  $C$  for various values of  $r$  have been plotted against  $r$  (or the equivalent volume units of the gradient tube) in Fig. 1. In this example a particle density of  $D_p=1.51$  and a top concentration of 15 per cent have been selected. Evidently, different isokinetic curves will be obtained for other top concentrations, particle densities and rotor dimensions.

For the experimental application of the principle of constant velocity sedimentation, a method of preparing the calculated gradients is needed. The constant velocity gradient in Fig. 1 exhibits a convex curvature. Convex exponential gradients in which the sucrose concentration  $C_v$  increases as a function of the volume  $V$  according to

$$C_v = C_R - (C_R - C_t) e^{-V/V_m} \quad (6)$$

can be generated by a simple gradient maker consisting of burette and mixing flask (Fig. 2). By a suitable choice of the mixing volume  $V_m$  and the concentration of the sucrose solution  $C_R$  in the reservoir, one of the curves described by equation (6) will (for a given  $C_t$ ) very closely approach the theoretical constant velocity sedimentation gradient of equation (5). By a simple mathematical procedure (outlined in the legend to Fig. 1) the experimental gradient can easily be made to match the theoretical curve within 1 per cent or less as illustrated by way of an actual numerical example (Fig. 1).



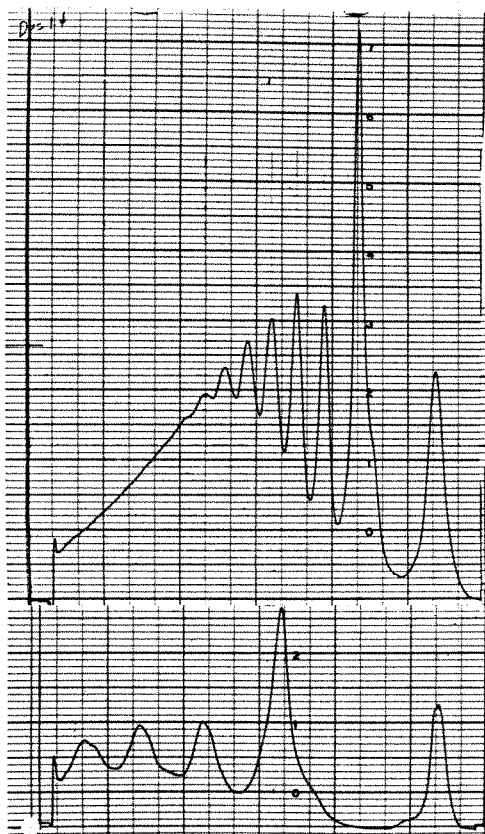


Fig. 3. Sedimentation patterns of mouse liver polysomes in isokinetic gradient. Input: 4  $A_{260}$  units in 0.2 ml. Recorded after 1 h (top) and 2 h (bottom) centrifugation at 40k r.p.m. and 2° C. Gradient calculated for  $D_p = 1.4$  and  $C_T = 15$  per cent sucrose. The strongest peak corresponds to the 80S monomer species. Centrifugations were carried out with the 6 × 14 ml. swinging bucket rotor No. 283 in a B-60 ultracentrifuge of the International Equipment Company. The centrifuge was set to the desired top speed and decelerated with the automatic brake on. The gradient tubes were punctured and the absorbancy of the effluent at 260 mμ was scanned and recorded automatically\* with the IEC-Gilford high resolution gradient analysing system. The design and components of this system, which is capable of resolving particles differing by less than 7 per cent in sedimentation coefficient, will be described in detail later. Sedimentation rates were determined from the strip chart recordings by measuring the distance between the peak centre and the top of the gradient. To indicate the top of the gradient, 0.2 ml. of a 0.2 molar sucrose solution containing 0.5 mg/ml. of RNA as an absorbancy marker was layered over the gradient immediately before collection (peak on extreme right).

Thus for a given top concentration, temperature and rotor only the particle density  $D_p$  is needed to select the proper mixing volume  $V_m$  and reservoir concentration  $C_R$  for preparing the desired isokinetic gradient. I have tabulated the parameters ( $V_m, C_R$ ) covering the useful range of particle densities from 1.2 to 1.8 (Table 1). These data have been calculated for a top concentration of 15 per cent, a temperature of 2° C and the 6 × 14 ml. swinging bucket rotors SB-206 and 283 of the International Equipment Company. The results of a computer programme giving the pertinent data for other top concentrations, temperatures and the variety of commercially available rotors are to be published.

Table 1. PARAMETERS FOR PREPARING CONSTANT VELOCITY SUCROSE GRADIENTS IN SWINGING BUCKET TUBES OF IEC ROTOR 283 AT 2° C\*

$D_p$ (g/ml.)	$V_m$ (ml.)	$C_R$ Per cent Molarity	$C_T$ Per cent Molarity	$C_b$ † Per cent Molarity
1.2	10.6	28.3	0.83	15
1.3	9.4	29.9	0.88	15
1.4	9.1	31.0	0.91	15
1.5	9.0	31.8	0.93	15
1.6	9.0	32.4	0.95	15
1.7	9.0	32.8	0.96	15
1.8	9.0	33.2	0.97	15

\* The volume added from the burette to the tubes is 12.0 ml.

† Concentration near the bottom of the gradient.

The most obvious advantage of the constant velocity sedimentation method is the ease with which it permits the determination of sedimentation constants. Thus the  $S$  value of an unknown macromolecule is immediately obtained from its mobility ratio with respect to an internal standard of equal density after a suitable period of centrifugation

$$S_{20,w}(\text{unknown})/S_{20,w}(\text{standard}) = R_U/R_S \quad (7)$$

According to equation (1) the method lends itself equally well to the determination of absolute sedimentation values. Because  $dr/dt$  is constant, the sedimentation coefficient is directly obtained by measuring the distance  $R$  the object has travelled from the start during the centrifugation time  $t$  at an average angular velocity  $\omega^2$

$$S_{20,w} = (R/t)(A/K)\omega^{-2} \quad (8)$$

in which

$$K = (D_p - D_t)r_t/\eta_t$$

The validity of the concept was tested in experiments illustrated in Figs. 3 and 4. Polysomes from mouse liver were centrifuged for 1.0 and 2.0 h at 2° C and 40k r.p.m. in the SB-283 rotor of a B-60 International ultracentrifuge. Mammalian ribosomes have been reported to have a density of 1.5 (ref. 12), and so constant velocity sedimentation gradients corresponding to  $D_p = 1.4, 1.5$  and 1.6 were used. The strip chart recordings of the sedimentation patterns in Fig. 3 show that the resolution is excellent and that with increasing time of centrifugation the separation between peaks continues to increase throughout the whole length of the tube, in contrast to the piling up of the components toward the bottom observed in conventional gradients.

According to theory, a plot of the distance travelled by the various polysome species against  $S \times t$  should give a straight line, if the sedimentation rate is constant throughout the gradient. The results in Fig. 4 are in excellent agreement with this prediction. To compute the  $S \times t$  values, an average of published  $S$  values<sup>6,12,14</sup> for mammalian polysomes (80S)<sub>n</sub>,  $n = 1-8$ , were used and the time factor was corrected for the contribution of acceleration and deceleration. There was no significant deviation from linearity in gradients calculated for  $D_p =$

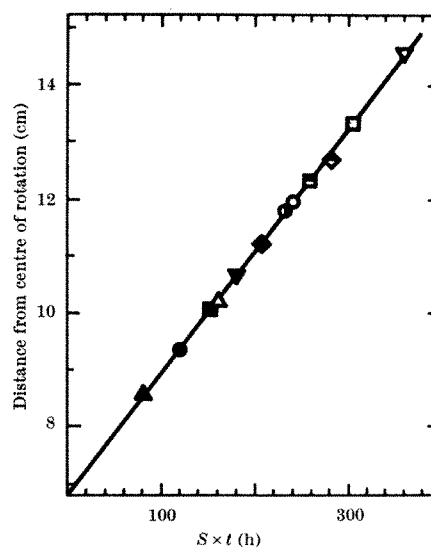


Fig. 4. Test for constancy of polysome sedimentation rates in isokinetic gradient. Mobilities of polysome peaks determined from recordings in Fig. 3 are plotted against  $S \times t$ . Monomer to tetramer: 80S  $\Delta$ , 119S  $\circ$ , 152S  $\square$ , 180S  $\nabla$  (solid symbols = 1 h, open symbols = 2 h); pentamer to octamer: 207S  $\blacklozenge$ , 230S  $\circ$ , 258S  $\blacksquare$ , 281S  $\blacklozenge$  (all 1 h).

1.4 and 1.6. As evident from Table 1, the precision of this parameter is not very critical, for the maximal concentration difference between these two gradients (at the bottom) is only 4 per cent.

An example illustrating the value of the method for the determination of absolute sedimentation coefficients is summarized in Table 2. The results obtained for repre-

Table 2. DETERMINATION OF  $S_{20,w}$  VALUES FOR RIBOSOMAL RNA FROM VARIOUS SOURCES

rRNA	Rat liver		Bean, cytoplasm		Bean, chloroplast	
	a	b	a	b	a	b
R (cm)	4.63	2.73	3.81	2.47	3.35	2.53
$S_{20,w}$	30.2	17.8	24.9	16.1	21.8	16.5

The  $S_{20,w}$  values were determined according to equation (8) using the following numerical values:  $t = 3.96 \times 10^4$  sec (11 h),  $\omega^2 = 1.75 \times 10^7$  (40k r.p.m.),  $r_1 = 6.8$  cm,  $D\rho = 1.7$ ,  $D_1 = 1.063$ ,  $D_{20,w} = 1.00$ ,  $\eta_1 = 2.83$ ,  $\eta_{20,w} = 1.00$ .

sentatives of the three known classes of ribosomal RNA<sup>7</sup> agree well with the values determined previously by the internal standard method in sucrose gradients<sup>7</sup> or by moving boundary analyses in the analytical ultracentrifuge<sup>15-17</sup>. It should be pointed out that the accuracy depends most critically on the precision of measuring  $\omega^2$  and good temperature control.

The method of constant velocity sedimentation introduced here describes a new type of gradient designed to keep the forces acting on the sedimenting particles constant over the entire path length. The theoretical shape of this gradient is specific for a given rotor, particle density and temperature, and can be approximated by a simple device consisting of burette and mixing flask. Once the appropriate parameters have been determined, isokinetic gradients can be prepared routinely with no more effort than conventional gradients. The new method greatly facilitates the quantitative evaluation of sedimentation behaviour and permits better separation of components with similar sedimentation rates, for differences in mobilities continue to increase at a linear rate throughout the whole length of the tube.

Although the gradients described here are strictly isokinetic only for spherical objects the density and shape of which are not affected by the change in concentration of

sucrose, no significant deviations from constant velocity were detected in sedimentation tests with ribosomes and ribosomal RNA. Furthermore, a comparison of curves corresponding to isokinetic gradients calculated for various densities reveals that relatively large changes in particle densities would have to occur during sedimentation to cause a detectable deviation from the constant velocity path. These observations suggest that isokinetic sedimentation would be of general usefulness for the characterization of a wide variety of macromolecules. Replacement of the many individual and largely arbitrary gradients now in use by the adoption of this method would eliminate much undesirable variation and provide a common standard for the comparison and interpretation of results obtained in different laboratories.

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## Diversity of RNA Components in Green Plant Tissues

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Gel electrophoresis of ribosomal RNA from green plant tissues provides evidence consistent with the suggestion that chloroplasts have evolved from symbiotic blue-green algae, while the plant cytoplasmic RNA is distinct from both the bacterial and mammalian.

THE presence of two distinct ribosome species, approximately 80S and 70S, has been demonstrated in leaf tissue from spinach<sup>1</sup>, Chinese cabbage<sup>2</sup>, peas<sup>3</sup>, tobacco<sup>4</sup>, and beans<sup>5</sup>. The 80S species, similar in size to animal ribosomes, is the normal cytoplasmic ribosome whereas the 70S, similar to bacterial ribosomes, is restricted to the chloroplast. These observations suggest that the ribosomal RNAs of the chloroplast may be smaller than those of the cytoplasm. Detailed information has not been obtained by conventional fractionation procedures, and so we have investigated this problem using the high resolution of RNA fractionation achieved by gel electrophoresis.

Previous work using fractionation on methylated-albumin, kieselguhr columns showed that total RNA prepared from green radish cotyledons was very different

from the RNA of root tips or other non-green tissues. Three or four high molecular weight components instead of the normal two ribosomal RNAs were partially resolved, and four low molecular weight components were present compared with the normal two from non-green tissues (our unpublished results). Although this method of fractionation only partially separates the high molecular weight RNAs it has been successfully used to investigate the cellular origin of the low molecular weight components. Two of these components were unique to the chloroplast, one to the cytoplasm, while the fourth, the transfer-RNA, was present in both<sup>6</sup>.

Sucrose density centrifugation has been used in several studies on the RNA of tissues containing chloroplasts. Chloroplasts from *Euglena* contained two RNA components, 19S and 14S, whereas the cytoplasm contained

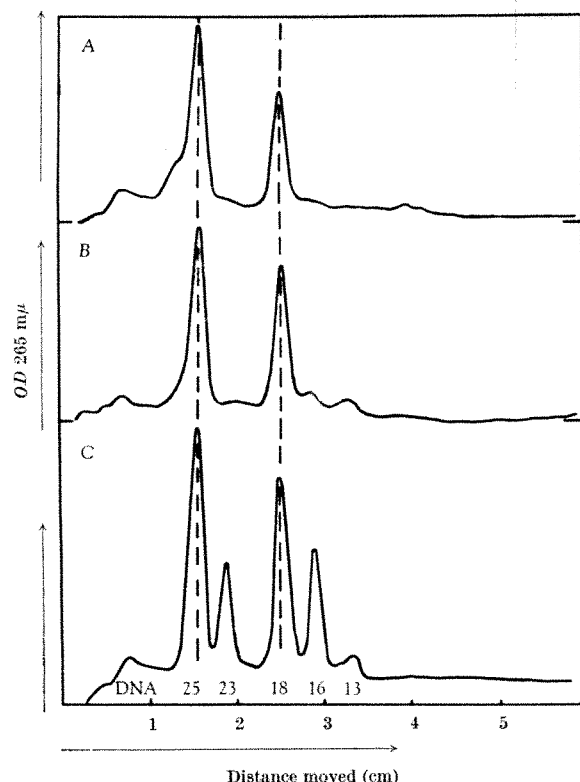


Fig. 1. Gel fractionation of nucleic acids from radish. Fifty radish seedlings, grown in the light for 6 days, were separated into roots (A), hypocotyls (B) and cotyledons (C), and total nucleic acids were prepared by method A. See Fig. 6 for the assignment of *S* values.

only the 19S component<sup>7</sup>. These sedimentation patterns, however, showed evidence of degradation. The chloroplast and cytoplasmic RNAs from *Euglena* had very different base compositions<sup>7</sup>. Chloroplast RNA from *Acetabularia* had two components similar in size to non-chloroplast RNA (ref. 8). RNA prepared from lettuce chloroplasts was not distinguished from the cytoplasmic ribosomal RNA by sucrose gradient centrifugation and only very small differences in composition were observed<sup>9</sup>. Sucrose gradient analysis of RNA prepared from chloroplasts of spinach, tobacco, radish, pea and tomato leaves showed only a single 16S component, whereas RNA from the whole leaf and from cytoplasmic ribosomes was resolved into 25S and 16S components<sup>10</sup>. Again, no significant difference in base composition was noted between chloroplast and cytoplasmic RNAs<sup>10</sup>. Stutz and Noll<sup>5</sup> have, however, been able to separate three high molecular weight RNA components from a mixture of cytoplasmic and chloroplast ribosomes from bean leaves. They suggested sedimentation constants of 25 and 16 for the cytoplasmic ribosomal RNAs, and 23 and 16 for the corresponding components from the chloroplast. It is interesting to note that in all these investigations the chloroplast preparations contained as much, or more, 16S than 23S RNA, whereas cytoplasmic ribosomal RNAs are present with a ratio of heavy to light components of approximately 2:1.

In the present investigation the very high resolution of RNA fractionation achieved by polyacrylamide gel electrophoresis<sup>11</sup> has been used to investigate this problem in relation to the high molecular weight components. Gels were prepared (in 7.5 × 0.63 cm 'Perspex' tubes) containing 2.4 per cent acrylamide (recrystallized from chloroform) and 0.12 per cent bisacrylamide (recrystallized from ace-

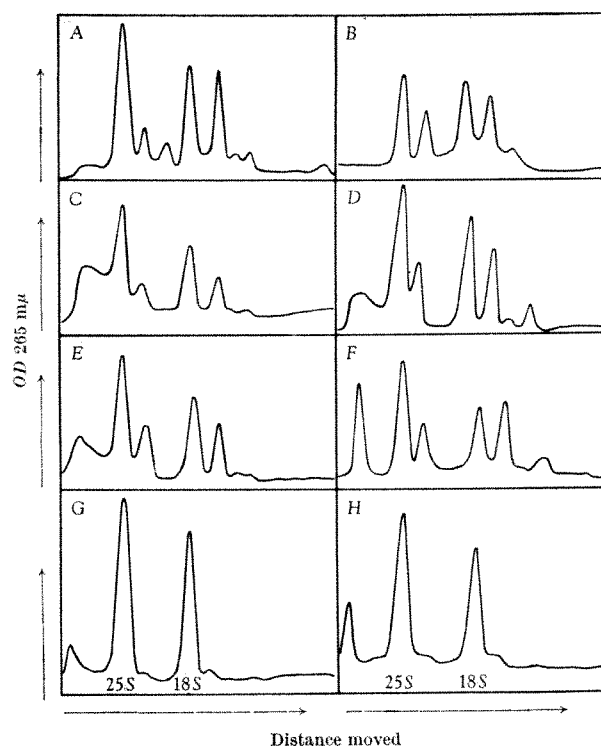


Fig. 2. Comparison of nucleic acids from green and non-green tissues. A, French bean leaf, 8 days (method A); B, lettuce leaf (method B); C, cocklebur leaf (method A); D, corn leaf (method A); E, pea leaf (method A); F, barley leaf (method B); G, pea root tips (method A); H, artichoke tuber tissue (method B).

tone). The buffer contained 36 mmolar *tris* ('Trisma', Sigma), 30 mmolar sodium dihydrogen phosphate and 1 mmolar ethylenediamine tetra-acetic acid. Sodium lauryl sulphate (B.D.H. 'specially purified' grade) was added to the buffer compartments at a concentration of 0.2 per cent, and allowed to enter the gels during pre-electrophoresis at 6.5 V/cm, 5 m.amp for each tube at room temperature (20° C) for 20–40 min. The gel tubes were completely immersed in the buffer for cooling,

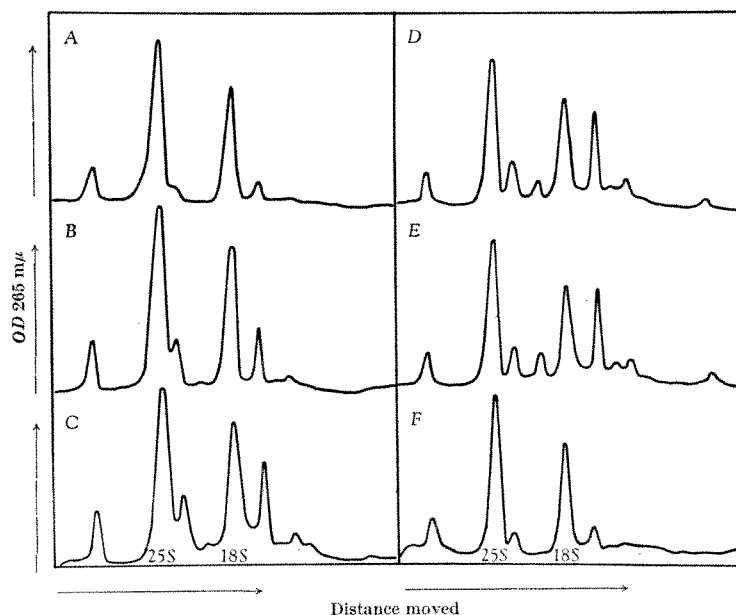


Fig. 3. Changes in nucleic acids during development of the French bean leaf. Nucleic acids were prepared by method B from the first pair of leaves from 4 (A), 5 (B), 6 (C), 8 (D) and 10 day old (E) light grown seedlings. Nucleic acids were also extracted from leaves of 7-day-old dark grown seedlings (F).

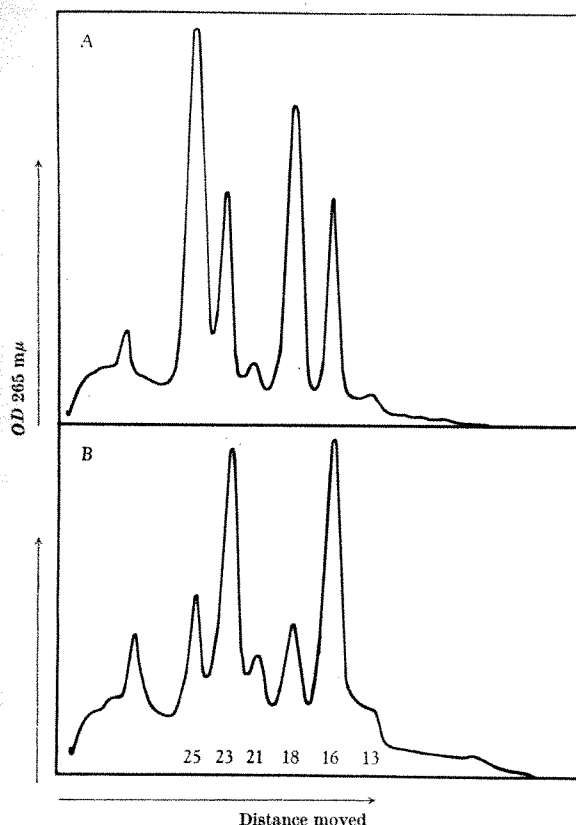


Fig. 4. Chloroplast nucleic acids. Nucleic acids were prepared from the whole leaf (A) and from the chloroplast fraction (B). Leaves from 8-day-old French bean seedlings were ground by pestle and mortar at 0° C in a medium containing 0.4 molar mannitol, 50 mmolar *tris*, 50 mmolar potassium chloride, 5 mmolar magnesium acetate, 0.1 per cent bovine serum albumin and 0.1 mg/ml. of Cleland reagent, pH 7.8 at 0° C. The homogenate was filtered through cheese cloth and centrifuged at 100g for 4 min. The supernatant was centrifuged at 1,000g for 5 min to pellet the chloroplasts. The chloroplasts were washed by resuspending in the medium and collected by centrifugation at 1,000g for 5 min. The pellet was suspended in a medium containing 1 per cent tri-isopropyl-naphthalene sulphonate, 6 per cent *p*-amino-salicylate and 1 per cent sodium chloride, and nucleic acid was extracted with phenol, as described in the text.

RNA was dissolved in the electrophoresis buffer supplemented with 0.2 per cent sodium lauryl sulphate and 5 per cent sucrose at a concentration of about 1 mg/ml., and 25  $\mu$ l. layered on the gel. Electrophoresis was continued for 2–3 h, by which time the soluble RNA had run off the gel. The gels were then removed from the tubes, rinsed in water for 5–30 min and scanned at 265 m $\mu$  using the Joyce Loebel 'Chromoscan'. The background optical density of the gels at this wavelength was 0.20.

Two main methods were used to prepare total nucleic acid from plant tissue. In method A the fresh tissue (approximately 1 g) was homogenized at 0° C for 10 sec at top speed (45,000 r.p.m.) in a Virtis '45' homogenizer in 10 mmolar *tris*, pH 7.4, containing 1 per cent sodium lauryl sulphate and 12 mg/ml. of bentonite plus an equal volume of phenol containing 0.1 per cent 8-hydroxyquinoline and 10 per cent *m*-cresol. After centrifugation the lower phenol layer was removed to leave the interphase and aqueous layer to which sodium chloride (final concentration, 0.5 molar) and an equal volume of phenol mixture were added. After mixing and centrifuging, the top aqueous layer was removed and extracted once more with phenol. Two volumes of ethanol were added to precipitate the nucleic acids from the final aqueous layer. The precipitate was dissolved in 0.15 molar sodium acetate, pH 6.0, containing 0.5 per cent sodium lauryl sulphate, and reprecipitated with two volumes of ethanol. After centrifugation the pellet was washed with cold 80 per cent ethanol, partially dried and dissolved in the electro-

phoresis buffer. In method B (modified from Kirby<sup>12</sup>) the tissue was homogenized in the Virtis as described here in 10 mmolar *tris*, pH 7.6, containing 50 mmolar sodium chloride and 0.5 per cent naphthalene 1:5 disulphonate. This medium inhibits nucleases while preventing the release and shearing of DNA. Tri-isopropyl-naphthalene sulphonate and *p*-amino-salicylate were then added to final concentrations of 1 per cent and 6 per cent respectively. An equal volume of the phenol mixture was added and thereafter the procedure was as described in method A. The two methods of preparation gave identical RNA patterns on gel electrophoresis, although the DNA was considerably sheared by method A, and gave a much broader peak.

Fractionation of total nucleic acid prepared from radish root and hypocotyl tissues showed the normal distribution of two ribosomal RNAs (Fig. 1A, B). The green cotyledons from the same radish seedlings contained, however, two additional major and one minor RNA component (Fig. 1C). These components have been assigned sedimentation constants of 25, 23, 18, 16 and 13 as described below (see Fig. 6D). Total nucleic acid has been extracted from radish cotyledons by the two methods described, and by several modifications of these procedures including milder homogenization with pestle and mortar and with 'Teflon' in glass. All extraction methods gave identical results for the electrophoretic pattern of the RNA components. The additional RNA components, 23S and 16S, were present in all of the green material examined (Fig. 2A, B, C, D, E, F) constituting about 30 per cent of the high molecular weight RNA, and were absent from non-green tissues (Fig. 2G, H). The minor components present in non-green tissues have not been investigated, but they may be plastid or perhaps mitochondrial RNA.

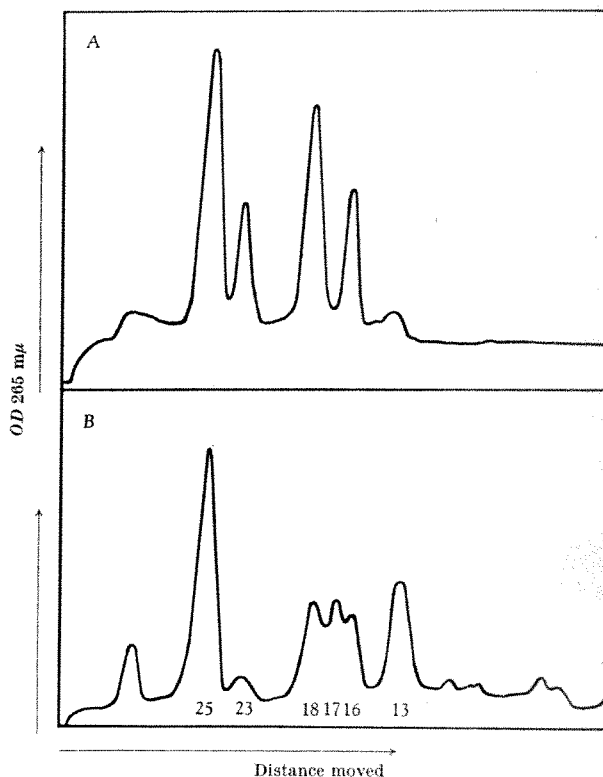


Fig. 5. Degradation of nucleic acids during cell fractionation. Nucleic acids were prepared from 6-day-old radish cotyledons (A) and from the same tissue after homogenization and centrifugation (B). The tissue was homogenized in the Virtis for 10 sec at top speed in the medium described by Stutz and Noll<sup>5</sup>. The homogenate was centrifuged at 200,000g for 90 min and nucleic acids were prepared from the pellet by method A.



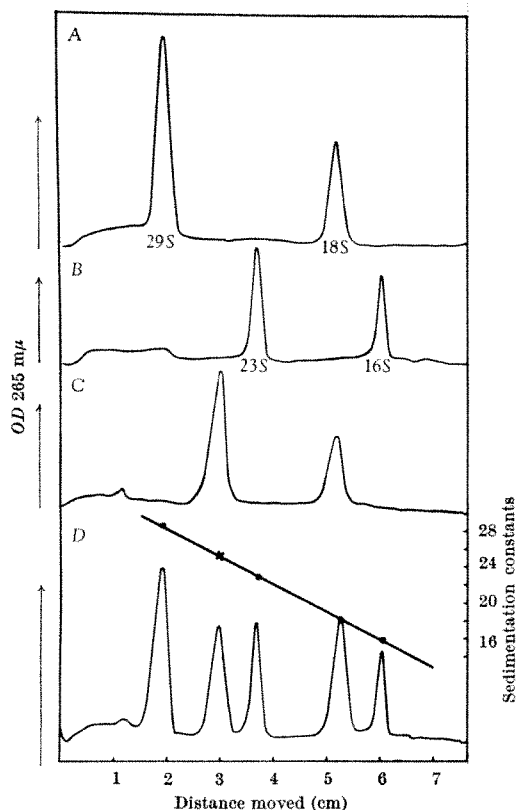


Fig. 6. Size of plant cytoplasmic RNAs compared with mammalian and bacterial ribosomal RNAs. A, HeLa cell RNA; B, *E. coli* RNA; C, pea root tip RNA; D, mixture of HeLa, *E. coli* and pea root tip RNAs. Ten centimetre gels were used, and electrophoresis was continued for 4 h to obtain greater separation. The electrophoretic mobilities of the RNA components were related to sedimentation constants, assuming values of 29/18 and 23/16 for the HeLa and *E. coli* RNAs respectively (D).

The relative proportions of the components varied with the developmental stage of the tissue. Fig. 3 shows the changes in total nucleic acid during development of the French bean leaf. The 23S and 16S components increased relative to the 25S and 18S RNAs during growth and maturation of the leaf, increasing from 10 per cent to 30 per cent of the total RNA. In addition, minor components of 21S, 15S and 13S appeared during development. These changes were not seen in leaves of plants grown in the dark (Fig. 3F). Similar developmental patterns have been observed with radish cotyledons and with cocklebur leaves.

The presence of these additional RNA components in green compared with non-green tissues suggests that they are associated with the chloroplast. RNA prepared from chloroplasts isolated from bean leaves showed an eight-fold enrichment of the 23S, 16S and minor components (Fig. 4). Considerable degradation of the RNA can occur during the separation of cell organelles by homogenization and differential centrifugation. Such degradation varied markedly with different tissues. So far it has been impossible to prepare RNA from isolated radish chloroplasts without loss of most of the 23S component. Such cases of degradation were usually accompanied by a large increase in 13S material (Fig. 5), and there is doubt therefore about the origin of this 13S component. Absence of a 23S component has also been noted with chloroplasts from spinach, tobacco, radish, pea and tomato leaves<sup>10</sup>. In the French bean, however, the relative proportions of the 23S, 21S, 16S, 15S and 13S components present in the total RNA preparation were maintained during isolation of the chloroplasts by a variety of methods. Consequently these components are thought to be true constituents of the bean chloroplast.

A peculiarity of the chloroplast is that there is more 16S than 23S RNA. This occurred in total nucleic acid preparations and with RNA from isolated chloroplasts, although the ratio of cytoplasmic 25S to 18S RNA was approximately two. This molar excess of 16S to 23S RNA has been observed in almost all chloroplast RNA studies<sup>5,7-10</sup>.

Gradual breakdown of the cytoplasmic RNA also occurred during cell fractionation. The first indication of degradation with leaf RNA was a broadening and loss of the 18S component accompanied by the appearance of a 17S peak (Fig. 5). The occurrence of such degradation, which could be missed by sucrose gradient analysis, was easily recognized because of the very high resolution of RNA fractionation by this electrophoretic method.

The sizes of the RNA components of the plants were compared with mammalian and bacterial RNAs (Figs. 6 and 7). A mixture of HeLa, pea root tip and *E. coli* RNA was fractionated (Fig. 6). The heavy ribosomal RNA from the plant cytoplasm (Fig. 6C) moved at a rate intermediate between those of the mammalian and bacterial components, while the lighter component moved at a rate similar to that from the mammalian ribosome. For convenience of comparison with other work we have related the electrophoretic mobilities to sedimentation constants, assuming mammalian and bacterial ribosomal RNAs to be 29S/18S and 23S/16S respectively. For this range of molecular size, and with the concentration of gel used, the sedimentation constants showed an inverse linear relationship to their mobilities in the gel (Fig. 6D). From this relationship the constants for the plant cytoplasmic components were found to be 25 and 18. The RNAs from the chloroplast had mobilities similar to the ribosomal RNAs of bacteria, namely, 23S and 16S (Fig. 7A, B). The resolution achieved in these conditions of electrophoresis (Fig. 6D) would show differences of 0.2S.

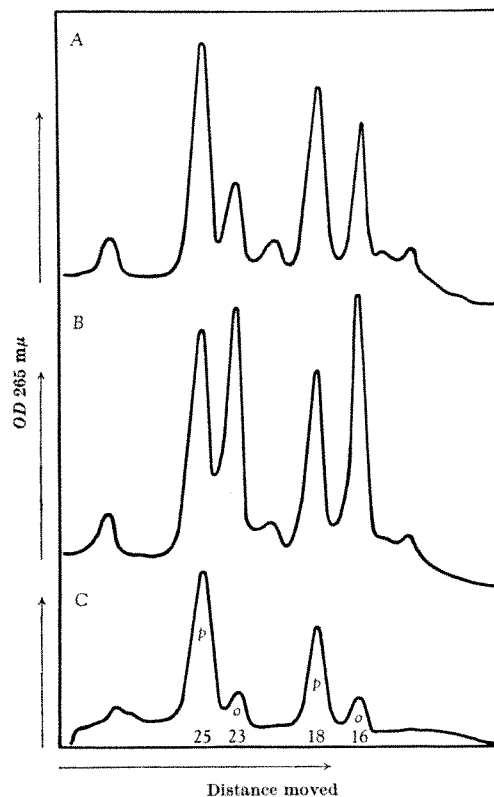


Fig. 7. Size of plant chloroplast RNAs compared with bacterial and blue green algal RNAs. A, Nucleic acids from 8-day-old French bean leaf; B, French bean leaf plus *E. coli* RNAs; C, pea root tip (p) plus a species of *Oscillatoria* (o) RNAs.

These results support the contention that three distinct classes of ribosomes exist: 80S in mammalian cells, containing 29S and 18S RNA; 80S in the plant cytoplasm, containing 25S and 18S RNA; and 70S in bacteria and plant chloroplasts, containing 23S and 16S RNA.

Our data contrast in part with those of Stutz and Noll<sup>5</sup>, who concluded that the lighter plant cytoplasmic component was smaller than the mammalian 18S RNA and similar to the bacterial and chloroplast 16S component. The resolution obtained by zone velocity centrifugation<sup>5</sup>, however, was not sufficient to show with any certainty whether degradation, such as the production of 17S from 18S (Fig. 5), had occurred during the long time involved in the preparation of the ribosomes. The discrepancy may also arise from the different methods used, for it is likely that the secondary structure of the molecule affects electrophoretic mobility and sedimentation in different ways. Such behaviour has been noted with tobacco mosaic virus RNA, which, with a sedimentation constant of 26–28, has a very low mobility on electrophoresis compared with 28S or 25S ribosomal RNA. Electrophoretic mobility on the gel is more directly related to log molecular weight than is the sedimentation constant<sup>13</sup>. We conclude that both components of plant cytoplasmic ribosomal RNA are distinct from, and larger than, bacterial RNAs.

Similarities in structure between the blue green algal cell and the chloroplast have led to the suggestion that this cell organelle may have evolved from the capture of such a lower organism. It has been demonstrated that blue green algae contain 70S ribosomes<sup>14</sup> similar to bacteria and chloroplasts. We have examined the RNA from a species of the blue green alga *Oscillatoria* and found it to be the same size as bacterial and chloroplast RNAs (Fig. 7C). This lends further support to the evolutionary hypothesis.

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## Effect of Anti-rat Lymphocyte Antibody on Humoral Antibody Formation

by

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Horse anti-rat lymphocyte IgG globulin suppresses and/or delays the primary response to alum-precipitated bovine serum albumin in hooded rats. This material does not have a marked effect on the secondary response of sensitized animals.

RECENT work has shown that anti-lymphocytic sera (ALS) are effective immunosuppressive agents prolonging the survival of skin homografts in rats<sup>1,2,15</sup> and mice<sup>3–5</sup> and renal homotransplants in dogs<sup>6–8</sup> and humans<sup>9</sup>. In addition, anti-lymphocytic antibody has been shown to suppress significantly the primary humoral antibody response of both rats<sup>9</sup> and mice<sup>3</sup> to sheep erythrocytes and possibly to exert a slight effect on the secondary response<sup>3,9</sup>. The present report is a detailed study of the effect of anti-lymphocytic IgG on the production of humoral antibody in hooded rats after primary and secondary stimulation with a further commonly used test antigen, namely alum precipitated bovine serum albumin (BSA). Throughout the investigations the humoral antibody response has been assessed by measuring the antigen binding capacity (ABC) of the rat sera by means of the sensitive ammonium sulphate method of Farr<sup>10</sup> which measures the primary interaction of antigen and antibody.

Details of the production of the horse antiserum to thoracic duct lymphocytes, and the isolation and assay of the IgG preparations used, have already been reported<sup>9</sup>. The reciprocal lymphocyte agglutination and lymphocytotoxic titres of the anti-lymphocytic IgG were both 256, while those of the normal IgG were less than 8.

The alum precipitated bovine serum albumin for injection (Armour Pharmaceutical Company, Lot Nos. C4101) was prepared as described by Pinckard, Weir and McBride<sup>11</sup>. Soluble BSA iodinated with iodine-131, for

use in the Farr procedure, was prepared by the chloramine-T method described by Hunter and Greenwood<sup>12</sup> using carrier-free iodine-131. The nitrogen content of the labelled BSA was determined by the micro-Kjeldahl procedure<sup>13</sup>. The labelled BSA preparations were used only if more than 96 per cent of the radioactivity was precipitated with 10 per cent trichloroacetic acid (TCA). The antibody content of the rat sera obtained following antigenic stimulation was assessed by the Farr procedure<sup>10</sup>. This technique was performed exactly as described by Pinckard, Weir and McBride<sup>11</sup>, using appropriate normal serum controls. The antigen binding capacities recorded in Table 1 and plotted in Figs. 1 and 2 were obtained with bovine serum albumin samples containing 0.2 µg nitrogen and are expressed in terms of the number of micrograms of nitrogen bound by 1 ml. of undiluted serum. In order to measure the relative binding affinity (a measure of the quality of the antibody produced) the antigen binding capacity was also determined using samples containing 0.02 µg of bovine serum albumin nitrogen. The latter ABC value was divided by the value obtained using 0.2 µg nitrogen and expressed as a percentage value. As the affinity of the antibody increases, the value obtained approaches 100 per cent. Throughout the investigations all samples were counted in a well-type scintillation spectrometer with a 2 in. sodium iodide crystal.

The effect of anti-lymphocytic IgG on the immune response to alum precipitated BSA was investigated in

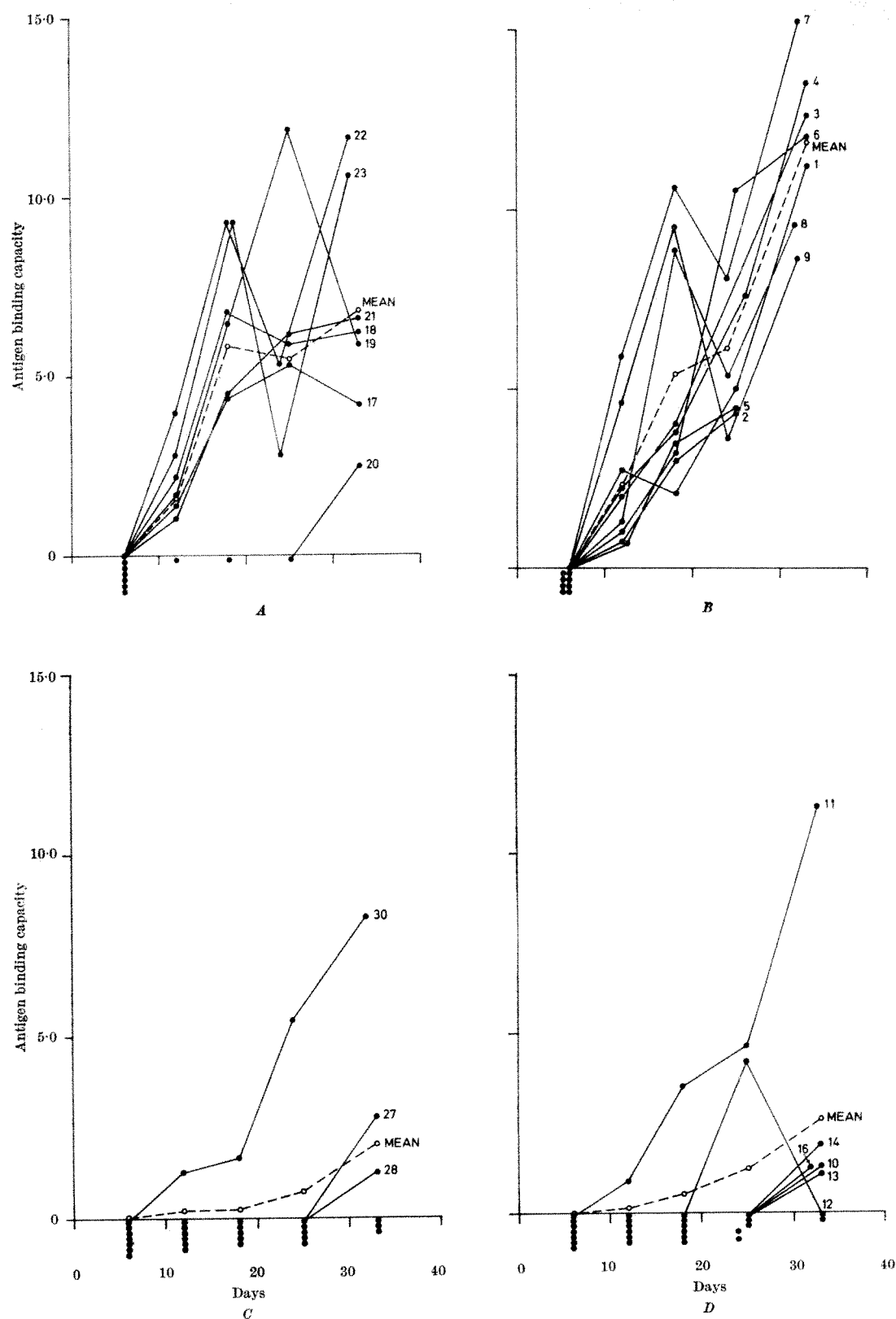


Fig. 1. Effect of normal IgG and anti-lymphocytic IgG on the primary immune response of hooded rats to alum precipitated bovine serum albumin. *A*, Experimental group 1A, male rats receiving normal IgG; *B*, experimental group 1B, female rats receiving normal IgG; *C*, experimental group 1C, male rats receiving anti-lymphocytic IgG; *D*, experimental group 1D, female rats receiving anti-lymphocytic IgG. The experimental groups were divided into male and female for ease of presentation of results. The circles under the base-line represent animals in which the immune response was so weak that ABC values could not be obtained using a bovine serum albumin test sample containing 0.2  $\mu$ g nitrogen. In some groups the number of samples varies slightly from bleed to bleed and this is due to deaths and the occasional inability to obtain sufficient sera for analysis.

hooded strain male and female rats. The animals received intraperitoneal injections of sterile normal horse IgG (experimental groups 1A and 1B—see Table 1) or anti-lymphocytic IgG (experimental groups 1C and 1D) on days -3, -2 and -1 and were injected intraperitoneally on day 0 with 5 mg of alum precipitated BSA. The animals were then bled at intervals of 6 days. After 30 days the rats in groups 1A and 1B were subdivided, half the rats in each group receiving a further course of normal IgG (experimental group 2A) and the other half anti-lymphocytic IgG (experimental group 2B). Similarly half the rats in group 1C and 1D received normal IgG (experimental group 2C) and the other half anti-lymphocytic IgG (experimental group 2D). These injections were given on days 30, 31 and 32 and the animals were rechallenged intraperitoneally with 5 mg of alum precipitated BSA on day 33. The animals were then bled at 7 day intervals. All the serum samples obtained were

stored at  $-20^{\circ}\text{C}$  until analysed. Further details on the animals used and the course of treatment are given in Table 1.

The effect of the normal and anti-lymphocytic IgG preparations on the primary response of individual hooded male and female rats is shown in Figs. 1A to 1D and summarized in Table 1. It is seen that anti-lymphocytic antibody causes a marked suppression and delay in humoral antibody formation in most of the rats studied (Figs. 1C and 1D). This suppression was also associated with a significant reduction in the peripheral blood lymphocyte count (Table 1). Anti-lymphocytic IgG did not markedly inhibit humoral antibody formation in all the rats studied, however, for two out of fourteen (that is, rat No. 30, see Fig. 1C, and rat No. 11, see Fig. 1D) showed a response of similar magnitude to that found in rats pretreated with normal IgG. It should also be noted that on day 33 all the sera from rats treated with anti-

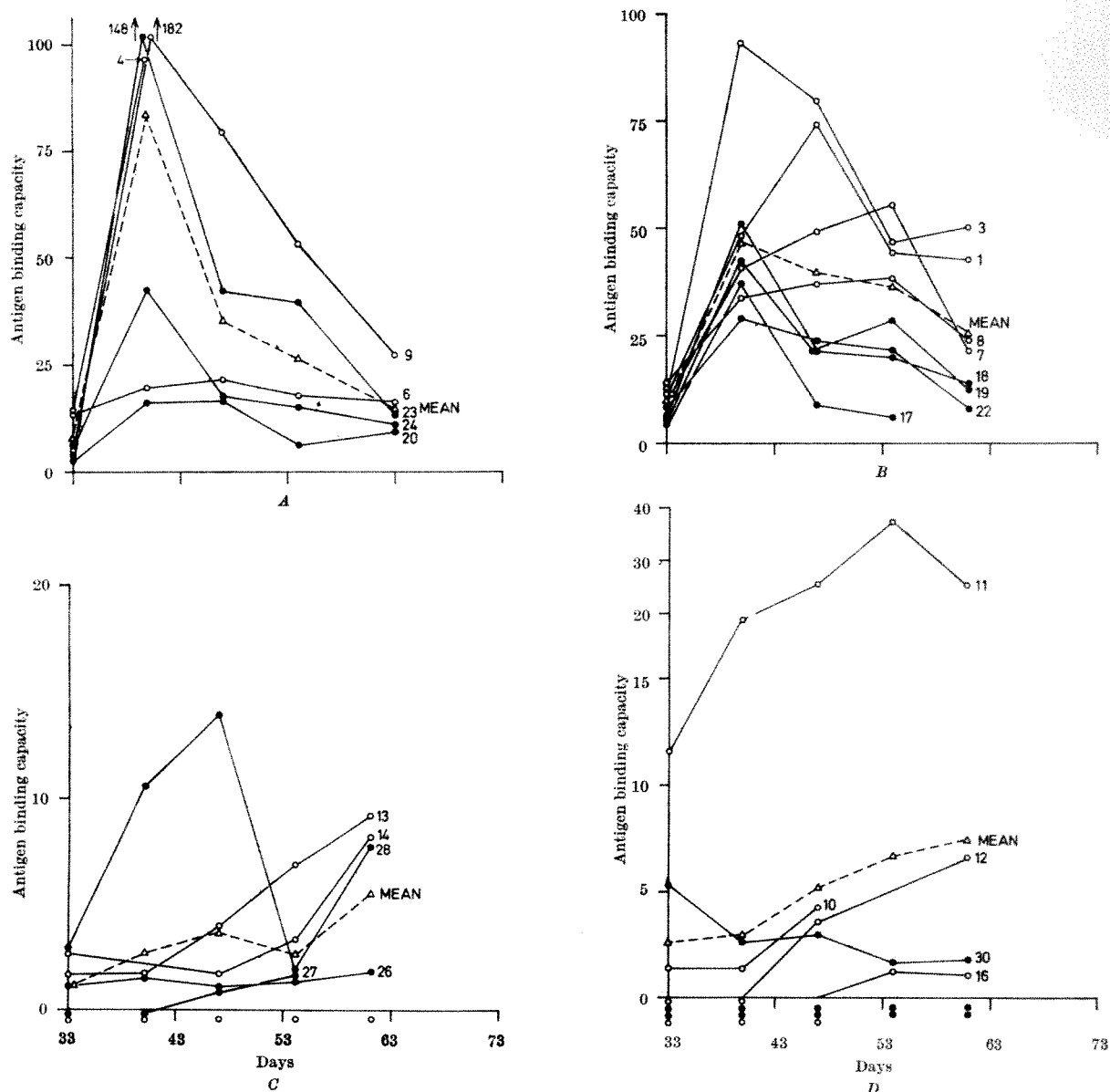


Fig. 2. Effect of normal IgG and anti-lymphocytic IgG on the secondary response of hooded rats (from groups 1A to 1D) to alum precipitated bovine serum albumin. ●, Male; ○, female. A, Experimental group 2A, rats receiving normal IgG before primary and secondary antigenic challenge with alum precipitated bovine serum albumin; B, experimental group 2B, rats receiving normal IgG before the primary and anti-lymphocytic IgG before the secondary antigenic challenge; C, experimental group 2C, rats receiving anti-lymphocytic IgG before the primary and normal IgG before the secondary antigenic challenge; D, experimental group 2D, rats receiving anti-lymphocytic IgG before the primary and secondary antigenic challenge. For further details see Fig. 1, Table 1 and text.



Table 1. PRETREATMENT OF HOODED RATS AND ITS EFFECT ON HUMORAL ANTIBODY PRODUCTION

Response	Experimental group		Rats				IgG treatment		Peripheral blood lymphocyte count (total and %)		Antigen binding capacity (0.2 µg nitrogen)	
			Sex	Nos.	Mean wt. (g)	Wt. range			-3	0	Initial	Maximum observed*
									Day or 30	Day or 33		
Primary	1A	{	Male	7	213	180-275	3 ml.	1 g% normal	3,720 (100)	3,550 (95)	0	7.9 ± 1.3
	Female		9	161	130-195	2 ml.	4,310 (100)		4,000 (93)	0	12.0 ± 0.7	
	1C	{	Male	7	221	185-330	3 ml.	1 g% ALS	3,900 (100)	2,200 (55)	0	2.1 ± 1.3
	Female		7	162	135-220	2 ml.	4,450 (100)		2,370 (53)	0	3.1 ± 1.5	
Secondary	2A	{	Rats from groups 1A and 1B	Male	{	6	3 ml.	1 g% normal	4,630 (100)	4,450 (96)	7.5 ± 2.4 (100 ± 32)	84.2 ± 22.1 (1,122 ± 294)
	2B			and		8			3 ml.	1 g% ALS	6,140 (100)	3,410 (56)
	2C	{	Rats from groups 1C and 1D	female	{	6	3 ml.	1 g% normal	3,075 (100)	3,135 (102)	1.2 ± 0.4 (100 ± 30)	5.8 ± 2.4 (580 ± 200)
	2D			7		3 ml.			1 g% ALS	3,850 (100)	2,960 (76)	2.6 ± 1.9 (100 ± 53)

\* The antigen binding capacities are expressed as mean values together with the standard deviation of the mean. In primary response studies these values were calculated only from rats which had survived the entire experimental period. In the secondary response all rats were considered because very few died and these had usually reached a maximum. The secondary values are also expressed as a percentage of the starting antigen binding capacity of the appropriate experimental group.

lymphocytic IgG contained detectable amounts of anti-BSA antibodies although the levels were not always high enough to allow the determination of antigen binding capacities (that is, a 1/10 dilution of the serum failed to bind 33 per cent of the test antigen<sup>10,11</sup>). In those cases in groups 1C and 1D where antigen binding capacities were measurable on day 33 the relative binding affinities were similar to those observed on day 12 in animals receiving normal IgG (experimental groups 1A and 1B). Thus although there was usually a decrease and delay in antibody production, the antibodies eventually produced appeared to be qualitatively comparable with those initially observed in "non-suppressed" animals.

As previously noted in experiments studying the response of rats and mice to sheep erythrocytes<sup>2,9</sup>, the effect of anti-lymphocytic IgG on the secondary response was not very marked. Indeed, it is quite apparent from Table 1 and Fig. 2B (experimental group 2B) that animals which had received normal IgG before the initial injection of alum BSA responded readily to a further injection of alum BSA whether or not this was preceded by injection of anti-lymphocytic IgG. Analysis of the results suggests, on the other hand, that anti-lymphocytic IgG may be influencing quantitative aspects of the secondary response in a number of animals. In support of this there was also a slight indication that the relative response to a secondary challenge in rats which had initially received anti-lymphocytic antibody was also reduced when they received a further course of anti-lymphocytic IgG before the secondary stimulus (experimental group 2D). In order to establish whether anti-lymphocytic IgG does influence the magnitude of the secondary response, experiments will have to be carried out using more animals.

The anti-lymphocytic IgG had no obvious effect on the relative binding affinities of the antibodies produced after secondary stimulation in animals which had received normal IgG before primary stimulation. In experimental groups 2A and 2B the relative binding affinities usually showed marked increases after secondary stimulation. On the other hand, the relative binding affinities in groups 2C and 2D (those that had received anti-lymphocytic IgG before primary challenge) rarely showed the high values observed in groups 2A and 2B, thus indicating qualitative as well as quantitative differences in antibody production.

It is clear from these results that anti-lymphocytic IgG suppresses and/or delays the primary response of hooded rats to alum precipitated BSA, in addition to inhibiting erythrocyte agglutinin formation in rats and mice<sup>3,9</sup>. Thus this material affects the onset of both humoral and cellular types of immunity (see homograft experiments with this preparation<sup>14,15</sup>).

It has still to be determined whether anti-lymphocytic antibody interferes with the sensitizing ("triggering")

phase of antibody production and/or the proliferative phase. A number of theories have been postulated to explain how this material could affect either, or both, of the processes. These include lymphocytolysis, the "sticky antigen" theory, blindfolding of lymphocytes<sup>1</sup> and sterile inactivation<sup>5</sup>. Whatever mechanisms are operating, it is clear that the present course of treatment, although it caused suppression, did not produce complete tolerance; all the animals receiving anti-lymphocytic antibody eventually produced detectable amounts of anti-BSA antibodies and also responded to a secondary challenge with BSA. Thus either the animals recovered their capacity to recognize and process antigen (the sensitization phase) and/or their ability to produce antibody after antigenic recognition (proliferative phase). This could result from the original cells recovering their immunological capacity or the maturation of additional immunologically competent cells or a combination of these and other phenomena. Cytological investigation<sup>16</sup> of those cells which do survive anti-lymphocytic treatment suggests that "deformation" may have occurred. This could render them incapable of producing normal levels of antibody or developing into cells with this potential. Whatever the explanation, it appears from measurements of relative binding affinity that the antibodies eventually produced after primary stimulation are qualitatively similar to those initially observed in animals receiving normal horse IgG. On the other hand, the affinities of the antibodies produced after secondary stimulation in group 2C and 2D were appreciably lower than those observed in animals which had received normal IgG before primary stimulation (group 2A and 2B).

The failure effectively to suppress the secondary response in "sensitized" animals (that is, those from group 1A and 1B) indicates that the course of treatment used did not "abrogate the pre-existing state of immunity to alum precipitated BSA", a phenomenon observed in relation to the survival of second set homografts<sup>4,5</sup>. Thus in these studies the anti-lymphocytic IgG failed to destroy immunological memory by inactivating memory cells. Nevertheless the results suggest that this form of treatment may be affecting the quantity, though not the quality, of the antibody produced after secondary stimulation in "sensitized" animals.

These investigations also indicate that gross lymphocyte depletion is not a prerequisite for effective immunosuppression<sup>4,9</sup>. Nevertheless, as previously suggested, the possibility that anti-lymphocytic antibody functions by destroying or inactivating a select proportion of vital cells cannot be precluded<sup>3,4,9</sup>. Indeed, it is also possible that this material may interfere with the activity of the macrophage, thus affecting sensitization.

In conclusion, it is apparent that anti-lymphocytic antibody is capable of suppressing or delaying primary

humoral antibody formation against alum precipitated BSA as well as cellular aspects of immunity, but the mechanisms involved in these important phenomena will require further investigation.

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## Crossover Model of Antibody Variability

by

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Several unsatisfactory models have been proposed to account for the variation in the primary structure of the polypeptide chains in the antibody molecule. A new model is proposed here; it is based on intrachromosomal crossing over during the development of plasma cells.

FOUR hypotheses have been suggested to account for the variation in the primary structure of the chains of polypeptides (one light and one heavy) which are thought to comprise the antibody molecule. Three models attribute the diversity of structure to variation in the DNA of the chromosome, and the fourth to abnormality in the mechanism of translation of messenger RNA into polypeptide.

The multiple gene hypothesis<sup>1,2</sup> seems untenable because the non-variable parts of the polypeptide chains show normal mutation and Mendelian segregation, implying determination by one gene. Dreyer and Bennett<sup>1</sup> explained this by supposing that the constant part of the polypeptide was determined by a virus or episome which became attached to one of the many alternative genes postulated to determine the variable part of the molecule. This seems unacceptable, however, because even within the variable part of the polypeptide chain there appears to be constant or relatively constant regions<sup>3</sup>. Doolittle<sup>4</sup> has pointed out that this hypothesis would require the same base substitutions to have occurred at corresponding positions in all the genes—a most unlikely occurrence.

The inverted duplication hypothesis<sup>5,6</sup> postulates somatic intrachromosomal crossing over between non-adjacent relatively inverted duplications within the gene for the light polypeptide chain (and similarly within the gene for the heavy chain). This was questioned by Milstein<sup>7</sup>, who pointed out that the observed variation in amino-acid sequence frequently fails to correspond with that expected from the reversed complements of triplets.

The hypermutation hypothesis has to account for the limitation of the variability to only a part of the light and heavy polypeptide chains. Some<sup>2,8</sup> favour separate genes for the constant and variable parts of the polypeptides, with enzyme joining of the resultant peptides. This seems untenable, however, because the light and heavy chains are synthesized as single processes<sup>9,10</sup>. This difficulty is avoided by postulating enzyme removal of one chain of the DNA in the appropriate part of the gene, followed by a defective repair mechanism, leading to mutations only in that part of it<sup>11</sup>. A similar model was proposed by

Dreyer and Bennett<sup>1</sup> but dismissed because the variable region of the light chain shows considerable resemblance between one antibody and another. This limited variability argues against hypermutation, because even single base substitutions within an average triplet will give rise to triplets coding for five to seven different amino-acids, yet the variation observed at any one position in the polypeptide seems often to be much less than this<sup>12</sup>. Furthermore, mutation must be controlled in such a way that addition or deletion of single nucleotides does not occur, because this would displace the reading-frame for the constant part of the gene. This is because the variable parts of both the light and heavy polypeptide chains are in the N-terminal halves, which are the first to be synthesized. A similar objection applies to some of the structural changes postulated by Smithies<sup>3,4</sup>.

The specialized translation hypothesis<sup>13</sup> involves unusual triplets in the DNA of the variable region, and changes in specificity in the activating enzymes of transfer RNA molecules, so that these triplets can code for diverse amino-acids. Particular amino-acids are not always replaced in other antibody molecules by the same one<sup>7</sup>, contrary to the prediction of this model, and several other reasons have been given for regarding the model as unlikely<sup>12</sup>.

Thus there is a paradox, and despite considerable progress in understanding the nature of the variability in antibody structure, all the hypotheses that have been put forward to account for it are improbable.

In attempting to resolve this paradox, there can be no doubt, as Watson<sup>14</sup> implied, that hypotheses of antibody variability based on recombination are the most attractive. The necessary variations in the DNA could then be of the kind, and at the positions, which selection had favoured. Furthermore, the potential variability would be inherited and would not depend for its origin in each individual on so unpredictable a process as mutation.

There is a relatively simple way in which antibody variation could arise through recombination and yet be inherited as if caused by single genes, one for the light chain and one for the heavy chain, as the data appear to require. The failure hitherto to consider a mechanism of the kind described here seems to be a result of the

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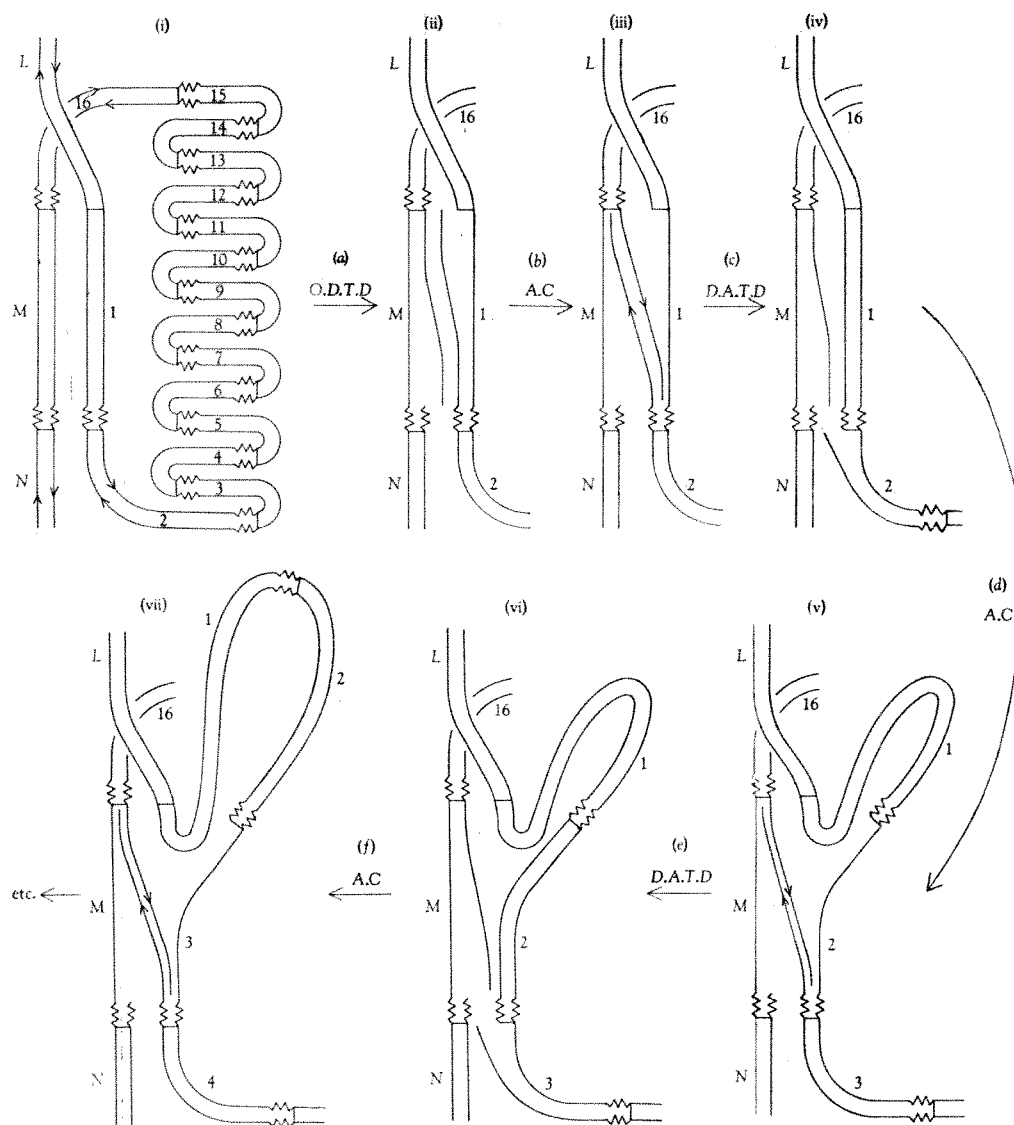


Fig. 1. Diagram to show the postulated behaviour<sup>17</sup> of a gene and sixteen copies of it when messenger RNA is to be synthesized. The gene and copies are arranged in a consecutive linear sequence within one DNA molecule. The copies are matched against the master gene and then extend as a lampbrush loop. The model is based on that proposed by Callan<sup>16</sup>. The lines represent nucleotide chains. Wavy lines show the position of the operator of the gene. (A) Annealing; (C) correction of mispairing to correspond with that in the chain with the descending arrow; (D) dissociation; (L, N) neighbouring genes to M and its copies. (M) Master copy of gene; (O) breakage at operator; (T) breakage at terminus. 1-16, Sixteen copies of gene M. I suggest that in the structural genes for the antibody polypeptides some modifications of the scheme shown would be necessary to allow both chains of all the other copies to be matched against one of the two copies which had taken part in an intrachromosomal crossover.

assumption that mammalian chromosomes are organized in the same way as the hereditary material of bacteria and viruses. This assumption, for example, is implicit in the misleading use of the term chromosome for the bacterial or viral DNA thread. Here I use the term chromosome in the strict sense, excluding the DNA thread of bacteria and viruses (for which I have suggested the term chromoneme<sup>15</sup>).

There is a considerable body of evidence, reviewed by Callan<sup>16</sup>, in support of the hypothesis that in chromosomes each gene is duplicated  $2^n$  times consecutively within one DNA molecule, where  $n$  may vary independently for each gene according to the species, sub-species or population, but may be of the order of six to twelve for many genes in *Triturus*<sup>17</sup>. The set of copies would form a chromomere. Callan<sup>16</sup> has proposed a mechanism whereby the copies of each gene are matched against that which has undergone recombination with the homologue, with the result that all acquire identical nucleotide sequences. I have suggested<sup>17</sup> a modification of this mechanism which facilitates the coiling and uncoiling of nucleotide chains (see Fig. 1).

It seems possible that the matching may precede the synthesis of messenger RNA whenever this occurs.

For genes which do not function in the germ-line and which are therefore presumed not to undergo matching of the copies in the germ-line, it is possible that in special circumstances selection might favour the maintenance of slight differences between the copies, and it is suggested that this may have happened in the evolution of antibody variability. In the germ-line there would then be a number of related and consecutive antibody-gene nucleotide sequences which differed from one another, as a result of mutations accumulated in the course of evolution, and all of which would be handed down in their diversity from generation to generation. For differences between the gene copies to be advantageous it would be necessary, however, for the matching process to be modified, because this would normally eliminate any differences between the copies before the gene could function. It is suggested that in the development of plasma cells, before antibodies are synthesized, a crossover takes place within the chromosome between two of

the copies, and that this is followed by matching of the remaining copies against one of these two. This course of events is postulated for both antibody genes, that is, within the copies of the gene for the light chain, and, independently, within those for the heavy chain.

This intrachromosomal crossing over is similar to the postulated normal behaviour for all genes at the pachytene stage of meiosis, except that at meiosis it is suggested that the crossover always occurs between the first and last members of the series of copies<sup>17</sup> (see Fig. 2). Such a crossover would detach the copies as a ring (Fig. 2 (v)) and allow the one copy remaining in the chromatid to undergo normal crossing over with its homologue. The ring of duplicates could be restored to the chromatid by a crossover between the gene in the chromatid and one of the copies in the ring (Fig. 2 (e)-(h)). This mechanism is comparable with that proposed by Campbell<sup>18</sup> for attaching or detaching circular phage DNA from the DNA of its host.

The abnormal behaviour proposed for antibody genes in plasma cell precursors would require some modifications of the steps shown in Figs. 1 and 2 to allow for crossing over between any two copies (not necessarily the first and last as shown), and to accommodate matching

of the remaining copies to one of these two, wherever they were located in the series. The copies situated between those which had undergone mutual crossing over would be detached as a ring, and a second crossover, not necessarily between the same two copies, would be needed to restore them to the chromosome. If it is assumed that both chains of all the other copies of the antibody gene are matched against a copy which has taken part in an intrachromosomal crossover, this would mean that the antibody gene was now specialized to produce the one kind of polypeptide determined by this recombinant gene. The specificity would reside in the chromosome, and so it would be passed on at mitosis to daughter cells and would account for immunological memory and for the origin of a clone of cells all specifying the same polypeptide. The existence of a number of copies of the gene would allow a fast rate of synthesis of messenger RNA in each cell.

The main differences from normal in the two antibody genes would be that the copies of each would not be identical, and intrachromosomal crossing over would occur in particular somatic cells, the plasma cell precursors. The non-identity of the copies would seem to require no special adaptations, as long as matching of the copies of

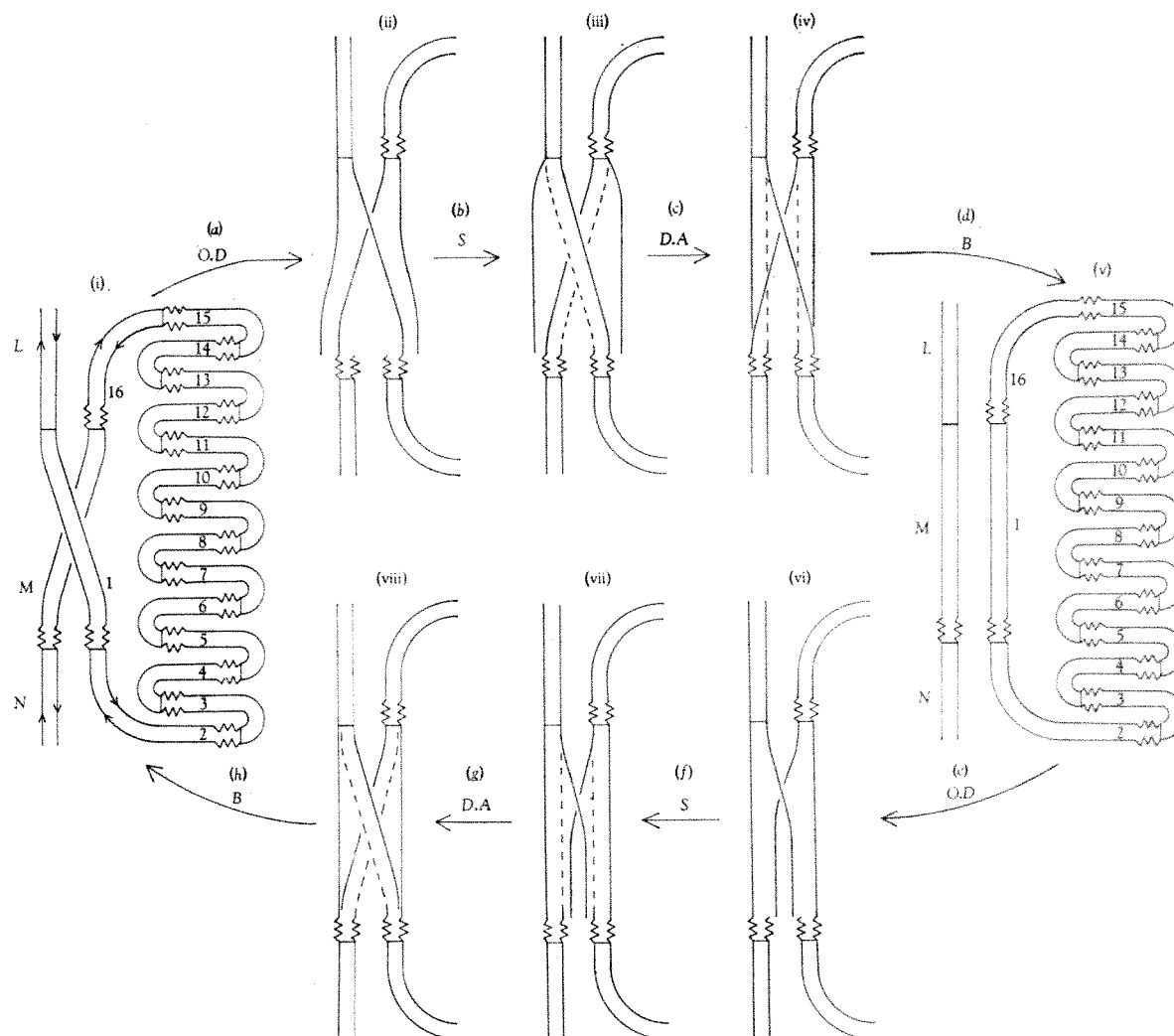


Fig. 2. Diagram to show the postulated behaviour<sup>17</sup> at meiosis of a gene and sixteen copies of it. (a)-(d) Detachment of the sixteen copies as a result of intrachromatid crossing over between the first and last members of the series, leaving the master gene, *M*, in the chromatid in a position to undergo crossing over with a homologous chromatid from the other parent. (e)-(h) Reincorporation of the copies into the chromatid by crossing over. The lines and symbols have the same meaning as in Fig. 1. (B) Breakdown of unpaired chains; (S) synthesis. I suggest that in the structural genes for the antibody polypeptides in the plasma cell precursors, a similar intrachromosomal crossover takes place, but that it may occur between any two of the copies of the gene. I also suggest that the second intrachromosomal crossover, to reincorporate the copies detached by the first one, might involve different copies.



a gene occurs only when a gene is about to function, and the antibody genes do not function in the germ line. The mutations by which the copies of each antibody gene differed would presumably all be base substitutions or additions or deletions or three nucleotides or a multiple of three, because otherwise the reading of the remainder of the gene would be affected (see previously). These mutations would have accumulated during many generations under the influence of natural selection. The intrachromosomal crossing over in the antibody genes of the plasma cell precursors would need to be followed, as already indicated, by a slightly abnormal matching process.

The postulates for antibody variation would thus be slight alterations of normal processes. The consequences, however, would be profound, for each plasma cell precursor would acquire a unique genotype with regard to both of its antibody polypeptides. Even if the number of copies of an antibody gene was as small as sixteen (as in Figs. 1 and 2) many different genotypes for the plasma cell precursors would be possible if each of the sixteen differed by several base substitutions. This assumes that a crossover could occur between any two of the sixteen and that the hybrid DNA segments of the crossover could vary in length. Such variation is thought also to occur with normal crossing over. I assume that crossing over between copies would occur by essentially the same mechanism as normal crossing over between homologous chromatids at meiosis, and that correction of mispairing would occur in the hybrid DNA. That normal crossing over appears to be specific to structural genes and to be controlled in a way similar to transcription<sup>18</sup> implies that a mechanism for bringing about crossing over between copies of the antibody genes would be available.

It seems to be significant that the variation in antibody structure is almost entirely confined to the *N*-terminal part of each polypeptide. This would correspond to the part of the gene nearest to the operator. It seems likely that in normal crossing over dissociation of the DNA and chain synthesis are both initiated from the operator end of the gene<sup>19</sup>, and so these processes would have to extend only to the middle of the gene in the plasma cell crossovers. That antibody variability is in the *N*-terminal rather than the *C*-terminal parts of the polypeptide chains may therefore be an adaptation to minimize the length of DNA occupied by the crossover. There might be a selective advantage in minimizing the amount of DNA synthesis required, and in shortening the time taken for crossing over to occur.

If the structural gene for the light polypeptide chain of the antibody molecule and its counterpart in the homologous chromosome both underwent intrachromosomal crossing over and copy matching, each cell could produce two different polypeptides for the light chain, and by the same argument another two for the heavy chain. Possibly, however, each antibody gene is active in only one of the two homologous chromosomes.

Immunological tolerance would be accounted for by an antigen present early in development combining with the antibody produced by a particular plasma cell before that cell had divided after the hypothetical intrachromosomal crossing over. This antigen-antibody reaction would also have to destroy the cell. Conversely, an antigen-antibody reaction later in development would evidently stimulate the cell producing it to divide.

Further investigation of the variations in amino-acid sequence of antibody polypeptides will provide information relevant to the hypothesis. The variation in amino-acid sequence would be expected to correspond to different combinations of certain nucleotides at each position. For example, Doolittle<sup>4</sup> has found the alternatives shown in Table 1 (a) for the first six residues at the amino end of the light chains in the rabbit. The corresponding triplets of nucleotides in messenger RNA would be those shown in Table 1 (b)<sup>20</sup>. Recombinations would be expected to

Table 1. PREDICTIONS OF THE RECOMBINATION HYPOTHESIS

Position	1	2	3	4	5	6
(a) Amino-acids observed	Ala Ile Asp Glu	Val Leu	Val Leu Gln/Glu	Val Gln/Glu	Gln Ala	Gln Ala Thr
(b) Coding triplets	GCX AUU/C or AUA GAU/C GAA/G	GUX CUX or UUA/G	GUX CUX or UUA/G CAA/G or GAA/G	GUX CAA/G or GAA/G	CAA/G GCX	CAA/G GCX ACX
(c) Minimum recombinant triplets	GUX ACX AAU/C AAA/G	—	—	—	CCX GAA/G	CCX GAA/G AAA/G
(d) Amino-acids predicted	Val Thr Asn Lys	—	—	—	Pro Glu	Pro Glu Lys

X = U, C, A or G.

(a) Data of Doolittle<sup>4</sup> for the first six residues at the amino end of the light chains in the rabbit. (b) The corresponding triplets in messenger RNA. (c) The minimum additional triplets expected to arise from recombination. (d) The amino-acid residues expected from (c). Ala, Asn, Asp, Gln, Glu, Ile, Leu, Lys, Pro, Thr and Val stand for alanine, asparagine, aspartic acid, glutamine, glutamic acid, isoleucine, leucine, lysine, proline, threonine and valine, respectively. A, C, G, U and X represent the ribonucleotides of adenine, cytosine, guanine, uracil and any of the bases, respectively, orientated with 5'-phosphates to the left.

give rise to at least to the additional triplets shown in Table 1 (c), and possibly to others depending on which of the alternatives in Table 1 (b) was present. The recombinant triplets in Table 1 (c) correspond to the amino-acids given at the bottom of the table. The finding of these amino-acid residues at these positions would therefore support the recombination hypothesis.

If the origin of antibody variability depends on somatic crossing over in the plasma cell precursors, as suggested, and if this crossing over requires DNA synthesis, it might be possible to detect this. Alternatively, it might be possible to inhibit the antibody variability by preventing the synthesis at the critical time.

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Since this paper was submitted for publication, a mechanism has been suggested for the origin of antibody diversity which has several features in common with that proposed here<sup>21</sup>. The model differs from mine primarily in the three following respects. (a) The duplicate genes all have equal status (not a master and slaves) and hence are all liable to undergo crossing over with a homologue at meiosis. (b) The somatic crossing over is not intrachromosomal but is between either sister-chromatids or homologous chromosomes. (c) There is no matching of the copies against one of their number.

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# Motivational Effects of Rewarding Intracranial Stimulation

by

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The theoretical definition of behavioural reinforcers has proved to be a difficult problem. Experiments with rats now suggest that a feature common to reinforcers may be the ability to generate or condition incentive-motivational properties in a previously neutral stimulus.

In studies of behavioural patterns, reinforcers are usually defined in terms of their effects on the probability of occurrence of a response on which they are contingent. Thus when a stimulus event contingently paired with a response reliably increases its probability of occurrence, the stimulation is called a (positive) reinforcer or reward. The widespread acceptance of this definition seems to have created the impression that a change in response probability is the only important consequence of reinforcing stimulation; indeed, theoretical questions about the basis of reinforcement are at present discussed (for example, in textbooks<sup>1-3</sup>) exclusively in terms of the effects of reinforcers on response probability. Yet it is obvious that reinforcers also have motivational effects on behaviour. After being given a single small food pellet to eat, a hungry animal becomes agitated and everyone knows that the reinforcing stimulations of normal life (such as an unexpected compliment or insult) may change a man's mood and outlook on life for several hours, affecting his behaviour in a variety of situations. Such motivational effects may be important in understanding the mechanisms by which reinforcers increase response probability.

The question we set out to study was whether stimulation considered reinforcing by the response probability criterion has any measurable effect on aspects of behaviour that are not in any way contingently associated with the stimulation. We examined the immediate effects on general activity of electrical stimulation of "positive" and "neutral" brain sites in the same rats. We chose intracranial stimulation as the reinforcing agent because it appears not to depend on any response for the reinforcement to be completed; in contrast, the reinforcing effect of food, for example, depends on the animal making appropriate consummatory responses. In order to ensure that the reinforcing stimulation was independent of the response, each rat was habituated to the experimental situation and the reinforcing stimulation was turned on when it was sitting quietly. In order further to reduce the chance of accidental reinforcement of a response provoked by a prior stimulation, the stimulations were turned on only briefly and were separated by long intervals.

Two bipolar stimulating electrodes were stereotactically implanted in each of eight adult male rats under sodium pentobarbital anaesthesia. One of the electrodes was placed in the lateral hypothalamic "positive region" and the other in a "neutral region" of the cortex. The electrodes and the procedure of implantation used have been described by Valenstein *et al.*<sup>4</sup>. Histological examination showed that the reinforcing electrodes were located either in the medial forebrain bundle—lateral hypothalamic area at the level of the ventromedial nucleus—or in the ventromedial nucleus itself. The cortical electrodes were distributed along a 3 mm sagittal plane centred on the bregma and 2 mm lateral to the mid-line; two of these electrodes penetrated to the corpus callosum.

The properties of stimulations in the two regions were confirmed by testing the rats in a lever-pressing situation to determine whether they would press the lever to obtain hypothalamic or cortical stimulation. A 30 cm square box, about 50 cm high, was fitted with a lever; each press on the lever delivered a 0.5 sec train of biphasic pulses (pulse duration, 0.2 msec; frequency, 100 pulse pairs/sec; Grass 'SD-5' stimulator). All animals were

first tested with hypothalamic stimulation with a current of 0.8 m.amp. After four to six 30 min sessions, one on each day, the rate of lever pressing was determined for each rat in a 10 min test, which came at the end of the 30 min session. After this test, each lever-press produced cortical stimulation; in cases in which the stimulation led to squealing the amplitude was reduced to the minimum that yielded some observable response to stimulation (for example, sudden turning of the head). In the test with hypothalamic stimulation all animals pressed the lever at a moderate rate (mean rate 39/min), but all animals stopped pressing when the cortex was stimulated (mean rate 0/min). Though described here first, the lever-pressing tests were actually conducted after the tests measuring activity, so that the animals received no intracranial stimulation contingent on their response before the activity tests.

After recovery from surgery, the rats were habituated to an activity measuring box, 30 cm long, 20 cm wide and 28 cm high, with grey walls and a metal grid floor. When introduced into the box the animals explored a good deal but, after two or three 30 min habituation sessions, they mostly sat quietly. After this habituation, a series of sessions was begun to measure activity. A mirror was so arranged above the box that the experimenter could observe the rat from his chair. A micro-switch, connected to a 0.1 sec timer, was used by him to record the duration of perambulation; the experimenter pressed the microswitch for as long as the rat walked, reared or explored. (For details of this method, see ref. 5.)

Each of the activity measuring sessions started with a habituation period of 9 min, which was followed by two 6 min observation periods separated by a 1 min interval. A 0.5 sec train of biphasic pulses of the type described here was given at the midpoint of the observation period (after 3 min of the period). In the ten initial sessions, each animal was stimulated in the hypothalamic site on five sessions and in the cortical site on the remaining five sessions; four animals were stimulated first in the hypothalamic site, and the remaining four were stimulated first in the cortical site. In these sessions the level of current was 0.2 m.amp. After this, each rat was tested in seven additional sessions; in the first three of these the current level was set at 0.5 m.amp and in the last four at 0.8 m.amp. With stimulation at 0.5 m.amp, four of the rats were tested with hypothalamic stimulation and the remaining rats with cortical stimulation. With stimulation at 0.8 m.amp, all animals were tested with hypothalamic stimulation on two sessions and with cortical stimulation on two sessions, half the animals receiving each treatment first.

The mean proportion of time spent perambulating was calculated for each rat for each type of stimulation at each value of current; the number of cases represented in the mean varied between five and eight because some of the electrodes failed. The most striking finding was that a momentary (0.5 sec) stimulation had a prolonged effect on general activity. The most unexpected finding was that both cortical and hypothalamic stimulations produced about the same effects on general activity, and this was true at all three current levels (see Fig. 1). The animal would typically be sitting before stimulation and would start sniffing and moving around after the

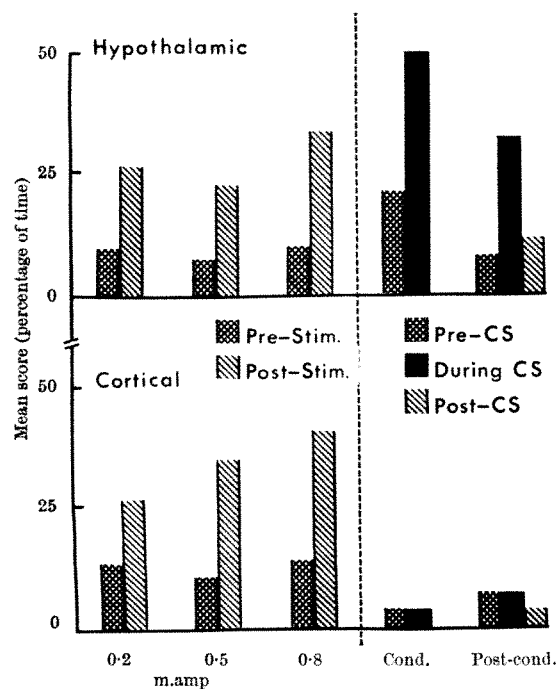


Fig. 1. The left part of the figure shows the mean perambulation scores in 3 min pre- and post-stimulation periods at each of three levels of current applied at hypothalamic and cortical electrode placements. The two sets of bars on the right show the mean perambulation scores obtained during the tests of incentive-motivation, the two final conditioning tests (15 sec observation periods) and the two post-conditioning tests (1 min observation periods), with hypothalamic and cortical stimulation. The high pre-CS scores during the conditioning tests in the case of hypothalamic stimulation probably represent the effects of conditioning to the experimental situation as a whole.

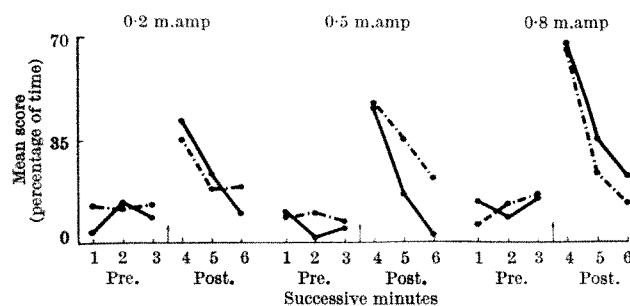


Fig. 2. Mean perambulation scores for the six successive 1 min sections of the activity tests conducted at each of three levels of current applied at hypothalamic and cortical electrode placements. The solid line represents hypothalamic stimulation and the broken line represents cortical stimulation.

stimulation. There were no obvious differences in the form of perambulation (for example, walking or rearing) after the two types of stimulation. The only notable difference was that cortical stimulation frequently produced squealing, suggesting that the stimulation was painful. The animals did not freeze or crouch, however, as is shown by their perambulation scores. The pre-stimulation and post-stimulation means of perambulation scores plotted over successive test sessions showed no evidence of habituation for either type of stimulation. Fig. 2 shows that the perambulation scores were highest in the first minute after stimulation, and then declined gradually, and this was true with both hypothalamic and cortical stimulations. Thus in spite of the marked differences between the reinforcing effects of hypothalamic and cortical stimulations, as measured by the lever-pressing test, the effects of the two on general activity were quite similar. Though differences in perambulation could certainly have been obtained by varying the parameters of stimulation, the point here is that differences in reinforcing value are not necessarily reflected in general activity.

In order to test the capacity of the two types of intracranial stimulation to generate conditioned incentive-motivational effects, a conditioned stimulus (metronome, 3 strokes/sec) was paired with intracranial stimulation. On each trial, the metronome was turned on 15 sec before the onset of intracranial stimulation, which consisted of five 0.5 sec stimulations set 1 sec apart; both the metronome and the stimulation were turned off simultaneously. The conditioning was carried on in the activity measuring box, and the activity of the animal was recorded for 15 sec before the onset of the metronome and then during the 15 sec metronome interval (before the onset of intracranial stimulation). All rats were first given seven sessions (four trials in each session) of this classical conditioning with hypothalamic stimulation. This was followed by two test sessions (without intracranial stimulation) in which activity was recorded during three 1 min observation periods; the metronome was sounded only during the middle period. All the animals were then given the same number of classical conditioning trials with cortical stimulation, and again this was followed by two test sessions. As seen in Fig. 1, the observations during conditioning, as well as the tests following the conditioning trials, showed that perambulation scores during the metronome periods were higher than during the adjoining control periods in the case of hypothalamic stimulation, but not in the case of cortical stimulation. Thus the metronome acquired conditioned incentive-motivational properties when it was paired with hypothalamic stimulation but not when it was paired with cortical stimulation. Not only was cortical stimulation incapable of generating incentive-motivational effects, it failed even to sustain them.

These experiments demonstrate under laboratory conditions that a positive reinforcing stimulation produces marked changes in behaviour even when the stimulation is not contingent on the prior occurrence of any particular response. Consistent with this demonstration is Miller's<sup>6</sup> proposal that a "go" or activating mechanism may act to intensify neural correlates of responses in progress and immediately preceding. One of his suggestions is that increased "overt motor activity" may serve to indicate the operation of the "go" mechanism. This particular suggestion is contradicted by the present finding that increased motor activity may occur as a result of non-reinforcing (cortical) stimulation as well. Enhancement of activity therefore cannot serve as a defining property of reinforcers.

Rather, our findings suggest that the capacity to generate (conditioned) incentive-motivational properties in an initially neutral stimulus may be a feature common to reinforcers. Certainly, the findings are consistent with the view that reinforcing stimuli, as well as the conditioned stimuli associated with them, create general neural states which have motivational properties that go far beyond the strengthening of particular stimulus-response connexions. That such neural states might also be created by natural reinforcers is indicated by the demonstration that a conditioned stimulus associated with the delivery of water to a thirsty animal raises the animal's level of general activity, and that the same stimulus associated with the delivery of a brief electric shock lowers the animal's level of general activity<sup>5</sup>. A systematic study of such reinforcer-induced motivational states may help to elucidate the neural basis of reinforcement.

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## LETTERS TO THE EDITOR

## ASTRONOMY

## Role of Impurities in Interstellar Graphite Grains

ACCURATE measurements of the wavelength dependence of interstellar obscuration in the spectral range from 10,000 Å to 3400 Å (refs. 1-4) show a distinct change in the slope of the extinction curve at 4300 Å. Extinction observations have been made for a number of galactic regions, and it is found that a change in slope always occurs at 4300 Å but that the magnitude of the change differs significantly from one region to another. At the same time, an unidentified interstellar band of half-width approximately 20 Å appears at 4430 Å, but the discontinuity in the slope of the extinction curve does not affect the profile of this band<sup>5</sup>.

The precise shape of the extinction curve in the region of 4430 Å is being investigated by Harris (private communication) at a higher dispersion (120 Å/mm) than that used by Nandy<sup>2</sup> (1000 Å/mm). The preliminary results show that (1) the "knee" in the extinction curve near 4300 Å is real; (2) it is independent of the 4430 Å band; and (3) the extinction curve is best represented by two straight lines intersecting at about 4300 Å over the wavelength range considered (6000 Å-3400 Å), confirming the results of Nandy<sup>2</sup>.

Interstellar extinction in the ultra-violet deduced from rocket observations<sup>6-8</sup> shows a maximum at  $2330 \text{ Å} \pm 50 \text{ Å}$ . Fig. 1 shows the mean ultra-violet extinction points and the mean extinction curve obtained by Nandy<sup>2</sup> for a region in the direction of Cygnus for reference. The peak in the ultra-violet provides a strong argument in favour of the graphite model originally proposed by Cayrel and Schatzman<sup>9</sup> and Hoyle and Wickramasinghe<sup>10</sup>. The predicted energy difference between the states of the

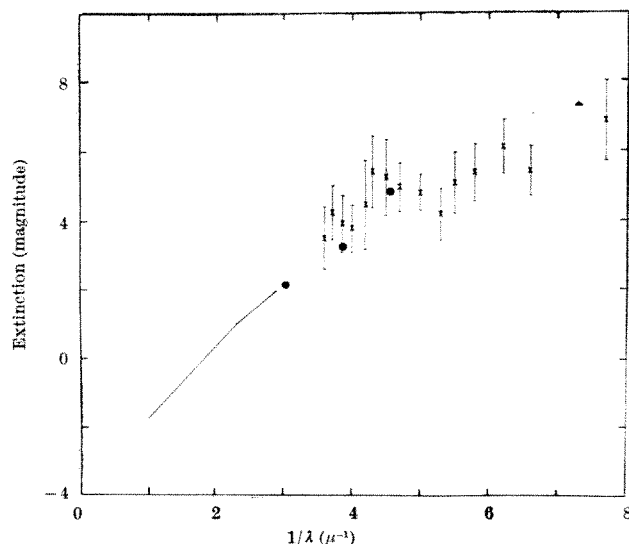


Fig. 1. Interstellar extinction curve. The solid line denotes the extinction law for Cygnus (after Nandy<sup>2</sup>). ●, ×, ▲, Mean ultra-violet extinction points (●, Boggess and Borgmann<sup>2</sup>; ×, Stecher<sup>4</sup>; ▲, Smith<sup>5</sup>).

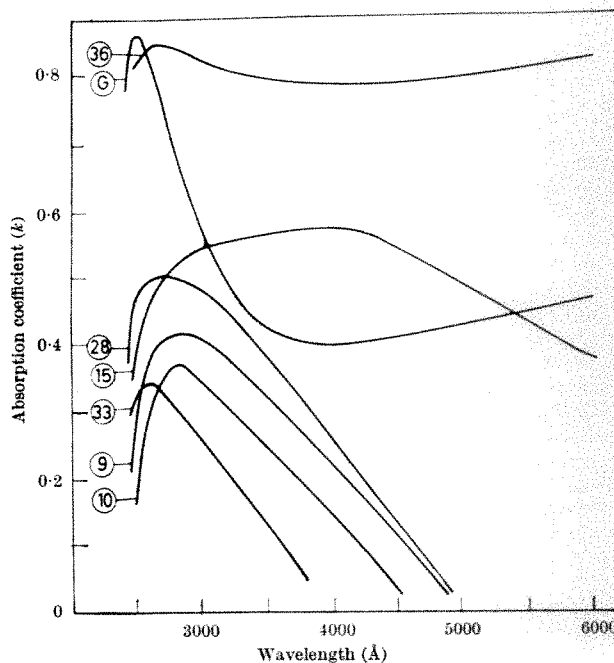


Fig. 2. Variation of absorption coefficient with wavelength (reproduced from Fuel (ref. 14)).

maximum density in the valence and conduction bands of graphite is close to 5 eV (ref. 11), which corresponds to a wavelength of 2480 Å, and the graphite peak indeed occurs in the neighbourhood of this predicted wavelength<sup>12,14</sup>. The observed "shoulder" of the extinction curve in the ultra-violet, however, is not sharp-peaked as is found for graphite. The purpose of this communication is to present some evidence that the knee of the extinction curve at 4300 Å may be produced by graphite particles containing small percentages of impurities.

The effect on its optical properties of impurities present in graphite can be investigated by examining the wavelength dependence of absorption coefficient and refractive index of coals. X-ray studies of high grade anthracites reveal that they are graphite-like and show three dimensional graphite crystallinity<sup>13</sup>. The variation of the absorption coefficient,  $k$ , and refractive index,  $n$ , with wavelength have been measured by Gilbert<sup>14</sup> for a series of coals of varying grades and for an artificial graphite in the spectral range from 6000 Å to 2400 Å. These studies have been made on coal in its bulk state, using a polarized light technique. The results show good general agreement with those of Ergun *et al.*<sup>12</sup>, who have measured the absorption of ultra-violet and visible light by coal and natural graphite in the form of flakes 500 Å-2000 Å thick.

The carbon content of the specimens used by Gilbert<sup>14</sup> varies from 81.5 per cent to 97 per cent for coals to 100 per cent for graphite. Spectral variations of the absorption coefficient and the real part of the refractive index are shown in Figs. 2 and 3. The absorption coefficient,  $k$ , is defined at a vacuum wavelength,  $\lambda$ , by  $I = I_0 \exp(-4\pi k x / \lambda)$ , where  $I_0$  is the intensity of the incident light, and  $I$  is the intensity of the emergent light after passing through a distance  $x$  in the medium. In Fig. 2, graphite shows a well defined absorption peak near 2500 Å. A peak also occurs for each sample, but it is not so well defined for high grade specimens. The  $n$  and  $k$  spectra of lower rank specimens run parallel to each other, but those of higher grade specimens depart from this trend. This is probably due to the fact that graphitization has not occurred in the lower grade specimens<sup>12</sup>. The shapes of the curves of wavelength dependence of  $n$  and  $k$  for specimens 36 and 15 (both high rank coals) behave very differently. Ergun *et al.*<sup>12</sup> have measured the extinction coefficient of sections 500 Å-2000 Å



thick of several grades of coals and natural graphite. The shapes of the curves of extinction coefficient versus wave energy (Fig. 4) for graphite and highest grade coals (anthracite and semi-anthracite) separate out from those for the lower grade ones, as found in Gilbert's  $n$  and  $k$  spectra of coals<sup>14</sup>. Also, like specimens 36 and 15 of Gilbert, the shapes for the anthracite and semi-anthracite are noticeably different in the blue-visible region. Ergun *et al.*<sup>12</sup> have reported that these high grade coals show X-ray scattering characteristic of two dimensional ( $hk$ ) graphite-like layers; and like graphite these coals show anomalous dispersion which is characteristic of a resonance phenomenon. Apparently, however, unlike crystalline graphite, they do not possess a well defined conduction band. Chemical analyses of these two coals show C, 92.4 per cent; H, 3.8 per cent; N, 0.5 per cent; O, 2.3 per cent; S, 1.0 per cent, for semi-anthracite, and for anthracite C, 94.1 per cent; H, 2.6 per cent; N, 0.4 per cent; O, 2.2 per cent; S, 0.7 per cent.

An interesting feature of the curve of absorption coefficient versus wavelength for specimen 15 (a high rank coal), shown in Fig. 2, is that a change in slope occurs near 4200 Å, which is close to the wavelength of the knee of the interstellar extinction curve. Such a change in slope also appears at about the same wavelength (4300 Å) for semi-anthracite in Fig. 4. This coincidence indicates that small amounts of impurities present in interstellar graphite particles can alter their optical properties so as to produce a change in slope of the interstellar extinction curve close to 4300 Å (see Fig. 1). It is our intention to examine whether the values of  $n$  and  $k$  for high grade coals like the specimen 15 of Gilbert<sup>14</sup> give a better fit to the observed interstellar extinction curve than the model of pure graphite covered with ice as used by Nandy and Wickramasinghe<sup>15</sup> to explain the Cygnus and Perseus extinction law.

Hoyle and Wickramasinghe<sup>10</sup>, and Donn *et al.*<sup>16</sup>, have examined in detail the growth mechanism of graphite grains in cool stars. They have concluded that crystalline graphite particles will condense in the photospheres of  $N$  stars and also in the outer atmospheres of ordinary  $M$  giants. These particles are ejected into interstellar space by radiation pressure, the critical radius for escape being of the order of  $10^{-5}$  cm. Impurity atoms can be incorporated in the grain structure in several ways. (1) If crystals grow at high supersaturation temperatures, some amount of foreign substances could be included in them. Furthermore, grains forming in the photospheres of  $N$  stars have to traverse stellar atmospheres before escaping; during this journey other substances could be adsorbed<sup>16</sup>.

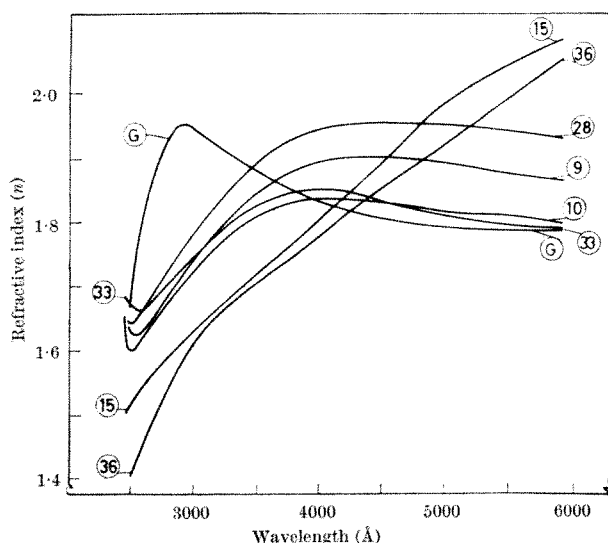


Fig. 3. Variation of refractive index with wavelength (reproduced from *Fuel* (ref. 14)).

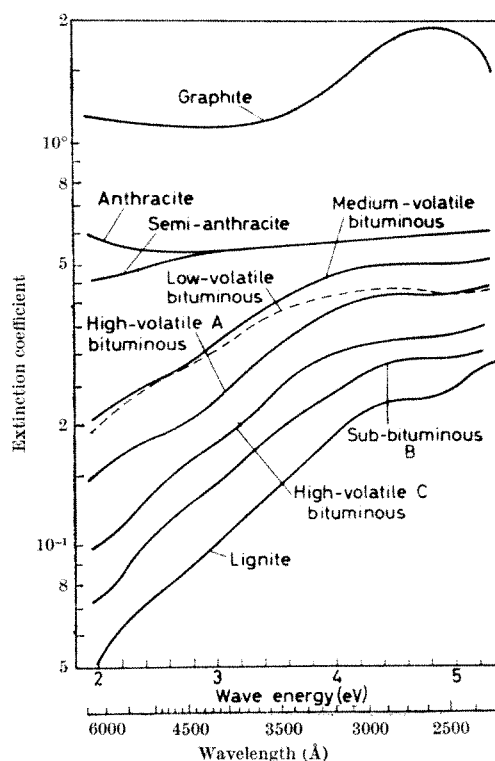


Fig. 4. Variation of extinction coefficient with wave energy (reproduced from *Fuel* (ref. 12)).

(2) During the encounters with clouds in interstellar space, energetic collisions between the grains may lead to melting and subsequent fusion incorporating other elements. (3) Sputtering can cause vacancies in the lattice, thereby enabling impurities to become incorporated more readily into the structure of the grain.

It is therefore highly improbable that interstellar graphite particles could exist in pure form. It is far more likely that these particles contain certain amounts of impurities. The observed knee at 4300 Å and the shoulder in the ultra-violet suggest that hydrogen and oxygen may be among the most abundant impurities, rendering the particles anthracitic. The variation in the amount and nature of these impurities may well explain the observed intrinsic scatter in the extinction curves.

We thank L. A. Gilbert of the BBC, London, for drawing our attention to his work on graphite and certain specimens of coals<sup>14</sup>.

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## PLANETARY SCIENCE

## The Goose Lake Meteorite and the Goose Lake Fragments

THE metallographic structure of the main mass of the Goose Lake meteorite has been described by Henderson and Perry<sup>1</sup>. The meteorite was discovered in the autumn of 1938 and was removed from the site in May 1939. The site was re-examined in 1960, when a large number of small, partly corroded, fragments were discovered in and around the place from which the main mass had been removed. The circumstances under which the fragments were found, together with a preliminary description of their structure and some suggestions about their origin, can be found in ref. 2.

We have examined a number of typical fragments weighing 0.2–0.4 g which were made available to us by Dr C. P. Butler and we have also examined the 72 g slice of Goose Lake [B.M. 1959, 951] which was made available to us by Dr Hey of the British Museum (Natural History).

The slice [B.M. 1959, 951] appears to have suffered distortion of the surface layers all round the smooth outside surface of the meteorite and this distortion extends inwards to a depth of about 0.5 mm. At a number of places within the distorted layer there appear to be relics of kamacite granulation, but the patches of granulation extend only to a depth of 0.1–0.2 mm below the present outer surface of the meteorite. The granular patches may be the relics of a heat alteration zone which was produced during atmospheric flight, in which case the zone of kamacite granulation has been almost completely removed by subsequent terrestrial corrosion and/or abrasion. In this context one source of terrestrial damage which must not be overlooked is the surface deformation which may arise when a heavy mass of iron is hauled about by chains during the recovery process.

In [B.M. 1959, 951] two small areas of non-metallic phase were present. The smaller was situated about 3 mm below the present surface of the meteorite and the larger was nearby but situated about 12 mm below the surface. Each consisted of schreibersite with swathing cohenite, and, as is shown in Fig. 1, the cohenite has undergone partial decomposition into graphite and ferrite at places where the cohenite was cracked. Furthermore, the range of plessite structures encountered within the slice included the spheroidal form which was figured by Henderson and Perry<sup>1</sup>, but more usually the large fields of plessite showed

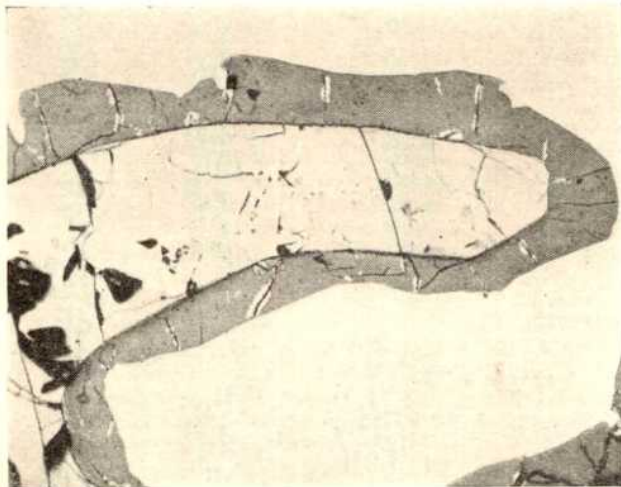


Fig. 1. Schreibersite surrounded by cohenite in [B.M. 1959, 951] ( $\times c. 75$ ). Etched in alkaline sodium picrate reagent. The schreibersite shows grey with cracks and holes (black); the cohenite has stained dark but shows unstained ferrite and black graphite along the radial cracks. The matrix is kamacite (smooth grey).

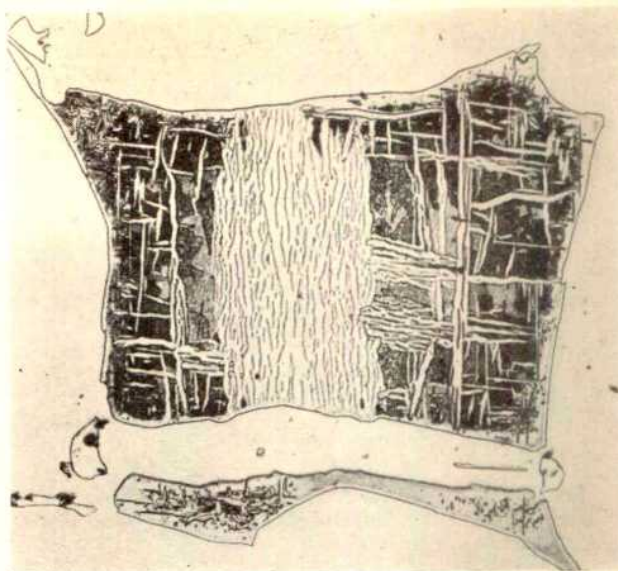


Fig. 2. An example of the complex decomposition morphology of plessite in the Goose Lake meteorite [B.M. 1959, 951] ( $\times c. 55$ ). Etched 2 per cent 'Nital'.

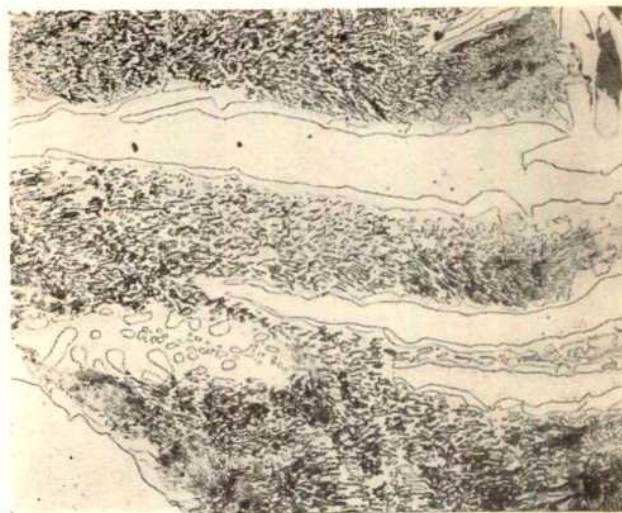


Fig. 3. Example of mechanically deformed fine pearlitic plessite from fragment (GLF)1 ( $\times c. 290$ ). Etched 2 per cent 'Nital'.

complex structures with comb-like, spindle, bainitic and fine pearlitic plessites coexisting to a greater or less extent as is shown in Fig. 2. Our own observations on the Canyon Diablo meteorite<sup>3</sup> suggest that fine pearlitic plessite is usually found in the vicinity of cohenite. The main kamacite bands of Goose Lake contained occasional rhabdites.

The Goose Lake fragments (GLF) are always corroded to a greater or less extent and seven of the least corroded fragments were examined metallographically. In no fragment was unambiguous granulation of the kamacite visible, but they all showed a greater or less degree of mechanical distortion of the type reported by Butler<sup>2</sup>. Moreover, mechanically distorted versions of all the types of structure noted above for the main Goose Lake mass have been found in the fragments. For instance, Fig. 3 shows a distorted area of fine pearlitic plessite in (GLF)1, while Fig. 4 shows cohenite in (GLF)12, and in the same fragment an earlier generation of relatively large hexagonal nodules of graphite was found embedded in the kamacite out of contact with the cohenite. A similar graphite nodule from (GLF)6 is shown in Fig. 5.



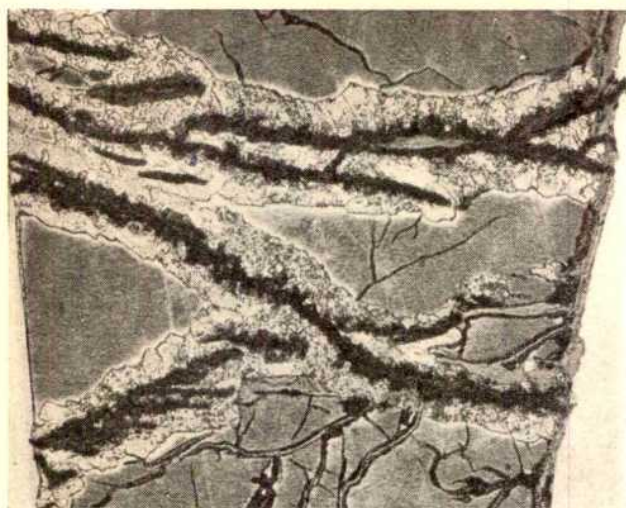


Fig. 4. Decomposed cohenite in fragment (GLF)12 ( $\times c. 290$ ). Etched in alkaline sodium picrate reagent. Undecomposed cohenite is stained dark. At cracks in the cohenite the compound has decomposed into black graphite and light polycrystalline ferrite. Some grey corrosion product is present at the interface between cohenite and the kamacite matrix (right side of photograph).

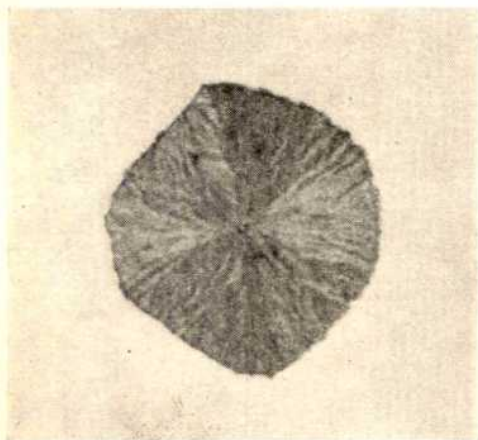


Fig. 5. Hexagonal nodule of graphite embedded in kamacite in fragment (GLF)6 ( $\times 500$ ). Polarized light.

From this examination it would appear that the structure of the Goose Lake fragments is not inconsistent with the structure of the main Goose Lake mass. Where there are differences they are differences of amount rather than of character. For instance, in the fragments the decomposition of cohenite to graphite and ferrite seems to be slightly more complete than is the case with the two examples of cohenite which are present near the surface of [B.M. 1959, 951] and, furthermore, while the presence of carbon shows itself as cohenite in the slice it is made manifest in some of the fragments by the appearance of both cohenite and nodular graphite. Thus some of the Goose Lake fragments appear to have arisen from carbon rich areas of the main Goose Lake mass, but it remains an open question where those areas of carbon enrichment were located in relation to the size and shape of the present Goose Lake meteorite and how they came to be distributed about the main mass at its place of discovery.

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## Large Surface Carbon Dioxide Anomalies in the North Pacific Ocean

DURING the trans-Pacific Lusiad expedition of R.V. Argo, May 18–June 9, 1962, Waterman observed a large inequality of the partial pressure of the carbon dioxide in the atmosphere and in the surface water in the centre of the North Pacific Ocean<sup>1</sup>. His traverse (Fig. 1) showed that the partial pressure of carbon dioxide ( $P_{CO_2}$ ) in the water was generally less than that in the atmosphere (320 p.p.m.), with a maximum anomaly of about 20 per cent. This difference in  $P_{CO_2}$  is too large to be accounted for solely by changes in temperature, salinity, or solution of calcium carbonate<sup>2</sup>. Our recent work shows that the carbon dioxide anomaly is of biological origin. It is similar to, but of greater dimensions than, the biologically induced carbon dioxide anomalies found by Teal and Kanwisher<sup>3</sup>.

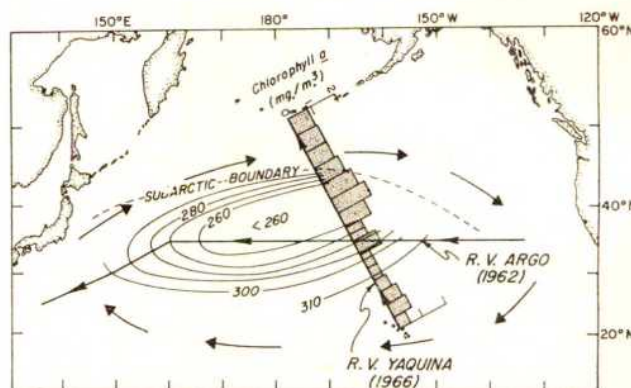


Fig. 1. Early summer  $P_{CO_2}$  contours in the North Pacific. Solid lines show the cruise tracks of R.V. Argo and R.V. Yaquina. The arrows indicate the general surface circulation pattern. Subarctic boundary, indicated by a dotted line, separates the ocean into the subarctic (north) and the subtropic (south) regions. Chlorophyll *a* histogram on the Yaquina traverse was prepared from average values per 2 latitudinal degrees: its concentration is in  $mg/m^3$ .

In June 1966, we studied the surface chemical conditions of the ocean along the Hawaiian–Aleutian line aboard R.V. Yaquina of Oregon State University<sup>4</sup>. From the surface pH, alkalinity, salinity, and temperature data, we calculated  $P_{CO_2}$  in sea water for our traverse<sup>5</sup>. Although the pH-alkalinity method for calculating  $P_{CO_2}$  in sea water enables us to estimate only in intervals of 10 p.p.m., we found a good agreement between the Argo and the Yaquina results where the traverses crossed each other. The summer (June) contours of  $P_{CO_2}$  based on these two traverses were plotted (Fig. 1). These contours follow the general surface circulation pattern of the North Pacific, as shown by arrows in Fig. 1.

A good correlation between the  $P_{CO_2}$  and the amount of chlorophyll *a* in the surface 1 m was observed in the Yaquina data. (The details of the analytical method used to measure chlorophyll *a* are given in ref. 5. In order to take into consideration variations in diel pigment, we have averaged the values for pigment over two latitudinal degrees to give the pigment distributions shown in Fig. 1.) The maximum pigment concentration of 1.5  $mg/m^3$  occurred at the minimum  $P_{CO_2}$ . The pigment concentration decreased with the increase in the partial pressure. The plant nutrient concentrations were quite low where  $P_{CO_2}$  was less than 260 p.p.m.: phosphate range was 0.2–0.5  $\mu moles/l.$ , nitrate 1–4  $\mu moles/l.$ , silicate 3  $\mu moles/l.$  It is probable that a phytoplankton bloom increased the pigment content and decreased nutrients in sea water. It is also possible that the organic matter production by phytoplankton lowered  $P_{CO_2}$  in the surface water, and maintained it by kinetically slow reactions involving the production and consumption of organic acids and bases.

The surface chemical conditions of oceanic water are quite different even from that 1 m below the surface.

Goering and Menzel<sup>6</sup> showed that dissolved organic carbon in the upper 3 mm of water was 0.4 mg/l. more than that at a depth of 1 m in the tropical Atlantic. This was attributed to the accumulation of decomposing organic material floating up to the surface. The increase in organic carbon indicates the possibility of increasing organic acid and base contents at the surface, which in turn affect  $P_{CO_2}$ , for the physical properties of the air-water interface may be extensively modified by the adsorption of surface active organic matter at the sea surface<sup>7</sup>. Because the air-sea exchange of carbon dioxide has to pass through this surprisingly different chemical "membrane" at the sea surface, we feel that a sustained anomaly of  $P_{CO_2}$  across the interface can be maintained biochemically.

We propose that the high anomaly in  $P_{CO_2}$  in the North Pacific surface water is produced by the combined effect of general oceanic circulation, biomass production and consumption. The organic matter could have been brought toward the area of low  $P_{CO_2}$  by the surface currents, and perhaps it is slowly utilized there by biomass. There is, however, reason to believe that the production of phytoplankton in the anomaly area was recent and may have been *in situ* rather than advected. Ratios of chlorophyll *a* to  $\beta$  carotene had maxima in the area of the high anomaly as well as near the Hawaiian and Aleutian Islands. High pigment ratios indicate a "healthy" population<sup>8</sup>. A population produced elsewhere and advected towards a convergence could be senile and the ratios may be low.

The carbon dioxide anomaly that Waterman<sup>1</sup> and we observed could be a seasonal rather than a permanent phenomenon, for we both made our observations in early summer. We believe that winter observations could show whether the anomaly is a seasonal or permanent phenomenon. We plan to study the winter condition in 1968.

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### Alkali Olivine Basalt dredged near St. Paul's Rocks, Mid-Atlantic Ridge

RECENT studies of deep sea basalts have revealed the great predominance of olivine tholeiites over both normative nepheline and normative quartz basalts<sup>1</sup>. An alkali basalt recently dredged between 2,950 and 1,975 m near St. Peter and St. Paul Rocks (St. Paul's Rocks) consequently seemed worthy of prompt description. Although mentioned in an abstract<sup>2</sup>, we have not previously published a chemical analysis of this basalt. This alkali basalt flow is of special interest because it is not part of a large submarine volcano, but rather evidently was erupted directly on a floor of spinel peridotite mylonites similar

to and contiguous with those described from St. Paul's Rocks, a probable high temperature intrusion derived from the mantle<sup>3</sup>.

Extensive dredging was carried out around St. Paul's Rocks during cruise 20 of the R.V. Atlantis II of the Woods Hole Oceanographic Institution in an attempt to delineate the outcrop of the ultrabasic mylonites which are exposed on the islets. Numerous rock types, in addition to the mylonites, were dredged within sight of the islets; although their study is not yet complete, some of the more interesting have been examined in the laboratory, and a detailed report is in preparation.

One of the most remarkable rock types, the subject of this report, is a vesicular basalt containing abundant small olivine nodules and partly "digested" mylonitized spinel peridotite inclusions. The dredge (No. 43) from which this basalt was obtained is located on Fig. 1.

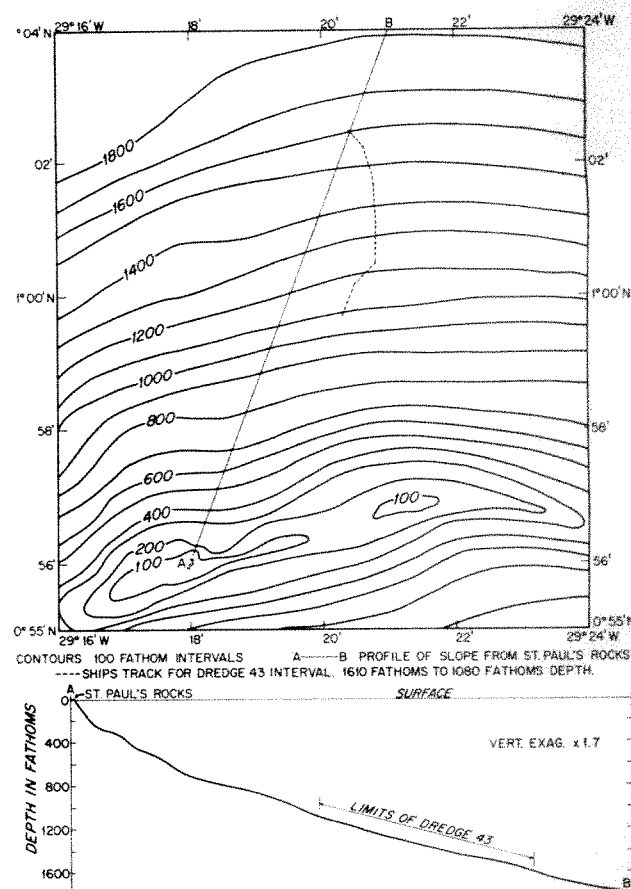


Fig. 1. Bathymetry and location of dredge A II-20, 43, St. Paul's Rocks.

The ultramafic intrusion exposed at St. Paul's Rocks extends beneath the sea along a ridge elongated in an E.N.E. direction. Dredge samples indicate that the submarine exposures of the intrusion are spinel peridotite mylonites and alkaline ultrabasic brown hornblende mylonites, the two major rock types on the islets<sup>3</sup>. A complex series of rocks which includes fresh and metamorphosed basalts, basaltic pyroclastic rocks, basic and ultrabasic plutonic rocks, and carbonate sedimentary rocks outcrop to the north and south of the intrusive mass. Some of the dredges, even those which only covered a short distance of the bottom, yielded a wide variety of rocks, suggesting that the rocks probably dip steeply around the margin of the intrusion. The alkali basalt described here probably occurs unconformably on the



intrusion, and is probably much younger than the commonly deformed and altered rocks which rim the intrusion. A large amount of talus derived from the islet was in the dredge on top of the alkali basalt fragments; evidently this talus was the last material to enter the dredge.

Table 1 gives the bulk analysis of a sample from which the nodules were extracted after coarse crushing. The basalt is perfectly fresh in hand specimen and in thin section. To remove any possible salt contamination, the sample was washed in distilled water after fine grinding. The high titania, soda and potash, and the low silica, are particularly significant in the analysis. These features, and the presence of nepheline in the norm, clearly place the basalt in the alkali olivine basalt series. Deep sea basalts may have "alkaline affinities"<sup>1</sup>, and may contain abundant normative and modal olivine, but rarely contain normative nepheline in the amount recorded here. The high barium (300 p.p.m.), strontium (500 p.p.m.) and zirconium (200 p.p.m.) further confirm the alkaline nature of this basalt, although the high nickel (270 p.p.m.) and chromium (250 p.p.m.) are anomalous for alkali basalts.

Table 1. COMPOSITION OF ALKALI OLIVINE BASALT\*, 4 II-20, 43-49, DREDGED A FEW KILOMETRES NORTH-EAST OF ST. PAUL'S ROCKS

		Norm	
SiO <sub>2</sub>	43.15	Or	9.63
Al <sub>2</sub> O <sub>3</sub>	13.46	Ab	9.67
Fe <sub>2</sub> O <sub>3</sub>	4.52	An	16.34
FeO	8.22	Ne	10.67
MnO	0.11	Di	22.23
MgO	10.80	Ol	16.76
CaO	9.80	Mt	6.55
Na <sub>2</sub> O	3.47	Il	5.13
K <sub>2</sub> O	1.63	Ap	1.64
H <sub>2</sub> O +	1.21		
H <sub>2</sub> O -	0.15		98.61
TiO <sub>2</sub>	2.70		
P <sub>2</sub> O <sub>5</sub>	0.75		
99.97		Analyst: E. Jarosewich	

\* The analysed basalt is extremely fine grained and contains olivine phenocrysts in a matrix of microlites of plagioclase (about An<sub>60</sub>), titan-augite, olivine and light brown barkevikitic hornblende. Accessories include an iron-titanium oxide, biotite, apatite and possibly haüyne. Small amounts of clear glass and alkali feldspar also occur. Modal nepheline is not present.

The predominance of low potash tholeiitic basalt on the ocean floor and the apparent restriction of alkali basalts to the top of high volcanic edifices has been noted by Engel *et al.*<sup>2</sup>. In the Hawaiian Islands, alkali basalts occur mainly as late, quantitatively minor extrusives. These relationships led some petrologists<sup>3</sup> to postulate, first, that alkali basalts are derived by low pressure differentiation of sub-alkaline olivine basalts of the "oceanic tholeiite" type in near surface magma chambers, and second, that "oceanic tholeiite" magma is the only basaltic magma derived from the mantle. Experimental data indicate that low pressure differentiation of tholeiitic magma should not yield normative nepheline liquids<sup>4</sup>, and thus argue against the first view.

The alkali basalt described here also suggests mantle derivation of alkali basalts. The basalt is clearly not part of a thick "oceanic tholeiite" pile (Fig. 1), and thus based on field occurrence alone is an unlikely low pressure differentiation of "oceanic tholeiites". It would appear that "parental" alkali basalt magma originates by some other, more deep-seated phenomena, such as partial fusion of eclogite of tholeiitic basalt composition—a process outlined by Yoder and Tilley<sup>5</sup>.

The St. Paul's Rocks situation clearly indicates that it is not always true that oceanic alkaline basalts occur as late eruptives in otherwise tholeiitic sequences. Thus caution should be used in tying the origin of all oceanic alkaline basalts to the situation exemplified, for example, by the Hawaiian Islands.

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## PHYSICS

### Identification of High Gain Laser Lines in Argon

BRISBANE has reported three high gain laser lines in a pulsed discharge in argon<sup>1</sup>.

The first line at 1.270  $\mu$ m is explained in Paschen notation as the transition  $3d_2-2p_6$ , which has the wavelength 12,638-480 Å (ref. 2). We believe that the observed line is the same that we have reported earlier<sup>3</sup> as a super-radiant line with the wavelength 12,702.21 Å. It corresponds to the transition  $3d'[1\frac{1}{2}]_1 \rightarrow 4p'[1\frac{1}{2}]_1$  in Racah notation ( $3s'_1 \rightarrow 2p_2$  in Paschen notation) and the wavelength in spontaneous emission is 12,702.280 Å (ref. 2).

The second line at 1.793  $\mu$ m is explained as the transition  $3d_5-2p_6$ , which has the wavelength 17,914.726 Å (ref. 2). It could also be explained as the transition  $3d_6-2p_7$  with the wavelength 17,914.629 Å (ref. 2) as pointed out by Patel *et al.*<sup>4</sup>, who observed the line in a CW operated laser.

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### Validity of the Debye Scattering Equation in Elastic Electron Diffraction

THE methods of Debye<sup>1</sup> are accepted in the scattering of electrons by gases, but despite the application to thin films by Germer and White<sup>2</sup> their use in kinematic electron diffraction from polycrystalline solids has seemed suspect and none of the recent texts on electron diffraction mention the methods. Possibly this is because of a pre-occupation with dynamical theory. Many real specimens, however, diffract kinematically and the Debye "gas" equation, if valid for closely packed small crystals, would enable the detailed intensity profile for polycrystalline films to be computed. The objection to the Debye theory is that it neglects interference between atoms situated in different crystals; while this is permissible for a gas of large molecules, it requires justification if the molecules (or small crystals) are densely packed.

The work of Menke<sup>3,4</sup> on molecular liquids in fact shows that the Debye gas equation must be valid for polycrystalline solids scattering X-rays kinematically. But the methods must be adapted if they are to be applied to electron radiation, for electron waves have a small coherence width  $2R_c$  of about 1000 Å, and the specimens are thin disks, perhaps 100 Å thick and 1 mm in diameter. Thus any one coherently irradiated volume will contain too few crystals for statistical methods to be valid. The specimen is, however, an assembly of about  $10^8$  of such volumes. One may therefore invent a "statistical coherence zone" having the properties of the coherently irradiated specimen volumes averaged over the assembly. To obtain the elastically diffracted intensity one must average phase factors between pairs of crystal centroids weighted by the probability of the occurrence of a given centroid-to-centroid spacing. This averaging can be carried out for a statistical coherence zone because it can contain very many points accessible to the crystal centroids.

Consider a disk shaped statistical volume of coherence containing  $N_c$  crystals, each of which is assumed to be similar and to possess  $n$  atoms. One may follow the X-ray procedure<sup>4</sup> and show that the theoretical elastic electron intensity is proportional to three terms. One of these depends on internal interference effects only

$$I_1 = nN_c f^2 \left( 1 + \frac{2}{n} \sum \frac{\sin 2\pi s r_{pq}}{2\pi s r_{pq}} \right) \quad (1)$$

It is the Debye gas equation,  $s = 2 \sin \frac{1}{2}\beta/\lambda$ .

The other two terms result from interference between atoms in different crystals. An approximation to one of them is

$$I_2 = -8f^2(N_c - 1)\Psi\Phi(2\pi s D) \quad (2)$$

where

$$\Phi(u) = 3(\sin u - u \cos u)u^{-3}, \Psi^{\dagger} = \sum \frac{\sin 2\pi s r_{cp}}{2\pi s r_{cp}}$$

and  $D$  is rather greater than the diameter of a crystal. The summation  $\Psi^{\dagger}$  is over every centroid-to-atom spacing in a crystal. The third term is a "Debye volume interference" term and contains a factor

$$I_3 = 4f^2 N_c(N_c - 1) \Psi \left\{ \frac{J_1(v)}{v} \right\}^2 \quad (3)$$

where  $v = 2\pi s R_c \cos \frac{1}{2}\beta$ , and  $J_1(v)$  is the first order Bessel function.

$I_1$ ,  $I_2$  and  $I_3$  all contribute to an intense maximum at the origin;  $I_2$  has a second weak maximum when  $s \approx 0.91 D^{-1}$ , and  $I_3$  when  $s \approx 0.82 R_c^{-1}$ . For example, if  $D = 50$  Å (small crystals and kinematic conditions) and  $R_c = 500$  Å, these weak maxima occur at  $s = 0.018$  and  $s = 0.0016$  Å<sup>-1</sup> respectively. For comparison the first maximum of the diffraction pattern of face centred cubic metals occurs at values of  $s$  greater than  $0.3$  Å<sup>-1</sup>.

In a real film there must be a spread of shapes and sizes which will add mathematical complexity without altering the physical principles. The remarkable and important fact is that the intercrystal interference effects do not contribute to the normal elastic electron diffraction pattern and therefore that the Debye equation (1) is valid for computing the elastic intensity profile in kinematic diffraction of electrons by polycrystalline solids.

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## Relationship between Wettability and Adhesion of Polyethylene

THE formation of a good adhesive joint requires intimate contact between the substrate and the adhesive. This requirement implies that at some point in the cure of a bonded joint the adhesive must become a liquid so that this contact can be obtained. Such contact can be termed the wetting of the substrate by the adhesive, depending on the relative roughness of the substrate<sup>1</sup>.

Wetting of a surface by a liquid is formally defined by the well known Young equation (equation (1))

$$\gamma_{sv} - \gamma_{sl} = \gamma_{lv} \cos \theta \quad (1)$$

where  $\gamma_{lv}$  is the surface tension at liquid-vapour interface;  $\gamma_{sl}$ , the surface tension at solid-liquid interface;  $\gamma_{sv}$ , the surface tension at solid-vapour interface; and  $\theta$  is the contact angle.

The relationship between bond strength and wetting can be found if one considers the reversible work of adhesion  $W$  needed to separate a liquid from a solid surface.  $W$  is given by an energy difference equation as follows

$$W = \gamma_{sv} + \gamma_{lv} - \gamma_{sl} \quad (2)$$

Combining equations (1) and (2) gives

$$W = \gamma_{lv} (1 + \cos \theta) \quad (3)$$

Because  $\gamma_{lv}$  is always positive, the theory predicts a direct linear relationship between bond strength and  $(1 + \cos \theta)$ .

The angle  $\theta$  is a measure of the degree of wetting and can be determined using a contact angle goniometer or, in the case of very small  $\theta$ , interferometry. Laboratory methods for obtaining  $\gamma_{lv}$  are well defined and no problems should be encountered in using methods involving the Du Nouy ring tensiometer. With  $\gamma_{sl}$  and  $\gamma_{sv}$ , accurate measurement is impossible. Zisman<sup>2</sup> has introduced  $\gamma_c$  (critical surface tension of a solid). This empirically determined parameter is a useful characterization of the surface in so far as it is proportional to  $\gamma_s$  (specific surface free energy of the solid). It will be seen from the definition of  $\gamma_c$  that it is a determination of the left side of equation (1) corresponding to  $\cos \theta = 1$  (complete wetting). Thus knowing  $\gamma_c$  eliminates the need to know  $\gamma_{sv} - \gamma_{sl}$  in order to find the value of  $\gamma_{lv}$  corresponding to complete wetting. Correspondingly  $\gamma_c$  can be used to predict lesser degrees of wetting. In brief, the method involved in a determination of  $\gamma_c$  for a solid is as follows. Using a set of liquids of varying surface tension (preferably a homologous series of organic liquids, for example,  $n$ -alkanes) contact angle measurements are taken on the surface in question. A plot of  $\cos \theta$  against the liquid surface tension,  $\gamma_{lv}$ , will reveal a straight line relationship. If extrapolated to  $\cos \theta = 1$  (which represents complete wetting) this graph will yield  $\gamma_c$  as the value of liquid surface tension at the point where the plot crosses the line  $\cos \theta = 1$ . In other words, liquids whose surface tensions are greater than  $\gamma_c$  will not give complete wetting on the surface, while liquids with surface tensions less than or equal to  $\gamma_c$  will give complete wetting.

The critical surface tension  $\gamma_c$  now makes available a method for classifying surfaces as far as their energy availability is concerned. Zisman has shown the value of  $\gamma_c$  for clean polyethylene to be about 31 dynes/cm. Thus polyethylene is a low energy surface in comparison with metals or glass, which have considerably higher values of  $\gamma_c$ . This difference in surface energy availability indeed makes it more difficult to bond to polyethylene than to metal. Polytetrafluoroethylene is reported to have a  $\gamma_c$  of about 18 dynes/cm, corresponding to the non-sticking property of "Teflon".

Any contamination on the surface will in general lower the value of  $\gamma_c$  apparent at the surface, and surface pre-

Table 1. CONTACT ANGLES OF EPOXY-POLYAMIDE IN 70/30 RATIO ON LOW DENSITY POLYETHYLENE (IN DEGREES)

No surface treatment	Acetone wipe	Acetone wipe and 20 min acid bath at room temperature	Acetone wipe and 60 min acid bath at room temperature	Acetone wipe and 1 min acid bath at 70° C	Acetone wipe and 5 min acid bath at 70° C
39	28	34	24	24	16
37	36	26	24	24	20
33	37	23	18	22	17
39	36	30	20	20	19
31	36	22	26	17	22
38	33	24	22	23	10
37	36	25	24	17	20
30	28	22	21	17	15
35		33	16	22	22
				19	17
				20	23
				20	26
				20	15
Average 35.4	33.8	26.5	22	20.4	18.5

paration before taking wetting measurements or bonding is therefore recommended in order to increase the surface energy available.

The question of whether an increase in wetting will give a proportional increase in bond strength was investigated for the case of polyethylene. The most direct approach to such a problem would be to limit the number of variables to a minimum by using only one type of substrate material and one adhesive, and attempting to keep all other conditions as constant as possible. The only variable used to influence the wetting was the type of surface treatment given the polyethylene<sup>3</sup>.  $\gamma_{LV}$  was therefore kept constant while  $\gamma_c$  was varied.

We used specimens of low density polyethylene measuring 1 in.  $\times$  4 in.  $\times$   $\frac{1}{8}$  in. The treatments given are shown in Table 1. The adhesive used was a mixture of a bisphenol type epoxy and a liquid polyamide resin in a 70/30 ratio by weight. This adhesive has a surface tension of 41.7 dynes/cm.

Table 1 gives the results obtained in measuring the contact angles formed by the adhesive on the polyethylene surfaces prepared by various means. The average value of contact angle at the bottom of each column decreased as the polyethylene was given better surface treatments. This indicates that the value of  $\gamma_c$  for the polyethylene was being increased by the treatments. Variation in the contact angles in any one column is evidence of inhomogeneity of the surface and/or the adhesive. The best wetting obtainable was about 18°, as is shown in the last column of Table 1. This is consistent with the theory because the adhesive surface tension is 41.7 dynes/cm, while the value of  $\gamma_c$  for polyethylene is 31 dynes/cm. Under no condition should such a system give complete wetting.

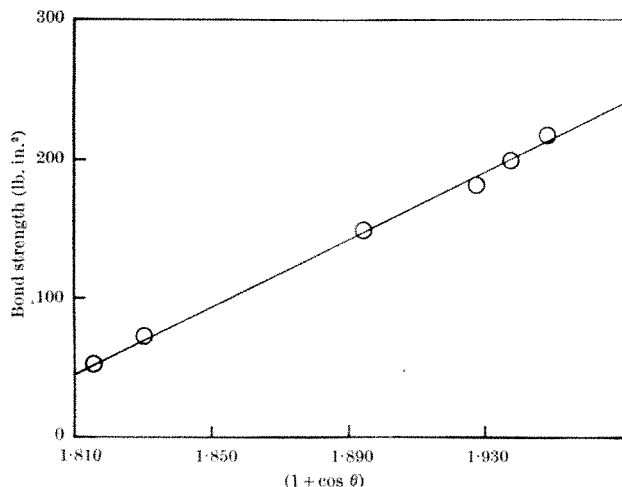


Fig. 1. Plot of bond strength against  $(1 + \cos \theta)$  on polyethylene using epoxy-polyamide adhesive in 70/30 ratio by weight.

Shear specimens with a bonded area of 1 in.  $\times$   $\frac{1}{2}$  in. were prepared from the different polyethylene surfaces. Once cured the samples were ruptured. The results are given in Fig. 1. The points on the plot represent the different types of treatment shown in Table 1. The experimental results agree excellently with the theory because they show bond strength and  $(1 + \cos \theta)$  to be linearly related with a positive slope. Such a plot would be useful in determining the strength values of bonds without having to destroy them.

It must be remembered that contact angles are influenced only by the surface. Any weak boundary layers beneath the surface would not contribute to the contact angle. Any break in such a region could not therefore be related to contact angles. Breaks occurring because of cohesive failure of the adhesive would also be irrelevant to surface energetics and therefore must also be avoided. If, however, one eliminates any weak boundary layer or has evidence that the break is adhesional (to the unaided eye) between the adhesive and the surface, as was the case with all the samples tested, then a plot similar to Fig. 1 can be obtained.

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## THE SOLID STATE

### Structure of High Modulus Carbon Fibres

In a recent letter we drew attention to the fact that carbon fibres with a range of mechanical properties<sup>1</sup> could be produced by a method developed at the Royal Aircraft Establishment<sup>2</sup>. These fibres are made from an oriented textile fibre and there is no evidence to suggest that the carbon fibres are sensitive to surface damage like glass fibres. Tensile fracture was tentatively attributed to internal flaws. This letter reports some observations of the structure made on thin sections cut from fibres.

The fibres were sectioned, after mounting in an acrylic resin, on an L.K.B. 'Ultratome 1', with a Leitz diamond knife, at a cutting speed of 1 mm/sec. Longitudinal sections 300 Å thick or less were cut.

Fig. 1 shows a longitudinal section of a fibre with a Young's modulus of  $60 \times 10^6$  lb./in.<sup>2</sup> after heating to 2,500° C. An internal structure of long narrow units lying parallel to the fibre axis can be seen. These units are about 100 Å across, but their length is difficult to define. The electron diffraction pattern (Fig. 2) shows the high degree of preferred orientation in which layer planes, indicated by the (002) reflexion, lie parallel to the fibre axis.

X-ray diffraction has shown that the crystallites are turbostratic with  $L_c$  at least twelve layer planes and  $L_a$  in the range 60–120 Å (R. J. Seed and J. N. Eastabrook, personal communication).

Sections were cut from fibres that had been carbonized to 1,000° C only. While being cut and collected these sections invariably ruptured along their length and opened to give the net-like structure shown in Fig. 3. The fibril units forming this network are about 800–1000 Å across and appear to run the full length of the section with no apparent terminations. From X-ray diffraction  $L_a$  in this 1,000° C material is about 30 Å. The net-like structure is perhaps a fundamental structure in carbon fibres because similar sections can be prepared from fibres heated to



2,500° C if attempts are made to cut thin sections less than 200 Å thick.

Thus from these sections the carbon fibres are seen to consist of long primary units lying parallel to the fibre axis and these primary units are bonded together to form a stretched network of branched fibrils that apparently run the full length of the fibre. The size and degree of branching of the fibrils may be derived from the fibril structure of the parent fibre.

In Fig. 4 a thin section of a high temperature fibre that was broken while being collected is shown. The irregular path of the fracture suggests a crack deflexion mechanism. The fracture behaviour of a fibre may be influenced by the size of fibrils and how well they are bonded together.

Bacon and Tang<sup>3</sup> have found long primary structures in carbon fibres made from oriented rayon fibres, and because the precursor fibre used here was not cellulosic it appears that the structure of the carbon fibres is a consequence of the structure of the parent organic fibre regardless of chemical constitution. We think that the modulus of the carbon fibre is determined by the orientation of the graphite crystallites within the carbon fibrils while the strength is a function of the inter-fibrillar bonding.

Other physical properties of the fibres of technological importance are a result of the orientation and small crystallite size. Because the crystallites are highly oriented, and the crystallite size, Young's modulus and density of the fibres are known, the thermal conductivity may be estimated, because graphite is a lattice conductor. The Debye equation can be used, namely  $K = \frac{1}{3} \rho C v l$



Fig. 1. Longitudinal section of a fibre. Young's modulus  $60 \times 10^6$  lb./in.<sup>2</sup>.



Fig. 2. Electron diffraction pattern of the fibre shown in Fig. 1.

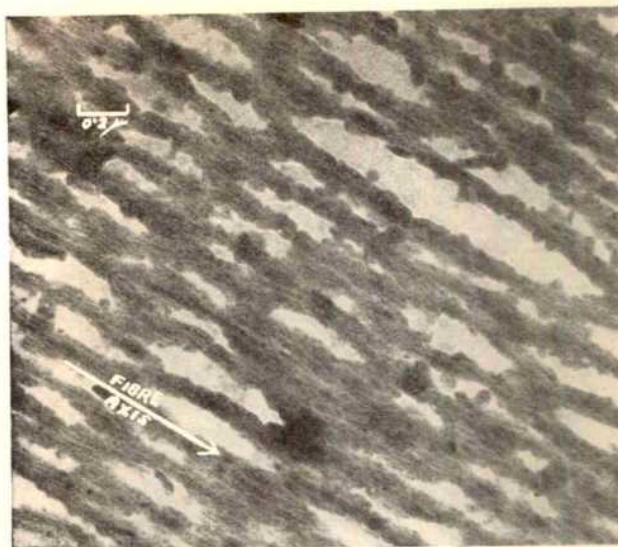


Fig. 3. Longitudinal section of a fibre carbonized to 1,000° C. The section has opened to give a net-like structure.

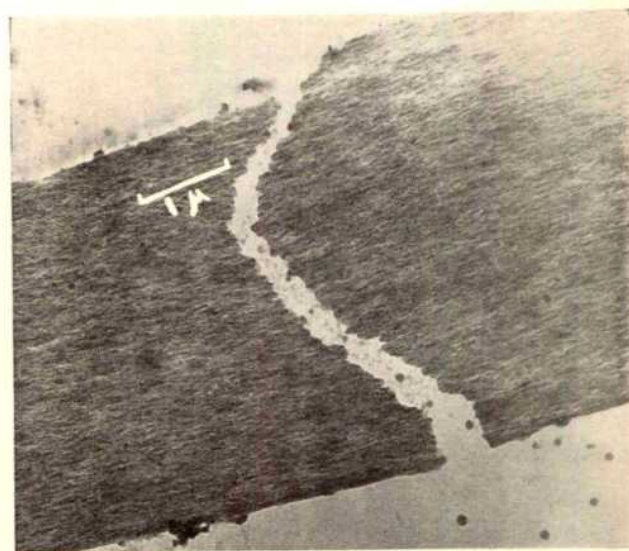


Fig. 4. Longitudinal section, of a high modulus fibre, broken while being collected.

where  $K$  = thermal conductivity,  $\rho$  = density,  $C$  = specific heat for unit mass,  $v$  = velocity of lattice waves transporting the thermal energy, and  $l$  = the mean free path for scattering of these waves. Taking the mean free path as equal to the  $L_a$ , that is, 100 Å, and calculating  $v$  from  $E$  and  $\rho$  gives a value for  $K$  of  $0.142 \text{ cal cm}^{-1} \text{ sec}^{-1} \text{ }^\circ\text{C}^{-1}$ . This is in reasonable agreement with the value calculated from measurements of the thermal conductivity of a composite bar at 77° C of 0.34 vol. fraction unidirectional fibres in a plastic matrix, that is,  $0.12 \text{ cal cm}^{-1} \text{ sec}^{-1} \text{ }^\circ\text{C}^{-1}$  (P. H. H. Bishop and L. N. Phillips, personal communication). This value is much lower than the thermal conductivity of a heat treated pyrolytic graphite (for example,  $3.4 \text{ cal cm}^{-1} \text{ sec}^{-1} \text{ }^\circ\text{C}^{-1}$  at 40° C (ref. 4)) and clearly shows the effect of crystallite size.

The same is true of the electrical resistivity which is  $3 \times 10^{-4} \text{ ohm cm}$  at room temperature for fibres of Young's modulus  $60 \times 10^6 \text{ lb./in.}^2$  whereas highly ordered pyrolytic graphite has a resistivity of about  $4 \times 10^{-5} \text{ ohm cm}$  parallel to the basal planes<sup>5</sup>.



Our observations on the structure of the carbon fibres are in agreement with those of D. V. Badami, J. C. Joiner and G. A. Jones which are published below.

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### Microstructure of High Strength, High Modulus Carbon Fibres

Watt, Phillips and Johnson<sup>1</sup> have reported the development of high strength, high modulus carbon fibres. More recently, Moreton, Watt and Johnson<sup>2</sup> have reported the variation of the tensile properties of these fibres with their heat treatment temperature. Shindo<sup>3</sup> did pioneer work on this class of fibre, including X-ray and electron diffraction and transmission electron microscopy on broken fragments, but he gave no clear picture of the structure within the fibre. This communication reports the results of our initial structural studies of fibres prepared by a similar process to that of Watt, Phillips and Johnson. These fibres, with diameters in the range 6–8  $\mu$ , had been heat treated to 2,600°C and had moduli of  $60 \times 10^6$  lb./in.<sup>2</sup> and ultimate tensile strengths of  $0.25 \times 10^6$  lb./in.<sup>2</sup>.

X-ray diffraction photographs (Fig. 1) taken of bundles of these fibres suggest that they consist of graphite crystallites about 50 Å in size—calculated from the line breadth of the (002) reflexion using the Scherrer equation—and that the basal planes are highly oriented along the fibre axis, with a spread of about  $\pm 10^\circ$ . A relation between orientation and modulus has been found which is similar to that reported by Watt, Phillips and Johnson<sup>1</sup>. The  $d$  spacing calculated from the (002) arcs was found to be 3.39 Å. This compares with 3.35 Å for natural graphite; it must be added that, as yet, no three-dimensional reflexions have been observed.

Transmission electron micrographs of ultramicrotomed sections<sup>4</sup>, replicas and cut ends of the fibres indicate that parts of the fibre are composed of fibrils running parallel to the fibre axis. Dark field micrographs<sup>5</sup> obtained using the (002) reflexions of the diffraction pattern confirm the X-ray estimate of crystallite size and show that the crystallites form chains along the fibre axis.

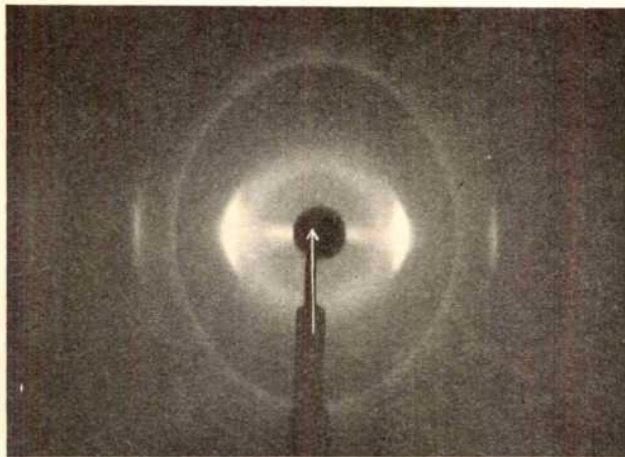
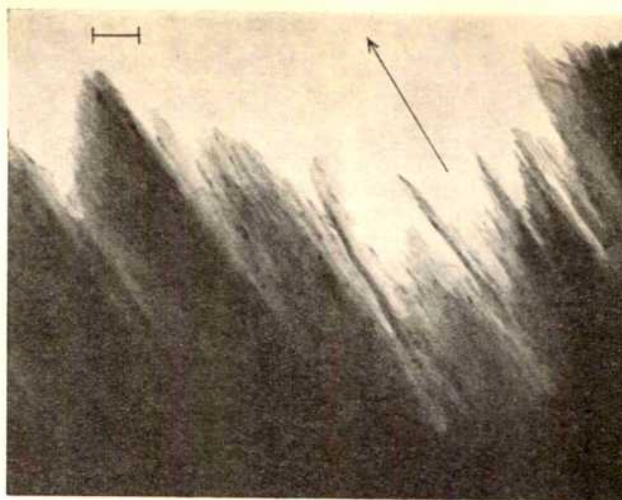


Fig. 1. An X-ray diffraction pattern from a bundle of high strength, high modulus carbon fibres; arrow indicates the fibre axis.



A



B

Fig. 2A. An electron micrograph of a cut end of a high strength, high modulus carbon fibre; arrow indicates the fibre axis. Scale length 1000 Å.

Fig. 2B. Same area as in Fig. 2A rotated about fibre axis by  $10^\circ$ .

Fig. 2 shows a cut end of a fibre in two orientations (separate and grouped fibrils with diameters in the range 250–1000 Å can be seen). As the fibre is rotated about its axis it is possible to see different groups of crystallites in the same fibril come into a position to reflect. Fibrils of similar diameter can also be seen in a two stage carbon-platinum replica (Fig. 3) of a fibre that has been embedded in a resin and longitudinally sectioned with a diamond wheel. A dark field micrograph of a longitudinal thin section is shown in Fig. 4. The areas contributing to one of the (002) arcs in the diffraction pattern are shown in light contrast. Crystallites of about 50 Å can be seen throughout the section and at the edges they are aligned in chains parallel to the fibre axis. Some of these chains are over a micron in length. Fig. 5 is an electron micrograph of a transverse ultramicrotomed section showing the distribution of diffracting crystallites—in bright field they appear in dark contrast.

It has been reported<sup>6</sup> that some elements of the morphology of rayon fibres are retained on carbonization and graphitization, and Bacon and Tang<sup>7</sup> have suggested that when cellulose is carbonized a "replica" of the original fibre structure is preserved. The same appears to be true with the raw textile fibres that we use in the production of high strength, high modulus carbon fibres, as fibrils have been reported in similar raw textile fibres<sup>8</sup> and we have also observed that the coarsely fluted outer surface



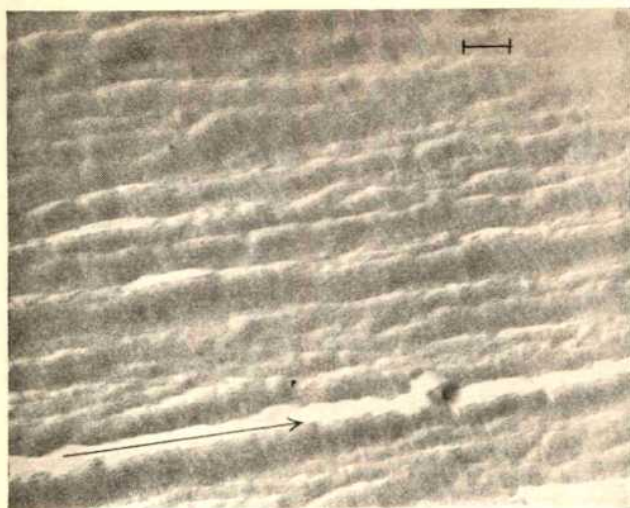


Fig. 3. An electron micrograph of a carbon-platinum replica from a longitudinally sectioned high strength, high modulus carbon fibre; arrow indicates the fibre axis. Scale length 1000 Å.

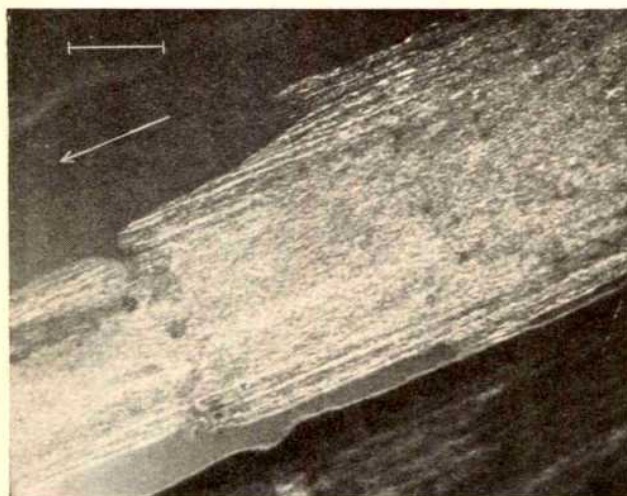


Fig. 4. An (002) dark field electron micrograph of a longitudinal thin section of a high strength, high modulus carbon fibre; arrow indicates the fibre axis. Scale length 1  $\mu$ .

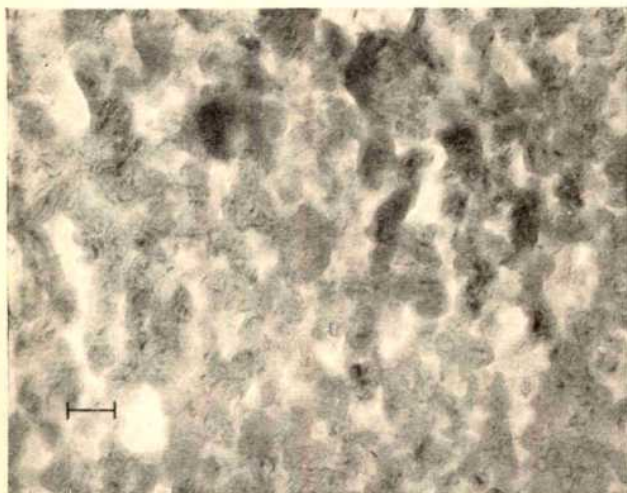


Fig. 5. An electron micrograph of a transverse thin section of a high strength, high modulus carbon fibre. Scale length 1000 Å.

of the raw fibre is retained throughout the manufacturing process. The influence of the morphology of the raw textile fibres on the physical properties of the carbonized fibres is now being investigated.

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### Voids in Solidified Iron Alloy Drops

THE surface tension of liquid metals can be readily measured by the drop-weight method, but Kelly has reported an unexpectedly wide scatter of values when he used this method<sup>1</sup>. He attributed the variation to the presence of internal voids in the drops which would alter either the contour of the drop, thus affecting measurements based on this parameter, or the presumed density of the drop used to evaluate its volume. Kelly believed that such voids could maintain themselves for an appreciable time in molten drops, and later accepted the cause to be gas present in his material. With electron bombardment devices as used by Kelly, the electron beam may impart angular momentum to the drop as a result of its being incorrectly aligned with respect to the drop surface. Thus the drops can spin about the interface between the metal drop and the rod, creating forces which, combined with the presence of dissolved gas, could lead to a stabilized void. Measurement of the rotation speed of a uniform liquid drop is virtually impossible, and thus comparison of the forces developed with the fracture stress of liquid iron has not been carried out, although Kelly confirmed in a personal communication that rotation was observed and was certainly present in the inductively heated drops in the present work to be described.

A similar observation of void formation in liquid metal drops was made by us in the course of an investigation into the oxidation of iron alloys where, in the main investigation, molten drops were allowed to fall through atmospheres containing various partial pressures of oxygen<sup>2,3</sup>. The presence of voids was investigated separately using 1.6 mm diameter iron wire of the following analysis

Element	C	S	P	N	H
Per cent	0.047%	0.03%	0.001%	0.003%	0.004 c.c./100 g

The wire was cleaned with emery paper and pickled in dilute hydrochloric acid (containing thiourea as an inhibitor) to remove any scale. Final electropolishing using a solution consisting of 60 per cent orthophosphoric acid and 40 per cent sulphuric acid with a stainless steel cathode provided a smooth polished surface.

The wire was then placed in a glass tube connected to a vacuum pump and a purified argon supply. The position of the wire could be altered through O-ring seals without breaking the vacuum or disturbing a gaseous atmosphere. The tip of the wire was then inductively melted under controlled conditions of power input and gaseous environment to form a drop the size of which was a function of the wire diameter and the liquid metal surface tension. The drop was then allowed to solidify and was sectioned for



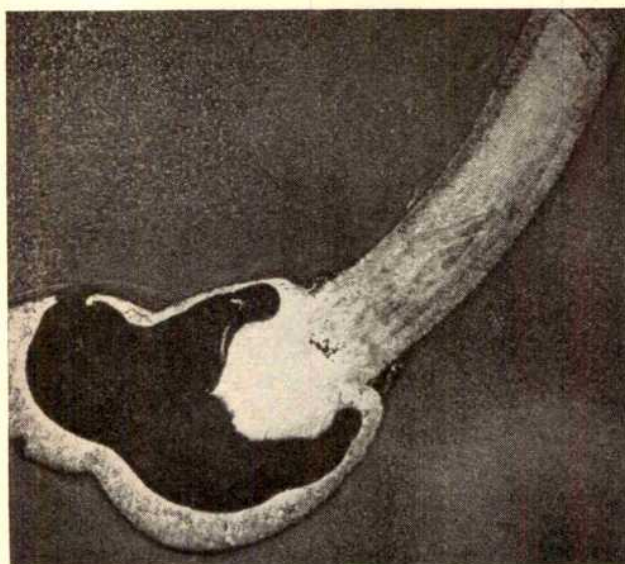


Fig. 1. Iron drop with void ( $\times 6,8$ ).

metallographic examination by spark machining to avoid mechanical deformation and any distortion of the interface between the metal and void.

During solidification the appearance of the surface of the drop altered, a "blick" or sudden brightening occurring, indicating the solidification of an undercooled liquid<sup>4,5</sup>. This was accompanied on occasions by rupture of the skin, which left a slit in the metal surface or an erupted "bubble" at a point which might itself burst to leave a hole at the surface. In section, the drops showed large smooth sided voids connected to the outer surface through a slit (Fig. 1). Although the amount of gas present would not have given rise to such porosity in normal solidification in the absence of oxygen, the presence of dissolved gases accompanying forces produced by drop rotation seemed to be a possible source of the voids and overall shrinkage against the gas void after solidification as the cause of surface rupture. A technique was therefore devised whereby both residual carbon and any gaseous elements, for example, hydrogen and nitrogen, could be further eliminated from the iron.

The iron was annealed in a stream of wet hydrogen for 24 h at 710° C to remove carbon and nitrogen and then cooled to room temperature in dry hydrogen. The pressure used in the subsequent vacuum annealing process for hydrogen removal had to be sufficiently low to prevent oxidation at the temperatures involved; 10<sup>-5</sup> mm of mercury at 650° C for 24 h was used. Typical analyses were

	C	S	P	N	H
Hydrogen treated	0.02%	0.03%	0.001%	0.001%	0.16 c.c./100 g
Vacuum treated	0.02	0.041	0.001	0.001	Nil

Compared with the starting material, the carbon activity was reduced by less than half, and the nitrogen reduced to a third; the amount of carbon monoxide which could be formed on the drop from traces of oxygen present in the argon atmosphere was thereby substantially reduced. Hydrogen, which might come out of solution at a low pressure void, was eliminated. Subsequent melting experiments on iron purified in this way produced only very small pin-hole cavities; these were probably due to minute amounts of residual gas or to internal solidification shrinkage, although the latter is unlikely to occur in small drops of this size (2–3 mm) unless nucleated by gas<sup>6</sup>, the overall change in specific volume being achieved by plastic deformation.

This investigation indicates the necessity of eliminating, as far as possible, any gas from metal to be used in droplet studies, for example, for surface tension determination,

particularly where the heating method causes high speed rotation. Kelly (1962, personal communication) confirmed the necessity for such a step while Cline and Ferriss<sup>7</sup> came to a similar conclusion in a study of tungsten and its alloys. Certain elements had to be removed by annealing *in vacuo* at 1,850° C for 3 h to prevent the formation of voids.

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## CHEMISTRY

### Reaction of Hydrogen with Graphite

In a recent communication<sup>1</sup> Gulbransen showed that reactions between graphite and hydrogen which are thermodynamically unfavourable can proceed to a measurable extent under conditions where the reaction products are rapidly removed or the system quenched. In view of this it is considered of interest to report certain observations made in these laboratories during experiments in which graphite was mechanically ground in the presence of hydrogen, and also of nitrogen. It was found that both these gases are taken up by graphite if present in the grinding chamber during the comminution process. The grinding was carried out using a vibratory ball mill fitted with gas-tight grinding chambers to which the gas was metered during grinding to maintain the pressure constant at 1 atm. The graphite was synthetic and contained less than 0.02 per cent of ash by weight, while the gases were of commercial quality at 99.9 per cent purity. The grinding medium was 0.25 in. steel balls. We concluded that the absorption of the gases must be due to some form of chemical reaction with the graphite for the following reasons. First, direct adsorption of the gases by the fresh graphite surface produced cannot wholly account for the uptake, because the extent of uptake is greater than when the graphite is first ground *in vacuo* and then placed in contact with the gas, even though the amount of fresh surface produced is greater in the latter case. Second, it is reasoned that neither hydrogen nor nitrogen reacts with the active (free radical) sites produced by fracture of the graphite planes, because we have shown that these are most readily neutralized by subsequent treatment with oxygen and that the amount of oxygen absorbed depends only on the fresh surface produced, irrespective of whether the graphite is initially ground in hydrogen, nitrogen or *in vacuo*. Finally, we have observed that graphite ground in the presence of nitrogen has a strong smell resembling that of hydrogen cyanide, whereas no such smell is detectable if the grinding is carried out *in vacuo*.

Following Gulbransen, we suggest that the reason these reactions proceed at measurable rates is the continuous subjection of the reactants to instantaneous high temperatures. These high temperatures will be generated in the frictional contacts between the steel balls used in the grinding process, and are known to be capable of reaching as high as 1,500° C (ref. 2). Because of the rapid dissipation of heat from the contacts, however, this high temperature is extremely localized, the temperature of the media

surrounding the contacts being actually less than 100° C. Thus the conditions indicated by Gulbransen for the accumulation of reaction products would appear to be fulfilled in this system.

It is proposed to publish fuller details of this work at a later date.

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### Geometry of Meisenheimer Salts

IN 1942 Wheland<sup>1</sup> proposed a model for the activated complex in the aromatic electrophilic, radical and nucleophilic substitution reactions. This model was the object of semi-empirical quantum mechanical calculations and was formulated as follows



where the asterisk stays for a plus charge, an unpaired electron or a minus charge, respectively.

Since then, the model has frequently been used as a basis for theoretical investigations on the mechanism of these reactions, in the frame of localization theory<sup>2-6</sup>. It has been pointed out, however, that this model more aptly describes a possible intermediate than an activated complex<sup>7-9</sup>.

In one example of nucleophilic substitution a stable intermediate has been isolated: this is the case of the complexes of alkyl picrates with alkaline alkoxides ("Meisenheimer salts")<sup>10</sup>. An investigation of the elec-

tronic and molecular structure of the complexes of ethyl picrate with potassium or caesium ethoxide by X-ray analysis was undertaken in this laboratory and crystallographic results have been published<sup>11</sup>. This communication describes the result of a preliminary determination of the structures, at a stage where the reliability index for the caesium salt is 12.1 per cent and for the potassium salt is 14.5 per cent on about half the three-dimensional results obtainable using copper *K* $\alpha$  radiation.

Because in both structures there are two non-equivalent molecules in the asymmetric unit, the geometry of this anion has, in fact, been measured in four different environments. The averages of the experimental values for bond lengths and angles in the ring are reported in Fig. 1.

The six carbon atoms lie on a plane (within 0.04 Å); the O-C(1)-O plane is about perpendicular to the ring and the O-C(1)-O angle is 99°. The C(6)-C(1)-C(2) angle is very close to the tetrahedral value and the C(1)-C(2) distance is not far from the "best" value of the  $C_{sp^2}$ - $C_{sp^2}$  bond: 1.501 Å (ref. 12). The C-O distance is 1.43 Å.

An appropriate aromatic molecule for a comparison is perhaps 2,4,6-trinitrophenetole, whose structure has recently been reported<sup>13</sup> (see Fig. 2). In this molecule, the C-O distance is 1.37 Å and the plane determined by the oxygen and the two carbon atoms of the O Et group is approximately orthogonal to the phenyl ring; consequently, the C-O bond in this molecule can be compared with the same bonds in Meisenheimer salts. The principal part of the observed shortening is presumably due to the change from  $sp^3$  to  $sp^2$  hybridization.

In Meisenheimer salts, the carbon-carbon distances in the conjugated chain show remarkable deviations from the standard value of 1.39 Å; however, this chain should not be compared with unsubstituted benzene, because of the  $sp^3$  hybridization of C(1) and the presence of three nitro groups bonded to C(2), C(4) and C(6).

For comparison, the carbon-carbon bond lengths in an unsubstituted pentadiene anion, as predicted by a Hückel molecular orbital calculation, with inclusion of the  $\omega$ -technique, are reported in Table 1, together with the same distances in the 1,3,5-trinitropentadiene anion, where standard values for the necessary parameters have been used<sup>6</sup>.

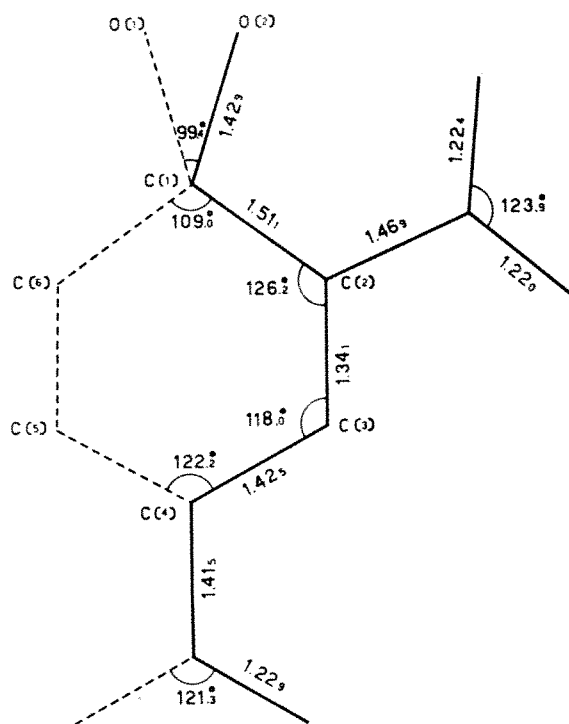


Fig. 1.

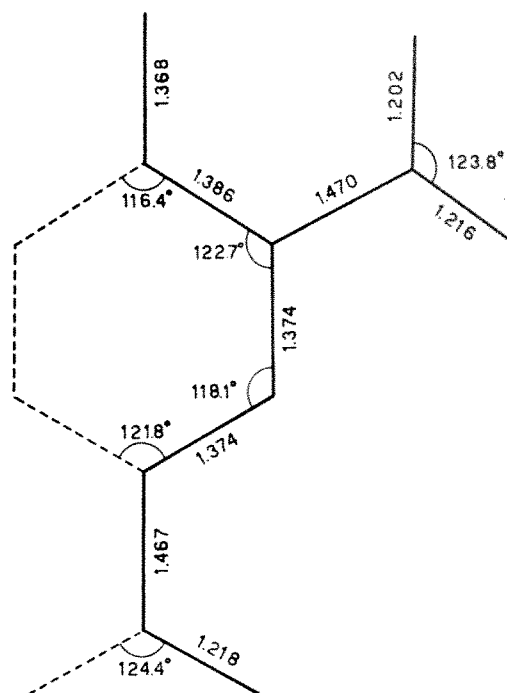


Fig. 2.



Table 1. CARBON-CARBON DISTANCES (IN Å)

	Pentadiene anion	Trinitropentadiene anion
C(1)-C(2)	1.376	1.374
C(2)-C(3)	1.414	1.420

In general, it can be said that the experimental results completely confirm Wheland's description of the model, at least for the case of an intermediate in a nucleophilic substitution reaction.

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## MOLECULAR STRUCTURE

### Transition in Cellulose in the Vicinity of $-30^{\circ}\text{C}$

THERE has been increasing interest in the investigation of thermal transitions in cellulose. It has been established that the glass transition temperature is in the region  $200^{\circ}\text{--}250^{\circ}\text{C}$ , and there is ample experimental evidence to indicate a transition at  $20^{\circ}\text{--}25^{\circ}\text{C}$ . This transition is also seen in glucose and cellobiose<sup>1-3</sup>. The purpose of this communication is to report a recently discovered transition at around  $-30^{\circ}\text{C}$ , observed in measurements of damping and thermal expansion of paper produced from wood cellulose.

Our measurements were made with an inverted torsion pendulum described elsewhere<sup>4</sup>. The paper sample (a strip,  $14.0 \times 1.5$  cm) was surrounded by a hollow brass cylinder of internal diameter 2.5 cm and length 18 cm, through which a thermostatic liquid (methanol) was circulated. The temperature was taken by thermocouples placed both in the thermostating liquid in the cylinder and at three points along the sample. The temperature gradient along the sample was less than  $0.1^{\circ}\text{C}$ . Readings were taken during both heating and cooling of the sample, and there was no obvious difference. The results shown below relate to measurements carried out during heating.

All experiments were performed in a vacuum of  $10^{-3}$  torr, which was applied 48 h before the start of the experiment. Before each reading of frequency and damping the temperature was usually kept constant for 20 min. The samples were cut from an isotropic sheet of paper of basic weight  $100\text{ g/m}^2$ , made in the laboratory. In the principal experiments a sheet made from bleached spruce sulphite cellulose of viscose quality was used. The pulp was mechanically treated (beaten, drainage resistance  $23^{\circ}\text{SR}$ ) to eliminate anomalies in mechanical behaviour known to occur with untreated pulp<sup>4</sup>. The  $\alpha$ -cellulose content of the pulp was 92.8 per cent, intrinsic viscosity was 584

$\text{cm}^3/\text{g}$  (DP 830), resin content was 0.24 per cent and ash content was 0.03 per cent. Other samples have also been investigated and their characteristics are given below.

The curves reproduced in Fig. 1 show the variation of the loss tangent and shear modulus with temperature in the bleached sulphite pulp. The maximum in the damping-temperature curves is well defined and the shape of its peak differs markedly from that of secondary maxima encountered in polymers. The appearance of a damping maximum is associated in the usual way with an inflexion of the modulus-temperature curve. The maximum occurs at about  $-30^{\circ}\text{C}$ , while the central part of the modulus inflexion lies somewhat lower. The points on curve A in Fig. 1 refer to a measurement where the sample was maintained at each temperature for 1 h, a time which was restricted to 20 min for the other measurements. The difference in the shape of the maximum may be the result of this time difference. The differences between the curves seem otherwise to be of the same order as that expected from the variation of mechanical properties between samples of paper. The increase in damping evident from curve A in Fig. 1 is caused by the maximum usually found at about  $20^{\circ}\text{--}25^{\circ}\text{C}$ . By the present method this maximum appeared at about  $35^{\circ}\text{C}$ .

For unbleached sulphate pulp with a lignin content of about 6 per cent, the maximum is no longer as pronounced as it is for a cellulose sheet of high purity. At the same time, it is shifted towards lower temperatures, as in Fig. 2. This figure also shows a  $\lg \delta$ - $T$  curve for pure cellulose II, that is, the bleached sulphite pulp of Fig. 1 which has undergone mercerization for 60 min in 18 per cent sodium hydroxide at  $20^{\circ}\text{C}$ . The transformation into cellulose II was checked by X-ray diffractograms. The position of the maximum was not changed by the transformation of cellulose I  $\rightarrow$  II. In sharp contrast to the other samples, commercial 'Cellophane', a cellulose II film softened with 15 per cent glycerol, exhibited no such transition in the interval investigated between  $-10^{\circ}$

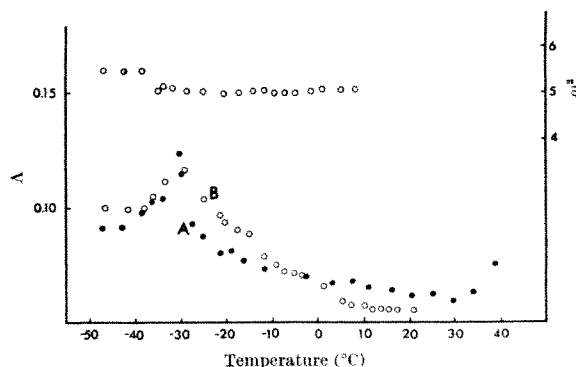


Fig. 1. Logarithmic decrement and square of frequency for two samples of pure  $\alpha$ -cellulose. Inverted torsion pendulum. The  $\omega^2(T)$  curves of the two samples coincided. Sample A was heated more slowly than sample B.

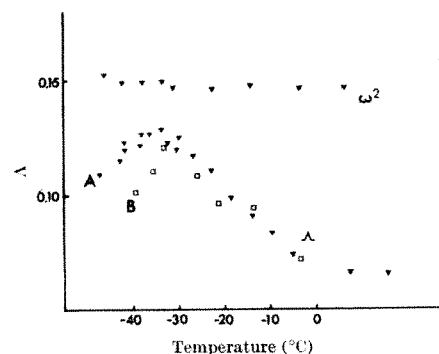


Fig. 2. Logarithmic decrement and square of frequency for a sulphate pulp (A) and a mercerized cellulose (B). Inverted torsion pendulum.

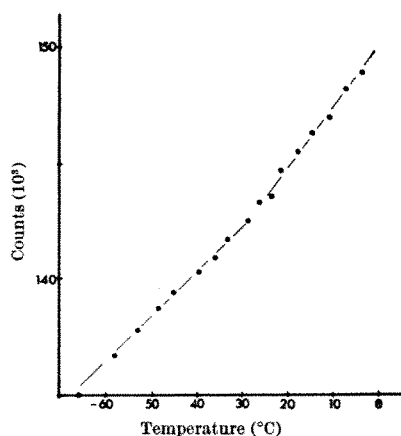


Fig. 3. Transmission of  $\beta$ -rays versus temperature for a sample of pure  $\alpha$ -cellulose. Radiometric dilatometry was employed.

and  $-40^\circ\text{C}$ . The decrement value increased linearly from 0.25 to 0.30 as the temperature was decreased. The simultaneous increase in the value of the modulus was also linear.

Independent experimental evidence for the transition was obtained by a dilatometric technique based on the dependence of the transmission of  $\beta$ -rays on the weight/unit area of the sample at varying temperatures<sup>5</sup>. Because of the discontinuity of the thermal expansion coefficient in the vicinity of a transition, the transmission-temperature curves have a shape equivalent to that of curves obtained dilatometrically. The line reproduced in Fig. 3 and obtained with an improved radiometric technique shows a discontinuity slightly below  $-30^\circ\text{C}$  (unpublished work of Klason, Kubát and de Ruvo). From the slope of the two rectilinear portions of this curve, the coefficients of thermal expansion for the regions above and below the transition point were calculated to  $6.0 \times 10^{-5}$  and  $4.5 \times 10^{-5}$ , respectively. The sample was prepared from bleached sulphite cellulose.

The physical background of the transition presented here is not known. Its disappearance in regenerated cellulose containing glycerol indicates that it is associated with the amorphous part of cellulose. Further work is in progress, with a view to investigating the molecular nature of this transition.

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## BIOPHYSICS

### Oriented Water in the Sciatic Nerve of Rabbit

A PRELIMINARY study has been made of the water found in the sciatic nerve of rabbit, which is one of the larger myelinated nerves, by proton magnetic resonance. Specimens from recently killed animals were stored in a refrigerator at about  $2^\circ\text{C}$  for periods of at least 1 h before use. In this process a certain amount of water was lost, as shown by condensation on the walls of

the containers. Approximately cylindrical samples of the nerve, about 4 mm long and 1 mm in diameter, were examined in a Varian 'A60A' spectrometer, the nerve axis being coincident with the cylindrical axis. The angle between the nerve axis and the applied magnetic field could be varied at will.

The spectra obtained are shown in Fig. 1. An appreciable amount of structure is observed and this structure depends on the orientation of the nerve within the applied field. It is consistent with the assumption that it is caused by dipolar interactions between the protons in individual water molecules. This interaction has the value  $-3\mu^2/2r^3(3\cos^2\theta - 1)$ , where  $\mu$  is the magnetic moment of the proton,  $r$  the interproton distance and  $\theta$  the angle between the proton-proton vector and the applied field direction. In normal isotropic water it is averaged to zero by molecular tumbling. This experiment suggests therefore that the bulk of the water inside the nerve is in a partially oriented state.

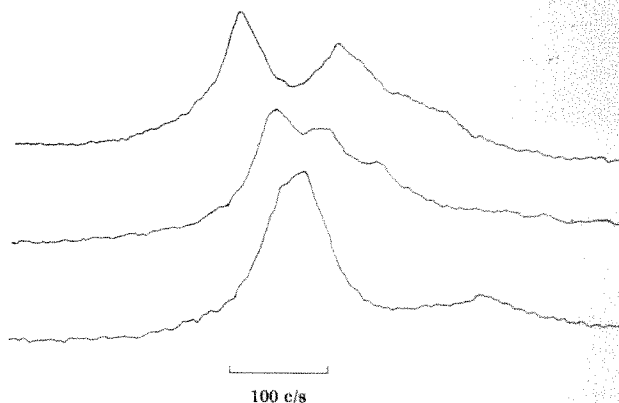


Fig. 1. The spectra of water in the sciatic nerve: (top) with nerve axis parallel to the applied field; (centre) the perpendicular orientation; (bottom) with the nerve axis at approximately  $54^\circ$  to the applied field direction.

The principal feature of the spectrum is a doublet the maximum splitting of which is observed when the nerve axis is parallel to the applied field. The splitting has half its maximum value in the perpendicular orientation and may be caused to be zero by setting the nerve axis at an angle of  $54^\circ 44'$  with the applied field. The orientation parameter  $S_{22}$  (ref. 1) has a value of about 0.004. The movement of the centre of the doublet as the orientation of the nerve is varied is too large to be caused by a chemical shift anisotropy and must originate in the anisotropy of the magnetic susceptibility of the nerve.

The other peaks in the spectra each show orientation dependent shifts much larger than this susceptibility shift. This suggests that these also originate in partially oriented water in a different environment and in a differently oriented state from the principal component.

It is interesting to postulate possible origins of these orientation effects. There are at least four possibilities. The water may simply be constrained by Van der Waals' forces to move in annuli which must have near molecular dimensions (about 10 Å). The orientation may be secondary orientation produced on the surface of protein molecules which are themselves aligned by the pores in the nerve. Orientation may be caused by electrostatic interactions near the fibre surface. A simple calculation suggests that the field strengths feasible are insufficient to explain the orientation observed. The water could be in a chain structure as postulated for the collagen system<sup>2</sup>. There appears to be no justification for this explanation, and the degree of orientation observed can be explained completely in terms of normal water.

In view of the normally accepted pore sizes in nerves, which are much too large to cause orientation, it is surprising that most of the water is not in its normal state.

Further work is in progress to assess the implications of these results on the structure of myelinated nerves.

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## IMMUNOLOGY

### Serological Expression of Antibody Specificity for a Single Determinant of an Antigenically Complex Molecule

THE immune response to an antigenically complex molecule results in the formation of antibodies specific for one or more antigenic determinants<sup>1</sup>. In all but a few instances<sup>2</sup>, antibodies specific for individual determinants cannot be identified, which precludes the evaluation of an immune response at this level of serological specificity. It is therefore usually assumed that antibodies specific for multiple determinants are regularly produced and participate in the serological reactivity of an antiserum. Such an assumption could obscure the possibility that a given antiserum consists of antibodies specific for a single determinant. The inability of such antisera to produce precipitation<sup>3</sup> may, in turn, lead to their being considered devoid of antibody activity or their serological properties being misinterpreted. Because of these practical considerations, we decided to describe our experience of this subject.

We became aware of antibody specificity for a single antigenic determinant during a serological examination of human tetanus antitoxin, when human sera of known toxin neutralizing activities were examined by both passive agglutination and immunoelectrophoresis. The initial examination was made by passive quantitative agglutination, a procedure previously used to compare antibody combining properties<sup>4</sup>. In its present application, tetanus toxoid was coupled to human group O red cells<sup>5</sup>. As we anticipated, there was no correlation between the magnitude of the quantitative agglutination curve and neutralizing activity (Fig. 1). Nevertheless, it was interesting that one antiserum with less than 1 U of neutralizing activity/ml. gave a strong agglutination curve. Additional examples were found among other available low-neutralizing sera (Fig. 2).

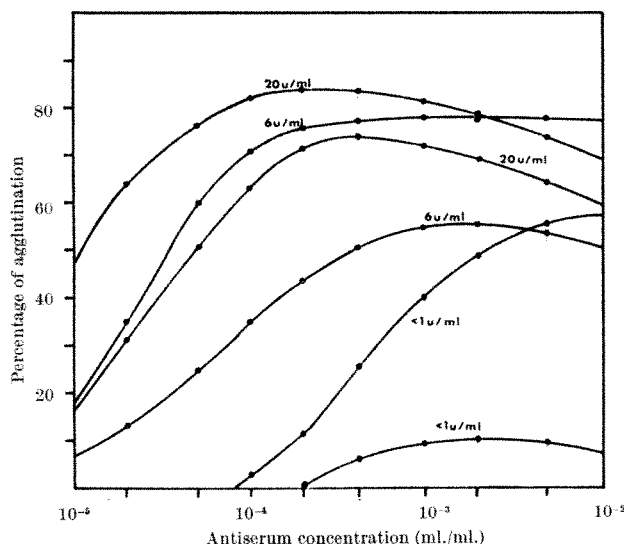


Fig. 1. Quantitative agglutination curves of human sera of different tetanus toxin neutralizing activities.

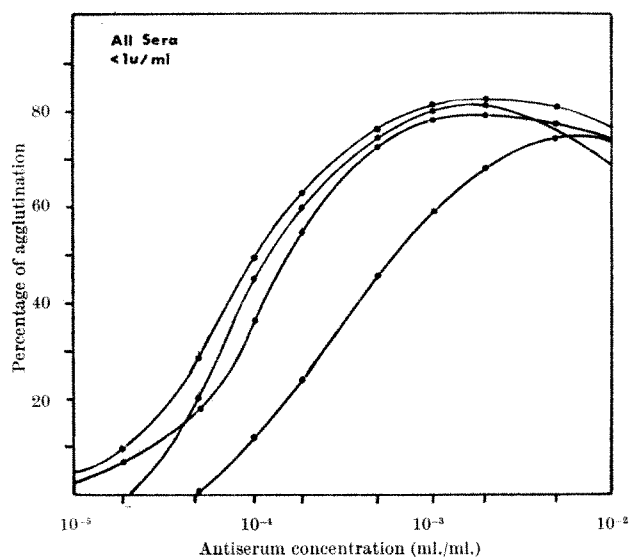


Fig. 2. Agglutinin activity of selected antisera in which little or no neutralizing activity was demonstrable.

Selected examples of both high- and low-neutralizing sera were compared by immunoelectrophoresis. All the low-neutralizing sera, and one of the high-neutralizing sera, failed to give a precipitin band. This was particularly surprising because some of the non-precipitating sera had given stronger agglutination curves than several of the precipitating sera. The failure of precipitation among the strongly agglutinating sera could not be reasonably ascribed to either low antibody concentration or affinity, and so we looked for an alternative explanation.

In comparing the mechanics of precipitation and passive agglutination, it became apparent that, although both reactions require lattice formation, antisera specific for a single antigenic determinant would behave quite differently; an antiserum specific for a non-repeating antigenic determinant would be incapable of lattice formation. It was, however, evident that if such an antiserum reacted with particles to which many antigen molecules were coupled, as in passive agglutination, then ample opportunity for lattice formation would exist. This principle, regularly considered in the assay of antibodies specific for simple chemical haptens<sup>6</sup>, seemed directly applicable to other observations. We concluded that the strongly agglutinating, non-precipitating antisera encountered in this investigation were made up largely of antibodies specific for a single, non-repeating, antigenic determinant. Evidence supporting this conclusion was obtained later.

Two related experiments were carried out in which advantage was taken of previously demonstrated properties of individual antisera. The first experiment consisted of mixing the one available example of a non-precipitating, high-neutralizing serum (>20 U/ml.) in varying proportions with a non-precipitating, low-neutralizing serum (<1 U/ml.) and testing with tetanus toxoid by immunodiffusion. Neither of these sera alone produced detectable precipitation of the tetanus toxoid. When combined in equal proportions, a comparatively strong precipitin band was formed, indicating that the two sera must have been specific for two different determinants, and that both determinants were present on the same molecule. In other circumstances, the non-precipitating character of such an antiserum would undoubtedly have been misinterpreted. In the second experiment a papain digest<sup>7</sup> of the  $\gamma$ -G antibody from a strongly agglutinating, non-precipitating, low-neutralizing (<1 U/ml.) serum was mixed with different precipitating, high-neutralizing sera in the hope of observing inhibition of precipitation with

tetanus toxoid. Inhibition of precipitation was observed in one of the first three high-neutralizing sera tested by immunodiffusion. An undigested sample of the same  $\gamma$ -G fraction did not alter precipitation. This precipitating, high-neutralizing serum was probably specific for no more than two determinants and inhibition was probably caused by the blocking of one of these determinants by the univalent antibody digest.

These experimental findings appear to agree with our previous conclusion. We therefore suggest that the combined use of precipitation and passive agglutination could be useful in the recognition of antisera specific for a single antigenic determinant.

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## Immunoglobulins in Tibetans

INFORMATION about increased gamma-globulins in certain ethnic communities is rare and as far as we know extends only to Navajo Indians, Puerto Ricans and certain American Negroes<sup>1-3</sup>. There has been no convincing explanation for this phenomenon. We discuss here racial differences in the synthesis of gamma-globulins, and increased exposure to chronic infections, the latter resulting in an increased production of humoral antibodies. Chronic liver disease can easily be excluded as a cause.

We have found low serum lipids as well as generally increased gamma-globulins in Tibetans in Nepal and Switzerland<sup>4</sup>. Now we have examined several serum components of a group of Tibetan refugees resettled in Switzerland by the Swiss Red Cross.

This communication presents the results of an examination of the immunological components of the serum proteins in seventy Tibetan males and females aged between 30 and 50. The results are compared with those of twenty-one matched Swiss controls of a similar age and without known illness.

Total proteins were determined in fasting serum samples; paper electrophoresis was performed, and immunoglobulins were separated on a semi-quantitative basis by double diffusion in agar-gel (differentiation of gamma-A and gamma-M).

Table 1

	Tibetans	Swiss	P-value
Serum samples (N)	41	22	—
Age ( $\bar{x}$ )	38.5	34.5	—
Total proteins (g per cent)	7.07 $\pm$ 0.46	6.86 $\pm$ 0.33	0.05
Gamma-globulins (g per cent)	1.58 $\pm$ 0.25	1.20 $\pm$ 0.14	0.001
Gamma-A-globulins (mean titre)	1 : 236	1 : 195	0.05
Gamma-M-globulins (mean titre)	1 : 37	1 : 31	0.05

Table 1 shows the results as mean values with the standard deviation. A further analysis was made according to age and sex, but this analysis did not yield any further information.

The total gamma-globulins in Tibetans are greater than in the Swiss controls, the difference being statistically significant ( $P < 0.001$ ). The mean value for the immunoglobulins gamma-A and gamma-M is also greater in the Tibetans but is less pronounced and these differences do not attain statistical significance. The more marked increase of the total gamma-globulins in comparison with the gamma-A and gamma-M values might be largely the result of a higher gamma-G subfraction.

We have no explanation for these findings. A substantial rate of manifest infection or liver disease in the Tibetans can be excluded. Racial and environmental factors require consideration, and further elucidation may be obtained by observation and re-examination of Tibetans abroad.

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## Post-embedding Staining with Ferritin Labelled Antibodies

THE ferritin labelled antibody technique<sup>1</sup> has been widely used for the location of antigens at the cellular and sub-cellular level<sup>2</sup>. There are two possible methods of applying the technique: (a) pre-embedding staining in which antigen is allowed to react with the labelled antibody before the cells are processed and sectioned in preparation for electron microscopy; and (b) post-embedding staining in which labelled antibody is applied to and reacts with the antigen in suitably fixed and embedded thin sections. Almost all published investigations have been carried out using the pre-embedding staining technique. Surface antigens are readily located by this method, but intracellular antigens can only be located after treatments such as freezing and thawing and disintegration which enable the labelled antibody to penetrate within the cell. The technical difficulties associated with the post-embedding staining technique are complicated by the non-specific attraction for ferritin of all commonly used embedding media, including butyl methacrylate-ethyl methacrylate co-polymer, polyglycol methacrylate, vestopal and epon<sup>3</sup>. The phenomenon was attributed by these workers to the non-ionic character of the embedding media, and they attempted to develop a new embedding medium with ionic charges which allowed wetting, thus reducing non-specific adsorption. Preliminary results with viral antigens in the polyampholyte embedding medium which they developed were very encouraging.

The present communication deals with our experience using this embedding medium in the location of bacterial antigens.

The strain used throughout this work was *Bacillus cereus*, variety terminalis. Antisera were prepared against both spores and vegetative cells<sup>4</sup> and coupled to ferritin using toluene 2 : 4 di-isocyanate<sup>5</sup>. A young vegetative cell suspension was prepared by thoroughly washing the deposit from a 12 h broth culture fixed overnight in 10 per cent buffered formalin, pH 7.2, or in 2.5 per cent buffered glutaraldehyde pH 7.2-7.4 for 2 h. After thorough washing in saline the organisms were embedded in the polyampholyte medium using the methods described by Singer and McLean<sup>3</sup>. Polymerization was initiated by heating at 60° C overnight and completed by a further period of curing for 3 days at room temperature. Sections



were cut on an L.K.B. 'Ultratome', using a cutting speed of 50 mm/sec, and collected on 400 mesh grids without supporting membrane. The grids were then stained with ferritin labelled antibody to the vegetative cell or ferritin labelled antibody to the spore by either of two methods. In the first method the grids were floated on to a drop of the labelled antibody and left for half an hour. After this the grids were lifted with forceps and rinsed by gentle agitation in a beaker of saline. In the second method, for which we are indebted to Dr S. J. Singer, the grids were held in forceps and immersed in a small receptacle containing approximately 1-2 ml. of phosphate buffered saline, pH 7.2, for 5-10 min. One drop of the appropriate ferritin labelled antibody was then added to the buffered saline, thoroughly mixed and left for a period of half an



Fig. 1. Electron micrograph of section of *B. cereus* in the polyampholyte embedding medium stained with method 1, fixed in 10 per cent buffered formalin.

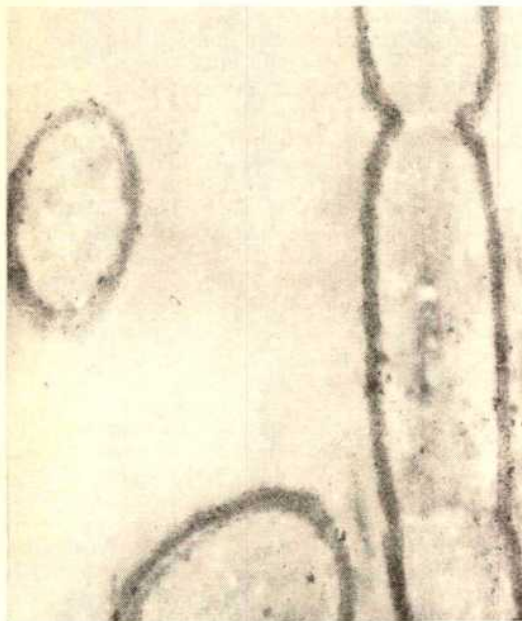


Fig. 2. Electron micrograph of section of *B. cereus* in the polyampholyte embedding medium stained with method 2, fixed in 10 per cent buffered formalin.

hour with occasional agitation. Phosphate buffered saline, pH 7.2, was then used to flush out the uncombined labelled antibody, after which the grid was washed for a further 10 min. At no time during the staining process was the grid allowed to dry. After a quick rinse in distilled water the grids were examined in a Philips 'EM 200' electron microscope at 60 kV.

Using method (1) ferritin particles were seen adhering to the embedding medium throughout the sections (Fig. 1), making specific staining difficult to differentiate. Using method (2) intense staining with antibody labelled with ferritin of the vegetative cell was observed only along the cell wall of the organisms, and background staining was minimal (Fig. 2). Contrast was very poor after formalin fixation, but rather better after glutaraldehyde fixation. No specific or non-specific staining of the vegetative cell was observed after treatment with ferritin labelled antibody to the spore.

The initial results obtained with the polyampholyte using method (1) were very disappointing and were no better than those obtained using normal butyl methacrylate-ethyl methacrylate co-polymer. Using method (2), however, background staining was reduced to a minimum and much better results were obtained. This method is designed to prevent surface-denatured antibody from coming into contact with the section (S. J. Singer, personal communication). Our results support those of Singer and McLean<sup>3</sup> and indicate that their approach to the problems of post-embedding staining in the development of an ionic embedding medium was valid. Unfortunately the method has not yet been developed to a routine stage. Some variation in results has been noted with different batches of component materials and with the age of the block; in general, the background staining decreases with increasing block age.

It would appear that antigenic sites survive embedding in the polyampholyte embedding medium and are available for combining with the labelled antibody. In view of the usefulness of the post-embedding staining techniques in solving problems of the location of intracellular antigens it is hoped that these preliminary results will provide the stimulus for further development of the method.

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### Antigen Stimulated DNA and RNA Synthesis in Spleen Cell Suspensions from Immunized Rabbits

SEVERAL metabolic inhibitors have been shown to inhibit the initiation of antibody synthesis in a variety of *in vitro* systems (see Table 1), and it has been suggested that certain of these agents may inhibit antibody formation by suppressing the formation of the messenger RNA which directs the synthesis of the antibody molecule, although other explanations have been considered<sup>3,4</sup>. In

Table 1. INHIBITION OF THE INITIATION OF ANTIBODY SYNTHESIS *in vitro*

References	Culture system	Inhibitor	µg/ml.
1	Spleen cell fragments	5 BUdR	6
2	Spleen cell fragments	Chloramphenicol	5-20
3	Spleen cell fragments	Chloramphenicol	20-50
	Spleen cell fragments	Actinomycin	8
4	Spleen cell fragments	Actinomycin	0.06

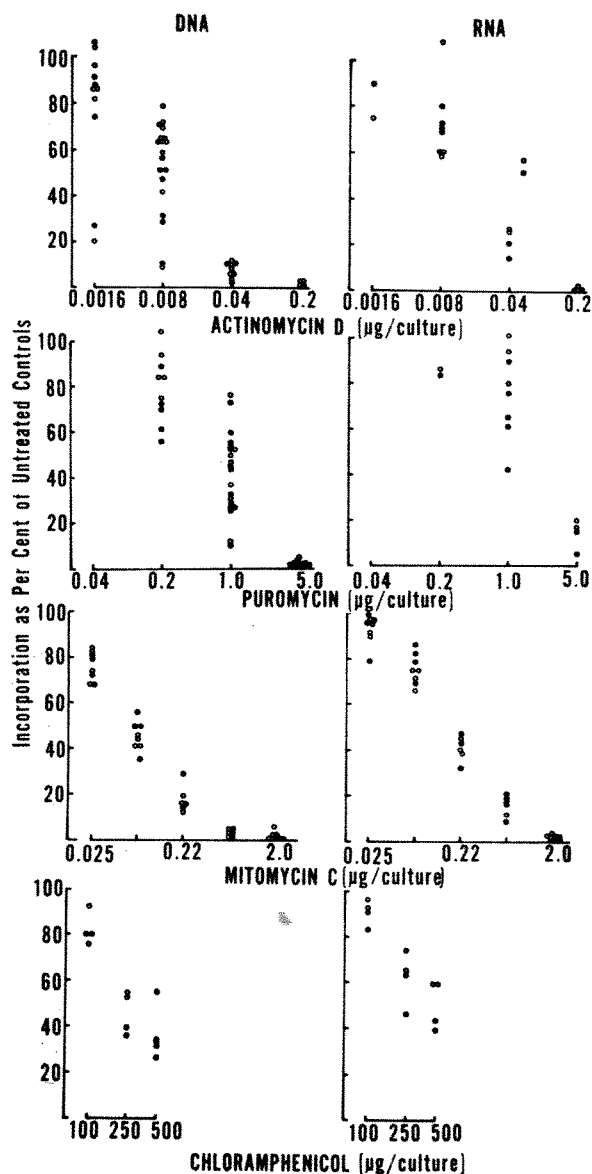


Fig. 1. Control and antigen stimulated cultures are represented by open and closed circles, respectively. Nucleic acid synthesis in spleen cell suspensions from previously immunized rabbits was stimulated by the addition of the specific antigen.  $1.5 \times 10^7$  spleen cells were incubated in 1.5 ml. of medium containing the inhibitor indicated. DNA and RNA synthesis were measured by the incorporation of tritiated thymidine (2  $\mu$ c. at 80 mc./mmole/culture) or cytidine (0.4  $\mu$ c. at 40 mc./mmole/culture) in the period 24–48 h after the start of incubation. The results are expressed as per cent inhibition of the incorporation seen in the absence of the inhibitor. Incorporation of thymidine or cytidine in the antigen containing cultures was, on average, approximately twice that observed in the control cultures.

these studies the effect of the inhibitor was assessed after several days' incubation with the cells. Subsequent studies in which much shorter time periods were used have suggested that this may be the case<sup>5,6</sup>. It has been shown, however, that the antibody forming cell population arises by a rapid proliferation of a much smaller number of precursor cells, both *in vivo*<sup>7-9</sup> and *in vitro*<sup>10-12</sup>, in both the primary and secondary response. It is clear that any agent which inhibits such proliferation will reduce the subsequent synthesis of antibody in experiments extended over longer periods of time. It is also probable that specific inhibitors of RNA and protein synthesis will subsequently affect DNA synthesis, providing the time of incubation with the drug is sufficiently prolonged. It was therefore of some interest to measure the concentration of a selected group of metabolic antagonists which would inhibit DNA, RNA and protein

synthesis in an appropriate *in vitro* system. The results of such a study are recorded in Fig. 1.

Spleen cell suspensions from normal or previously immunized rabbits were incubated<sup>13</sup> in a modified Eagle's medium in the presence or absence of the metabolic antagonists, as indicated. The rate of DNA synthesis was determined by the uptake of tritiated thymidine in the period of 24–48 h after the start of incubation, and the inhibitor was present for the whole incubation period. The rate of RNA synthesis was similarly determined using tritiated cytidine. In this system the addition of very low concentrations of metabolic antagonist, except chloramphenicol, can markedly inhibit DNA and RNA synthesis. The concentration of drug required to effect an inhibition is comparable with previously reported values when cultures of other mammalian cells were treated with actinomycin D<sup>15-17</sup>, puromycin<sup>17,18</sup>, mitomycin<sup>17,19</sup> and chloramphenicol<sup>17</sup>. Similar inhibitory effects were seen in antigen stimulated and control cultures. Inhibition of DNA synthesis is known to depress the initiation of antibody formation<sup>1</sup>. It is therefore probable that the inhibition of the initiation of antibody synthesis seen in other studies<sup>3,4</sup> was a secondary consequence of the suppression of cell division.

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### Immunological Reactivity of Peptides related to Angiotensin

THE production of antibodies to angiotensin II has made measurement of this peptide by radioimmunoassay possible<sup>1</sup>. While this procedure is extremely sensitive, the specificity of the method is determined by the degree to which related forms of the molecule will react with the same antiserum and thus register as angiotensin II in the assay. Because the measurement of angiotensin II in body fluids is of cardinal importance in the elucidation of the role of the renin-angiotensin system in physiological processes such as vascular reactivity and aldosterone biosynthesis, it is essential to determine whether the biologically active octapeptide is specifically estimated by the results obtained from radioimmunoassay.

This problem was examined in a radioimmunoassay system for angiotensin II which uses antibodies to val 5



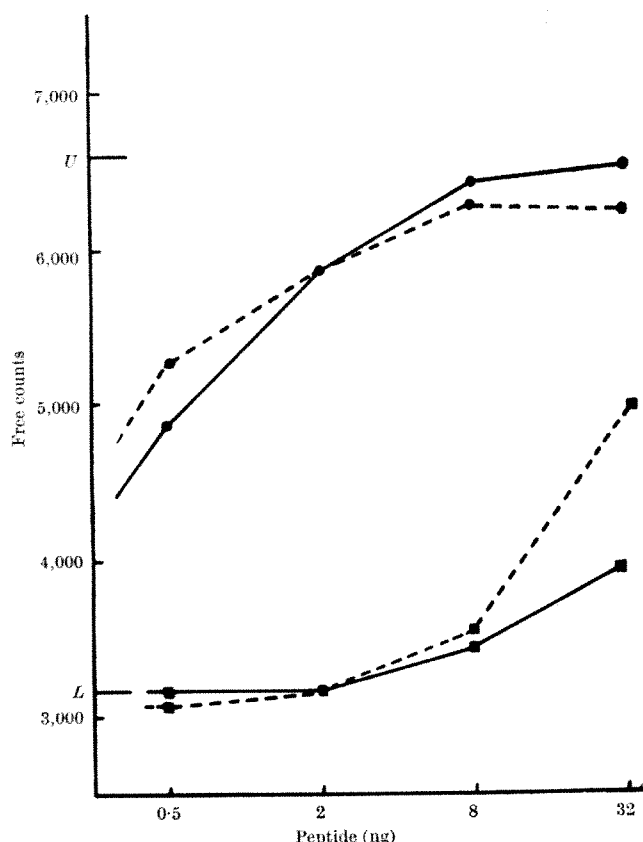


Fig. 1. The displacement of  $^{125}\text{I}$ -angiotensin II amide from combination with its antiserum by angiotensin II amide (●—●), the hexapeptide des-asp, arg, angiotensin II (○—○), angiotensin I (■—■), and the tetradecapeptide renin substrate (□—□). "Free counts",  $^{125}\text{I}$ -angiotensin II amide displaced from antiserum to the ion exchange resin 'CG-400' (OH<sup>-</sup>) by unlabelled peptide. U, Upper limit of assay (no antiserum present). L, Lower limit of assay (no peptide present).

angiotensin II amide, and ion exchange to achieve separation of bound and free tracer angiotensin II<sup>2</sup>. The antibodies were generated by immunization of rabbits with a conjugate consisting of angiotensin II amide coupled to porcine gamma-globulin, and were detected by their ability to bind radioiodinated angiotensin II amide. In the assay, the presence of unlabelled angiotensin II results in displacement of  $^{125}\text{I}$ -angiotensin from the antibody bound form to the "free" form, which is then adsorbed to the anion exchange resin 'CG-400' (OH<sup>-</sup>) and counted. The radioactivity of the ion exchange resin (or "free" tracer) thus rises toward a maximum with the addition of increasing quantities of angiotensin II or related peptides capable of reacting with the antiserum. The  $\alpha$  and  $\beta$  aspartyl forms of angiotensin II were found to be equally reactive as the amide, indicating that configurational changes around the N-terminus of the molecule do not affect immunological reactivity and that the naturally occurring form of the peptide was detected by these antibodies. On the other hand, the decapeptide angiotensin I showed very little cross reaction in the assay, possessing only 2.5 per cent of the immunological potency of angiotensin II. Of the other peptides which occur in blood, bradykinin, vasopressin and adrenocorticotrophic hormone were tested and found to be completely unreactive.

Because the biological inactivation of angiotensin II proceeds in part by the removal of amino-acid residues from the N-terminal end of the molecule<sup>3</sup>, it is essential

to determine the reactivity of fragments of the molecule which may occur *in vivo* during this process of degradation. The hexapeptide formed by the removal of the two N-terminal amino-acid residues from angiotensin II has been shown to be biologically inert<sup>4</sup>. The effect of this peptide on the radioimmunoassay system for angiotensin II is shown in Fig. 1. It is apparent that considerable cross reaction occurs, the hexapeptide being of the same order of immunological potency as angiotensin II amide at low concentrations, though of decreased potency at higher concentrations. To extend the previous finding on the reactivity of angiotensin I, the effects of this peptide and the tetradecapeptide renin substrate were also examined. The earlier observation on the low reactivity of angiotensin I was confirmed, and the immunological potency of the tetradecapeptide shown to be of an even lower order. From the results of five experiments similar to that shown in Fig. 1, the following conclusions were reached. Angiotensin I exhibited only 2.4 per cent of the immunological potency of angiotensin II. The tetradecapeptide showed only 35 per cent of the immunological potency of angiotensin I. These two peptides did not usually begin to register in the assay until at least 8 ng was present. 0.5 ng of angiotensin II was well detected by the assay, occupying approximately 50 per cent of the total range. The hexapeptide was more reactive than angiotensin II when less than 2 ng was present, and less reactive when greater quantities were present. This information is illustrated in Fig. 2.

It is apparent that the "antigenic site" of angiotensin is more affected by additions to the C-terminal end of the molecule than by alteration of the N-terminus. Goodfriend *et al.* used inhibition of complement fixation to show that the immunological reactivity of angiotensin was little affected by modifications at position 1 (ref. 5) but in contrast to the present results detected only quite low reactivity (3 per cent) in the 1, 2 des val 5 angiotensin analogue which we found to react quite strongly with antibodies to angiotensin. Apart from the possibility of differing specificities of the two antisera used for these studies, it is possible that the different nature of the two assays could contribute to this difference in apparent reactivity of the hexapeptide. It should also be emphasized that although low concentrations of the hexapeptide react quite strongly with anti-angiotensin in the radioimmunoassay, there is a considerable fall in potency relative to angiotensin II at higher concentrations in this assay.

Because of the low immunological potency of angiotensin I and the tetradecapeptide renin substrate, these peptides are unlikely to interfere with the measurement of angiotensin II extracted from body fluids and incubation media. The strong reactivity of the hexapeptide at low concentrations, however, could possibly comprise the measurement of angiotensin II by this radioimmunoassay. To avoid invalidation of the assay results by the presence of this peptide, it may be necessary to include a chromato-

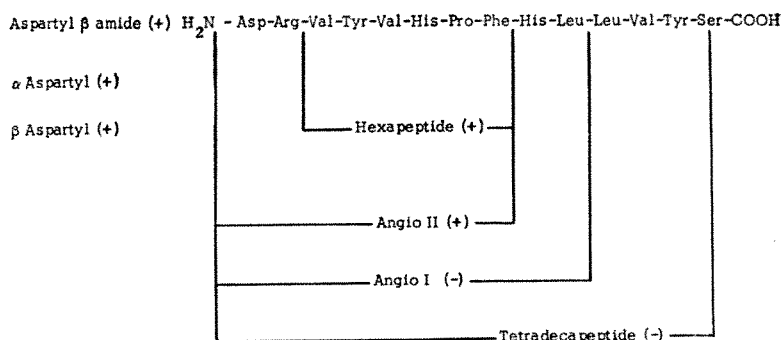


Fig. 2. Diagram of the immunological reactivity of the angiotensin series of peptides (+), Strongly reactive; (-), weakly reactive.

graphic or electrophoretic step to separate the hexapeptide from angiotensin II in blood extracts before the immunoassay is performed. Depending on the rate of degradation of the hexapeptide relative to that of its formation from angiotensin II, the measurement of the level of this fragment following such a fractionation step could provide a useful reflexion of angiotensin II metabolism *in vivo* and *in vitro*.

We thank Dr B. Riniker and Dr F. Gross of Ciba, Basle, for angiotensin, 1,2 des-angiotensin and tetradecapeptide. This work was partly supported by a grant from the National Heart Foundation of Australia.

*Note added in proof.* Dietrich<sup>6</sup> has recently reported that the C-terminal of angiotensin II is of the utmost importance for immunological specificity, whereas the N-terminal is relatively unimportant in this regard, and has suggested that antibody and biological receptors for angiotensin II may not require the same structural features.

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## Relationship between the Subunits and "T" Antigens of Adenovirus Type 12

In previous reports, we have described the separation of the group-specific ("A" antigen) and type-specific ("C" antigen) subunits of adenovirus 12 (ref. 1) and the "T" antigens<sup>2</sup> of the highly oncogenic group A adenoviruses, and we have described, using indirect methods, the production of type-specific antigen in virus free hamster tumour cells induced by adenovirus type 12 (ref. 3).

This communication describes the separation of the tumour cell antigens induced by adenovirus 12, in order to compare these components with those found in the

infected cells, because such components might differ in form, content or amount if present in the tumour cell. The tumour antigens were prepared in the following manner. A primary hamster tumour induced by adenovirus 12 of a size greater than 50 mm was excised, and debrided of all necrotic tissue. The tissue was suspended in Eagle basal media with penicillin and streptomycin at 4° C, and the tissue was broken up mechanically with a syringe and successively smaller needles, with repeated washing. When fine enough to be drawn into a 17 gauge needle, 1 c.c. was inoculated into each animal above the right flank. When the tumour was larger than 40 mm, the animal was bled and killed. The tumour was removed and debrided of all necrotic tissue. It was then minced, washed and spun at 1,500 r.p.m. for 19 min at 4° C three or four times. The tumour was weighed and a 16.7 per cent w/v suspension in Eagle basal media was prepared. This suspension was homogenized with a 'Virtis' homogenizer at low speed for 15 min and at medium speed for 15 sec at 4° C. The bulk of the material was frozen at -60° C and a portion of each tumour was frozen separately and tested for complement-fixing (CF) reactivity with hamster tumour antisera. Those tumours with a CF titre of more than 1:16 with carefully standardized hamster tumour sera were used in the present experiments. The method used to separate the tumour antigens was the same as that described previously for the separation of adenovirus 12 "T" antigens from the infected cell<sup>2</sup>, except that the tumour was homogenized by hand before separation: 3 ml. of homogenized antigen preparation was layered on top of a 1.3 × 61 cm 'Sephadex G-100' column under 2.9 ml. buffer and eluted (15 cm water pressure above the top of the column) with 0.01 molar phosphate buffer, pH 7.0, at a flow rate of 0.4 ml./min. Because centrifugation resulted in antigenic loss, we eliminated fast-sedimenting particles and a cell debris from unclarified starting materials by means of a preliminary partial separation on 'Sephadex' carried out as follows. The void volume of each column, about 25 ml., was collected, followed by consecutive collections of 20 ml. (Pool A), 12.5 ml. (Pool B), and 35 ml. (Pool C). Similar collections from each of eleven columns were pooled and concentrated ten times. Pool A was sonicated in a sonic oscillator (50 W, 9 kc/s) for 1 min at an output voltage of 180 V. A portion of each of the three pools was then set aside to be used as a control. Eight fresh 'Sephadex' columns, three labelled A, two labelled B, and three labelled C, were then loaded with 3.0 ml. of the respective concentrated A, B and C pools. Each was applied directly on to the top of the gel and void volume (25 ml.) plus four fractions (12.5, 5.0, 12.5 and 20.0 ml., respectively) were

Table 1. SEPARATION AND RECOVERY OF ADENOVIRUS 12 T ANTIGENS FROM CRUDE LATE HARVESTS OF INFECTED KB CELL PACK MATERIAL<sup>3</sup>

Material tested—second cycle	Volume of fluid	Concentration*	CF antigen titre as measured with indicated sera (reciprocals)			Total No. of CF antigen units in stock put on columns and in recovered fractions as measured with sera†		
			I-1:32	II-1:20	III-1:20	I	II	III
Stock put on column A	3 ml.		512	256	16	1,536	768	48
Column A fraction I	12.5	1.25	1,024	128	16	1,180	160	20
Column A fraction II	5.0	1.0	16	16	0‡	16	16	—
Column A fraction III	12.5	1.25	0	8	0	—	10	—
Column A fraction IV	20.0	2.0	0	0	0	—	—	—
			Total units recovered			1,196	186	20
			% of total recovered			77%	24%	42%
Stock put on column B	2.85 ml.		64	128	0	182.4	364.8	—
Column B fraction I	12.5	1.25	128	16	0	160	20	—
Column B fraction II	5.0	1.0	0	32	0	—	32	—
Column B fraction III	12.5	1.25	0	32	0	—	40	—
Column B fraction IV	20.0	2.0	0	0	0	—	—	—
			Total units recovered			160	92	—
			% of total recovered			88%	25%	—
Stock put on column C	3 ml.		32	32	16	96	96	48
Column C fraction I	12.5	1.25	128	8	0	160	10	—
Column C fraction II	5.0	1.0	4	0	0	4	—	—
Column C fraction III	12.5	1.25	0	32	32	—	40	40
Column C fraction IV	20.0	2.0	0	0	0	—	—	—
			Total units recovered			164	50	40
			% of total recovered			171%	52%	83%

\* Concentration on UM-1 'Diaflo' membranes.

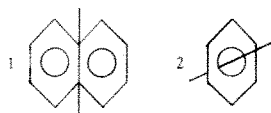
† Serum I, human adenovirus LID number 26186—measures group antigen; serum II, adenovirus 12 hamster tumour sera pool LID number 13—measures T antigen complex and virion antigens; serum III, adenovirus 12 hamster tumour sera pool LID number 15—measures only one component of the T antigen complex.

‡ Less than 1:2.



Table 2. SUMMARY OF KNOWN CHARACTERISTICS OF THE HIGHLY ONCOGENIC GROUP A ADENOVIRUS 12 SUBUNITS AND T ANTIGENS

Adenovirus 12 T antigen (type-specific)	—molecular weight about 80,000. —heat unstable. —does not react with rabbit anti-group (A) or anti-type (C) specific virion antisera or with narrow-reacting hamster tumour sera. —does react with hamster tumour sera which is reactive both with adenovirus 12 T complex and with purified C antigen. —is sensitive to FUDR. —is probably the form C antigen takes in the tumour. —is inseparable of component parts on DEAE when purified T antigen is used. —is separable of component parts on DEAE when purified 80,000 tumour antigen is used. —is present in nuclear, microsomal subcellular particulates. —separated from washed, infected cells by triple-cycling on 'Sephadex G-100', followed by gel electrophoresis.
Adenovirus 12 T antigen (group-specific)	—molecular weight of approximately 20,000. —heat stable. —sensitive to ether and to several freezings and thawings. —reacts with all group A adenovirus hamster tumour sera. —is not sensitive to FUDR. —is associated with or contains a phospholipid protein. —does not react with rabbit anti-group or anti-type specific virion antisera. —is present in nuclear subcellular particulates. —separation from washed, infected cells by double-cycling on 'Sephadex G-100' and a third cycle on one of the lower-numbered 'Sephadexes', followed by gel electrophoresis.
Adenovirus 12 type-specific subunit (C antigen)	—molecular weight of approximately 63,000. —heat stable. —double-cycled on DEAE is semi-sensitive to ether, quad-cycled is not. —does not react with rabbit anti-group specific sera or with narrow-reacting hamster tumour sera. —does react with rabbit anti-type specific sera and with certain hamster tumour sera demonstrated to have C antibody. —is often associated with a component with a mol. wt. of 137,000 which has not been characterized. —separated from late harvested, infected cell supernates by five cycles on DEAE or by double cycles on DEAE followed by gel electrophoresis.
Adenovirus 12 group-specific subunit (A antigen)	—heat stable, reacts with rabbit anti-group specific sera. —not sensitive to ether, partially sensitive to trypsin. —separated in same manner as C antigen. —exists naturally as a dimer (mol. wt. 376,000) and in ratio of one dimerized A antigen to C antigen, which suggests hook-up type 1 over type 2.



collected. Concentration was carried out with 'Diaflo' model 50 ultrafiltration cells, using UM-1 'Diaflo' membranes, operated at a pressure of 30 lb./in.<sup>2</sup>.

For comparison, a typical second cycle separation and recovery of adenovirus 12 "T" antigens from crude late gathered infected KB cells<sup>2</sup> is shown in Table 1. The antigen which was recovered from the second cycle of pool B (fractions II and III) was found to have a molecular

weight of about 80,000 and the antigen recovered from column C (fraction III) was found to have a molecular weight of about 20,000 (ref. 2).

A summary of what we know so far about two compounds from infected cells which react with hamster tumour antisera and about subunits of adenovirus 12 is shown in Table 2.

The second cycle separation and recovery of adenovirus 12 hamster tumour antigens from 'Sephadex' columns are shown in Table 3. If one compares the separation of the infected cells in Table 1 with the separation of the tumour cells in Table 3, it can be seen that a component of lower molecular weight from column C pool III is present in both the infected cells and in the tumour cells. This tumour fraction is likewise sensitive to ether and to freezing and thawing and is stable at 56° C for 30 min. It can be seen that there was no CF reacting component from column B pool II. After this fraction was cycled on DEAE (ref. 1) a whole type-specific (C antigen) was present, as measured in complement-fixation tests with specific anti-C rabbit sera. Thus it might be concluded that there was present in the tumour cell a partially fragmented type-specific antigen which was in some manner covered or interrelated with the "T" antigen. The separated "T" component (column C fraction III) was measured more strongly by the narrow-reacting hamster sera (III). The partially fragmented or unstable type-specific component was not measurable in the separated fraction II of column B, but, apparently, it was reassembled or brought together or altered in some manner on recycling over DEAE and gave a complement fixing reaction with type-specific antisera.

We suggest that the type-specific antigen was somehow covered by or attached to the phospholipid-containing or -associated "T" antigen in the tumour cell. In the tissue culture system there may have been greater interaction and a more cohesive attachment due to the large number of virion components in that system. In addition, the higher molecular weight "T" component in the infected cell was collected from both early and late cells<sup>2</sup> while the type-specific antigen was separated only from cells collected late<sup>1</sup> in amounts sufficient for detection by complement-fixation. Thus it is possible that the higher molecular weight "T" component in the infected cell may be some form of precursor to the type-specific subunit. Or, in the tumour cell, the "T" coat may have been more easily removed. We were unable to break apart the higher molecular weight component derived from the infected cells by using the same methods; however, when a similar fraction was separated from the tumour cell on 'Sephadex', it was possible to extract a CF reacting

Table 3. SEPARATION AND RECOVERY OF ADENOVIRUS 12 HAMSTER TUMOUR ANTIGENS FROM 'SEPHADEX' COLUMNS

Material tested	Volume of fluid	Concentration	CF antigen titre as measured with indicated sera* (reciprocals)			Total No. of CF antigen units in stock put on columns and in recovered fractions as measured with sera*		
			I	II	III	I	II	III
Stock put on column A	9.0 ml.		0	32	32	0	288	288
Column A fraction I	37.5 (12.5 × 3)	3.75	0	32	> 32	0	288	> 288
Column A fraction II	15.0 (5 × 3)	1.50	0	8	16	0	12	24
Column A fraction III	37.5 (12.5 × 3)	3.75	0	32	32	0	120	120
Column A fraction IV	60.0 (20 × 3)	6.0	0	0	0	0	0	0
				Total units recovered		0	420	432
				% of total recovered		—	146%	150%
Stock put on column B	6.0		0	4	8	0	24	48
Column B fraction I	25 (12.5 × 2)	2.5	0	8	8	0	20	20
Column B fraction II	10 (5 × 2)	1.0	0	0	0	0	0	0
Column B fraction III	25 (12.5 × 2)	2.5	0	0	0	0	0	0
Column B fraction IV	40 (20 × 2)	4.0	0	0	0	0	0	0
				Total units recovered		0	20	20
				% of total recovered		—	83%	42%
Stock put on column C	9.0		0	4	4	0	36	36
Column C fraction I	37.5 (12.5 × 3)	3.75	0	0	4	0	0	15
Column C fraction II	15.0 (5 × 3)	1.50	0	0	0	0	0	0
Column C fraction III	37.5 (12.5 × 3)	3.75	0	4	8	0	15	30
Column C fraction IV	60.0 (20 × 3)	6.0	0	0	0	0	0	0
				Total units recovered		0	15	45
				% of total recovered		—	42%	125%

\* Sera I, II, III: see footnote Table 1.

Starting material: GWU No. L HR<sub>3-3</sub>, 9, 10  
(First cycle: 33 ml. on 11 columns)

I 0 II 32 III > 32

type-specific subunit by using our method of separation on DEAE (ref. 1) followed by the 'Diaflo' ultrafiltration concentration method. We are not sure whether "T" antigen is internally connected to virion antigen; indeed, this may be a matter of semantics: one would call any internal component a "virion" antigen. It is noteworthy, however, that in the 'Sephadex' experiments with infected cells<sup>2</sup> a portion of the lower molecular weight component always remained associated with group-specific antigens (pool A fraction 1) and was not released by sonication or by repeated separation on 'Sephadex'.

*In vivo*, it is possible that the type-specific "T" antigens detach from the virion capsid and remain associated in the tissue cells for indefinite periods of time. We plan to explore this hypothesis further using an electron microscope, with other sera prepared against separated whole and disintegrated antigens and virion, and by purification and chemical analyses of these, and perhaps other, separated antigens.

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### Heat-labile Macroglobulin revealed in Adenovirus 12 Tumour Sera by Gel Electrophoresis

THERE are two important differences in the way serum proteins separate by zone electrophoresis which employs gels, when compared with paper or moving boundary electrophoresis. First, the sieving effect of the gel alters the mobilities of the proteins and, second, more fractions are resolved. Varying the pore size of the gels excludes from or permits movement of the proteins based on the molecular size and shape relative to the gel pore size.

Previously, we reported the location of complement-fixing antibodies to adenovirus 12 tumour and "T" antigens in the 7S gamma-globulin fractions of tumour bearing hamsters<sup>1</sup>. The "T" and tumour antigen complexes previously had been shown by separation studies to have at least one heat stable and one heat labile component<sup>2</sup> and some quantitative differences in reactivity of the 7S fraction with tumour and "T" antigens from different sources were noted. In addition, the location of antiviral complement-fixing antibody in gamma-globulin fractions of sera from a child at three different times before and after infection with adenovirus type 31 was found almost entirely in the 7S fraction and this was later confirmed in neutralization tests and in other test sera (unpublished results of Vargosko and Hollinshead). This second paper in our series of investigations dealing with adenovirus-related antibodies is a study of the whole serum, 19S and 7S fractions of normal and adenovirus 12-tumour bearing hamsters using polyacrylamide gel disc electrophoresis.

The sera from two normal and four tumour bearing hamsters were investigated. At the time the sera were

obtained the animals were 45–90 days old, and the tumour sizes were 35–70 mm across. Of the tumour bearing animals two were litter mates inoculated at birth with adenovirus type 12 and two were unrelated adult animals that received tumour cells from the fifth passage of an adenovirus 12-induced tumour. Inoculations were given subcutaneously and both males and females were used. Sera from the tumour bearing animals had complement-fixing titres of  $>1:64$  using LID adenovirus 12 tumour antigen pool No. 10 diluted 1:4. Sera were stored at  $-70^{\circ}\text{C}$  in samples of 1–3 ml. and no serum was more than 9 months old.

Separation of the whole serum into the 19S and 7S fractions was by the method of Kunkel<sup>3</sup> using 10 per cent to 37 per cent (w/v) sucrose. The 7S fraction was removed by puncturing the side of the tube and the 19S fraction by bottom puncture. A sample of 1 ml. of each fraction was concentrated to 0.2 ml. by the method of Kohn<sup>4</sup>. Electrophoresis of the whole serum and the 7S fraction on polyacrylamide gel was performed as described by Ornstein<sup>5</sup>. When the 19S fraction was investigated, a separating gel of 4.2 per cent was made by diluting the standard 7 per cent acrylamide gel solution with distilled water. By letting the gel stand overnight in the column at room temperature a more rigid workable material developed. The stacking and sample gel were then applied before electrophoresis. In order to obtain clear bands with the 19S fraction a modified sample gel was prepared. A saturated solution of sucrose was used instead of the standard 40 per cent stock solution generally recommended. A 3:5 ratio of sample to gel was employed. A *tris* glycine buffer, pH 9.5 and ionic strength 0.5 molar, was used and all the samples were run at a current 2.5 m.amp/tube for 1 h.

To test for the presence of the  $\text{C}_1$  macroglobulin component of complement, 0.15 molar disodium ethylene tetra-acetic acid (EDTA) was prepared and titrated to pH 7.4 with 0.1 molar sodium hydroxide. Equal parts of EDTA and serum were mixed and allowed to equilibrate for 30 min at  $4^{\circ}\text{C}$  before sucrose density separation.

When 5  $\lambda$  of undiluted whole serum was separated by electrophoresis the number and location of the bands obtained were strikingly similar for normal and tumour sera. Between nine and eleven bands were found in each, with the most prominent disks being albumin and transferrin. There were, however, quantitative differences when normal and tumour sera were compared (Fig. 1).

Twenty-five  $\lambda$  of the 7S sucrose fraction was used and again there was no difference between tumour and normal hamster sera except in the thickness of the bands. Because the 7S fraction migrated as a diffuse band in the upper gel and was obscured by other unrelated proteins, 7S

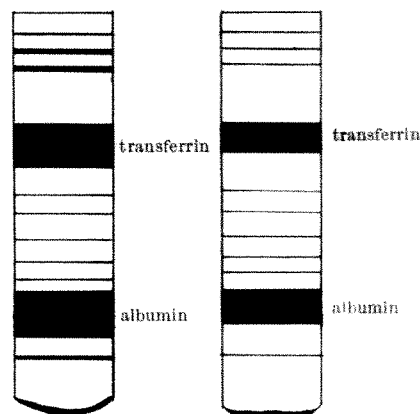


Fig. 1. Electrophoresis of normal hamster serum (left) and adenovirus type 12 tumour serum (right) using 7 per cent polyacrylamide gel. The origin (cathode) is at the top.

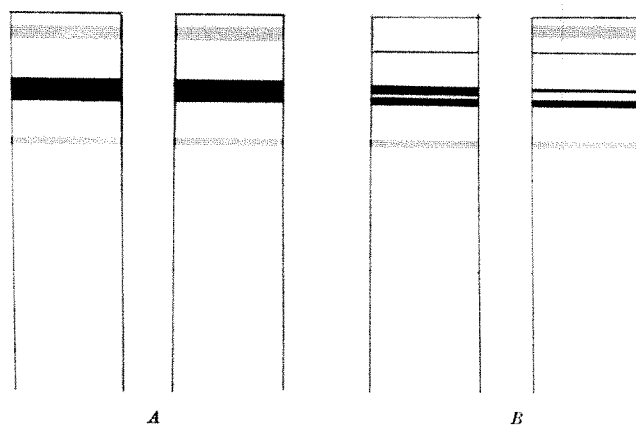


Fig. 2. Separation of unheated and heated serum of the 19S fractions of normal hamster (A) and hamster with an adenovirus type 12 tumour (B) using 4.2 per cent gel. There is no difference in normal heated hamster serum when compared with unheated normal serum. In the unheated tumour serum (B), however, two dark bands are present instead of one, and the upper dark band almost completely disappears on heating.

fractions were extracted with rivanol before electrophoresis and again only a quantitative difference was noted between normal and tumour sera.

Reduction of the separating gel concentrations to 4.2 per cent allowed the macroglobulins to migrate readily into the gel. A marked difference between the 19S fractions of normal and tumour sera was noted. As can be seen in Fig. 2, the four bands which appeared in the upper part of the separating gel in the normal sera differed from the four bands identified in the tumour sera. When normal serum was heated to 56°C for 30 min there was no alteration in the bands after electrophoresis compared with the unheated normal serum. When the tumour serum was heated in the same conditions, however, one band almost completely disappeared with only a trace of the protein being identified. Another disk appeared as a hazy band at the very top of the separating gel (Fig. 2), and probably represents heat-agglutinated protein. These differences between the 19S fractions could be better delineated by using a 50λ sample.

Although there was no proof that the heat labile macroglobulin of the tumour bearing hamsters contained the 19S antibody, it was possible that this represented the C<sub>1</sub> component of complement. Naff *et al.*<sup>6</sup> have shown that C<sub>1</sub> is a macroglobulin with a sediment coefficient of 19S. They have also found that this macroglobulin in the presence of EDTA is broken down into three subunits that sediment as 11S, 7S and 4S proteins. Treating our sera with 0.15 molar EDTA did not alter the 19S bands of the unheated tumour or normal sera; thus by indirect evidence, the macroglobulin bands were not part of the complement system.

In conclusion, in the hamster with a tumour induced by adenovirus 12 there is a change in the proteins in the whole serum and in the 7S fraction as determined by gel electrophoresis when compared with normal serum. At the time the complement-fixing antigens in the tumour and 7S serum antibodies were at their peak a new serum protein in the 19S fraction appeared that was not present in normal serum. This fraction was almost completely destroyed by subjecting the serum to a temperature of 56°C for 30 min and may account for the low titres of 19S antibody in the tumour sera<sup>1</sup>.

Takemoto *et al.*<sup>7</sup>, using a fluorescent antibody test, found a heat labile fraction in polyoma virus-induced tumour antiserum responsible for nuclear fluorescence. This heat labile serum component was also not entirely destroyed by heating to 56°C for 30 min, for minimal fluorescence persisted. We are investigating adenovirus 12 and SV40 virus to determine whether the heat labile serum fractions of sera of tumour bearing hamsters have

similar characteristics and whether they are located in the 19S fraction.

Of more importance, Takemoto's findings suggest to us that there may be a difference between the fluorescent and complement fixing antigens, because the latter reacts with a heat stable antibody.

We thank Dr R. J. Huebner and Mr H. C. Turner for the hamster sera and complement-fixation tests.

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### Delayed Hypersensitivity *in vitro*

DURING investigations of the effects of induced immune paralysis on delayed hypersensitivity *in vivo* and *in vitro*, evidence has been found<sup>1,2</sup> for a diffusible factor which is released from the sensitive cells in contact with the antigen; this factor inhibits migration of cells from a normal lymph node explant. These investigations have been extended and the results will be summarized here.

Delayed hypersensitivity to bovine gamma-globulin was produced by injecting 20 μg of the substance in the pierylated state (38 moles of pieryl/mole of bovine gamma globulin), in Freund's complete adjuvant into each hind footpad. In another group of guinea-pigs delayed hypersensitivity to a urea extract<sup>3</sup> of sheep red blood cells was produced by injecting 0.2 ml. of a mixture of equal volumes of 30 per cent sheep red blood cells and Freund's complete adjuvant into each hind footpad. 6-14 days after the injections of pierylated bovine gamma-globulin and 4-6 days after the injections of sheep red blood cells the popliteal and inguinal lymph nodes were excised. After the excisions skin tests showed delayed hypersensitivity to bovine gamma-globulin in thirty-four out of thirty-six animals in the first group, and to a urea extract of sheep red blood cells respectively in six out of eight animals in the second group.

Small fragments weighing about 5 mg each were prepared from individual lymph nodes and were cultured according to the method of Ferraresi and Halpern<sup>1</sup> with or without antigen (50 μg/ml. of bovine gamma-globulin or 15 volumes per cent of sheep red blood cell extract, respectively). The culture medium consisted of 80 per cent normal guinea-pig serum and 20 per cent guinea-pig spleen extract. The radii of cell migration were measured after 24 and 48 h of incubation. Antigen inhibited cell migration from fragments obtained from certain lymph nodes by 30-53 per cent as compared with cultures without antigen. The migration of both lymphocytes and macrophages was inhibited, but macrophages were affected to a greater degree. Bovine gamma-globulin (50 μg/ml.) did not inhibit the cell migration from lymph node explants of normal guinea-pigs or guinea-pigs injected with complete Freund's adjuvant. The sheep red blood cell urea extract stimulated the migration of normal cells slightly.

Only rarely were the cells of all inguinal and popliteal lymph nodes inhibited by antigen. This may be because

Table 1. INHIBITION OF CELL MIGRATION IN "MIXED" CULTURES

Mean cell migration in mixed cultures measured in  $\mu$ 

Without antigen				In presence of antigen					
Immunized lymph nodes No.	Migration*	Normal lymph nodes No.	Migration*	No.	Immunized lymph nodes Migration*	Percentage inhibition	No.	Normal lymph nodes Migration*	Percentage inhibition
1a	342	1	296.4	1a	342	—	1	319.2	—
b	353.4	2	256.5	b	279.3	21% $P > 0.2$	2	205.2	20% $P > 0.2$
c	330.6	3	250.8	c	205.2	38% $P < 0.01$	3	157.3	37.2% $P < 0.02$
2a	285	4	171	2a	273.6	—	4	164.1	—
b	319.2	5	167.5	b	199.5	37.5% $P < 0.02$	5	114	32% $P < 0.08$
3	262.2	6	296.4	3	148.2	43.4% $P < 0.01$	6	199.5	32.6% $P < 0.01$
4	216.6	7	285	4	148.2	31.5% $P < 0.01$	7	199.5	30% $P < 0.05$

(1-4) From different guinea-pigs with delayed sensitivity; (a-c) different lymph nodes. Nos. 1 and 2 are animals sensitized with sheep red blood cells (the antigen added to the cultures was sheep red blood cell extract); Nos. 3 and 4 are animals sensitized with picrylated 38 bovine gamma-globulin (in cultures, with the bovine gamma-globulin).

\* Each figure represents the average migration radius of 5 to 8 explants.

of uneven distribution of the injected antigen and/or on variations in the stage of sensitization exhibited by individual lymph nodes at the time of sampling. Evidence was obtained by the immunocyto-adherence test<sup>4</sup> that lymph nodes, containing significantly increased numbers of cells carrying anti-sheep red blood cell antibody, were not sensitive to sheep red blood cell urea extract, although the guinea-pigs, from which the lymph nodes were excised, showed delayed hypersensitivity to a skin test with the same antigen.

To test for the presence of a soluble factor which could inhibit the migration of normal lymph node cells, we proceeded in two ways. (a) Explants from lymph nodes from sensitive animals were cultured in the same chamber with lymph node explants from normal animals or from animals injected with Freund's complete adjuvant. The explants were at least 5 mm from each other. Cell migration from the sensitive lymph node explants and from adjacent normal explants was inhibited in both cases to about the same degree in the presence of the specific antigen (Table 1). When no inhibition of cell migration occurred in the lymph node explants of a sensitive animal, then no inhibition was observed in the adjacent normal explants. (b) Culture media from bovine gamma-globulin-sensitive and control cultures were freed from cells by centrifugation, stored at  $-20^{\circ}\text{C}$  for 1-5 weeks and were then diluted 1:1 with fresh culture medium and added to the explants of control lymph nodes from guinea-pigs injected with adjuvant. The "inhibitory supernates" were obtained from cultures of explants containing Ag and taken from sensitive lymph nodes. "Control supernates" were taken from antigen-free cultures of sensitive and normal lymph nodes, and from antigen containing cultures of normal lymph nodes and of non-inhibited lymph nodes from sensitive animals. Cell migration of normal lymph node explants was inhibited 17 per cent to 54 per cent (average 37 per cent) in seven out of nineteen experiments in the presence of supernates from antigen sensitive cultures as compared with control supernates. This difference was significant ( $P < 0.02$ ). The migration inhibition in the donor cultures was 31 per cent to 53 per cent, with an average of 40 per cent. In nine of the remaining experiments inhibition was induced in normal explants ranging from 11 per cent to 40 per cent (average 23 per cent). These inhibitions were not significant at the 5 per cent level. Here the inhibition of migration in the donor cultures was between 22 per cent and 47 per cent, with an average of 30 per cent. In one experiment no inhibition of migration was conferred by supernate from sensitive culture inhibiting 22 per cent. In the other two experiments the supernates from antigen sensitive cultures stimulated 28 per cent and 29 per cent the migration of normal lymph node cells, although the media were taken from sensitive cultures inhibited 47 per cent and 34 per cent, respectively.

These findings confirm and extend to two additional antigens the results of Bloom and Bennett<sup>5</sup> and David<sup>6</sup>.

Our results indicate that the release of inhibitory material from sensitive cells in the presence of the antigen also occurs when the architecture of the lymph node is partially maintained.

That the induction of migration inhibition in normal lymph node cells by inhibitory supernates was not as strong and consistent as before<sup>5,6</sup>, could be caused by the dilution of the culture supernates from inhibited cultures with fresh medium. David<sup>6</sup> indicated that 1:5 dilutions of the inhibitory material were not effective in his system. In cultures of mixed lymph node explants, however, inhibition was conferred to normal explants, whereas David *et al.*<sup>7</sup> did not observe the same phenomenon in tests on capillary tubes filled with peritoneal exudate cells. It is likely that in the lymph node explants the variety and the number of interacting cells, especially of lymphocytes, are more complex than in the peritoneal exudates.

Our investigation leaves several points for further elucidation. (a) Why were several of the more remote local lymph nodes not sensitive to antigen? This may reflect the progression of the immune process through the lymphoid system. Similar observations have been made by Carpenter<sup>8</sup>, who also used the explant-culture method. There are at least two possibilities; either that these lymph nodes were not yet sensitive or that sensitive cells were no longer present in these lymph nodes. (b) The cells which were not inhibited by antigen were apparently unable to release inhibiting factor. This agrees with previous observations<sup>5,6</sup> that non-sensitive lymph nodes may not possess sufficient lymphocytes capable of secreting inhibitory factor. This is apparently not a sampling artefact, for all explants cultured from the entire node behaved similarly. (c) Finally, the relationship of sensitive and antibody-carrying cells to delayed skin reactivity needs further study.

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## HAEMATOLOGY

## Surface Ultramicroscopy of Human Blood Cells

This communication describes the preliminary results of an investigation of the surface appearance of blood cells. Alterations in the surface contour of these cells were demonstrated using the Cambridge 'Stereoscan' electron microscope, which provides a three-dimensional view of the surface structure.

Early work<sup>1</sup> on individual cells, using the 'Stereoscan' electron microscope, has shown the different depression patterns from ameloblast processes on the surface of developing dental enamel in the manatee *Trichechus latirostris* and the monkey *Rhesus macacus*. More recently reports have illustrated the surface picture of simple and compound eyes<sup>2</sup>, details of the leg of a young shore crab<sup>3</sup> and the three-dimensional appearance of buccal squames and endothelial cells<sup>4</sup>.

Blood cells were prepared for examination by fixing sequestrenated whole blood or bone marrow in 0.5 per cent phosphate buffered glutaraldehyde (pH 7.4) (Lewis, S. M., personal communication), washing the cells in distilled water, and making a thin film on a small cover glass which was attached to the aluminium specimen stub. The specimens were covered with a layer of gold and palladium 500 Å thick to render their surfaces conducting. They were examined in the Cambridge 'Stereoscan' electron microscope and the micrographs recorded with a 20 kV scanning electron beam on Ilford 'HP3' film with an exposure time of 40 sec.

The normal red blood cell appeared exactly as would have been expected from the appearance of unstained fresh blood under the light microscope, that is, as a biconcave disk 7–8  $\mu$  in diameter (Fig. 1). The surface of the majority of red cells was relatively uniform and smooth, even at magnifications of 115,000. In patients with a reticulocytosis, a proportion of red cells about the same percentage as the percentage of reticulocytes, had a slightly more granular surface than usual (Fig. 2). These findings were broadly in agreement with those of Danon and Marikovsky<sup>5</sup>, who studied separated red cell membranes with a transmission electron microscope. In blood

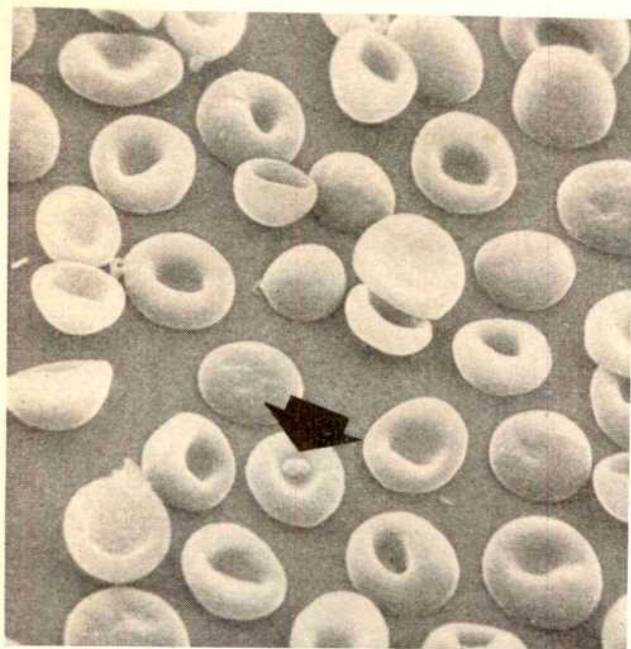


Fig. 1. Normal red blood cells. Note the platelet lying in the concavity of a red cell in the foreground. ( $\times 2,850$ .)

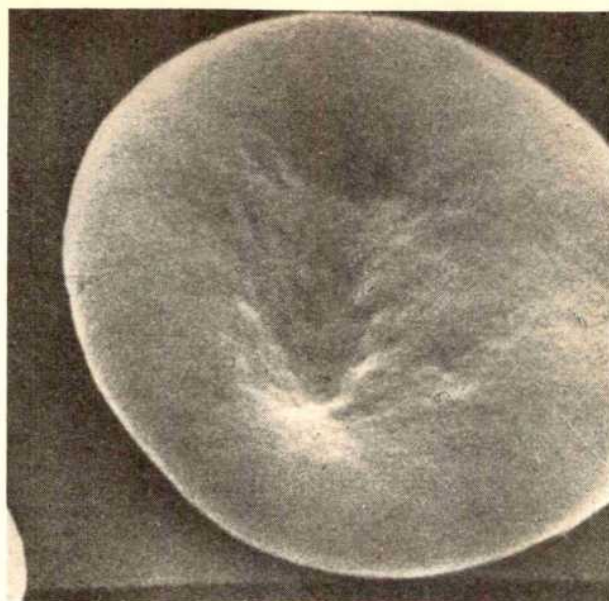


Fig. 2. Reticulocyte from a case of systemic lupus erythematosus with haemolytic anaemia. The central concavity is much less pronounced and much of the red cell surface is finely granular. ( $\times 12,500$ .)

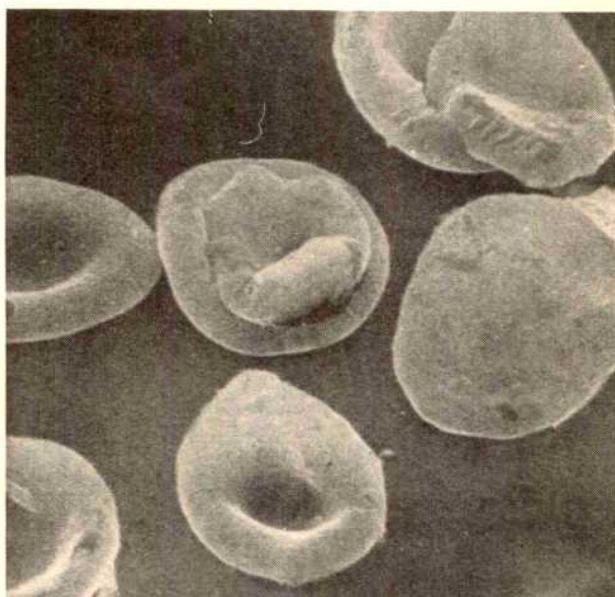


Fig. 3. Red blood cells showing patchy denudation of the nuclear membrane from a case of idiopathic thrombocytopenic purpura following splenectomy. ( $\times 4,850$ .)

from patients who had undergone a splenectomy, many red cells showed denudation of the outer cell membrane in small, irregular patches, often with detached membrane around the margin of such areas (Fig. 3). This is probably an ageing process.

Various pathological red blood cells, such as hypochromic cells, spherocytes, macrocytes, elliptocytes, poikilocytes, schistocytes, sickle cells and target cells, were identified with relative ease. For example, target cells were obtained from cases of thalassaemia, liver disease and steatorrhoea and from patients after splenectomy. Regardless of the underlying disease process, the target cells were of two types; they showed either a central hemispherical protrusion (Fig. 4) or a roughly linear protrusion extending across the cell almost as far as the peripheral cell thickening (Fig. 5). The majority



of cells in cases of sickle cell anaemia were considerably elongated, a linear fold extending along the entire long axis of the cell (Fig. 6). The surface of pathological red blood cells at high magnifications did not vary much from normal in the majority of cases. In certain cases of haemolytic anaemia and dyshaemopoietic anaemia, large pores were noted in the membrane of some red cells. This phenomenon is being investigated further. The complement holes described in the membranes of red cells from cases of paroxysmal nocturnal haemoglobinuria<sup>6</sup> could not be seen. Such holes have been reported to have mean diameters of about 103 Å—the resolution of the 'Stereoscan' microscope is generally 200 Å.

White blood cells were examined in preparations of nucleated cell concentrates of blood and of bone marrow. The surface appearance of white cells fixed by glutaraldehyde in suspension was less striking than that of red cells. Granulocytes were approximately 10  $\mu$  in diameter, were covered with multiple pseudopodial projections, and had smooth surfaces. Lymphocytes were 8–9  $\mu$  in diameter,

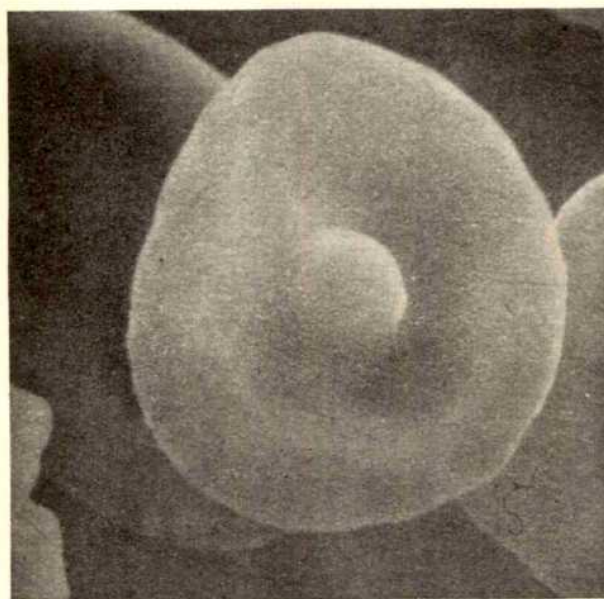


Fig. 4. Target cell from a case of thalassaemia major. ( $\times 11,500$ .)

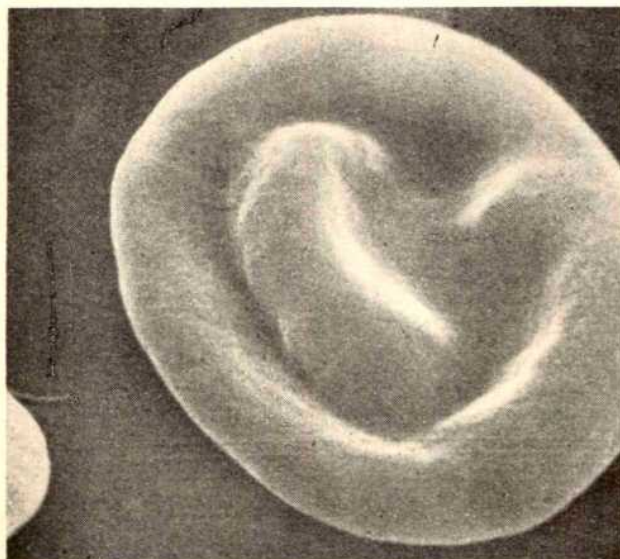


Fig. 5. Target cell from a case of phenacetin-induced haemolytic anaemia. ( $\times 11,000$ .)

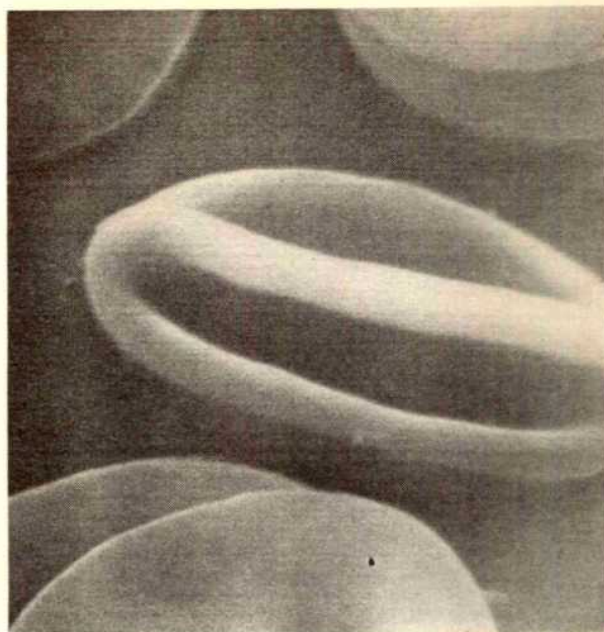


Fig. 6. Sickle cell. Sickle cell anaemia. ( $\times 10,000$ .)

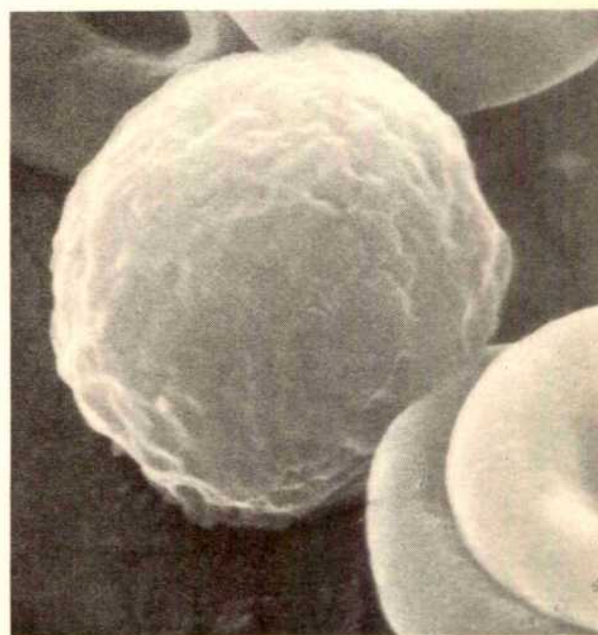


Fig. 7. Lymphocyte from normal blood. ( $\times 9,500$ .)

spherical, and with a rough, "shaggy" surface (Fig. 7). Monocytes were 12–15  $\mu$  in diameter, irregular in shape, and with a rough surface similar to that of the lymphocyte. White cell precursors were remarkably uniform and featureless in surface appearance, varying only in size.

Platelets were irregular in outline, with no characteristic surface appearances. Megakaryocytes in preparations from bone marrow were readily identified as large cells 30–50  $\mu$  in diameter with evidence of active platelet formation on their surface (Fig. 8). The smallest platelets appeared to be completely spherical and smooth: only large platelets which were almost completely detached from the cytoplasm of the megakaryocyte showed the irregular outline described already.

We thank the Cambridge Instrument Company for access to their instrument, and Miss Farrow for her tech-



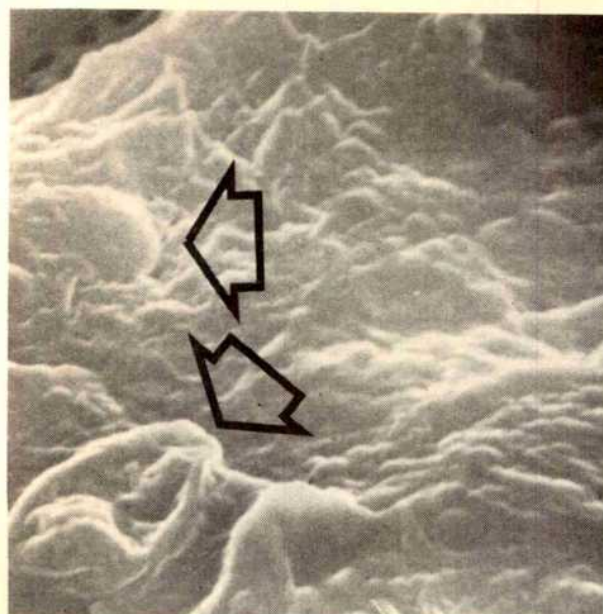


Fig. 8. Platelet formation on the surface of a megakaryocyte. Note the small spherical platelet with a smooth surface; below it a large irregular platelet almost completely detached from the megakaryocyte. ( $\times 9,000$ .)

nical assistance. Mr Tredinnick, of the Department of Medical Photography, St. Bartholomew's Hospital, assisted with the illustrations. Professor D. L. Mollin kindly advised us during this study and made many helpful suggestions.

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## Haemoglobin Types in Deer

HAEMOGLOBIN types have been examined in only a few species of deer. Single fractions without variants have been found in chital deer (*Axis axis*) and Indian muntjac (*Muntiacus muntiacus*)<sup>1</sup>, red (*Cervus elephas*) and fallow deer (*Dama dama*)<sup>2</sup>, and in sika (*Cervus nippon*) and barasingha deer (*Cervus duvanceli*)<sup>3</sup>. Haemoglobin which separates electrophoretically into one to three fractions has been found in white-tailed deer (*Odocoileus virginianus*)<sup>3-6</sup>. We have examined the haemoglobin types in feral British deer and single samples of Père David (*Elephurus davidianus*), chital and barasingha deer by paper and Poulik's discontinuous buffer starch gel electrophoresis<sup>7</sup>, pH 8.8, and by alkali denaturation<sup>8</sup>. The numbers of these species are listed in Table 1.

Table 1

Deer species	No. examined
Red, <i>Cervus elephas</i>	32
Sika, <i>Cervus nippon</i>	44
Barasingha, <i>Cervus duvanceli</i>	1
Fallow, <i>Dama dama</i>	62
Roe, <i>Capreolus capreolus</i>	80
Chinese muntjac, <i>Muntiacus reevesi</i>	40
Père David, <i>Elephurus davidianus</i>	1
Chital, <i>Axis axis</i>	1
Chinese water, <i>Hydropotinae inermis</i>	21

The electrophoretic mobilities of haemoglobin from red, sika, barasingha, Père David and chital deer are identical in the methods used. Fallow deer haemoglobin moves faster than these, with roe haemoglobin lying between fallow and *Cervus* species. All these species yield a single haemoglobin fraction, and no variants were found among them except for a single fast moving component in a roe fawn replacing the normal haemoglobin of this species.

The Chinese muntjac (*Muntiacus reevesi*) has a polymorphic haemoglobin, the different samples examined having two or three fractions. The different patterns obtained were separable as two slower moving fractions occurring together provisionally identified as type A, and two faster moving fractions as type B, the faster moving fraction of type A corresponding in mobility to the slower moving fraction of type B. A third pattern of separation was also found after starch gel electrophoresis in which haemoglobin fractions of all three mobilities were found together. Electrophoresis of a mixture of types A and B yielded a pattern indistinguishable from the naturally occurring three bands. Assuming that the distribution of the genes from the two forms A and B are allelic, the presence of three bands would appear to represent the heterozygous form AB.

Determination of the gene frequencies according to Wiener and Vaisberg<sup>9</sup> yields

	Type A	Type AB	Type B	Total
No. of samples	11	22	7	40
Proportion per cent	27.5	55	17.5	100
Frequency of gene A = 0.55				
Frequency of gene B = 0.45				

Incidence in sample examined if AB is the heterozygote of AA and BB

	Expected	Observed
AA	12	11
AB	19.4	22
BB	8.5	7

$\chi^2 = 0.494$ ,  $P = 0.5$  supporting the suggestion that types A and B are allelic.

In eighteen of the Chinese water deer (*Hydropotinae inermis*) the haemoglobin moved as a single fraction provisionally identified as A, with the same mobility as the *Cervus* species. A second, slow moving, minor component (B) was present in three of them. No example of the second component occurring alone was seen, but calculation of the gene frequencies, assuming them to be allelic, gives type A as 0.92 and type B as 0.07.

Samples from all species showed more than 50 per cent resistance to alkali denaturation. All blood samples examined except for one muntjac foetus (type B) were taken from adult or late juvenile animals and no evidence was found for a foetal haemoglobin component. The

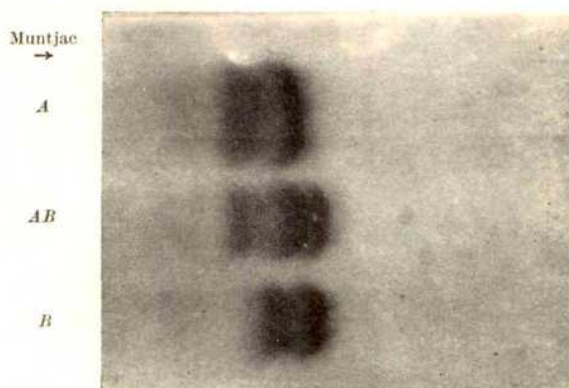


Fig. 1. Reeve's muntjac haemoglobin. Poulik's discontinuous starch gel electrophoresis, pH 8.8.

sickling phenomenon in 0.9 per cent saline solution was demonstrated in red, sika, barasingha, fallow, roe and muntjac. Père David and chital deer red cells were not examined for this phenomenon. None of the twenty-one samples of Chinese water deer red cells sickled in physiological saline or in 2 per cent sodium metabisulphite.

The sika samples examined included two of the Manchurian variety of the species. The haemoglobins of these were indistinguishable from the type species.

As the haemoglobin of the Indian muntjac was found by Naik<sup>1</sup> to separate into one fraction only, it appears likely that the muntjacs feral in south-east England are of the Chinese species. Genetic admixture with Indian muntjacs which escaped from parks many years ago is a possibility and haemoglobin analysis may provide a point of taxonomic separation of the species. It may also help in strain separation of the Chinese water deer, but appears to be of no value among the genera of larger deer.

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## GENETICS

### Another XYY Phenotype

ON seeing the webbed neck of a 24 year old mentally retarded male, one of us (M. B.) was prompted to question the cytogenetic sex. The man in question was of average height, well built, with adult genitalia, descended testes, and male hair distribution. The inguinal lymph nodes were palpable. He was living in an institution among mental defectives and had a history of suspected arson, seizures, emotional disturbances and familial mental retardation of marginal degree.

Chromosomes were counted in fifty-four, and karyotypes were assembled from twenty-two, metaphases of cultured leucocytes<sup>1</sup> stained lightly with orcein and photographed by phase microscopy. The Y and G group chromosomes were further distinguished by shadowing with platinum-carbon<sup>2</sup> and by autoradiography of chromosomes labelled with tritiated thymidine 3 h before mitoses were arrested with colchicine<sup>3</sup>.

Ninety per cent of the cells contained forty-seven chromosomes. The karyotype showed an extra small acrocentric that fitted morphological criteria for the Y chromosome<sup>4</sup>. The aneuploidy of the few cells with more or less than forty-seven chromosomes was variable and unrelated to the sex or G group chromosomes. One endoreduplicated metaphase contained ninety-four chromosomes.

Shadowing (carried out by courtesy of Dr M. R. Edwards) emphasized the satellite distinctions, and autoradiography the differential labelling pattern of the Y

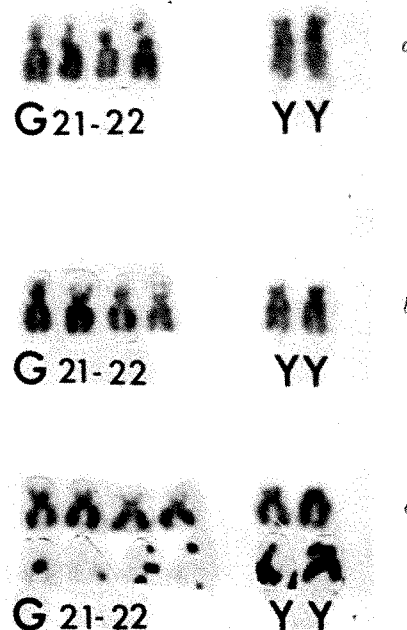


Fig. 1. Three partial karyotypes of G group and Y chromosomes. (a) Stained with aceto-orcein; (b) shadowed with platinum-carbon; (c) autoradiographed after 4 h of exposure to tritiated thymidine.

and G group chromosomes (Fig. 1). Relative asynchrony in labelling of the two Y chromosomes occurred, as has been noted before<sup>5,6</sup>.

This phenotype is unique among the twenty-five or so XYY genotypes recorded for his striking webbed neck. Its clinical significance is not clear, however, other than to trigger associations, because its concomitance in "male Turner's" syndrome is usually accompanied by a normal male karyotype<sup>7</sup>. Nor is there much likelihood of our patient having been a cytogenetic Turner's mosaic, because recorded XO/XYY phenotypes are female<sup>8,9</sup>.

Our case adds to the variable phenotype presented by XYY genotypes, and a recent dissenting report of synchronous labelling of YY chromosomes in two normal males<sup>10</sup> suggests that phenotypic differences are reflected in a variable autoradiographic pattern. Reference to the phenotype as the "YY syndrome" is, indeed, inadvisable<sup>11</sup>.

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## Heredity of Two Types of Normal Colour Vision

THE anomaloscope is used to diagnose red and green colour blindness and its four or six sub-types. In the apparatus the lower half of a circular plane is illuminated by a yellow light of wavelength 589 m $\mu$ , and in the upper half red (670 m $\mu$ ) and green light (546 m $\mu$ ) are mixed. It is possible, by changing the proportion of red and green illumination, to make the two halves look the same; the proportion of red and green lights required is called the Rayleigh equation, which is accepted by individuals with normal colour vision, but colour blind people react in different ways. On the anomaloscope which I used (model II, Schmidt and Haensch, Berlin) it is possible to change the wavelength of the monochromatic yellow light to make it more and more yellow green, or more and more orange, while the red and green lights are shifted in a similar manner. Rayleigh equations can be found for these wavelengths. I have built up a system of nine equations with the yellow light varying from 574 m $\mu$  to 603 m $\mu$ .

When these nine equations are recorded for males it appears that some of them show a series of smaller values, while others show a series of larger values. There might be some overlapping in places, but on the whole the males can be divided into two groups, with two normal properties, which I have called protopia and deuteropia, denoted by P and D (both for the property and for the gene which is supposed to be responsible for the property). With the same anomaloscope it can be shown that the protopic individuals have their greenpoint (when they see green and not yellow or blue) at about 515 m $\mu$ ; the others have their greenpoint at 525 m $\mu$ .

Females can be divided into the same two groups, but about fifty out of a hundred of them show a third type of reaction. They behave as deuteropics in the orange region (the D dominates, but not completely), and as protopics in the green tinted region (the P dominates, but not completely).

I propose that these properties derive from hereditary factors in the X chromosome, from two mutant allelomorphs in one cistron. This would mean two genotypes in the male, P and D, with frequencies—which are also the gene frequencies in the population—of 60 per cent and 32 per cent to 8 per cent, which is the frequency of colour blindness in males, and there would be the two phenotypes, protopia and deuteropia. In females there would be three genotypes, PP, PD and DD, and three phenotypes, protopia, intermediary (supposed to be the heterozygotes P/D) and deuteropia. The heterozygous group has a greenpoint at about 520 m $\mu$ , with some overlapping with the other groups of greenpoints.

Because of the intermediate nature of the reactions of the supposed heterozygous group, diagnosis might not be so easy for females as for males. It is made more difficult—it is almost impossible to be accurate—in the group, expected to be about 15 per cent of all females, who are heterozygous for the genes for colour blindness. These females (eight (12) groups with P or D in the one X chromosome and one of the four (6) genes for colour blindness in the other X chromosome) show atypical reactions with respect to the nine equations, atypical reactions which suggest colour blindness.

Results of family examinations are given in Tables 1 and 2.

These two tables show agreement with the hypothesis discussed previously for the mode of inheritance. There was one exception: a P "father" and D "daughter". I cannot be sure that the determinations are correct, but the most likely explanation is that the P male is not the true father to this eldest daughter, although he could be the father to the younger daughter, who was PD. Even with this exception the two tables are convincing evidence of the validity of my hypothesis.

Table 1. THE P AND D DIAGNOSES FOR MOTHERS AND THEIR SONS

Mothers	Sons	
	P	D
20 P mothers	31	
27 PD mothers	26	21
8 D mothers		11

Table 2. THE P, PD AND D DIAGNOSES FOR GROUPS OF PARENTS AND THEIR DAUGHTERS

Parents	Daughters	
	P	D
21 P $\times$ PP	38	
15 P $\times$ PD	10	15
1 P $\times$ DD		15
8 D $\times$ PP		4
8 D $\times$ PD		8
6 D $\times$ DD		9

Based on these facts, I suggest that these hereditary factors have one locus in one cistron in the X chromosome, with two mutants for normal colour vision and four (6) for defective colour vision.

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## No Simple Pattern of Inheritance in Ability to smell Solutions of Cyanide

IN 1950, at the Cold Spring Harbor Symposium on the origin and evolution of man, Mourant commented: "It is most important that more new markers be sought and found for the human chromosomes." As an example of a possible new marker he remarked that: "One person out of four fails to smell hydrogen cyanide, a proportion suggesting a balanced polymorphism".

Since then the ability to detect the odour of hydrogen cyanide has been studied in populations in Europe<sup>2</sup>, Africa<sup>3</sup>, India<sup>4</sup>, Japan<sup>5</sup> and Australia<sup>6</sup> with some evidence of differences in the frequency of "non-smellers". Kirk and Stenhouse<sup>6</sup> and Fukumoto *et al.*<sup>5</sup> investigated families, and suggested that the trait "inability to smell hydrogen cyanide" was inherited as a sex linked recessive, but their families showed some exceptions to the expected results of that hypothesis<sup>7</sup>. Huser *et al.*<sup>2</sup>, by similar studies, showed that this trait is neither recessive nor sex linked, and they concluded that it is inherited as a dominant, probably autosomal.

Published reports on the detection of hydrogen cyanide odour are based on a variety of methods of detection and arrangements for presenting the gas to the subject. These variations have made exact comparison between the various reports impossible.

In an attempt to evaluate further the trait for detection of this odour, we have carried out a series of studies using a serial dilution technique similar to that of Harris and Kalmus<sup>8</sup> for phenylthiocarbamide taste testing except the solutions are smelled rather than tasted. A solution of 10 g of potassium cyanide in 100 ml. of distilled water was diluted by a factor of four in eleven serial steps. Of the resulting twelve solutions the strongest was a million times more concentrated than the weakest. This range of concentrations has been found to cover the sensitivities of most adult humans in our test system.

In the test procedure, the subject was presented with a rack of twelve 1 in. diameter, stoppered, test-tubes. Eleven contained 50 ml. of a serial dilution, and one identical tube contained distilled water. The subject was instructed to smell the water and then each of the eleven tubes in succession and report if the tube smelled like the water or smelled different. The tubes were presented to the subject in order from the most dilute to the most concentrated. The first tube which provoked a response difference from water was considered to be the threshold and its rank is used as the score in tabulation and calculation.

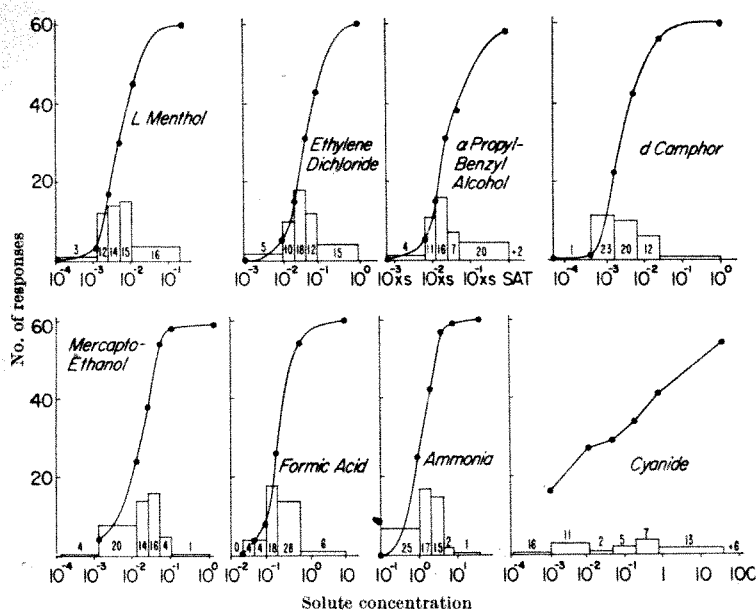


Fig. 1. The distribution of thresholds of detection of odour for water solutions of eight compounds in sixty unrelated adults.

A pretest of the system on sixty healthy adults showed (Fig. 1) that the distribution of the threshold of hydrogen cyanide was different from a wide variety of other compounds tested in a similar manner. Hydrogen cyanide had a much wider range of thresholds and the distribution was trimodal rather than unimodal. The unimodal distribution of "good smellers", "fair smellers" and "non-smellers" gave a perfect fit to a binomial distribution assuming an autosomal pair of additive alleles with the non-smell gene having a frequency of 0.316. There was no evidence for any difference between the sexes in the

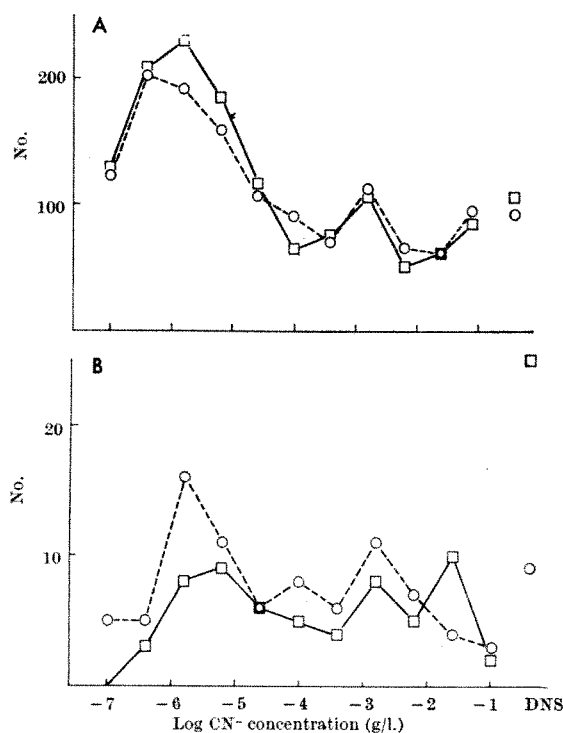


Fig. 2. (A) The distribution of perception threshold for hydrogen cyanide odour in 1,515 boys and 1,370 girls aged 10-18 yr. (B) The distribution of perception threshold for hydrogen cyanide odour in eighty-six pairs of parents. DNS does not detect a smell in 10 g/l. of potassium cyanide. □, Male; ○, female.

frequency of the phenotypes, which suggested that sex linkage was not involved.

The test system was then used to collect threshold data on 2,885 school children of age 10-18 yr, and all of European ancestry. Data were available for assigning them to sibships. The thresholds of the children were distributed similarly to the pretest series (Fig. 2A). There was a trimodal distribution with modes at solution concentrations of  $10^{-6}$ ,  $10^{-3}$  and  $10^{-1}$  of potassium cyanide/l. or more. There was no overall difference in the distribution of males and females as would be predicted on the basis of sex linkage. The apparent excess of males in both the end groups may be a function of the differences in age distribution of the sexes with respect to age in the school population studied because of a decrease in frequency of girls in the older age groups. There was also a significant difference in the distribution of thresholds with respect to age which may be compounded with the differences between the sexes of the modes of the sensitive group.

The population distribution could not be fitted as a genetic equilibrium, if there were only two alleles at one locus. Assuming that the three modal classes represent genotype groups, rather confused by other factors, a variety of autosomal additive models involving three alleles or two independent loci will fit it very well.

Eighty-six pairs of parents were also tested (Fig. 2B); their distribution was significantly different from that of the children. The fathers showed a decrease in frequency of "good smellers" and a large increase in the "non-smellers" group. The frequency of non-smeller mothers was about the square of the frequency of non-smeller fathers as would be predicted by the hypothesis of simple sex linked recessive inheritance. Such a hypothesis does not explain the "fair smeller" modal group and is not compatible with the distribution of the thresholds of the children.

Further evidence about the genetics of hydrogen cyanide thresholds can be obtained from the limited segregation data provided by eighty-six families (Table 1). These data indicate that there is no simple segregation pattern and that the children are distributed within families roughly in proportion to the population distribution rather than in relation to parental phenotypes.

The difficulty in finding compatible genetic hypotheses to explain both parent and child threshold distributions and the lack of expected genetic segregation patterns in the different classes of sibship raises a question about the existence of any genetic component in the control of hydrogen cyanide odour threshold. An examination of the correlations of thresholds between different types of related individuals showed little correlation even between twins of the same sex (Table 2). The only significant positive correlations were between like sex siblings and there was a significant negative correlation between

Table 1. SEGREGATION OF THE ABILITY TO SMELL HYDROGEN CYANIDE IN EIGHTY-SIX SIBSHIPS CONTAINING 156 CHILDREN

Parents		Children		
Father group	Mother group	Group 1	Group 2	Group 3
1	1	26	5	2
2	1	14	1	2
3	1	28	11	7
1	2	15	3	0
2	2	9	8	2
3	2	1	1	3
1	3	3	3	0
2	3	0	0	1
3	3	8	2	1

Group 1 detects less than  $16^{-4}$  g/l. of potassium cyanide; group 2 detects  $10^{-2}$  but not  $10^{-4}$  g/l. of potassium cyanide; and group 3 does not detect  $10^{-2}$  g/l. of potassium cyanide.

Table 2. CORRELATION OF HYDROGEN CYANIDE ODOUR THRESHOLD SCORES IN RANDOM CHILDREN, SIBLINGS AND PARENTS

	No.	Correlation
Random pairs of children	240	0.03
Consecutive siblings	264	0.07
Mother and daughter	72	0.05
Father and son	59	0.34
Same sex twins	20	0.30
Male sib pairs	209	0.20**
Female sib pairs	188	0.23**
Male-female sib pairs	210	-0.43**

\*\* Significant at 0.01

siblings of different sexes. These results are not compatible with a simple Mendelian hypothesis. The pattern of correlations was suggestive of a familial environmental factor affecting males differently from females. Possible environmental factors that are compatible with this type of distribution are tobacco smoking and contact with chemicals such as fertilizers and pesticides.

The existence of some genetic component similar to that in other complex physiological traits such as blood pressure or blood sugar concentration is suggested by the increase of the correlation coefficients from siblings through same sex siblings to same sex twins. This is, however, confounded with the high degree of correlation between members of the same sex in the same family.

The threshold for hydrogen cyanide odour does not seem to be a simple, segregating, genetically controlled trait. A similar conclusion has been independently reached by Kirk (personal communication). The genetic proportion of the control of the threshold seems to be small compared with the total variation. Investigation of the hydrogen cyanide odour threshold seems to be of limited use in genetic studies of populations or families, because of the important part that age and environmental factors play in its control.

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## CYTOLOGY

### Genesis of Hassall's Corpuscles

HASSALL's corpuscles, a characteristic structure of the thymus, are composed of acidophilic concentrically arranged cells. Only two hypotheses<sup>1,2</sup> for the genesis of these corpuscles account for the epithelioid character of their cells, their extensive branching<sup>3</sup>, the presence of a lumen at different stages of their development, and the presence of vascular elements within them. If the Hassall's corpuscles have a vascular origin<sup>4,5</sup>, this suggests that they are derived from the occlusion of capillary segments followed by inspissation of their contents, and necrosis of the vascular endothelium with proliferation of the neighbouring epithelium to form their typical "onion-like" structure. The other hypothesis<sup>3,6</sup> suggests that Hassall's corpuscles develop from persistent thymopharyngeal ducts lined with epithelium and that the frequent vascular elements are derived from the associated venules. Our evidence—obtained during an investigation of antigen localization in organs—supports the blood vessel origin of Hassall's corpuscles.

Polymerized flagellin labelled with iodine-125, a protein constituent of the flagella of *Salmonella adelaide*, was injected intravenously or into the hind foot-pads of 10-12 week old Wistar albino rats of both sexes in a quantity of 10 µg. Details of the procedures for both the preparation and iodination of polymerized flagellin are published elsewhere<sup>7</sup>.

The rats were X-irradiated at 235 kV, 15 m.amp, half value layer of 1.0 mm of copper, at a dose rate of 68 rads/min 1 day before the injection of polymerized flagellin.

To prepare autoradiographs, the thymus was fixed in 10 per cent formalin and histological sections, 5µ thick, were prepared by the paraffin method. Sections were dipped in Kodak 'NTB/2' emulsion, exposed for 60 days, developed and stained with methyl green and pyronin.

The animals were divided into three groups: (a) unirradiated; (b) sub-lethally irradiated with 450 r.; and (c) lethally irradiated with 950-16,000 r. Results of the examination of these autoradiographs of the thymus, for localization of polymerized flagellin, are shown in Table 1.

Table 1. LOCALIZATION OF LABELLED POLYMERIZED FLAGELLIN IN AUTORADIOGRAPHS OF THE RAT THYMUS

X-ray* dose	No. of † rats	Hours after injection of labelled polymerized flagellin	
		3	48-72
None	7	Label in wall and lumen of blood vessels in all rats	No label in any rat
450 r.	7	Label in wall and lumen of blood vessels in all rats	Occasional Hassall's corpuscle labelled in three rats
950-16,000 r.	11	Not done	Label in the wall and lumen of many blood vessels and Hassall's corpuscles in all rats.

\* Rats were irradiated 1 day before the injection of the labelled material.

† This refers to the number of rats in each sample.

Autoradiographs of the thymus from groups a and b, 3 h. after injection of polymerized flagellin, showed that the label was restricted to the wall and lumen of blood vessels (Fig. 1). Two or three days after injection of antigen there was no detectable label in any thymus from seven unirradiated rats that had received labelled polymerized flagellin either intravenously or into the hind foot-pad. In three of seven similarly injected rats that were irradiated with 450 r., label was confined to occasional Hassall's corpuscles (Fig. 2). In all eleven rats exposed to radiation between 950 and 16,000 r. and examined 1 or 2 days after injection of antigen, label was found in vessels ranging from those with a patent lumen through a series of intermediate stages to the developed Hassall's corpuscle (Fig. 3).

The suggestion that the Hassall's corpuscles are derived from vascular elements is supported by my observations on localization of protein labelled with iodine-125 and indirectly by the observations of vascular changes in irradiated animals<sup>8</sup>. The small vessels in a section of skin from rats exposed to large doses of X-ray were found to be fewer in number than in normal rats and showed varying degrees of vascular occlusion not normally seen. Thus the larger doses of radiation induced both a greater incidence and an increase in the rate of change in the endothelium that led to occlusion. These effects, in turn, would lead to the trapping of more antigen in more vessels. Treatment with agents, such as X-ray or cortisone<sup>2</sup>, which cause thymic involution increased the uptake of carbon particles by perivascular macrophages. A process such as described by the proponents for the blood-vessel origin of Hassall's corpuscles therefore might occur with greater frequency and speed after irradiation.

The proposal that Hassall's corpuscles arise from persistent thymo-pharyngeal ducts, with the accidental inclusion of endothelial elements<sup>3,7</sup>, cannot account for the presence of polymerized flagellin in these structures. It does not explain how this protein gets from the blood



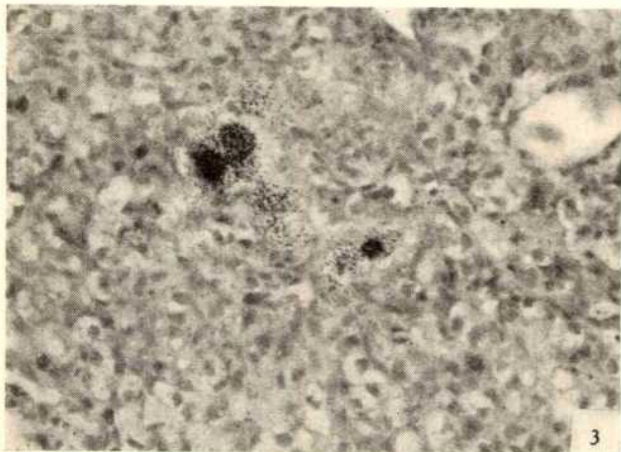
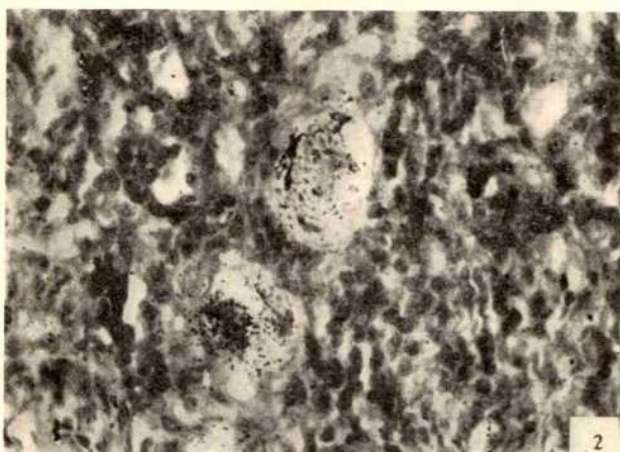
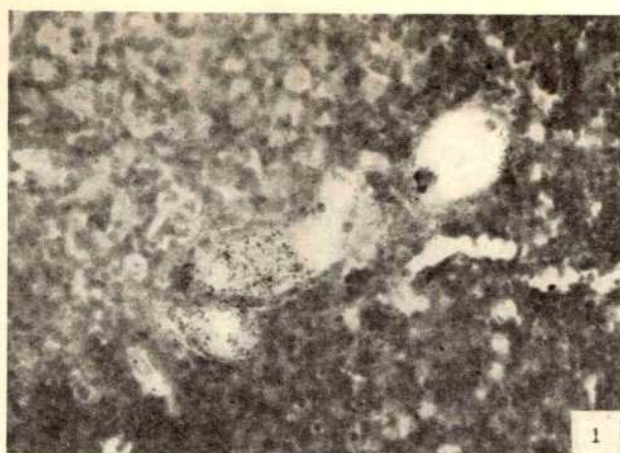


Fig. 1. Section of rat thymus 3 h after intravenous injection of polymerized flagellin labelled with iodine-125. Note the label associated with the patent blood vessel.

Fig. 2. Section of thymus taken 2 days after foot-pad injection of polymerized flagellin labelled with iodine-125 into a rat exposed to 450 r. Note the label directly over typical Hassall's corpuscles.

Fig. 3. Section of thymus taken 2 days after foot-pad injection of polymerized flagellin labelled with iodine-125 into a rat exposed to 8,000 r. Note that the label is associated with an occluded vessel which terminates in a typical Hassall's corpuscle.

vessels to the corpuscle. The ducts are not patent in adult life, and the polymerized flagellin is seen only in association with the walls of patent vessels (not lined with epithelium), in the Hassall's corpuscles, and in the intermediate stages (Figs. 2 and 3).

Hassall's corpuscles in the thymus could represent no more than a common end-product of diverse origin. Development around an occluded vascular segment in this case would represent only one way in which the

corpuscle develops. The present evidence and observations from thymuses of patients with systemic lupus erythematosus<sup>6</sup> strongly suggest a vascular origin for this structure.

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## BIOCHEMISTRY

### Steroids of Invertebrates: Production of Oestrogens by an Accessory Reproductive Tissue of the Slug *Arion ater rufus* (Linn.)

A PREVIOUS communication<sup>1</sup> described the endogenous production of 11-keto-testosterone, testosterone and 17 $\alpha$ -hydroxyprogesterone during the *in vitro* incubation of the eggs of *Arion ater*. The present study concerns the *in vitro* production of C<sub>18</sub> steroids from endogenous precursors by an accessory reproductive organ of this animal, the bursa copulatrix or spermatheca gland. The function of the spermatheca is believed to be storage of sperm acquired during copulation<sup>2</sup>.

Spermatheca glands containing sperm (1.62 g wet weight) were obtained from ten adult animals and incubated *in vitro* for 4 h; the conditions of incubation and methods adopted for steroid isolation and purification were similar to those described previously<sup>1</sup>. Steroid fractions obtained after repeated thin-layer purification on silica gel GF<sub>254</sub> were chemically modified by derivative formation, and the products submitted to gas chromatographic investigation. All gas chromatographic data were obtained with an 'F and M 400' biomedical gas chromatograph using a 2 per cent 'XE-60' column; eluted substances were detected by hydrogen flame ionization. Quantitative assessments of the steroids produced were based on their individual mass/peak area response graphs.

After *in vitro* incubation of the spermatheca gland tissue and (presumably) its sperm content, no detectable amounts of progesterone, pregnenolone, 17 $\alpha$ -hydroxyprogesterone, 17 $\alpha$ -hydroxypregnenolone, testosterone, androstenedione or dehydroepiandrosterone were revealed by gas chromatography. Two substances formed during the incubation, however, were shown to be identical with authentic oestrone and oestradiol-17 $\beta$ .

The presence of oestrone was tested as follows. Acetylation afforded a product the *R<sub>F</sub>* of which was identical with that of oestrone acetate; gas chromatography of the acetate alone or mixed with authentic oestrone acetate gave a single peak of the correct retention value; alkaline hydrolysis and sodium borohydride reduction yielded a substance which appeared as a homogeneous Turnbull blue positive spot, the *R<sub>F</sub>* of which was identical with that of authentic oestradiol-17 $\beta$ .



For oestradiol-17 $\beta$  the following procedure was adopted. The substance obtained after acetylation had an  $R_F$  value which corresponded with that of oestradiol-17 $\beta$  diacetate; individual or mixed gas chromatography with authentic oestradiol-17 $\beta$  diacetate gave retention data which suggested the identity of the two substances; alkaline hydrolysis and chromic acid oxidation furnished a product the similarity of which to authentic oestrone was indicated on the basis of a single Turnbull blue spot of the correct  $R_F$  value. Oestrone and oestradiol-17 $\beta$  were produced in amounts (uncorrected for losses incurred during processing) of 115 and 77  $\mu\text{g/h/g}$  of tissue, respectively.

We have found that the two naturally occurring mammalian  $C_{18}$  steroids, oestrone and oestradiol-17 $\beta$ , are also present in an accessory reproductive tissue of this invertebrate. Hitherto no  $C_{18}$  steroids have been chemically demonstrated as occurring in an invertebrate phylum. It is yet to be determined whether  $C_{18}$  steroids, which have clearly defined oestrogenic activity in vertebrates, also participate in invertebrate reproductive physiology. The fact that no precursors of oestrogenic steroids were detectable might be a result of one or all of the following alternatives. Spermatheca tissue may selectively concentrate oestrogens produced at other sites; the turnover of precursors may be very rapid so that end-products only are detectable; immediate precursors such as androstenedione and testosterone may be produced at other sites, concentrated selectively by the spermatheca, whereupon oestrogen synthesis occurs with rapid disappearance of the substrate.

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### Neutron Activation as a Means of Detecting Phosphoryl Peptides

AMINO-ACID sequences about "active-centre" residues, which react with organophosphorus inhibitors or which are the site of attachment of phosphate in phosphoryl-enzyme intermediates, have been a subject of interest in

recent years<sup>1</sup>. In these sequence studies inhibitor or reactant labelled with phosphorus-32 is generally used and the peptides, after degradation of the protein, are detected and distinguished from other ninhydrin-positive material by their radioactivity. Sanger and co-workers<sup>2-5</sup> have developed a method for the determination of sequence about a labelled residue in which the use of ninhydrin is eliminated and considerably less material is required. In their work the labelled protein is subjected to partial acid hydrolysis and the phosphoryl peptides are separated by high voltage electrophoresis on paper. The pattern of peptide bands detected by autoradiography is characteristic of the amino-acid sequence present in the protein.

This note reports a new procedure in which the protein is reacted with unlabelled reagent, the phosphorylated protein hydrolysed and the peptides separated by electrophoresis. The peptides are then subjected to neutron irradiation on paper, whereby the phosphorus is made radioactive and can thus be detected by autoradiography or scanning techniques. This procedure avoids any inconvenience of working with the radioactive form of the reagent, and yet utilizes the advantages of labelling with phosphorus-32 in comparing the pattern of phosphoryl peptides with that from known sequences.

In the test experiments chymotrypsin and subtilisin which had been inhibited by DFP (diisopropylphosphoryl-fluoridate) to yield the DIP (diisopropylphosphoryl)-enzymes were used, together with pepsin, a natural phosphoryl protein. Samples of the phosphoryl proteins were partially hydrolysed with acid (6 normal hydrochloric acid at 100°C for 30 min) and the resulting peptides separated by electrophoresis on Whatman 3 MM paper (pH 3.5, pyridine-acetic acid, 40 V/cm, 2h). After drying, marker spots of serially diluted *O*-phosphorylserine in the range 4.0–0.02  $\mu\text{g}$  phosphorus/cm<sup>2</sup> were applied to the paper. The electrophoretogram was rolled between aluminium foil and sent to the Australian Atomic Energy Commission Research Establishment, Lucas Heights, where it was irradiated with a neutron flux of  $3 \times 10^{12}$  neutrons/cm<sup>2</sup>/sec for 4 h. A longer time of irradiation was not recommended by the A.A.E.C. as the paper is liable to damage and may be difficult to unroll. After irradiation autoradiography was carried out using Kodak medical X-ray film.

Autoradiographs completed within 2 days of irradiation showed a uniformly dark background caused by activation of impurities in the paper. The main impurity was apparently sodium which had been activated to

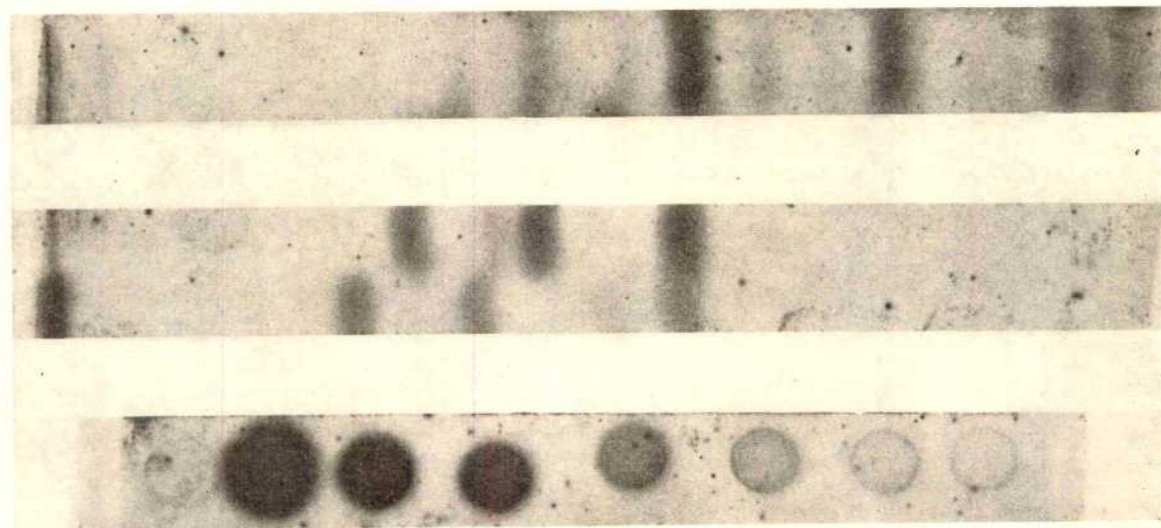


Fig. 1. Autoradiograph of sections of electrophoretogram after neutron irradiation. Top section: hydrolysate of DIP-chymotrypsin; the origin is on the left and the band on the extreme right is *O*-phosphorylserine. Middle section: comparison of hydrolysates of pepsin (top) and DIP-subtilisin. Bottom section: marker spots of *O*-phosphorylserine; the concentration of phosphorus from left to right is 4.0, 0.8, 0.4, 0.2, 0.1, 0.04 and 0.02  $\mu\text{g}$  phosphorus/cm<sup>2</sup>.

sodium-24, with a half-life of 15 h, so that 4 days after irradiation the sodium-24 had very largely decayed and, on an autoradiograph prepared after that time, bands were visible against a lighter background. Carbon, hydrogen, oxygen and nitrogen would not be expected to yield any significant amount of radioactive material on neutron bombardment under the conditions used, and sulphur did not give rise to sulphur-35 because no darkening on the autoradiograph was observed in the position of cysteic acid containing peptides derived from oxidized insulin. The radioactive bands were later cut out and counted in a scintillation counter at various times over 2 weeks; they showed a half-life of 14.3 days which is characteristic of phosphorus-32.

Fig. 1 shows an autoradiograph of sections of the electrophoretogram; the film and electrophoretogram were in contact for 10 days starting 6 days after the irradiation. The patterns of phosphoryl peptides obtained from DIP-chymotrypsin and DIP-subtilisin are the same as those previously observed using  $^{32}\text{P}$ -DFP and are characteristic of the sequences Gly.Asp.SerDIP.Gly<sup>2</sup> and Thr.SerDIP.Met.Ala<sup>3</sup> respectively. Pepsin is reported<sup>6</sup> to contain the sequence Thr.SerP.Glu, but the electrophoretic pattern of phosphoryl peptides obtained after partial acid hydrolysis has not been examined previously. Approximately 0.8 mg of the phosphoryl protein hydrolysates was applied over 2 cm for separation on the electrophoretogram and a concentration equivalent to 0.04  $\mu\text{g}$  phosphorus/cm<sup>2</sup> was clearly visible.

While in the example above peptides were produced by partial acid hydrolysis, the procedure could be applied to an enzyme hydrolysate of a suspected phosphoryl-enzyme intermediate isolated from an enzyme catalysed reaction. Study of such peptides would enable determination of the stability of the phosphoryl bond. If a metallo-enzyme is used in such experiments the metal may give rise to a radioisotope in the neutron flux, but such radioisotopes should be distinguishable from phosphorus-32 by their decay characteristics.

A disadvantage of this technique is that irradiation could cause destruction of the amino-acids and so, unlike the work starting with the radioactive form of a reagent, the peptides, once activated, could not be used for further determination of structure. Detection could be performed on marker strips, however, as is normal for staining techniques. Sensitivity of detection would be enhanced by the use of a chromatogram scanner, but even by autoradiography it is at least ten times better than molybdenic acid sprays<sup>7</sup> and is independent of the chemical form of the phosphate. The use of neutron activation in the characterization of small quantities of nucleic acids has been reported by Murray and Offord<sup>8</sup>.

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### Cardiac Monoamine Oxidase in Rat

THE monoamine oxidase (MAO) of the rat heart exhibits the unusual property of increasing in activity with increasing age and weight<sup>1</sup>. This property is not shared by the liver or brain; these organs reach a maximum MAO activity early in life and presumably maintain these levels

Table 1. DEVELOPMENT OF MAO ACTIVITY IN LIVER AND BRAIN OF MALE AND FEMALE RATS

Age in weeks	Liver		Brain	
	Male	Female	Male	Female
2	20.2 (17.5-23.0)	24.7 (23.7-24.9)	6.5 (5.8-7.2)	8.5 (8.1-8.8)
4	32.2 (30.0-33.0)	29.6 (28.0-31.0)	8.9 (8.0-9.9)	10.6 (9.9-11.4)
6	28.2 (26.0-31.0)	30.2 (29.0-32.0)	10.5 (9.9-11.2)	9.5 (8.7-10.2)
8	24.8 (24.0-26.0)	30.6 (27.4-35.0)	8.7 (6.6-11.2)	10.1 (8.7-10.8)
10	26.2 (21.0-29.0)	25.8 (23.7-28.5)	9.9 (8.7-10.6)	10.0 (9.4-10.8)
13	36.2 (34.0-40.0)	31.0 (28.0-33.0)	10.1 (9.9-10.5)	10.1 (9.3-10.8)
16	28.0 (25.8-30.0)	26.0 (24.0-29.0)	9.2 (7.7-11.1)	9.0 (7.8-11.3)
19	27.4 (24.0-31.0)	30.5 (27.9-33.6)	9.1 (8.3-9.7)	9.4 (9.1-9.8)
22	29.1 (26.6-30.0)	30.0 (28.5-31.6)	9.4 (8.9-9.7)	8.7 (7.6-9.2)

Results are expressed as the mean values ( $\mu\text{moles}$  serotonin metabolized per gram in one hour) obtained from tissues of five animals. Figures in parentheses indicate the ranges of MAO activity.

for the rest of the animal's life span. This communication presents further information about the properties of MAO in the rat heart and its comparison with other tissues and species of animals.

Novick<sup>1</sup> first observed the relationship between the weight of the rat and cardiac MAO activity. There exists, however, a difference in the growth rate between the male and female rat, and this might affect the levels of cardiac MAO. A study was therefore made to determine the possible sex difference of MAO in the heart, liver and brain of the rat. Sprague-Dawley rats from ages 2 weeks to 22 weeks were employed in this study. MAO activity was measured by determining the rate of breakdown of 5-hydroxytryptamine (5HT) by tissue homogenates or mitochondria. The 5HT was assayed employing the nitroso-naphthol reaction as described by Udenfriend *et al.*<sup>2</sup>.

Table 1 demonstrates the MAO activity of the liver and brain of male and female rats during ages 2-22 weeks. Maximum MAO activity of both organs was evident by 6 weeks. Some degree of sex difference appeared to be present in liver MAO activity during the first 8 weeks of development, but its significance is questionable because of the variation from week to week. The heart exhibits a completely different picture in that at 2 weeks essentially no activity is present, but thereafter MAO progressively increases during the following 16-20 weeks (Fig. 1a). A plateauing appears at about the twentieth to twenty-fourth week in the male. At this time the MAO activity is equal to or exceeds that of the liver. The female exhibits a similar age-dependent relationship of cardiac MAO activity, but within 4-8 weeks shows a slower rate of increase and maintains a lower activity throughout the experimental period than that of the male.

If the cardiac MAO activities of the male and female rats are plotted against their weights as in Fig. 1b, one finds a greater degree of linearity than when related against age. Male rat heart MAO activity progressively increases up to 500-550 g while in the female the line stops at approximately the 250-300 g level, because further weight gain does not occur with increasing ages up to 30 weeks. It would appear that on a weight basis there is no difference between the male and female rat heart MAO, but on the basis of age there is a considerable difference.

The fact that the rat heart exhibits this progressively increasing MAO activity with age while the brain and liver does not led us to determine whether other organs and tissues behave in this manner. Assays of the intestines, kidney, spleen, aorta and skeletal muscle for MAO activity from rats of varying ages indicated that these structures acted more like the brain and the liver in that the maximum activity was already present in male rats weighing 100-150 g (8-12 weeks). This would lead us to believe that the rat heart was specific in this property. Further investigations have also disclosed that the age and weight dependent increase of cardiac MAO occurred not only in the heart, but also that MAO was the only enzyme that showed this relationship. Succinic dehydrogenase and cytochrome oxidase, both mitochondrial enzymes and abundant in heart tissue, did not



follow the age dependent relationship of MAO. Aldehyde dehydrogenase, a soluble fraction enzyme which is responsible for the second step of the MAO system (that of converting the aldehyde formed from the initial deamination of a substrate amine to the corresponding carboxylic acid), also did not follow the MAO activity curve with increasing age. Finally, determination of total protein content (according to the method of Lowry *et al.*<sup>3</sup>) of heart mitochondria from rats of varying ages showed no significant difference, again failing to follow the increase in cardiac MAO activity of the male rat.

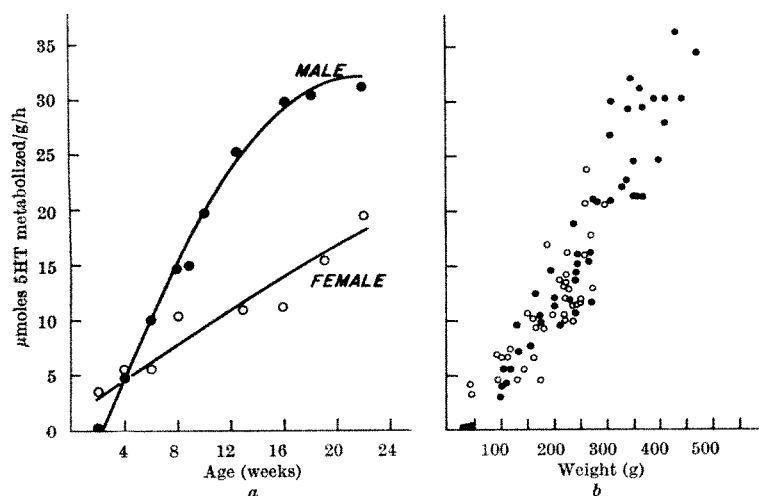


Fig. 1. *a*, Graph representing the development of monoamine oxidase in male and female rat heart during ages 2–24 weeks. Each point represents the mean values from four to five animals. *b*, Relationship between cardiac monoamine oxidase activity and weight gain in male (black circles) and female (open circles) rats. Each point represents data from individual animals.

The MAO activity of hearts of several species of animals has also been examined at different age and weight levels, and it appears that of those animals investigated (mouse, guinea-pig, rat, rabbit and cat) the rat is the only species possessing this peculiar quality. This was also demonstrated histochemically by Studer<sup>4</sup>.

These experiments were carried out to establish and extend the observations of Novick. The results emphasize the presence of a sexual dimorphism in rats to the increase of MAO activity with age and it appears to be related to the rate of growth of the animals. The process of MAO increase as described herein is also specific for the heart and is species specific for the rat. Other enzymes in the rat heart do not follow the same relationship as MAO. Gey *et al.*<sup>5</sup> also noted that other enzymes involved in the metabolism of catecholamines, such as catechol-O-methyl transferase and dihydroxyphenyl-alanine-ketoglutarate transaminase, did not change with age.

The importance of changes in MAO activity of rat heart with age and sex differences is obvious in pharmacological studies which employ sympathomimetic amines which are substrates of MAO.

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## Tryptophan Residue in Aspergillopeptidase A

AMINO-ACID analysis has shown that the acid proteinase of *Aspergillus saitoi* (aspergillopeptidase A, EC, 3.4.4.17) contains one tryptophan and about twenty tyrosine residues in a molecule (average molecular weight, 34,550) (refs. 1–4). In this communication we describe the effect of chemical modification of aspergillopeptidase A and discuss the role of the single tryptophan residue in the molecule.

Aspergillopeptidase A, purified as before<sup>1</sup>, was removed on 'Sephadex G-100' at pH 4.0 to separate minor autodigestive products, and then lyophilized. Proteinase was estimated and amino-acid was determined, also as before<sup>1,2</sup>. Tryptophan was determined by the *p*-dimethylaminobenzaldehyde method<sup>3</sup>.

The concentration of aspergillopeptidase A is inversely proportional to the amount of *N*-bromosuccinimide<sup>4</sup> added at pH 4.1, and the enzyme is completely destroyed if about 40 moles of the compound is present for each molecule of enzyme at 0° C for 20 min. When the oxidized enzyme was fractionated with 'Sephadex G-100' the tryptophan, tyrosine and histidine residues were modified, but the other residues remained almost unchanged within the limit of experimental error (Table 1).

Aspergillopeptidase A was inactivated by photo-oxidation in the absence of sensitizers at pH 5.5. The enzyme solution was placed 20 cm from the centre of a Toshiba 'SHL-100' ultra-violet mercury lamp<sup>7</sup> at 11° C for 20 h. Amino-acid analysis of the modified and fractionated enzyme showed that only tryptophan and tyrosine residues had changed, even when inactivation had reached 60 per cent (Table 1).

Table 1. AMINO-ACID MODIFICATION WITH *N*-BROMOSUCCINIMIDE OR PHOTO-OXIDATION

Experimental condition	Amino-acid residues/molecule of aspergillopeptidase A				Activity presented (per cent)
	Histidine	Lysine	Tyrosine	Tryptophan	
Native	2.5	10.7	17.7	1.0	100
Oxidation with <i>N</i> -bromosuccinimide	1.8	10.0	7.4	0.4	14
Native	2.6	11.4	17.8	1.0	100
Photo-oxidation	2.5	10.7	15.6	0.4	40

Acetylation of the proteinase was performed at 0° C by adding excess of 100 molar *N*-acetylhydrazole<sup>10,11</sup> to a solution of  $2 \times 10^{-5}$  molar aspergillopeptidase A in 0.033 molar phosphate buffer, pH 7.0, containing 2 molar sodium chloride. The number of tyrosine residues acetylated in the modified enzyme molecule was 3.9 moles, calculated using a molar extinction coefficient of 1,160 at 278 mμ<sup>10</sup>. This value was obtained even when inactivation had reached 90 per cent of the control. We assume that the loss of the proteolytic activity is attributed to the modification of tyrosine residues.

A 50 molar excess of 2-hydroxy-5-nitrobenzylbromide<sup>8,9</sup> dissolved in 0.5 ml. of methyl alcohol was stirred with 4.5 ml. of native or denatured with 7 molar urea aspergillopeptidase A solution at 0°–2° C in acetate buffer, pH 4.1. After 75 min a sample of the treated enzyme solutions was passed through a column of 'Sephadex G-25' (1.8 cm × 72 cm) in 0.01 molar ammonium formate buffer, pH 4.1, to remove the uncombined 2-hydroxy-5-nitrobenzylbromide. The eluted solutions were dialysed and lyophilized. The number of tryptophan residues reacting with the 2-hydroxy-5-nitrobenzylbromide was calculated using a molar extinction coefficient of 18,900 (ref. 8) at 412 mμ in 0.1 normal potassium hydroxide. In the absence of urea, there was no reaction and the treatment had no

effect on the enzyme activity. On the contrary, calculation of the molar ratio of 2-hydroxy-5-nitrobenzylbromide to aspergillopeptidase A modified with 7 molar urea gave a value of 1.2. These findings agree with the previous conclusion that the single tryptophan residue is buried or strongly bound in the hydrophobic regions<sup>4</sup>. It therefore seems that the single tryptophan residue in aspergillopeptidase A is not at the active site of the enzyme, and that it has the function of stabilizing the conformation of the enzyme molecule.

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### Kinetics of Acetylsalicylic Acid Disposition in Man

ALTHOUGH acetylsalicylic acid (ASA) has been in extensive clinical use since 1900, there is surprisingly little known with accuracy about the kinetics of absorption, metabolism, distribution and excretion of this drug in man and animals. Also, much of the present information has been indirectly estimated from the studies of the metabolite salicylic acid (SA). This has been a result of the lack of specific and sensitive methods for the determination of ASA in biological fluids. Further, while ASA has been shown to be present in the blood after an oral dose<sup>1-7</sup>, only the data of Leonards<sup>8</sup> allow an assessment of the elimination half-life of ASA in man (17 min). The latter data were obtained utilizing a method which relies on measuring ASA as the difference between free and total salicylate, however. We therefore decided to investigate in more detail the pharmacokinetics of ASA in man.

The study was conducted in four male subjects who each received an intravenous dose of ASA (650 mg) as the *N*-methylglucamine salt. Plasma levels of ASA were measured specifically using a gas-liquid chromatographic method and free SA was determined spectrophotofluorometrically.

The results obtained showed that the plasma concentration time curve of ASA can be described by a biexponential equation (see Fig. 1), with a very rapid half-life for both exponents (Table 1). The elimination half-life of ASA is 13–19.5 min, and therefore nearly all the ASA is eliminated from the body within 2 h of an intravenous dose. Also, with one subject who received various intravenous doses of ASA, there was a direct proportionality between the area under the ASA curve and dose, while the elimination half-life remained constant.

Oral administration of the same dose of ASA (650 mg), in solution, to these subjects resulted in a peak ASA plasma level occurring 15–25 min after the dose, indicating that the absorption is predominantly from the stomach. In some cases the half-life of the descending part of the ASA curve was longer than that obtained with the intravenous dose (Table 1). This, we think, is because absorption of ASA still occurs during the decline of the ASA plasma levels.

Table 1. TABLE SHOWING THE HALF-LIVES AND THE AREA UNDER THE ACETYSALICYLIC ACID CONCENTRATION TIME CURVES AFTER INTRAVENOUS AND ORAL ADMINISTRATION OF 650 MG ACETYSALICYLIC ACID

Subject	Route	Half-life (min)		Area ( $\mu\text{g/ml. min}$ )	Ratio of areas $\times 100$
		$\alpha^*$	$\beta^*$		
A	Intravenously	2.7	14.5	948	72.5
	Oral		15.0	687	
B	Intravenously	2.2	14.0	904	74.4
	Oral		17.0	673	
C	Intravenously	4.0	19.5	923	71.3
	Oral		19.5	658	
D	Intravenously	3.0	14.0	956	64.8
	Oral		18.0	620	

\*  $\alpha$  and  $\beta$  are the half-lives of the first and second exponent of the acetylsalicylic acid.

Without exception, however, the area under the ASA concentration time curve was lower (65–74 per cent) than that obtained after the intravenous dose (Table 1). In contrast, the SA plasma level after 1 h (when most of the ASA had been converted to SA) was the same after an oral or intravenous dose of ASA (Fig. 1). The latter finding shows that while ASA is totally available after an oral dose, as measured by SA plasma levels, only a part of the intact drug reaches the tissues and circulating fluids. Thus during the absorption of ASA, appreciable hydrolysis to SA must occur either in the gastrointestinal fluids, across the gut wall or during the first passage of this drug through the liver. It may be recalled that after an oral dose, all the drug passes through the liver via the hepatic portal vein; however, after an intravenous dose, the drug-containing blood goes to the heart and only 30 per cent of the cardiac output reaches the liver.

The influence of the gastrointestinal fluids on the hydrolysis of ASA is probably very small, as we showed that the drug was essentially stable in gastric and duodenal juices. On the other hand, a preliminary experiment in a dog indicated that the liver clearance of ASA is important. Thus, when ASA was infused into the dog via the jugular vein, hepatic portal vein, and given orally, the relative areas under the ASA curves were 100, 60 and 40 per cent, respectively. This result also suggests that esterases may be present in the gut wall. In addition, these data place some doubt on the use of the SA levels to assess the availability and absorption rate of ASA.

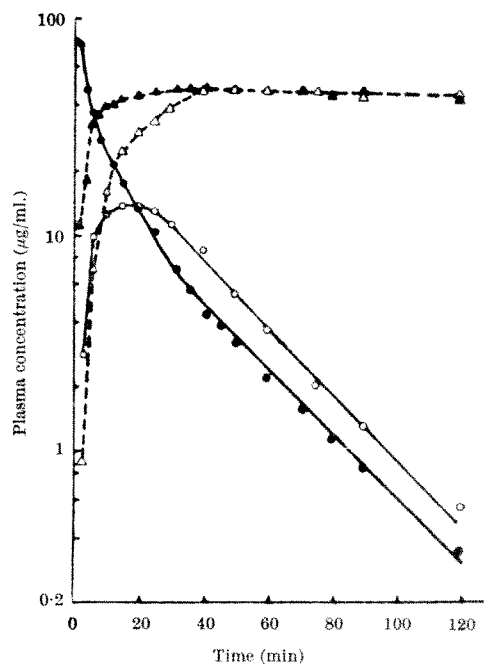


Fig. 1. Acetylsalicylic acid and salicylic acid plasma levels after intravenous and oral administration of 650 mg acetylsalicylic acid. Subject C. Intravenous study: ●—● acetylsalicylic acid; ▲—▲ salicylic acid. Oral study: ○—○ acetylsalicylic acid; △—△ salicylic acid.



Evidence to date shows that ASA is a more potent analgesic agent than SA (refs. 8 and 9) and thus it is important to have a knowledge of those factors that influence the availability of ASA, the route of administration being one. The formulation of the oral dosage form may be another factor.

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### Ribonucleases of Chronic Granulocytic Leukaemia Leucocytes

It has already been found that the ribonuclease activities of serum and urine of patients with chronic granulocytic leukaemia show characteristic variations related to this disease<sup>1</sup>. The behaviour of this enzyme in leukaemic leucocytes, however, has not been thoroughly studied. Recently, Ressler *et al.*<sup>2</sup> have demonstrated the presence of two electrophoretically separable ribonuclease fractions in leukaemic leucocytes. The present work is connected with the isolation and preliminary characterization of ribonucleases from chronic granulocytic leukaemia leucocytes. Some data concerning purification procedure have been presented earlier<sup>3</sup>.

A leucocytic mass containing about 75 per cent of mature granulocytes was used as the starting material for the isolation procedure. This mass (100 ml. in volume) was homogenized with 10 volumes of water and centrifuged for 10 min at 3,000*g*; from the supernatant inactive protein was precipitated by addition of solid sodium chloride to a concentration of 6 per cent (w/v). The precipitate was separated by centrifugation and rejected, and the supernatant was dialysed and applied to a column of 'CM-Sephadex C-50'.

The eluted material contained two overlapping ribonuclease peaks, as demonstrated by spectrophotometric determination of ribonuclease activity<sup>4</sup> in collected fractions. The active fractions corresponding to the individual peaks were pooled, lyophilized, and rechromatographed on 'Sephadex G-75' column. As a result, three active peaks, designated A, B and C, were obtained.

Repeated chromatography of the three peaks separately, and of pooled fractions corresponding to all three peaks, on 'CM Sephadex C-50' and 'Sephadex G-75', resulted in no increase in the number of peaks obtained. It was concluded from these experiments that the three ribonuclease fractions A, B and C are present in this material.

Table 1. SUMMARY OF ISOLATION PROCEDURE OF RIBONUCLEASES FROM LEUKAEMIC LEUCOCYTES

	Total activity (μg)	Purification ratio	Recovery (per cent)
Crude homogenate	7,500	1	100
Extract 6 per cent NaCl	7,300	1.7	97
First 'CM Sephadex' chromatography			
Ribonuclease I	2,040	26	27
Ribonuclease II	3,060	35	40
First 'Sephadex G-75' chromatography			
Ribonuclease A	820	45	11
*Ribonuclease B	1,750	94	23
*Ribonuclease C	1,520	109	20
Second 'CM Sephadex' chromatography			
*Ribonuclease A	464	56	6
*Ribonuclease B	1,115	180	15
*Ribonuclease C	975	207	13
Second 'Sephadex G-75' chromatography			
*Ribonuclease A	213	50	3
*Ribonuclease B	900	221	12
*Ribonuclease C	512	214	7

\* Only from peak fractions.

It was found that none of the three fractions showed any activity towards *bis-p*-nitrophenylphosphate nor do they depolymerize DNA. No effect was observed with lithium, sodium and potassium ions. Copper, zinc and manganese ions, however, produce some decrease in activity.

Ribonuclease present in fraction A shows an apparent molecular weight of about 24,000, as determined with a 'Sephadex G-75' column. It has an optimum pH of 5.8 and, in contrast to bovine pancreatic ribonuclease, is clearly unstable at a temperature of 95°C and a pH of 2.5.

Ribonucleases from fractions B and C (with molecular weights of 19,000 and 15,500 respectively) are both more

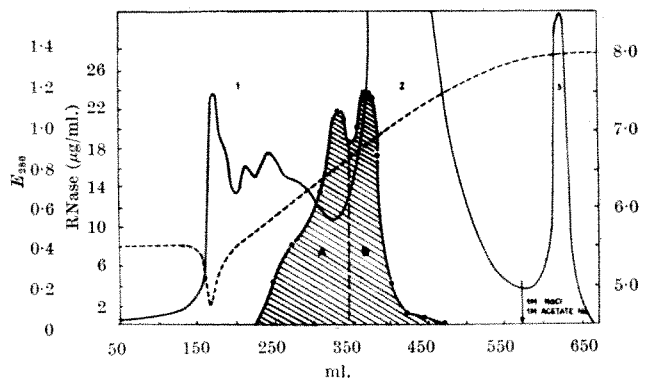


Fig. 1. Chromatography of proteins from 6 per cent sodium chloride extract on 'CM Sephadex C-50' column (2.2 × 25 cm), equilibrated with 0.05 molar acetate buffer pH 5.6. Gradient elution was performed by increasing the acetate concentration to 1 molar and increasing the pH to 7.8. Hatched area, ribonuclease activity.

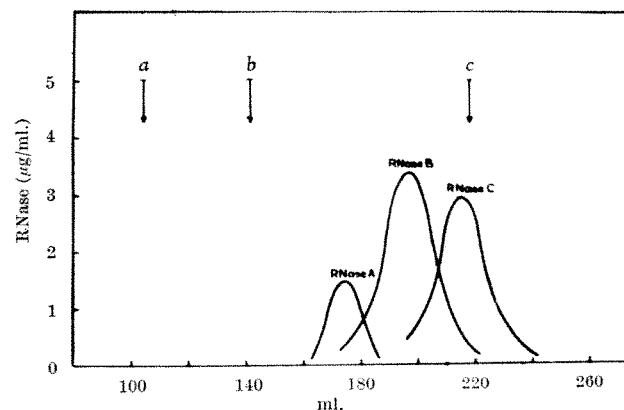


Fig. 2. Elution of RNases from human leukaemic leucocytes on 'Sephadex G-75' (3.5 × 65 cm) column. The arrows show elution from this same column of γ globulin (a), pepsin (b), and bovine pancreatic RNase (c).

thermostable than fraction *A* of the enzyme, but still less so than bovine pancreatic RNase. The optimum pH is 6.8 for fraction *B* and 7.2 for fraction *C*.

Separation of the products released from yeast RNA by the action of isolated ribonuclease fractions revealed that RNase *A* differs markedly from RNases *B* and *C*, which are more or less similar. RNase *A* produced only a few products of low electrophoretic and chromatographic mobility, whereas RNases *B* and *C* formed one relatively immobile core with a number of smaller products showing much higher mobility.

The results obtained demonstrated the existence of at least two leucocytic ribonuclease fractions, one acidic and thermolabile, and the second neutral and thermostable.

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### Metabolism of Orally Administered Dextran and 'Sephadex' Derivatives in the Rat

DEAE 'Sephadex A-25' has oral hypocholesterolaemic activity in cockerels and dogs<sup>1</sup>. This material is thought to interrupt the enterohepatic circulation of bile acids by binding them in the intestinal lumen and enhancing their excretion in the faeces. DEAE 'Sephadex A-25' is a cross-linked tertiary amine derivative of dextran with an average molecular weight of 25,000 and is insoluble in water and salt solutions. As such it would not be expected to be absorbed intact from the gastrointestinal tract. The possibility that the polymer might be hydrolysed by intestinal enzymes and the hydrolysis products absorbed has not, however, heretofore been investigated.

Chemical methods are not readily available for detecting hydrolysis products of DEAE 'Sephadex', or of the intact polymer, in biological fluids or tissues. It has, however, been shown that oral administration of dextran leads to an early increase in blood sugar and liver glycogen in rats and man<sup>2</sup>. This is presumably the result of hydrolysis of dextran to glucose by intestinal enzymes<sup>3</sup>. To determine whether or not DEAE 'Sephadex' is metabolized in a similar manner, the hyperglycaemic and glycogenic effects of dextran and DEAE 'Sephadex', together with other dextran derivatives, were compared in fasting rats. The extent of hydrolysis of these materials by homogenates of small intestinal mucosa was also determined.

Male Sprague-Dawley rats, 243–325 g, which had been maintained on a semisynthetic diet, were fasted for 24 h. Animals then received by stomach tube 500 mg of the material to be tested, dissolved or suspended in 5 ml. of distilled water. 4 h later the animals were anaesthetized with an intraperitoneal injection of 'Cyclopal' [5-allyl-5(2-cyclopenten-1-yl)barbituric acid] and blood samples were taken from the abdominal aorta in heparinized syringes for analysis of glucose. Livers were excised, rinsed in saline, blotted dry and weighed. Samples of about 500 mg were dropped immediately into glass centrifuge tubes containing 1 ml. of 30 per cent potassium hydroxide. The tubes were heated in boiling water for 30 min. 3 ml. of distilled water and 5.5 ml. of 95 per cent ethanol were added and the tubes were placed

Table 1. HYPERGLYCAEMIC AND GLYCOGENIC EFFECTS OF DEXTRAN AND 'SEPHADEX' DERIVATIVES IN FASTED RATS

Material administered	Blood glucose (mg/100 ml.)	Liver glycogen (mg/g)
Distilled water	85 ± 2.7	1.0 ± 0.33
Glucose	104 ± 2.7	6.8 ± 0.61
Dextran 60-C*	101 ± 1.8	6.2 ± 1.47
DEAE dextran†	91 ± 6.3	5.6 ± 1.46
'Sephadex G-25'	87 ± 3.6	4.7 ± 1.97
DEAE 'Sephadex A-25'	81 ± 4.6	0.6 ± 0.37

Glucose was determined in whole blood by the potassium ferrioxalate-potassium ferrocyanide method of Hoffman<sup>4</sup> adapted to the 'Technicon' auto-analyser. Although this method is sensitive to other blood reducing substances, normal concentrations of these substances are relatively insignificant compared with glucose levels. The results represent means ± standard error of the mean, ten animals in each group.

\* Sigma Chemical Company, average molecular weight 75,000.

† Pharmacia Fine Chemicals, Inc., average molecular weight 2 × 10<sup>6</sup>.

Table 2. HYDROLYSIS OF DEXTRAN AND 'SEPHADEX' DERIVATIVES BY HOMOGENATES OF SMALL INTESTINAL MUCOSA

Substrate	Hydrolytic activity (μmoles maltose equiv./60 min)
Dextran 60-C	8.66
DEAE dextran	2.44
'Sephadex G-25'	0.14
DEAE 'Sephadex A-25'	0.06

Twenty mg of substrate dissolved or suspended in 1 ml. of 0.1 molar maleate buffer (pH 6.0) and 1 ml. of homogenate were incubated for 60 min at 37° C. Hydrolytic activities were corrected for reducing substances present in the homogenate. The results are the means of duplicate samples.

again in boiling water to precipitate the glycogen. The tubes were cooled in tap water for 10 min and centrifuged. Supernatant fluid was decanted and the glycogen was dissolved in distilled water. Glycogen was determined by the anthrone method of Seifter *et al.*<sup>4</sup> using a glucose standard.

Intestinal mucosa homogenates were prepared from rats fasted for 24 h. The animals were killed by a blow on the head and the proximal two-thirds of the small intestine was excised, rinsed in cold saline and slit longitudinally on an iced glass plate. Mucosa was scraped off with the edge of a microscope slide and homogenized in 4 ml. of cold saline per g tissue in a 'Lourdes' tissue homogenizer at full speed for 1 min. The homogenate was centrifuged for 10 min at 2,000g at 0° C. The supernatant fraction was used to assay for hydrolytic activity according to the procedures of Dahlqvist<sup>3,5</sup> for determining amylase and dextranase activity. Hydrolytic activity is expressed as the increase in reducing power equivalent to 1 μmole of maltose for 60 min at 37° C.

Blood sugar concentration in rats dosed with glucose and dextran was significantly increased after 4 h (Table 1). In animals dosed with DEAE dextran, 'Sephadex G-25', or DEAE 'Sephadex A-25' blood sugar was not significantly different from controls given distilled water. Liver glycogen increased between five and seven times in rats given glucose, dextran, DEAE dextran, or 'Sephadex G-25'. DEAE 'Sephadex A-25', on the other hand, produced no noticeable change in liver glycogen. An apparent discrepancy exists because DEAE dextran and 'Sephadex G-25' were markedly glycogenic but did not increase blood sugar. On the other hand, in a previous study<sup>2</sup> maximum elevation of blood sugar occurred in rats 0.5 to 2 h after dosing with 450 mg of dextran, and blood sugar concentration had returned to control values after 4 h. It is therefore probable that blood sugar was elevated by DEAE dextran and 'Sephadex G-25' also, but that this was not detected when samples were taken after 4 h.

The results in Table 2 show that DEAE dextran is extensively hydrolysed *in vitro* by an enzyme preparation previously shown to hydrolyse dextran itself<sup>3</sup>. 'Sephadex G-25', an insoluble cross-linked dextran, is also hydrolysed, although to a much smaller extent, while DEAE 'Sephadex A-25' is not affected significantly.

These experiments indicate that DEAE 'Sephadex A-25' is resistant to degradation in the intestinal lumen. It seems unlikely therefore that any appreciable amount

of this material could be absorbed after oral administration.

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### Free Amino-acids in Human Sweat from Different Parts of the Body

THE presence of free amino-acids in human sweat has been known since 1910 when Embden and Tachau<sup>1</sup> isolated serine from this secretion. In 1946, Hier, Cornbleet and Bergeim<sup>2</sup> identified arginine, histidine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophane, tyrosine and valine in human sweat; they were also able to demonstrate that the amount of amino-acids secreted in sweat is independent of the diet and of variations in amino-acid levels in the blood; Rothman and Sullivan<sup>3</sup> found two additional amino-acids to be present in sweat, ornithine and citrulline. This observation, together with the high urea level of sweat<sup>4</sup>, led to the hypothesis that urea is formed in the sweat glands<sup>2,3</sup>. Hamilton<sup>5</sup>, and Oró and Skewes<sup>6</sup>, using ultra-micro methods, found the relative concentrations of amino-acids on the surface of the fingers of ten subjects to be essentially constant. Other authors<sup>7</sup> who sampled sweat from the whole body surface, rather than from a single area, reported greater individual differences.

For the study of regional differences, sweat was collected from the hands and (or) backs of forty-two children and adults of both sexes, aged from 2 months to 62 yr. Among them twenty-eight individuals each furnished a sample from both sites.

For the collection of sweat, the following procedure was adopted: the skin of the hands and back was washed with alcohol, ether and warm distilled water and plastic bags were fixed with adhesive tape to the wrists (enclosing the hands) and backs (in the interscapular region between the seventh cervical and the twelfth thoracic vertebra). Strips of weighed filter paper were introduced into the bags and removed after 5 h; 0.5–2 g of sweat was normally obtained. The amino-acids were eluted from the paper with distilled water and the eluate was evaporated to dryness. The dry material was dissolved in a measured volume of buffer solution and samples containing approximately 20  $\mu$ moles of ninhydrin positive substances were used for amino-

acid analysis by the method of Spackman, Stein and Moore<sup>8</sup>. With the method of sweat collection used, it is difficult to eliminate completely evaporation of water during the sampling period. The results were therefore expressed as relative concentrations; threonine, which was found in almost equal amounts in sweat from both sites, was used as the reference substance (Fig. 1 and Table 1).

Very significant differences between sweat from hands and backs were found for ornithine and citrulline, the relative concentration of ornithine being approximately eight times higher in sweat from the hands than in sweat from the back and that of citrulline approximately five times higher in sweat from the back than in sweat from hands. Arginine and urea, like citrulline, were significantly higher in sweat from backs than in sweat from hands. Of the other seventeen amino-acids, the threonine quotients of valine, isoleucine, leucine, tyrosine and proline were significantly higher in sweat from hands than in sweat from backs, but these differences were small compared with the differences mentioned here in ornithine citrulline, arginine and urea. No differences were found for threonine, lysine, histidine, tryptophane, aspartic

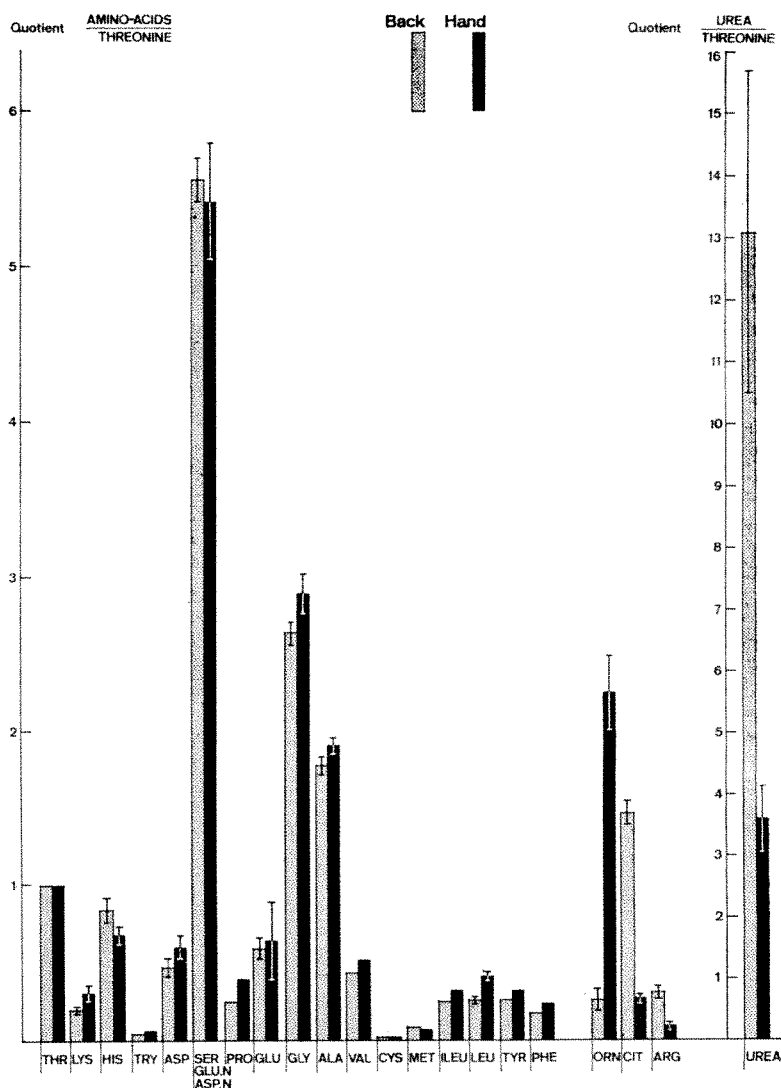


Fig. 1. Threonine quotient ( $\mu$ moles amino-acid or urea/ $\mu$ moles threonine) for twenty amino-acids and urea in sweat from children and adults of series I (Table 1). Black columns: sweat from hands; white columns: sweat from backs. The height of the columns indicates the mean values. One standard deviation (S.D.) is graphically represented for most of the amino-acids; where this is not the case, S.D. was  $< 0.02$ . The relatively high S.D. for urea might partially be caused by the small extinction coefficient of the ninhydrin coloration<sup>8</sup>.

acid, serine-glutamine-asparagine (no separate determinations), glutamic acid, glycine, alanine, cystine, methionine and phenylalanine. The means, ranges and standard deviations of values for ornithine, citrulline, arginine and urea are given in Table 1.

For the study of amino-acid patterns in sweat from patients with anomalies of amino-acid metabolism, sweat was collected from two adult male individuals with cystinuria, one girl aged 4 with cystine storage disease and Fanconi syndrome, one boy aged 1 with the non-ketotic type of hyperglycinaemia, one boy aged 2 with phenylketonuria and one boy aged 10 months with oculocerebro-renal syndrome (Lowe syndrome). When the values for the amino-acids in the sweat of these patients were compared with those from healthy controls, no significant differences in the relative concentrations were observed, but all showed the characteristic differences between sweat from hands and backs. These values were therefore included in the study of regional differences.

Table 1. THREONINE QUOTIENTS ( $\mu$ MOLES AMINO-ACID OR UREA/ $\mu$ MOLES THREONINE) FOR ORNITHINE, CITRULLINE, ARGININE AND UREA IN SWEAT FROM CHILDREN AND ADULTS

			Threonine quotient			UREA
			ORN THR	CIT THR	ARG THR	THR
Hand	Series I n = 12	Mean	2.26 ± 0.26	0.26 ± 0.03	0.09 ± 0.02	3.59 ± 0.54
		Range	1.54 - 4.79	0.13 - 0.44	0.03 - 0.29	1.82 - 7.07
	Series II n = 20	Mean	1.74 ± 0.12	0.19 ± 0.03	0.13 ± 0.02	—
		Range	1.10 - 2.81	0.07 - 0.55	0.01 - 0.27	—
Back	Series I n = 18	Mean	0.26 ± 0.07	1.48 ± 0.08	0.31 ± 0.04	13.08 ± 2.60
		Range	0.05 - 0.91	0.82 - 2.19	0.03 - 0.51	1.51 - 49.90
	Series II n = 20	Mean	0.09 ± 0.01	1.72 ± 0.12	0.27 ± 0.04	—
		Range	0.06 - 0.12	0.88 - 2.58	0.01 - 0.57	—
P = Level of significance		Series I	< 0.001	< 0.001	< 0.002	0.01 - 0.002
		Series II	< 0.001	< 0.001	< 0.050	—

The values of series I are included in Fig. 1. The thirty samples were taken from twenty-two individuals, whereby eight persons furnished samples of both sites. Confirming results from a later and independent set of determinations are represented in series II, where each individual provided a sample from both sites. The only difference between the two series concerns the P-values for ARG/THR.

The values for P were obtained with the X-test<sup>11</sup>. n = Number of sweat samples.

From the data presented, the following conclusions can be drawn. The relative concentrations of free amino-acids in human sweat from a specific area show only minor individual variations. This finding is in agreement with the data of Oró and Skewes<sup>6</sup>, who found little individual variation in amino-acid patterns from fingers. Some authors<sup>7</sup> have reported higher individual variations for ornithine, citrulline, arginine and asparagine; such variations may be obtained when the two types of sweat are mixed (for example, in thermal sweat from the whole body surface).

Notable regional differences occur in the relative concentrations of the amino-acids involved in the urea cycle and in those of urea: ornithine is high in sweat from the hands (mainly palmar sweat) but low in sweat from the back; in contrast, the relative concentrations of citrulline, arginine and urea are higher in sweat from the back than in sweat from hands. With the present state of knowledge it is difficult to suggest a possible mechanism for these differences, because not enough is known about the metabolism of the eccrine sweat glands and about the secretion mechanism of amino-acids of sweat. The observation that palmar and back-sweat show marked differences in urea cycle amino-acids suggests, however, that the eccrine sweat glands in the two locations, although morphologically identical<sup>8</sup>, have different properties with respect to the metabolism of urea and the amino-acids involved in the urea cycle. It is most unlikely that the differences are artefacts (results of bacterial action, admixture of apocrine sweat or secretion from sebaceous glands, different degree of keratinization) because the differences occurred with great constancy in all subjects regardless of age, sex, degree of keratinization

or presence of hairs. Furthermore, such artefacts would probably also affect other amino-acids.

In some anomalies of amino-acid metabolism (cystinuria, cystine storage disease, hyperglycinaemia, phenylketonuria, Lowe syndrome) the pattern of sweat amino-acids appears to remain unchanged, despite marked alterations in plasma and/or urine amino-acid levels. This finding is in agreement with the observation of Hier, Cornbleet and Bergeim<sup>2</sup> that levels of the amino-acids of sweat are independent of changes in plasma amino-acids and that amino-acids do not appear in the sweat merely as a result of filtration from the blood plasma. Because, however, anomalies of amino-acid metabolism and amino-acid transport may affect the sweat glands as well as other organs, it is possible that such alterations will be found when more "inborn errors" of amino-acid metabolism are investigated. Coltman, Rowe and Atwell<sup>10</sup> have, in fact, recently reported a generalized increase of amino-acid concentrations in sweat in patients with Wilson's disease.

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## Stabilization of Enzyme Activity by an Organic Solvent

BIOCHEMICAL work with unstable enzymes is often handicapped by the loss of their enzyme activity during preparation and storage, and the instability of the enzymes makes their further purification and study difficult. A number of enzymes can be stabilized with the use of reducing agents, chelating compounds, competitive inhibitors, co-enzymes, and substrates and their analogues. Recently, Nozaki, Kagamiyama and Hayaishi<sup>1</sup> have found that metapyrocatechase from *Pseudomonas sp.*, which is very unstable, particularly in the presence of air, is stabilized by the presence of a low concentration of an organic solvent such as acetone or ethanol, and this observation led to its purification and crystallization. This has led us to investigate whether organic solvents have a stabilizing effect on a wide variety of enzymes. In this communication we report the effects of acetone and ethanol in stabilizing benzylalcohol dehydrogenase from *Pseudomonas sp.*, homogentisicase from bovine liver, and alcohol dehydrogenase from yeast.

Benzylalcohol dehydrogenase, which catalyses the oxidation-reduction of benzylalcohol and benzaldehyde with stoichiometric amounts of nicotinamide adenine dinucleotide and reduced nicotinamide adenine dinucleotide,



respectively, was observed to be markedly unstable<sup>2</sup>. The dehydrogenase had a half-life of a few hours in 0.05 molar phosphate buffer, pH 7.5 at 5° C, and reducing or chelating agents had no significant retarding effect on the loss of activity. An organic solvent such as acetone or ethanol, however, was found to cause significant and often complete stabilization of the dehydrogenase. Experiments collected in Fig. 1A indicate that incubation at 5° C and pH 7.5 of the dehydrogenase caused rapid inactivation, but the presence of 10 per cent acetone was found to protect the dehydrogenase from inactivation almost completely. As shown in Fig. 1B, the protective effect of ethanol was similar to that observed in acetone. Similar protection was also observed in ammonium sulphate (Fig. 1C). A further point of interest is whether the inactivation is prevented by maintaining the dehydrogenase anaerobically. To this end incubation was carried out in an atmosphere of nitrogen with a Thunberg tube at 5° C and it was found that anaerobic conditions do not stabilize the dehydrogenase (Fig. 1C).

Yeast alcohol dehydrogenase is relatively unstable compared with liver alcohol dehydrogenase, although the former is quite stable in a crystalline suspension in ammonium sulphate or under conditions in which oxidation of -SH groups cannot occur. The dehydrogenase was markedly unstable at a low concentration of the protein

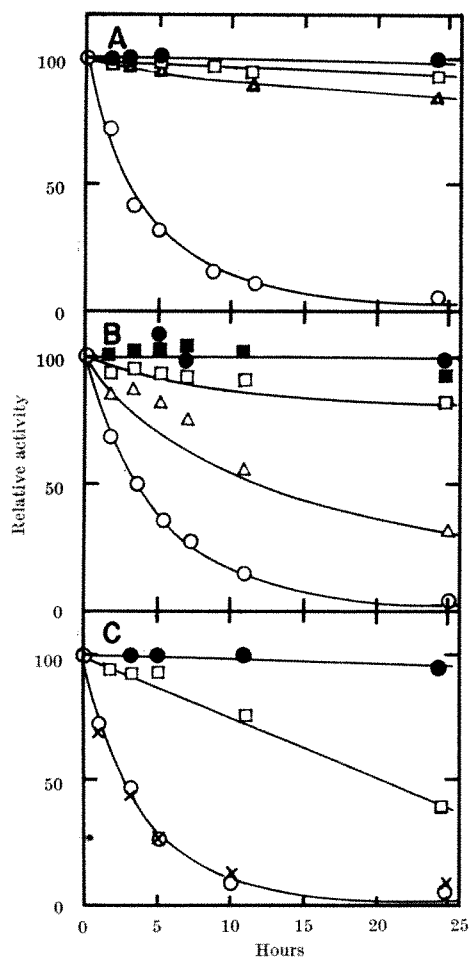


Fig. 1. A and B, Stability of benzylalcohol dehydrogenase in an organic solvent. Enzyme (46  $\mu\text{g}/\text{ml}$ ) was stored at 5° C in 0.05 molar phosphate buffer, pH 7.5, which contained different concentrations of acetone or ethanol. At the times indicated aliquots were transferred to the reaction mixture and assayed under standard conditions<sup>2</sup>. A, Acetone; B, ethanol;  $\blacksquare$ , 20 per cent;  $\bullet$ , 10 per cent;  $\square$ , 5 per cent;  $\triangle$ , 2 per cent;  $\circ$ , none. C, Stability of benzylalcohol dehydrogenase in ammonium sulphate and under anaerobic conditions. Enzyme (46  $\mu\text{g}/\text{ml}$ ) was stored at 5° C in 0.05 molar phosphate buffer, pH 7.5, which contained different concentrations of ammonium sulphate. Anaerobic experiments were carried out under nitrogen in a Thunberg tube.  $\bullet$ , 1 molar;  $\square$ , 0.5 molar;  $\circ$ , none;  $\times$ , nitrogen atmosphere.

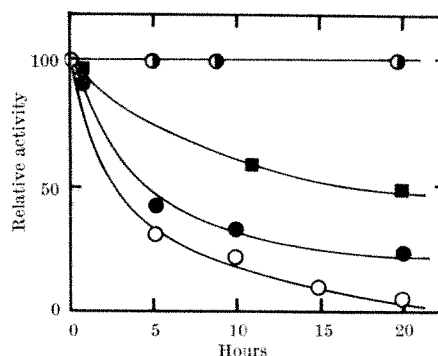


Fig. 2. Stability of yeast alcohol dehydrogenase in acetone and in glutathione. The enzyme was dissolved in 0.05 molar phosphate buffer, pH 7.5, at the protein concentration of 22  $\mu\text{g}/\text{ml}$ , and was incubated with acetone at concentrations of 20 per cent ( $\blacksquare$ ) and 10 per cent ( $\bullet$ ), respectively. The control system was without acetone ( $\circ$ ). Likewise, the enzyme was incubated with 1 mmolar glutathione ( $\circ$ ). The incubation was carried out aerobically at 26° C. At the times indicated, aliquots were transferred to the reaction mixture and assayed as previously described by Racker<sup>4</sup>.

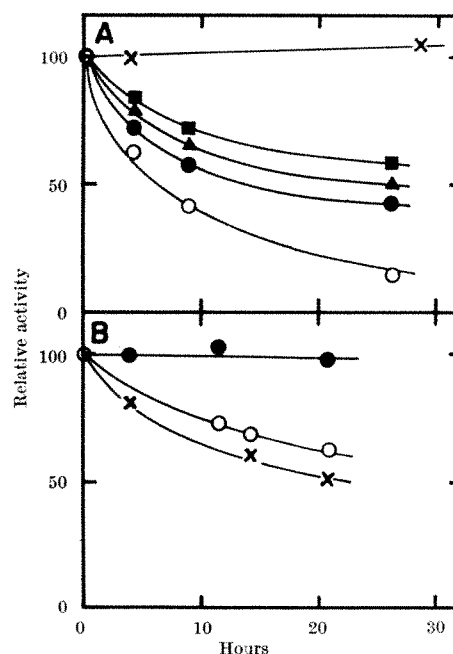


Fig. 3. Stability of homogentisicase in an organic solvent. The apo- and holoenzymes of homogentisicase were prepared as described previously<sup>3</sup>. Enzymes (106  $\mu\text{g}/\text{ml}$ ) were in 0.01 molar phosphate buffer, pH 7.0, which contained different concentrations of acetone and were incubated at 4° C in air. Anaerobic experiments were carried out under nitrogen in a Thunberg tube. At the times indicated, aliquots were transferred to the reaction mixture and assayed as described previously<sup>3</sup>. A, Holoenzyme; B, apoenzyme.  $\blacksquare$ , 20 per cent;  $\blacktriangle$ , 15 per cent;  $\bullet$ , 10 per cent;  $\circ$ , none;  $\times$ , nitrogen atmosphere.

in 0.05 molar phosphate buffer, pH 7.5 at 26° C, as shown in Fig. 2. When the dehydrogenase was incubated in the presence of 20 per cent acetone, the inactivation was considerably reduced. Likewise, in the presence of 1 mmolar glutathione the enzyme suffered no loss of activity, even after 20 h.

Bovine liver homogentisicase, which is generally known as dioxygenase and catalyses oxidative cleavage of the aromatic ring of homogentisic acid, was observed to be highly unstable, particularly in the presence of air. The presence of a low concentration of acetone protected the holoenzyme from inactivation by air<sup>3</sup> (Fig. 3A). Ethanol also retarded inactivation. The apoenzyme (which is completely inactive in the absence of ferrous iron) was found to be completely stable in the presence of 10 per cent acetone, even after 24 h storage in air. In contrast to the holoenzyme, no appreciable protective effect of the apoenzyme was observed under anaerobic conditions.

These phenomena of the apoenzyme were quite similar to those observed in the case of benzylalcohol dehydrogenase.

It appears that the inactivation in the case of benzylalcohol dehydrogenase or the apoenzyme of homogentisicase is not caused by the destruction of any oxy-labile groups in the enzyme protein, and that the stabilization of the enzyme activity by the organic solvent is probably caused by maintenance of the "native state" of the protein conformation. It is noteworthy that the organic solvent such as acetone or ethanol is effective for the stabilization of unstable enzymes, and can be used in practice without significant interference in chromatographic systems during the process of enzyme purification. The mechanism of the protection by the organic solvent will be better interpreted by further investigations on physico-chemical properties of the protein molecule in aqueous organic solvents.

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### Structural Basis for Phosphorylation of Adenosine Congeners

DURING the past few years the metabolism by mammalian cells of a number of natural and synthetic nucleosides structurally related to adenosine has been investigated in this<sup>1-5</sup> and other laboratories<sup>6-10</sup>. In our studies with Ehrlich ascites cells, we have tried to establish a correlation between the structural variations and the utilization by cells of these nucleosides and have observed the following salient features: (a) In order to be phosphorylated to the 5'-triphosphate level, the adenosine analogue must have a primary amino group at the C-6 position of the purine moiety. Compounds that belong in this category are 3'-deoxyadenosine<sup>2,6</sup>, 3'-amino-3'-deoxyadenosine<sup>4</sup> and 2-fluoroadenosine<sup>3</sup>. These three nucleosides were also found to be potent inhibitors of nucleic acid synthesis in Ehrlich ascites cells. Work in other laboratories has shown that 7-deazaadenosine<sup>7</sup>, xylosyladenine<sup>8</sup>, arabinosyladenine<sup>9</sup> and the bases 2-azaadenine and 2,6-diaminopurine<sup>10</sup> were also phosphorylated to 5'-triphosphate. 2,6-Diaminopurine is also metabolized to the triphosphate level by microbial cells<sup>11,12</sup>. Removal of the 6-amino group as in 3'-deoxynuclearine resulted in complete absence of phosphorylation by ascites cells<sup>4</sup>. (b) 6-Methylaminopurine ribonucleoside—an adenosine analogue with a 6-methylamino group—was metabolized to the monophosphate level only<sup>4</sup>. This was also found to be so for 6-methylaminopurine-2'-deoxyribonucleoside, 6-methylaminopurine-3'-deoxyribonucleoside and 6-methylaminopurine<sup>5</sup>. (c) Replacement of the 6-amino group by 3'-deoxyadeno-

sine with an ethylamino or dimethylamino group completely suppressed the capacity of the nucleoside for phosphorylation. This relationship was exemplified by 6-ethylaminopurine-3'-deoxyribonucleoside and 6-dimethylaminopurine-3'-deoxyribonucleoside<sup>4</sup>. Furthermore, 6-dimethylamino-3'-amino-3'-deoxyribonucleoside was also inactive, while its parent compound, as already mentioned, was readily phosphorylated. Nucleosides that were not phosphorylated had almost no effect on nucleic acid synthesis.

This work has been continued with several new synthetic adenosine analogues, and the structure-activity relationships and structural specificity for phosphorylation of these compounds are described and discussed in this communication.

2-Fluoro-3'-deoxyadenosine, 5'-5'-dimethyladenosine, 3'-deoxy-3-isoadenosine and tritiated 3'-C-methyladenosine<sup>13</sup> were supplied by Drs E. Walton and F. Holly. 2',3'-Dideoxyadenosine was prepared and supplied by Dr R. K. Robins<sup>14</sup>.

The procedures used to study the phosphorylation of adenosine analogues have already been described<sup>2-4</sup>. In brief, the perchloric acid-soluble portion of ascites cell incubated with the test nucleoside was fractionated by means of 'Dowex-1'-formate chromatography. The materials obtained were characterized by measurements of ultra-violet absorption, phosphate determination and by paper electrophoresis or chromatography. The methods used to determine deamination or cleavage of nucleosides have been described<sup>4</sup>.

A study of the metabolism of 3'-C-methyladenosine was especially interesting because this compound is the first derivative of adenosine with a branched-chain pentose. This nucleoside was not deaminated or cleaved by intact Ehrlich ascites cells. When tritiated 3'-C-methyladenosine was incubated with ascites cells for 3 h at 37° C and the substance soluble in hydrochlorous acid was fractionated by ion-exchange chromatography, a radioactive material which absorbed in the ultra-violet was eluted from the region corresponding to nucleotide triphosphate. This substance had an absorption spectrum similar to that of adenosine or 3'-C-methyladenosine ( $\lambda_{\max}$  258,  $\lambda_{\min}$  227, 250/260 = 0.79, 280/260 = 0.16) at pH 7 and contained 3.2  $\mu$ moles of phosphorus/ $\mu$ mole of adenosine. On paper electrophoresis at pH 3.5, the mobility of the substance was found to be comparable with that of ATP. These results, in addition to the observation that this analogue was not deaminated or cleaved, indicated that the radioactive

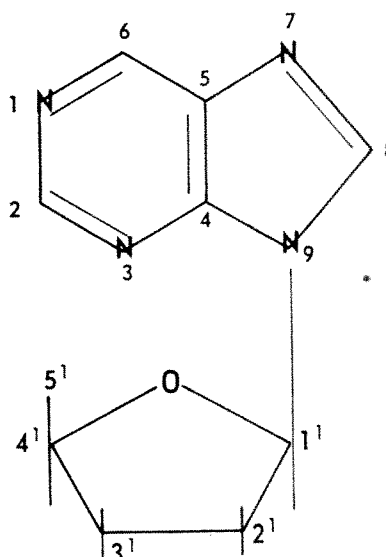


Fig. 1.

\* A possible exception to this rule was demonstrated by Gordon *et al.* (*J. Amer. Chem. Soc.*, **79**, 3256; 1957) when they showed that 9- $\beta$ -D-ribofuranosyl purine was phosphorylated in the adult rat.

Table 1

	Mono-phosphate	Tri-phosphate	Ref.
(1) 3'-Deoxyadenosine 6-Amino-9-(3'-deoxy- $\beta$ -D-ribofuranosyl)- purine	+	+	2, 6
(2) 3'-Amino-3'-deoxyadenosine 6-Amino-9-(3'-amino-3'-deoxy- $\beta$ -D- ribofuranosyl)purine	+	+	4
(3) 2-Fluoroadenosine 6-Amino-2-fluoro-9-( $\beta$ -D-ribofuranosyl)- purine	+	+	3
(4) 3'-C-methyladenosine 6-Amino-9-(3'-C-methyl- $\beta$ -D-ribofuranosyl)- purine	+	+	*
(5) 2-Fluoro-3'-deoxyadenosine 6-Amino-2-fluoro-9-(3'-deoxy- $\beta$ -D- ribofuranosyl)purine	-	-	*
(6) 2',3'-Dideoxyadenosine 6-Amino-9-(2',3'-dideoxy- $\beta$ -D- ribofuranosyl)purine	-	-	*
(7) 3'-Deoxynebularine 9-(3'-Deoxy- $\beta$ -D-ribofuranosyl)purine	-	-	4
(8) 6-Methylaminopurine ribonucleoside 6-Methylamino-9-( $\beta$ -D-ribofuranosyl)purine	+	-	4
(9) 6-Methylaminopurine-2'- deoxyribonucleoside 6-Methylamino-9-(2'-deoxy- $\beta$ -D- ribofuranosyl)purine	+	-	5
(10) 6-Methylaminopurine-3'- deoxyribonucleoside 6-Methylamino-9-(3'-deoxy- $\beta$ -D- ribofuranosyl)purine	+	-	4
(11) 6-Dimethylaminopurine-3'- deoxyribonucleoside 6-Dimethylamino-9-(3'-deoxy- $\beta$ -D- ribofuranosyl)purine	-	-	4
(12) 6-Ethylaminopurine-3'-deoxyribonucleoside 6-Ethylamino-9-(3'-deoxy- $\beta$ -D- ribofuranosyl)purine	-	-	4
(13) 6-Dimethylaminopurine-3'-NH <sub>2</sub> -3'- deoxyribonucleoside 6-Dimethylamino-9-(3'-NH <sub>2</sub> -3'-deoxy- $\beta$ -D- ribofuranosyl)purine	-	-	4
(14) 5',5'-Dimethyladenosine 6-Amino-9-(5',5'-dimethyl- $\beta$ -D- ribofuranosyl)purine	-	-	*
(15) 3'-Deoxy-3-isoadenosine 6-Amino-9-(3'-deoxy- $\beta$ -D-ribofuranosyl)- purine	-	-	*
(16) 7-Deazaadenosine 4-Aminopyrrolo (2,3-d)pyrimidine- $\beta$ -D- ribofuranoside	+	+	7
(17) Xylosyladenine 6-Amino-9( $\beta$ -D-xylofuranosyl)purine	+	+	8
(18) Arabinosyladenine 6-Amino-9-( $\beta$ -D-arabinofuranosyl)purine	+	+	9
(19) 2-Azaadenine 6-Amino-2-azapurine	+	+	10
(20) 2,6-Diaminopurine	+	+	10

\* This communication.

triphosphate obtained from the acid-soluble fraction was 3'-C-methyladenosine triphosphate. Similar experiments showed that the following new adenosine analogues were not phosphorylated at all: 2-fluoro-3'-deoxyadenosine, 5',5'-dimethyladenosine, 2',3'-dideoxyadenosine and 3'-deoxy-3-isoadenosine. The chromatographic patterns of the acid-soluble fractions from these experiments were similar to those in the control experiments<sup>4</sup>.

These results thus show that another adenosine analogue (3'-C-methyladenosine) with a 6-amino group was phosphorylated to the triphosphate level and it is also noteworthy that a methyl group on the 3'-position of the ribose moiety did not interfere with the utilization of the nucleoside. The results obtained with the other new adenosine analogues also substantiate the view<sup>4</sup> that although the 6-amino group is essential for phosphorylation, its mere presence does not necessarily ensure phosphorylation. The fact that 2-fluoro-3'-deoxyadenosine was inert while both 2-fluoroadenosine and 3'-deoxyadenosine were phosphorylated suggested that substitution of 3'-OH with 3'-H altered the property of the 2-fluoro-adenine moiety of the nucleoside. The inactivity of 2',3'-dideoxyadenosine indicated that at least one hydroxyl group is required at either the 2'- or 3'-position of the pentose moiety. Steric hindrance caused by the relatively bulky methyl groups at the site of phosphorylation and diminished reactivity of the tertiary hydroxyl group may explain the inactivity of 5',5'-dimethyladenosine. Transfer of the 3'-deoxyribosyl group from the N-9- to the N-3-position also hindered phosphorylation.

Thus adenosine can be made to undergo certain structural modifications and yet retain its capacity for conversion to the triphosphate. The structural alterations that are known to permit phosphorylation can be summarized as follows (Table 1 and Fig. 1).

- Position 2: Fluorine could replace hydrogen (2-fluoroadenosine)
- NH<sub>2</sub> could replace hydrogen (2,6-diaminopurine)
- Nitrogen could replace CH (2-azaadenine)
- Position 7: CH could replace nitrogen (7-deazaadenosine)
- Position 2': Either epimer (ribose or arabinose)
- Position 3': Either epimer (ribose or xylose)
- Hydrogen for hydroxyl (3'-deoxyadenosine)
- NH<sub>2</sub> for hydroxyl (3'-amino-3'-deoxyadenosine)
- Methyl for hydrogen (3'-C-methyladenosine)

Modifications of adenosine or 3'-deoxyadenosine that do not allow phosphorylation to triphosphate can be outlined as follows (although much of this work was done with 3'-deoxyadenosine analogues, it is assumed that the structure-activity relationship of these nucleosides can be extrapolated to adenosine because 3'-deoxyadenosine was phosphorylated nearly as well as adenosine).

- Position 6: NH<sub>2</sub> replaced by H, CH<sub>3</sub>NH, C<sub>2</sub>H<sub>5</sub>NH or (CH<sub>3</sub>)<sub>2</sub>N
- Position 1': Hydrogen replaced by CH<sub>2</sub>OH (psicofuranine, ref. 15).
- Position 5': 2H replaced by (CH<sub>3</sub>)<sub>2</sub>
- Positions 2 and 3': Simultaneous changes at C-2 (F for H) and 3' (H for OH) as in 2-fluoro-3'-deoxyadenosine.
- Positions 2' and 3': Simultaneous changes at 2' (H for OH) and 3' (H<sub>2</sub> for OH) as in 2',3'-dideoxyadenosine.
- Position 9: Transfer of 3'-deoxyribosyl group from N-9 to N-3.

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### Reversal by Phosphate of Glucose Repression of Catalase Synthesis in *Saccharomyces cerevisiae*

It has long been known that glucose represses many inducible enzyme systems<sup>1</sup>. The mechanism responsible for this specific effect has elicited wide interest although none of the suggestions put forward has received unqualified acceptance. Neidhart and Magasanik<sup>2</sup> and Mandelstam<sup>3,4</sup> have concluded from their studies on  $\beta$ -galactosidase that any compound utilized by the organism as a source of energy and carbon can act as repressor and that it may not be glucose itself but a catabolite derived from it that is the active repressor. According to the "feedback hypothesis" of Neidhart and Magasanik<sup>5</sup> and Vogel<sup>6</sup>, the formation of each catabolic enzyme is controlled by the intracellular concentration of some particular metabolite which is an immediate or ultimate product of that enzyme and which is also produced from glucose at a rate faster

than its utilization for synthetic reactions in the cell. A possible site of glucose action is the release of the enzyme from the RNA template<sup>7</sup> or ribosome<sup>8</sup>.

In the course of our work on the induction of catalase, when anaerobically grown cultures of *Saccharomyces cerevisiae*<sup>9,10</sup> were aerated, we observed (Table 1) that glucose had a stimulatory effect at low concentrations but a repressive effect at concentrations higher than 56  $\mu$ moles/ml. Of the various catabolites of glucose which we tested, lactate and acetate showed a similar behaviour; however, succinate and citrate were highly inhibitory whereas pyruvate was stimulatory. Of the other sugars and related compounds tested, fructose, mannose and galactose gave a response qualitatively similar to that with glucose while sorbose and glycerol stimulated the induction with increasing concentrations. The repressive effect of glucose diminished as the sugar was used up so that by extending the time of aeration the induction could again be demonstrated.

Table 1. EFFECTS OF SUGARS AND GLUCOSE METABOLITES ON INDUCED SYNTHESIS OF CATALASE

Addition to aeration medium	$\mu$ moles/ml.		
	14	140	280
	Percentage of alteration in catalase activity		
Glucose	+15.0	-24.6	-42.9
Pyruvate	+10.9	+18.1	+20.8
Lactate	+3.0	-7.2	-21.5
Acetate	+12.3	+3.1	-62.2
Succinate	-5.1	-25.0	-100.0
Citrate	-20.6	-30.3	-100.0
Fructose	+3.7	-24.8	-34.0
Mannose	+9.4	-24.9	-42.3
Galactose	+19.2	+7.8	-15.5
Sorbose	+11.1	+31.6	+36.0
Glycerol	+9.4	+24.3	+29.2

Cells of *Saccharomyces cerevisiae*, grown anaerobically in glucose-peptone-yeast extract medium, were collected and washed. Quantities of about 200 mg dry weight were suspended in 10 ml. of the aeration medium ( $\text{KH}_2\text{PO}_4$ , 1.0 per cent;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.01 per cent;  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , 0.01 per cent, pH 6.8) and aerated for 4 h. At the end of the period the cells were centrifuged, washed, treated with a few drops of chloroform and catalase activity was assayed as described previously<sup>9</sup>. The glucose catabolites tested were used at double the stated concentrations.

The inhibition by glucose of catalase induction may be a case of "catabolite repression". On the other hand, the possibility cannot be dismissed that glucose inhibits the respiratory enzymes with a consequent decrease of the *in situ* production of hydrogen peroxide which appears in the present case to be the inducer. In fact, it was observed that at higher concentrations glucose inhibited the respiration of the yeast cells, an observation analogous to the Crabtree effect observed with tumour cells<sup>11</sup>. In view of the report that the Crabtree effect is reversed by inorganic phosphate<sup>12</sup>, we studied its effect on the induction of catalase (Table 2).

Table 2. REVERSAL BY PHOSPHATE OF GLUCOSE REPRESSION OF CATALASE

Phosphate in aeration medium ( $\mu$ moles/ml.)	Catalase activity Kat f units in the presence of glucose in aeration medium ( $\mu$ moles/ml.)	
	14	280
73	718	396
146	723	670
219	727	730
292	734	840
365	740	915

We observed that an increase in the concentration of phosphate in the aeration medium did not appreciably affect the synthesis of catalase when glucose was present at a low concentration. The inhibition produced by high concentrations of glucose could, however, be overcome by increasing the concentrations of phosphate. The inhibition of respiration by glucose, on the other hand, was not reversed by increased concentrations of phosphate. It is interesting to recall in this context the observations of Mandelstam<sup>3,4</sup>, during his studies on the formation of  $\beta$ -galactosidase in *Escherichia coli*, that any carbon source metabolite will cause repression when the cells are starved of nitrogen, magnesium ions or some specific auxotrophic factor. The present observation demonstrates that

glucose inhibits the induction phenomenon by creating a deficiency of inorganic phosphate.

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### Multinucleated Muscle Fibres: Induction of DNA Synthesis and Mitosis by Polyoma Virus Infection

In many cell types differentiation is closely associated with the end of DNA synthesis. The differentiation of muscle cells is a typical example. The formation of muscle fibres takes place by the successive fusion of mononucleated myoblasts into ribbon like cells, containing as many as several hundred nuclei. After fusion, the nuclei within the fibres do not synthesize DNA, do not undergo mitosis<sup>1-4</sup> and apparently are also restricted in RNA synthesis<sup>5,6</sup>.

The aim of the present work was to test whether the formation of multinucleated muscle fibres is associated with an irreversible block of DNA synthesis, or whether the nuclei within the fibres can be induced to resume DNA synthesis and undergo mitosis. Previously it was shown that the oncogenic viruses polyoma (PV) and SV40 can induce host DNA synthesis and cell division in contact inhibited cells and also induce DNA synthesis in X-irradiated cells<sup>7-11</sup>. It was thus decided to test whether an oncogenic virus can induce host DNA synthesis in rat multinucleated muscle cells. Cells of these species were selected because rat fibroblasts had previously been shown to undergo malignant transformation by PV in the absence of the synthesis of infectious virus particles. It was hoped that rat muscle cells would also not support PV multiplication and thus facilitate observation of host DNA synthesis and eliminate the complication of cell lysis.

Cells obtained by trypsinization of newborn rat thigh muscle cells were cultured as previously described<sup>4,6</sup>. The small plaque mutant of polyoma virus<sup>12</sup> was used throughout this investigation. Infection was carried out at input multiplicity of 100 P.F.U./cell. Unless otherwise stated, 4 day old cultures were infected. At this stage the cultures consisted of multinucleated fibres, myoblasts in the process of fusion and undifferentiated mononucleated cells.

In order to measure DNA synthesis in differentiated fibres, duplicates of infected and non-infected cultures were exposed to 2.5  $\mu$ Ci/ml. <sup>3</sup>H-thymidine (18,000 mCi./mmole) for 6 h at various times after introduction of the virus. The cultures were processed for autoradiography and stained for detailed cytological examination as previously described<sup>6</sup>.

DNA synthesis was not observed in multinucleated fibres exposed to virus for less than 30 h, as judged by



incorporation of tritium into their nuclei. More than 30 h after infection, however, an increasing proportion of the fibres were found to synthesize DNA. After 40 h as many as 15 per cent of the fibres had labelled nuclei. Usually when DNA synthesis was observed in a fibre all of the nuclei were found to have incorporated the labelled precursor (Fig. 1); however, fibres were found in which

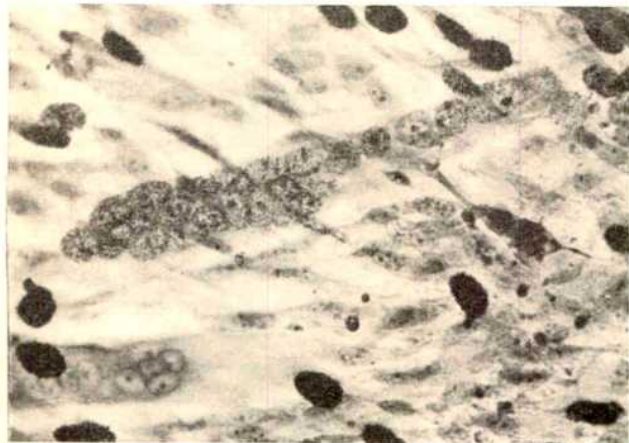


Fig. 1. Autoradiograph of PV infected multinucleated muscle fibre. 4 day old cultures were infected with PV; 48 h after infection the cultures were exposed to  $^3\text{H}$ -thymidine for 6 h. Notice labelling of all nuclei in one fibre and absence of labelling in a neighbouring fibre ( $\times 300$ ).

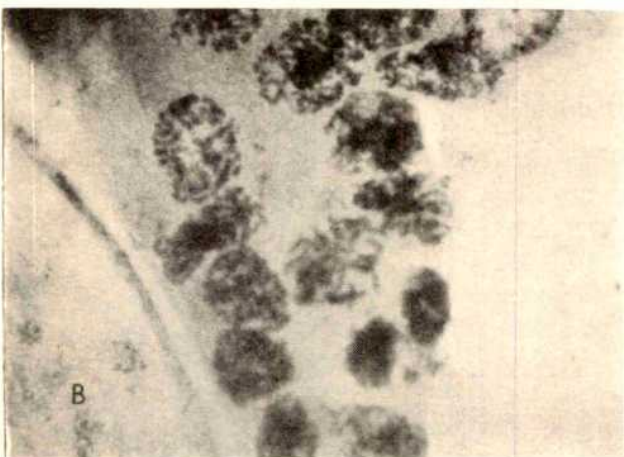


Fig. 2. A, Dividing nuclei in a PV infected muscle fibre. Note gradient of progressive stages of mitosis: from non-dividing nuclei and early prophase (left) to clumping of chromosomes in late metaphase (right). Culture conditions and infection identical to those described in the legend to Fig. 1. Fixed at 72 h after infection and stained with Giemsa. ( $\times c. 275$ .) B, Portions of Fig. 2A at higher magnification ( $\times 1000$ ).

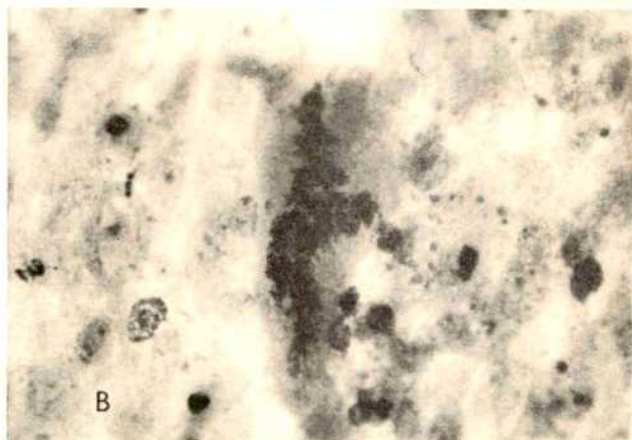
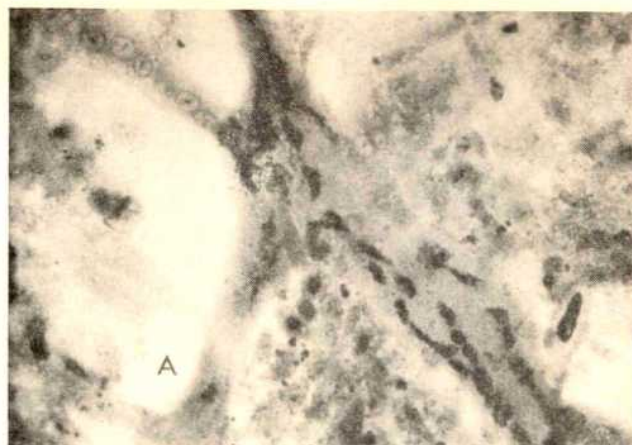


Fig. 3. Stages in the formation of abnormal giant nuclei after PV infections, as a result of aggregation and clumping of groups of chromosomes within the multinucleated fibres. Culture conditions and infection were identical to those described in the legend to Fig. 1. A fixed at 72 h, B and C at 82 h after infection. Stained with Giemsa. ( $\times c. 235$ ,  $\times c. 425$ ,  $\times c. 265$ .)

only a fraction of the nuclei was labelled. In the latter case the labelled nuclei were clustered in one section of the fibre.

Mitotic figures began to appear in multinucleated fibres about 50 h after infection. After 70 h about 30 per cent of the fibres contained nuclei in various stages of mitosis. In many such fibres advanced metaphases could be observed at one end of a group of dividing nuclei; distal to these nuclei progressively earlier stages of mitosis were observed which terminated with early prophase and resting nuclei (Fig. 2). In other fibres all the nuclei undergoing mitosis were found to be in metaphase.

Division of nuclei within the fibres did not proceed beyond late metaphase. Instead the fusion of several metaphase figures into large clumps of chromosomes was observed. This phenomenon was frequently associated with rounding of the fibres and loss of their elongated form. In cultures fixed 90 h after infection or later, fibres and rounded cells containing huge abnormal nuclei were frequently found (Fig. 3). These are most probably the result of the clumping of the dividing nuclei observed in earlier stages. Mitotic figures were not observed in fibres of uninfected cultures.

The appearance of mitotic figures within the infected fibres suggested that the observed incorporation of thymidine into nuclei of muscle fibres represented the synthesis of cellular type DNA. A more direct approach was taken to determine the nature of the newly synthesized DNA, however. Rat muscle cells were infected with PV and were exposed to  $^3\text{H}$ -thymidine after 24 h. After 72 h cells were collected and their DNA was extracted. This DNA was mixed with PV DNA labelled with carbon-14 and fractionated on a methylated albumin kieselguhr (MAK) column as previously described<sup>11</sup>. From the results (Fig. 4A and B) it can be seen that the newly synthesized DNA is mostly of cellular and not of viral origin. This observation is in accordance with previous ones made on X-irradiated PV infected rat embryo cells<sup>10</sup>.

In order to determine whether there is synthesis of infective particles in PV infected rat muscle cultures, the following assay was performed. At various times after infection duplicate cultures were frozen and their virus content was assayed by the plaque technique<sup>14</sup>. Virus titre 2 h after infection was  $3.1 \times 10^7$  and at 24, 72 and 120 h titres were  $3.0 \times 10^7$ ,  $2.1 \times 10^7$  and  $1.8 \times 10^7$  plaque forming units respectively. Although the synthesis of minute amounts of virus cannot be excluded by this method, we interpreted these data as showing that virus synthesis does not play a significant part in this system.

At the time of infection the cultures used in the present study were in the stage of cell fusion, that is, myoblasts were still joining the multinucleated fibres. It was found that the number of fibres induced by PV to synthesize DNA dropped considerably if infection was delayed by a few days. This may be caused by the impermeability or insensitivity of multinucleated fibres to PV. Thus the infection of the differentiated cells appears to occur via the introduction of nuclei of pre-infected myoblasts into the growing fibres.

Incorporation of  $^3\text{H}$ -thymidine into infected cultures in the first 30 h after infection was into mononucleated cells, although an occasional fibre was observed to contain a labelled nucleus among many unlabelled nuclei. Somewhat later, all nuclei in affected fibres were observed to be labelled. This suggests that the introduction of the virus had induced postmitotic nuclei to resume DNA synthesis. In other words, it appears that the effect of PV infection is an induction of DNA synthesis in post-mitotic cells rather than merely the prevention of the formation of a mitotic block. The few labelled nuclei within the fibres most probably originated from myoblasts which incorporated labelled thymidine during the 6 h exposure to the radioactive nucleoside, and subsequently joined the fibres<sup>3</sup>.

These findings indicate that the formation of multinucleated fibres is not necessarily associated with an irreversible change in the capacity of the nucleus to synthesize DNA and undergo mitosis. The fusion of dividing nuclei into abnormal clumps of chromosomes and subsequently the formation of giant nuclei may not be caused by aberrations in the chromosomes, but rather by the close proximity of many mitotic apparatuses within the same cytoplasm. There is also a possibility of interference of the contractile proteins of the specialized muscle cells with the formation of mitotic spindles. Formation of giant nuclei has been observed in artificially induced polykaryons<sup>15</sup>.

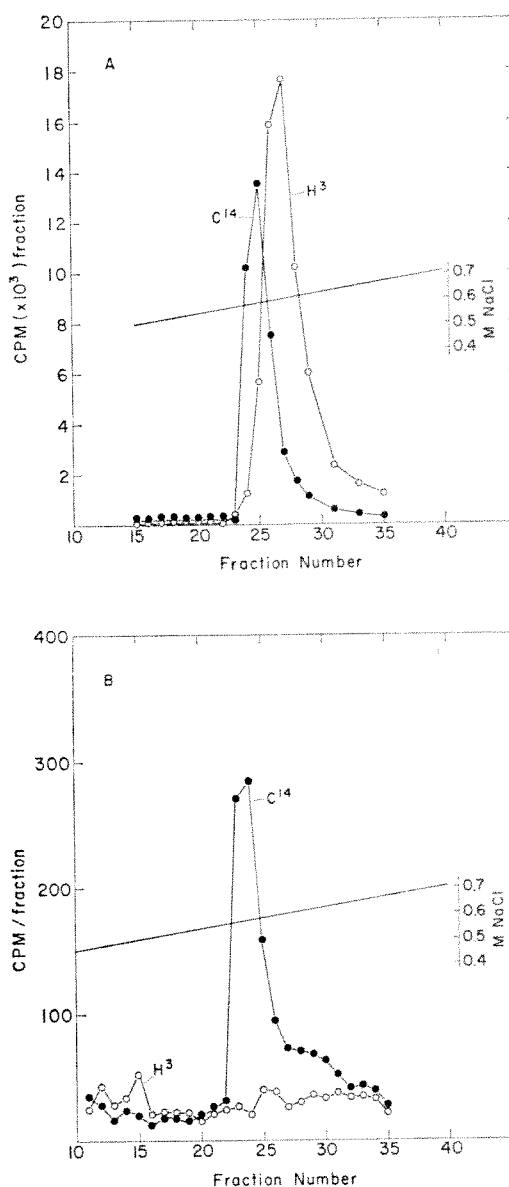


Fig. 4. MAK column chromatography of DNA from PV infected rat muscle cells 72 h after infection. The infected cultures were exposed to  $^3\text{H}$ -thymidine between 17 and 72 h post infection. A, Without heating. An aliquot of  $^3\text{H}$ -thymidine rat muscle cell DNA was mixed with PV marker DNA labelled with carbon-14 and chromatographed on an MAK column as previously described<sup>11</sup>. Recovery, tritium, 89.1 per cent; carbon-14, 96.8 per cent. B, With heating. The same mixture as used in A was boiled for 10 min in low salt concentration. After quick cooling in an ice bath, the mixture was chromatographed on the MAK column. Heat treatment was used because it had been shown for PV<sup>16</sup> that the heat-treated virus DNA eluted at the same salt concentration as native virus DNA, whereas there was practically no elution at this salt concentration of heat-denatured cellular DNA. Recovery, tritium, 0.55 per cent; carbon-14, 30.1 per cent. The lack of coincidence of the peaks of tritium and carbon-14 radioactivity in A and the extremely low recovery of tritium radioactivity in B indicate that the labelled DNA from the PV infected rat muscle cells is predominantly of cellular origin.

In most of the fibres which were induced to synthesize DNA, all the nuclei were synthesizing DNA and undergoing mitosis. Because the probability of infection of all the nuclei is very low, this phenomenon leads one to speculate that there are two possible mechanisms by which a few PV transformed nuclei within a fibre induce DNA synthesis and mitosis in all the nuclei: (a) by directly supplying the genetic information for the synthesis of proteins necessary for DNA synthesis and mitosis; (b) by producing a substance which suppresses the effect of an inhibitor of DNA synthesis present in normal multinucleated muscle cells.



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## PHYSIOLOGY

### Entry of Ferritin into Human Red Cells during Hypotonic Haemolysis

A NUMBER of small molecules have been shown to enter red cells during hypotonic haemolysis. The membrane has been shown to be permeable to ATP (refs. 1, 2) and ghosts containing a number of organic compounds of metabolic interest, such as adenosine, cytidine, ATP and G6P, have been prepared<sup>3</sup>. The entry of dextran, with molecular weights from 10,000 to 250,000, into the red cell has been reported<sup>4</sup>, and albumin labelled with iodine-131 and haemoglobin labelled with iron-59 have been introduced into ghosts, and it was shown that these molecules could enter the cell only during haemolysis<sup>5</sup>. The red cell membrane is normally impermeable to these molecules, as well as to haemoglobin, and becomes impermeable again after release of haemoglobin, and so it is possible that the foreign molecules make their entry through the same channels which allow the escape of haemoglobin. The diffusion radius of haemoglobin is not less than 32 AU (ref. 6) and so the pores in the slightly stretched membrane of the haemolysing cell must be at least 64 AU in diameter during release of haemoglobin.

I have found that the protein ferritin with a molecular weight of 600,000 and a diameter of 100 AU will also diffuse across the membrane at the time of osmotic haemolysis and can be seen inside the ghosts in thin sections of a pellet of embedded ghosts. Red cells from a finger puncture were washed twice in isotonic saline and divided into three aliquots each of 0.1 ml. of packed cells. The cells of one aliquot were haemolysed with 5 ml. of phosphate buffer, pH 7, made 30 mmolar with distilled water and containing ferritin (Worthington, twice crystallized) in a concentration of 2 mg/ml. The second aliquot was treated in the same way, except that the ferritin was added to the hypotonic haemolysate 30 sec after the dilution with haemolysate. The third aliquot was not haemolysed, but 2 mg/ml. of ferritin was added to the

cell suspension. After 15 min at room temperature the cells of all aliquots were fixed in 1 per cent buffered osmium tetroxide, concentrated by centrifugation, dehydrated and embedded in 'Vestopal W'. Thin sections were examined in an RCA 'EMU3F' microscope without staining.



Fig. 1. Sectioned human red cell haemolysed in the presence of ferritin (2 mg/ml.) showing ferritin on both sides of plasma membrane. Unstained, fixed with osmium tetroxide, embedded in 'Vestopal'. ( $\times 195,000$ .)

The ghosts of the first aliquot showed ferritin molecules on both sides of the membrane (Fig. 1), and sometimes apparently within the membrane. Micrographs taken at high angle specimen tilt have not determined whether the ferritin is indeed within the membrane. Ferritin was seen only on the cell exterior in the pellet made from the second aliquot (Fig. 2). Results were the same in a modification of the treatment given to the second aliquot in which the ghosts were restored to an isotonic environment before the ferritin was added. The control cells (unhaemolysed, third aliquot) showed only some ferritin attached to the outside of the cell.



Fig. 2. Ferritin was added to the haemolysate 30 sec after haemolysis in 30 mmolar phosphate buffer. Ferritin is seen only on the exterior of the cell. Unstained, fixed with osmium tetroxide embedded in 'Vestopal'. ( $\times 195,000$ .)

In order to assess the role of electrostatic charge the experiment was repeated at a pH less than the isoelectric point of ferritin (pH 4.5), thus reversing the net charge on the ferritin from negative to positive. Net charge is most probably not a decisive factor because positively charged ferritin also penetrated the membrane.



In an attempt to set an upper limit on the pore size, colloidal gold (Matheson) with an average particle size of 250 AU was substituted for ferritin. The gold did not enter the cells (Fig. 3), indicating either that the pore diameter is less than 250 AU or that possibly the presence or absence of a protein coat on the probing particle is a crucial factor influencing entry.

It is well established that hypotonic haemolysis involves a prelytic swelling of the cell caused by the entry of water and that at a critical volume about 60 per cent greater than the initial volume haemoglobin escapes to a diffusion equilibrium with the external medium<sup>2</sup>. It is not at all certain whether the haemoglobin emerges through pre-existing pores which have been stretched, and, if so, whether these pores are the same pores which regulate water and small anion transport. The pore size for water transport in the normal cell was measured indirectly as 4 AU (ref. 7) and the total pore area was calculated to correspond to 0.01 per cent to 1 per cent of the cell surface area. Thus, there must be  $10^5$ – $10^8$  pores/cell. A single red cell contains about  $10^8$  molecules of haemoglobin, and so complete haemolysis must involve the passage of 1–1,000 molecules/pore. Fast rates of transport in the outward direction would be expected to have an inhibitory effect on entry, especially of large molecules. Thus the number of molecules such as ferritin entering the cell could be limited.

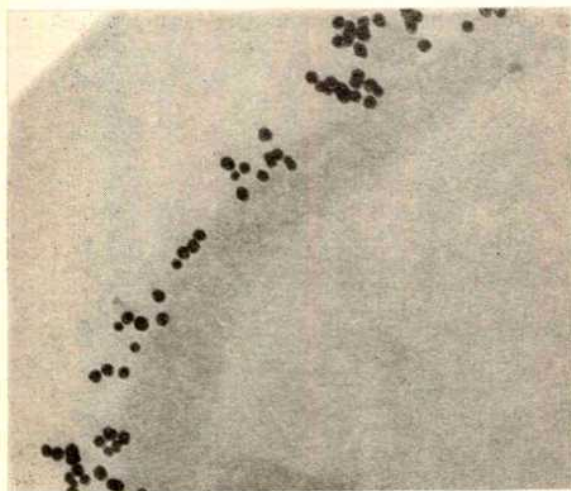


Fig. 3. Tangential cut through red cell membrane. Haemolysed in the presence of colloidal gold. The gold particles were never seen inside the ghosts. Unstained, fixed with osmium tetroxide embedded in "Vestopal". ( $\times 68,000$ .)

In an experiment designed to test this point the concentration of ferritin was varied in a 10:1 range. While the amount of extracellular ferritin seen varied approximately in the ratios added, the number of ferritin molecules seen inside the cells was little different for this range of concentrations, suggesting that some rate limiting factor was in effect.

Since this work was completed Seeman<sup>8</sup> has reported that, in agreement with our results, the red cell membrane is permeable to ferritin only at the time of haemolysis. He used mixtures of ferritin and colloidal gold (25–300 AU) and observed both kinds of particle inside cells if they were present in the haemolysate. In addition to the demonstration of the entry of ferritin and gold, Seeman demonstrated the existence of transient pores 300–500 AU in diameter in thin sections of the cells through which the ferritin presumably entered the cell. Reasons were given for believing that haemoglobin makes its escape through the same pores, although the possibility of other routes could not be excluded.

In summary, ferritin molecules have been shown to diffuse across the red cell membrane at the moment of release of haemoglobin, indicating a lower limit on pore size of at least 110 AU. Colloidal gold (250 AU) was excluded. Impermeability to ferritin immediately after haemolysis is indicative of a labile element in the membrane, in agreement with other results using smaller molecules, and with Seeman's results, also using ferritin.

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### Transneuronal Cell Degeneration in the Olfactory Bulb shown by the Golgi Method

THE Golgi technique has been used extensively to investigate the detailed morphology of individual neurones in normal material of the central nervous system, but the lack of understanding of the principles which underlie the selective impregnation of only a few cells and the well known capriciousness of the method have tended to preclude its experimental application. This report concerns the change in the appearance of nerve cells and their processes shown by this method during the early stages of transneuronal degeneration. Cells in the olfactory bulb which have undergone this form of degeneration because of interruption of the olfactory afferent fibres do not impregnate as well with the Golgi method as the cells in the opposite normal bulb<sup>1</sup>, and when the pyramidal neurones in certain areas of the cerebral cortex are deprived of some of their afferent fibres they may show diminished impregnation of either their finer dendritic branches or of a proportion of their spines<sup>2–4</sup>. In most of these investigations the appearance of the cells has been studied after relatively long survival periods, and there is little evidence<sup>5</sup> to indicate the time of onset of these changes and how they are correlated with the observations made in sections stained by the Nissl and reduced silver method.

The impregnation of the constituent neurones of the olfactory bulb has now been studied in rabbits at varying intervals after unilateral removal of the olfactory mucosa. The rabbits were operated on at the age of 6 weeks, and two animals were killed at each of the survival times. Ten days after operation there is little difference between the olfactory bulbs of the two sides, but by 17 days a striking difference has developed; very few mitral, tufted and periglomerular cells are seen on the operated side, and those that are present are scattered over the entire cross-sectional area of the bulb. The characteristic dense felt-work of dendrites and recurrent collaterals in the external plexiform layer is almost completely absent on the operated side. The difference in impregnation of the mitral cells is also clearly shown in the accessory bulb. The same changes are found at survival periods of 24, 32 and 49 days, and the only mitral cells found on the operated sides of these brains are in small groups, usually in the ventro-lateral parts of the bulbs, subjacent to where



some of the olfactory fibres are preserved. At these longer periods the bulb as a whole is considerably shrunken, and a new type of cell is found at the level of the mitral cell layer. The soma of this cell is the same size as a mitral cell, but it has numerous fine, irregular processes on both its superficial and deep aspects. It is difficult to determine whether this cell is glial in nature or an altered mitral cell. At all these survival times the unimpregnated periglomerular, tufted and mitral cell bodies can be readily stained, on the same Golgi section, with thionin, and in other Bodian-stained material the dendrites of these cells, although attenuated, are clearly seen. In many of the experiments involving long survival the neurones of the anterior olfactory nucleus and of the pyriform cortex on the operated side show a less extensive dendritic arborization. The reasons for considering that these changes are the result of the experimental denervation and not simply an expression of the unreliability of the Golgi method have already been discussed<sup>1</sup>.

These findings confirm the earlier observations that cells undergoing transneuronal degeneration in the olfactory bulb are more resistant than normal cells to impregnation with the Golgi method, and extend them in showing that this change in reaction to the Golgi impregnation occurs at the same time as shrinkage of the cell body becomes evident in sections stained with the Nissl method. In the olfactory bulb the absence of staining, either of the neurone as a whole or of the dendritic branches, does not mean that these cells or processes have been lost, for the cell-bodies of the affected neurones can be demonstrated with the Nissl stain, and their dendrites with the Bodian method. In the pyriform cortex, where Golgi material showed a marked loss of dendritic spines after interruption of afferent fibres from the olfactory bulb, electron microscopy showed the persistence of the spines even in association with degenerated presynaptic endings<sup>5</sup>. It is possible, therefore, that the dendritic spines in other areas of the cerebral cortex which do not impregnate with the Golgi method after partial deafferentation<sup>4</sup> are also not lost but are altered so that they do not react. The basis for this change in staining properties is not known, but may be elucidated by electron microscopic studies of transneuronal cell degeneration, and this in turn may provide an explanation for the selective impregnation of normal neurones.

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### Growth Characteristics of Mouse Thymus in the Neonatal Period

ALTHOUGH renewed efforts have been made towards an understanding of the function(s) of the thymus, the exact mechanisms involved remain unclear. It is not known whether the thymus serves primarily as a source of immunologically competent cells, or whether it produces a substance or substances and/or contact factors which may control proliferation and immunological maturation of peripheral lymphoid cells. Combinations of such suggestions have been made repeatedly<sup>1,2</sup>. A meaningful evaluation of the existence and magnitude of thymic cell migration during the perinatal period of life requires more information on at least the following thymic growth characteristics as a function of age before and after birth: (a) the rate of thymic growth; (b) proliferative activity of thymic lymphoid cells; (c) the generation time of thymic lymphoid cells; and (d) a quantitative estimate

of intrathymic cell death. We report here an investigation of growth and proliferative activity of the thymus of mice ranging in age from 3 days before to 6 days after birth.

Specific pathogen-free Swiss albino mice (Hale-Stoner strain) of both sexes were used. Mating time was limited to 8 h to allow a more accurate estimate of the gestation time. One pregnant female in each cage was allowed to deliver on term and served as a reference for determination of the age of foetuses killed before birth. Tritiated thymidine (specific activity 1.9 c./mmole, diluted in buffered saline, pH 7.4, to contain 100  $\mu\text{C}/\text{ml}$ .) was administered as a single intra-abdominal injection of 0.5  $\mu\text{C}/\text{g}$  of body weight. Injections were given to four animals in each age group 3, 2 and 1 day before birth (0.5  $\mu\text{C}/\text{g}$  of body weight of tritiated thymidine given to the mother by the intra-abdominal route), immediately after birth, and 1, 2, 3, 4, 5 and 6 days after birth. The animals were killed with ether 30 min after the injection of tritiated thymidine. The thymus was removed and minced in a drop of normal mouse serum. Smears were made with a fine brush. The air-dried preparations were fixed in absolute methanol for 10 min, dipped in liquid 'NTB-2' emulsion (Kodak, Rochester, New York), and exposed at 4°C in dehumidified air for 51 days. The developed preparations were stained with buffered Giemsa solution, 1:100, pH 6.0. An average of 1,000 cells was evaluated from each mouse. Lymphoid cells were classified according to their morphological and tinctorial characteristics as described previously<sup>3</sup>. Mitotic figures were recorded separately. As a correction for background, only cells with four and more grains were considered to be labelled. In a total of 260 additional

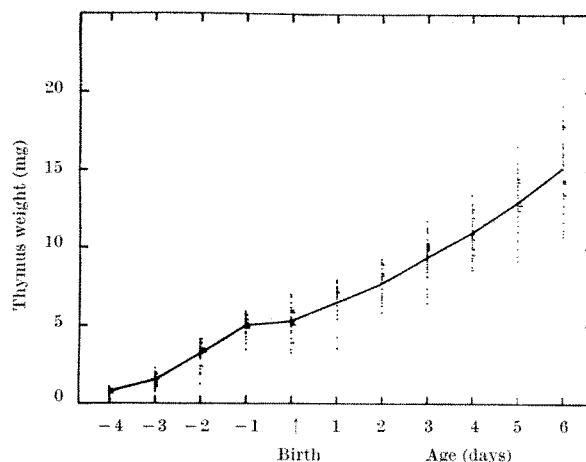


Fig. 1. Thymic weights of Swiss mice in relation to age.

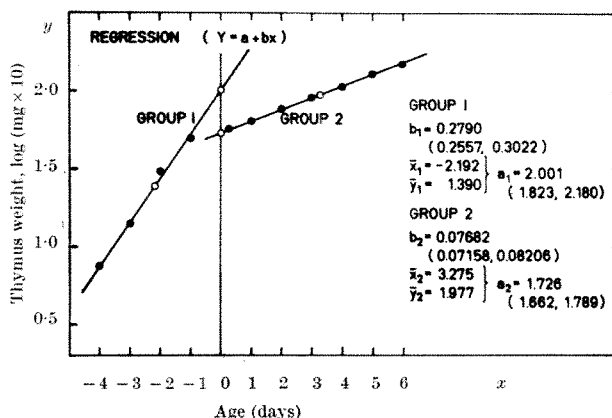


Fig. 2. Regression lines calculated from logarithmically transformed individual thymic weights.

Table 1. ANALYSIS OF VARIANCE

Variance:	Group 1				Group 2				Significance
	Sum of squares	Degrees of freedom	Mean squares	F	Sum of squares	Degrees of freedom	Mean squares	F	
resulting from regression	6.08	1	6.08	608**	4.27	1	4.27	804**	Significant
of means of sub-groups about regression	0.08	2	0.04	4 <sup>-</sup>	0.01	5	0.002	1 <sup>-</sup>	Not significant
within sub-groups	0.76	74	0.01	—	0.93	175	0.005	—	—
Total variance	6.92	77	—	—	5.21	181	—	—	—

animals (from twenty to thirty in each age group), wet weights of thymus were recorded.

Wet thymic weights of mice aged from 4 days before to 6 days after birth are given in Fig. 1. Thymic weights increased at a steady rate from 4 to 1 day before birth; from that time thymic weights continued to increase steadily but apparently at a diminished rate. Calculation of regression lines based on logarithmically transformed individual weight measurements (Fig. 2) made it possible to test whether or not the relative weight increase before birth (group 1) was statistically different from that observed after birth (group 2). Based on the slopes of the regression lines we calculated that before birth thymic weights increased daily by a factor of 1.911 (95 per cent confidence limits, 1.812, 1.996) whereas the relative daily weight gain after birth was 1.195 (95 per cent confidence limits, 1.189, 1.208). A *t* test on these two figures showed that the difference was very significant ( $P < 0.001$ ). An analysis of variance of the two regressions proved both to be linear to a significance level of 1 per cent (Table 1). The body weights measured in similar age groups did not allow the calculation of linear regressions, and so the increase in body weight could not be directly compared with that of thymic weight.

The number of mitotic figures counted in 1,000 thymic lymphoid cells for each age group before and after birth is plotted at the top of Fig. 3. Throughout the period of observation the number of thymic cells in mitosis ranged from nine to thirteen/1,000 cells with the exception of a

peak number of mitotic figures of seventeen and nineteen/1,000 cells at 3 and 4 days, respectively. Combined initial labelling indices of all classes of thymus cells are shown in the middle part of Fig. 3. The overall labelling index did not fluctuate appreciably from 3 days before to 2 days after birth: from 8 per cent to 11 per cent of thymic cells contained four grains or more. Three and four days after birth, however, initial labelling indices of almost 20 per cent were observed, with a reduction to about 15 per cent on days 5 and 6. Overall mean grain counts of initially labelled thymic lymphoid cells with four grains or more are shown at the bottom of Fig. 3. Mean grain counts remained at less than twenty grains/labelled cell up to the age of 2 days. An increase to thirty and forty-eight grains/labelled cell was noted 3 and 4 days after birth. Two days later, the mean grain count had diminished to twenty grains. These data show that the thymic growth rate decreases sharply immediately before birth, and that a marked increase in the relative proportion of proliferating cells was observed after 3–4 days without a concomitant increase in the weight of the organ.

The observation of a prenatal change in the rate of growth of the thymus is consistent with and extends the findings of Ball<sup>4</sup>, who investigated the total number of thymic lymphoid cells in a limited number of mice of different age groups (3–10 mice/group) with the aid of a Coulter counter. He found that the total lymphoid cell population increased approximately ten-fold between 4 and 2 days before birth: after a short stationary period, only a five-fold increase in the total number of cells was noted between 2 and 14 days after birth. This break in the weight curve of the thymus<sup>5</sup> can be explained by (a) an increased migration of cells out of the thymus; (b) a prolongation of the mean generation time of proliferating lymphoid cells in the thymus; (c) a diminished influx of cells; (d) a rate of intrathymic cell death after birth which is greater than that occurring before birth; or (e) two or more of these possibilities combined.

In evaluating the autoradiographic data the possibility has to be considered that a certain number of actively proliferating cells with a low labelling intensity may have been missed because of the relatively low specific activity of the injected tritiated thymidine; the same small doses were used in a later experiment for multiple injections and permitted thus to remain below concentrations causing radiation damage. Autoradiographic inefficiency may have been another reason for missing weakly labelled cells, for only cells with four and more grains were considered to be labelled. Fig. 2 shows that with a progressive background correction the shape of the curve did not change appreciably. On the other hand, during the proliferative peak 3–4 days after birth the number of cells in mitosis increased from ten/thousand to nineteen/thousand cells, while the number of cells incorporating tritiated thymidine initially, at the same time, increased from 10 to 18 per cent. This clearly indicates that at that particular time only a small percentage of cells could have been missed as being unlabelled because of autoradiographic inefficiency. It therefore seems justified to say that a greater number of thymic lymphoid cells were in proliferation 3–4 days after birth than at any time during the period of observation. Although on brush preparations no distinction could be made between cells of cortical and medullary origin it may be assumed, nevertheless, that most of the proliferation takes place

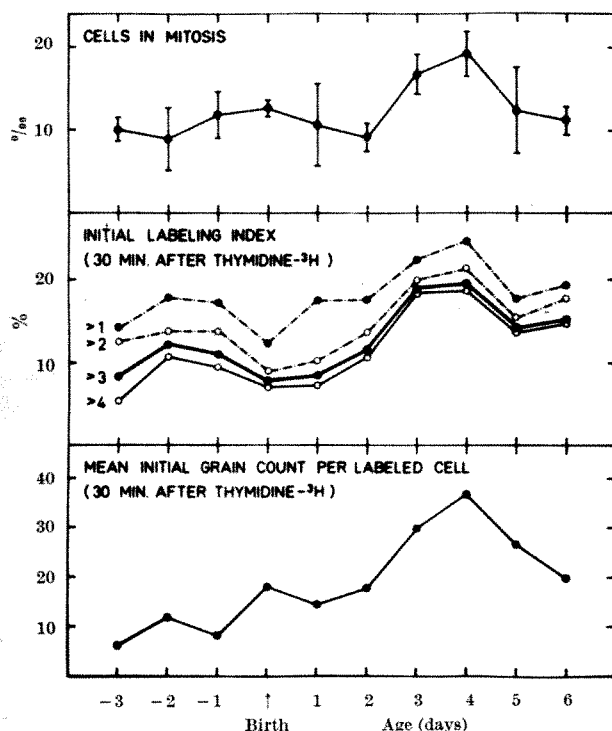


Fig. 3. Top, number of mitotic figures/1,000 cells of mouse thymus in relation to age. Middle, initial labelling indices of thymic cells in relation to age (progressive background correction:  $>1$  = two and more grains;  $>2$  = three and more grains;  $>3$  = four and more grains;  $>4$  = five and more grains). Bottom, mean grain counts of initially labelled thymic cells (four and more grains) in relation to age.

in the thymic cortex as visualized by autoradiography of histological sections and because of a cortex : medulla ratio of 9 : 1.

No change in the thymic weight curve was observed during the peak in proliferative activity. The following explanations may apply: (a) an increased migration of unlabelled small lymphoid cells out of the thymus causes a relative increase of initially labelled medium and large cells; (b) an increased rate of intrathymic cell death compensates for increased proliferation; or (c) a postnatal increase in generation or mitotic time of thymic lymphoid cells explains the unchanged growth rate at a time of increased proliferation. A combination of any of these explanations could be the answer, and discussion has to await further results.

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## Organization of Somatic Afferents to the Diencephalon

THREE main areas within the diencephalon are known to be concerned with somatosensory mechanisms; these are the ventrobasal complex, the intralaminar complex, and the posterior group. It is generally accepted that the afferents to the ventrobasal complex are organized in a simple somatotopic fashion<sup>1,2</sup>, whereas the input to the intralaminar complex<sup>3,4</sup> and to the posterior group<sup>4,5</sup> is non-somatotopic and generally of long latency. The arrangement of afferents to the ventrobasal complex may be more complex than previously supposed<sup>6,7</sup>, and the lateral thalamic nuclei may also be involved in somatosensory activity<sup>7</sup>. Furthermore, the properties of the nuclei of the dorsal column show a rostro-caudal differentiation<sup>8,9</sup>, and it has been suggested that this is reflected in a differential termination of the medial lemniscus<sup>10</sup>. We have investigated the distribution of degeneration in the rat diencephalon using the Nauta method<sup>11,12</sup> after lesions made separately in the gracile and cuneate nuclei, the spinal cord at different levels from the spinomedullary junction to the lumbar region, and the trigeminal nuclear complex.

The projection from the caudal half of the nuclei of the dorsal column conforms to the classical distribution of the medial lemniscus, except for a small projection to the contralateral medial (magnocellular) part of the medial geniculate complex. Within the contralateral ventrobasal complex pars externa the lemniscus is distributed in a topographical pattern corresponding to the somatotopy

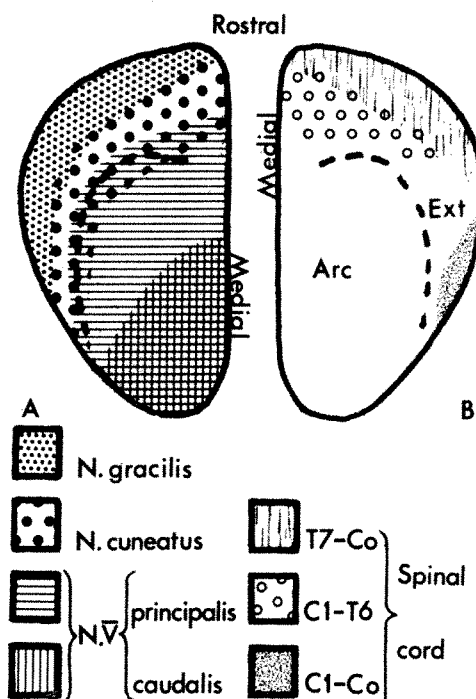


Fig. 1. Scheme of afferents to the rat ventrobasal complex as viewed from above. The curving hatched line represents the boundary between the arcuate (Arc) and external (Ext) parts of the complex (A). The distribution of fibres from the nucleus gracilis and nucleus cuneatus is shown. The representation of afferents from the trigeminal nuclei (N.V.) is drawn to emphasize the difference between the subnucleus caudalis and the other subnuclei. B shows the projections from the spinal cord. The first cervical to sixth thoracic (C1-T6) and seventh thoracic to coccygeal (T7-Co) segments project rostrally. All segments (C1-Co) have an additional projection caudally, but this arises largely in the first two cervical segments.

revealed by the evoked potential method<sup>6</sup> (Fig. 1A). The projection from the rostral half of the nuclei of the dorsal column to the ventrobasal complex is organized similarly. In each case axosomatic endings are found. In addition, an unorganized projection from the rostral parts of the nuclei of the dorsal column ends contralaterally in the medial part of the medial geniculate complex, in the deep part of the anterior pretectal nucleus, in the zona incerta and in the dorsal part of the posterolateral thalamic complex. With the exception of the zona incerta all these regions also receive fibres from the spinothalamic tract which in addition distributes diffusely to the nucleus centralis lateralis and the lateral part of the nucleus medialis dorsalis. Within the ventrobasal complex the pattern of termination differs from that of the medial lemniscus in that the spinothalamic tract ends predominantly in the rostral one third of the complex, although showing the expected mediolateral topography (Fig. 1B), and the endings are not axo-somatic. Each segment of the cord also projects to the caudal part of the ventrobasal complex in an area restricted almost entirely to the field of distribution of the nucleus gracilis (Fig. 1B). This latter projection, however, arises largely in the first two cervical segments, but shows some slight evidence of topographical ordering with more caudal segments projecting ventrally. Lesions restricted to the dorsal horn of the second and third cervical segments do not reveal the more heavy (cervical) component of this projection, and so it is thought to arise in the lateral cervical nucleus. These same most cranial two segments also send a very small projection to the parafascicular-centrum medianum complex, ending predominantly in its dorsolateral part. Degeneration in this complex after high cervical cordotomy has been attributed to damage to the subnucleus caudalis of the trigeminal nucleus<sup>13</sup>, but we have failed to produce such degeneration by making lesions confined to either the subnucleus caudalis or to the second and

third segment dorsal horns. This projection has also been considered to be to the most caudal parts of the nucleus centralis lateralis and not to the parafascicular-centrum medianum complex proper<sup>14</sup>. Our results are largely, but not completely, in accord with this explanation. We have found no evidence of a projection from any of the above systems to the homologue of the posterior group, unless the posterolateral complex and medial part of the medial geniculate complex are included in this term. The rat posterior group (in the narrow sense of the term) does, however, appear to receive fibres from the medullary and pontine reticular formation. In no experiment have we found degeneration in the thalamic reticular nucleus.

It is widely accepted that the spinothalamic system has two components, only one of which reaches the ventrobasal complex. It is now clear that the same is true of the dorsal column-medial lemniscus system, although the diencephalic distribution of the extra-ventrobasal components of the two systems is not identical. While the observation that all parts of the dorsal column nuclei send afferents to the ventrobasal complex is interesting (in view of their afferent functional subdivision<sup>8</sup> and the fact that the properties of ventrobasal neurones are not identical with those of neurones in any part of the dorsal column nuclei<sup>15</sup>), even more striking is the complexity of organization of the long afferents to the ventrobasal complex we have revealed. The caudal distribution of the afferents from the spinal cord which we believe to arise partly in the lateral cervical nucleus is compatible with observations in the cat<sup>16</sup>, and this region of the rat ventrobasal complex appears to be that in which a "second somatic sensory area" has been described<sup>6</sup>. In the cat this area may correspond at least in part to the "zone of convergence" of dorsal column-medial lemniscus and spino-cervico-thalamic activity in the posteroventral nucleus<sup>7</sup>. The extension of this zone into the lateral thalamic complex is compatible with our findings. The differential distribution of the direct spinothalamic system within the ventrobasal complex has not been noted in primates<sup>13</sup>, and although a rostrocaudal organization has been described in the cat<sup>17</sup> it does not at all correspond with our findings. Further work must determine whether these species differences are real. Our present work suggests that any differences in the mechanisms of cutaneous sensibility in different species are reflected in a changing pattern of organization of the spinothalamic system rather than of the medial lemniscus.

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## Projection to the Spinal Cord from the Medial and Descending Vestibular Nuclei of the Cat

THE principal projection from the vestibular nuclei to the spinal cord of the cat is the vestibulospinal tract, which originates in Deiters's nucleus<sup>1</sup>, but fibres from the vestibular nuclei also reach the cord through the descending medial longitudinal fasciculus (MLF). There is uncertainty concerning the origin of this bundle: some authors have suggested that some of its fibres originate in the medial and descending nuclei<sup>1</sup>, while others believe that the descending nucleus does not contribute to this projection<sup>2</sup>. Many descending MLF fibres may be branches of dichotomizing axons, the other branch of which enters the ascending MLF<sup>1</sup>. The descending MLF fibres terminate in parts of lamina VII and lamina VIII of the spinal cord<sup>2</sup>, but their exact connexions and function are not known; impulses in the descending MLF may produce depolarization of primary afferent terminals, perhaps by relays through propriospinal pathways<sup>3</sup>.

In the present experiments we have recorded from cells in the medial and descending vestibular nuclei in acutely decerebellated cats anaesthetized with chloralose-urethane, paralysed with 'Flaxedil' and artificially respired. Throughout the experiment the expired carbon dioxide was maintained at normal physiological levels and the blood pressure was maintained above 80 mm mercury. The stimulating and recording equipment and much of the dissection and preparation of the animal were as previously described<sup>4</sup>. The descending MLF was antidromically stimulated by an array of two concentric steel electrodes. The protruding inner electrode was inserted perpendicular to the spinal cord near the midline at C3 so as to lie near the fibres which are along the anterior median fissure<sup>5</sup>. The ascending MLF was also antidromically stimulated by a similar electrode array, inserted at an angle of about 45° into the midline of the floor of the fourth ventricle, about 2 mm rostral of Deiters's nucleus. Stimuli to the membranous labyrinth were delivered through a fine platinum ball electrode, inserted as deeply as possible towards the ipsilateral fenestra vestibuli and held in place with dental cement. Glass micropipettes, filled with 2 molar sodium chloride saturated with fast green FCF and selected for initial resistances of 2-5 megohms, were used to record the activity of single units extracellularly and to mark the position of the electrode tip<sup>6</sup>; a mark was made in almost every track. Numerous tracks were made over the whole extent of the medial nucleus (except for its rostralmost tip) and in the descending nucleus, usually within 400  $\mu$  of its border with the medial nucleus. Cells were found by searching in the nuclei while stimulating the ascending and descending MLF at a rate of 5 stimuli/sec. Antidromic impulses were identified by their fixed latency at varying strengths of stimulation, by their response to high frequency stimulation and by collision with spontaneous impulses<sup>4</sup>. It was often easy to determine from the shape and size of the potentials whether responses evoked by stimulation of different inputs were those of the same unit. In questionable cases the identity of the doubtful response was tested by attempting to produce collision block of a known antidromic response (Fig. 1B). At the end of the experiment the animal was perfused and the brain stem was removed, fixed, sectioned serially at 100  $\mu$  on a freezing microtome and stained for cells and fibres<sup>6</sup>. The position of the cells in each track was estimated from the location of the mark in that track.

290 cells, with spike amplitudes ranging from 50 to 1,000  $\mu$ V, have been studied in thirteen experiments. Of 146 medial nucleus cells tested with the descending MLF stimulus, 24 (16 per cent) were activated with latencies ranging from 0.5 to 2.2 msec and hence projected into the descending MLF. In contrast, 85 of 211 (40 per cent) medial nucleus cells projected into the ascending MLF.



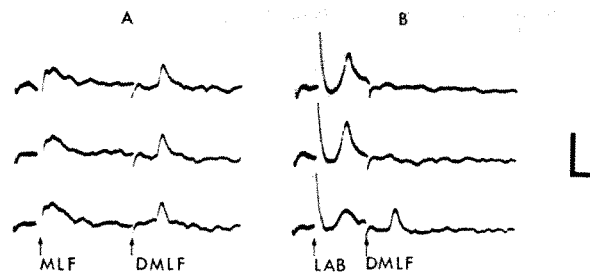


Fig. 1. Responses of cell located in the rostral half of the descending nucleus, 200  $\mu$  from its border with the medial nucleus. A, This unit does not respond to stimulation of the ascending medial longitudinal fasciculus (MLF), but is antidromically driven by stimulation of the descending MLF (DMLF). B, The cell is fired monosynaptically by stimulation of the labyrinth (LAB). The stimulus is near threshold, and in the lowest sweep the cell fails to fire; the LAB stimulus produces only a focal potential, and the DMLF stimulus evokes an antidromic response. In the two upper sweeps LAB stimulation evokes a monosynaptic response on top of the focal potential and the DMLF response is blocked by collision. Calibration, 500  $\mu$ V; time, 1 msec. Upward deflexion negative.

Although cells with axons in the descending MLF were found throughout the medial nucleus, eighteen out of twenty-four were found rostral to its mid-point. Cells were found dorsally, ventrally, medially and laterally and in each of the quadrants the fraction of units driven antidromically by the cord stimulus was similar to the fraction driven antidromically in the population as a whole. In the area of the descending nucleus which we explored, nine out of thirty-one cells had axons in the ascending MLF and thirteen out of twenty-five units projected into the descending MLF (antidromic latencies, 0.9–2.0 msec). The response of one of the latter units is shown in Fig. 1. Of all units projecting to the spinal cord, five of twenty-two medial units and three of twelve descending units were also invaded antidromically as a result of a stimulus to the ascending MLF. While it is very likely that not all axons, ascending and descending, were activated by our stimuli, this finding suggests that many of the axons of cells in the medial and descending nuclei projecting to the spinal cord are not branches of dichotomizing fibres.

Many units in the medial and descending vestibular nuclei were fired by stimulation of the ipsilateral labyrinth at a repetition rate of 1/sec. Of the total sample, 36 per cent (62/171) of the medial and 40 per cent (12/30) of the descending cells were fired monosynaptically (Fig. 1) at a latency, to strong shocks, of 0.9–1.5 msec after the stimulus artefact<sup>7,8</sup>. Most driven cells responded once to every stimulus. Of the medial cells with axons in the ascending MLF, 30 per cent (18/61) were monosynaptically activated by stimulation of the labyrinth. Of the medial cells that could not be identified antidromically but were found because they were firing spontaneously, 14 per cent (7/51) were monosynaptically activated. In contrast, labyrinthine stimulation caused monosynaptic firing of 73 per cent (16/22) of those medial nucleus units, and 64 per cent (7/11) of those descending nucleus units with axons in the descending MLF. Many medial (55/153) and descending (17/32) cells were driven polysynaptically by stimulation of the labyrinth; a few of these cells also responded monosynaptically. But only three out of twenty-one medial and four out of twelve descending units antidromically activated from the descending MLF were polysynaptically fired by stimulation of the labyrinth. The units projecting to the cord as a group therefore received a particularly marked monosynaptic input from the labyrinth. These results are similar to those obtained with Deiters's units projecting to the cervicothoracic cord by way of the vestibulospinal tract: while 51 per cent (31/61) of these cells were monosynaptically driven from the labyrinth, only 5 per cent (3/61) were driven polysynaptically<sup>8</sup>.

The results of our experiments confirm anatomical findings<sup>1,2</sup> and show that the medial and lateral parts of the medial vestibular nucleus send fibres to the spinal cord in the descending MLF and that this projection is a modest one compared with both the spinal projection of the lateral vestibular nucleus and the ascending projection of the medial nucleus. It is quite certain that the medial part, at least, of the descending nucleus also contributes to the descending MLF. This system, considered by all authors to reach no further caudally than the mid-thoracic cord<sup>1,2</sup>, has a strong direct input from labyrinthine receptors. This finding, together with our previous report that the cervicothoracic component of the vestibulospinal tract is relatively more subject to labyrinthine control than the lumbosacral component<sup>8</sup>, provides an explanation for observations that the motor activity of the cervicothoracic cord is more influenced by the labyrinth than the motor activity of the lumbosacral cord<sup>1</sup>.

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### Effect of Tetrodotoxin on the Electrical Activity of the Smooth Muscle of the Vas Deferens

THE action potentials of vertebrate smooth muscles differ from those of nerve and fast skeletal muscles in several ways. For example, replacement of up to 80 per cent of the sodium salts of the bathing solution with sucrose has little or no effect on the action potential<sup>1</sup>. Provided that allowance is made for the possible redistribution of Na<sup>+</sup> ions in a low Na<sup>+</sup> environment and if it is assumed that relatively few channels are available for the inward movement of Na<sup>+</sup>, it is possible to account for this finding in terms of the ionic theory of Hodgkin and Huxley<sup>2</sup>. On the other hand, the slow rate of depolarization of the spikes of the taenia coli<sup>3</sup>, vas deferens<sup>4</sup> and uterus<sup>5</sup>, and the graded nature of the spikes in response to electrical stimulation<sup>3,4</sup>, are characteristics which resemble the properties of crustacean skeletal muscle. Fatt and Ginsborg<sup>6</sup> have shown that the spike mechanism in crustacean muscle involves an increase in conductance for divalent cations rather than Na<sup>+</sup> ions. It has been suggested that Ca<sup>++</sup> ions may also be involved in the action potentials of smooth muscle<sup>7</sup>.

The puffer-fish poison, tetrodotoxin, does not block the spikes of crayfish muscle<sup>8</sup> and evidence is accumulating that this toxin acts specifically on the voltage dependent increase in Na<sup>+</sup> conductance which is characteristic of cells specialized for the conduction of excitation<sup>9</sup>. It was of

interest therefore to study the action of tetrodotoxin on smooth muscle. The vas deferens was used for the present experiments because, despite its dense innervation, individual cells are electrically excitable and many cells respond to intracellular stimulation with self-regenerative spikes<sup>4</sup>.

Test solutions of tetrodotoxin were freshly prepared from stock solutions which were made by dissolving the buffered toxin obtained from Sankyo (Tokyo) in distilled water. Stock solutions were kept at about 5° C for no longer than one month. The potency of the toxin was tested by determining the concentration which blocked the action potential of toad skeletal muscle ( $10^{-7}$  g/ml.).

Isolated preparations of vas deferens supplied by the hypogastric nerve were dissected from guinea-pigs and mice which had been stunned and bled out. Preparations were mounted in a constant temperature bath (35° C) which was perfused with a modified Krebs solution, as previously described<sup>4</sup>.

A single micro-electrode was used to pass current and to record the membrane potential of individual smooth muscle cells by means of the bridge circuit described by Martin and Pilar<sup>10</sup>. The voltage displacement due to current flow across the membrane could be distinguished from that due to the electrode tip by the relatively long membrane time constant of the smooth muscle cell (2–6 msec).

Fig. 1A shows a typical spike in response to intracellular stimulation with a current of 1.7 n.amp. Fig. 1B was recorded from the same preparation (guinea-pig vas deferens) after exposure to tetrodotoxin,  $10^{-7}$  g/ml. for 40 min. At this time the response to nerve stimulation was completely blocked.

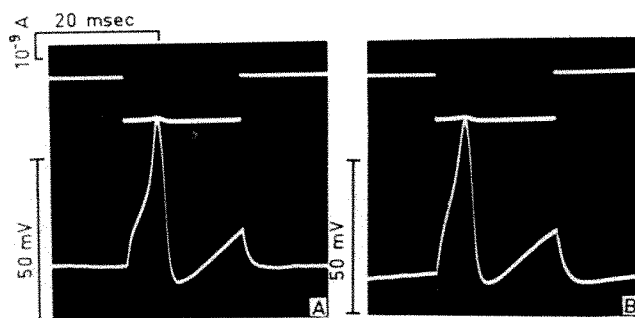


Fig. 1. Action potentials in response to intracellular stimulation (lower traces) by current pulses (upper traces) recorded from a preparation of guinea-pig vas deferens; record A taken in normal solution, and record B after 40 min exposure to tetrodotoxin  $10^{-7}$  g/ml.

In normal conditions the amplitude and time course of the spikes in response to intracellular stimulation vary from cell to cell. The currents needed to elicit spikes of maximum amplitude (up to 75 mV) also vary, probably due to the non-uniformity of the dimensions of individual cells and the variable position of the micro-electrode<sup>4</sup>. It was not always possible to maintain an impalement for long enough to compare the spikes recorded in the same cell before and after tetrodotoxin. The characteristics of smooth muscle cells impaled in the presence of the toxin were, however, indistinguishable from those of normal cells. Self-regenerative spikes—spikes which continued to develop after the cessation of the current pulse—were just as common in the presence of the toxin as in control experiments. The concentrations of toxin studied in these experiments (up to  $10^{-6}$  g/ml.) did not appear to have any

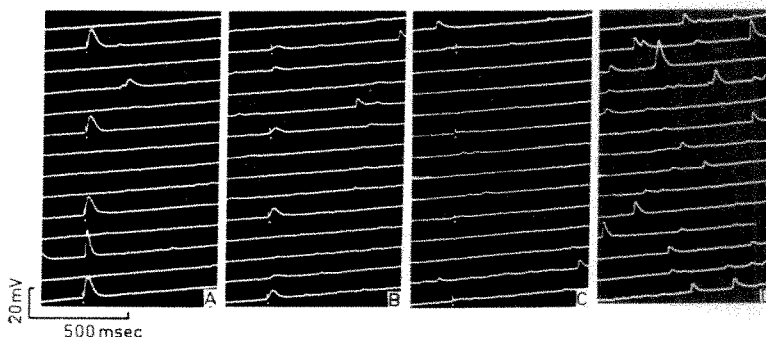


Fig. 2. Effect of tetrodotoxin,  $10^{-7}$  g/ml., on spontaneous junction potentials and junction potentials in response to intramural nerve stimulation (white dots) recorded from a preparation of mouse vas deferens. Record A taken in normal solution, records B, C and D, 3 min, 5 min and 11 min after exposure to the toxin. Note the increase in frequency of the spontaneous junction potentials in record D.

effect on the electrical excitability of either mouse or guinea-pig vas deferens.

Thus the action of tetrodotoxin on these smooth muscles is similar to its action on muscles which normally give graded responses to stimulation<sup>9</sup>. It may be tentatively concluded that an increase in  $\text{Na}^+$  conductance is not essential for spike generation. The vas deferens is, however, a densely innervated tissue and conduction of excitation from cell to cell probably does not occur in normal circumstances. It may be that an  $\text{Na}^+$  mechanism is involved in the generation of action potentials in other smooth muscles where conduction occurs over long distances (for example, the ureter).

Fig. 2 shows the effect of tetrodotoxin ( $10^{-7}$  g/ml.) on the junction potentials recorded from a preparation of mouse vas deferens. Fig. 2A, recorded in normal solution, shows a series of junction potentials in response to stimulation of intramural nerve fibres, together with spontaneous junction potentials occurring in the absence of nerve stimulation. In this experiment the strength of nerve stimulation was just above threshold and the junction potentials were too small to trigger action potentials. Fig. 2B was recorded 3 min after exposure of the test solution at a time when the response to nerve stimulation was failing. In Fig. 2C, 5 min after exposure, the junction potentials were completely blocked. The spontaneous junction potentials persisted in the presence of tetrodotoxin, however, and 11 min after the response to nerve stimulation was blocked their frequency was markedly increased (Fig. 2D). It is interesting that a similar increase in the frequency of miniature end-plate potentials was observed by Kao and Nishiyama<sup>11</sup> in experiments on the frog skeletal neuromuscular junctions in the presence of saxitoxin which also has a specific blocking action on  $\text{Na}^+$  conductance.

In order to test whether blockade of transmission by tetrodotoxin in the vas deferens resulted from failure of the nerve impulse, action potentials were recorded from the hypogastric nerve. The proximal and distal ends of the nerve were held in oil for stimulating and recording respectively, while the central portion was immersed in modified Krebs solution to which the toxin was added in concentrations ranging from  $10^{-7}$  to  $10^{-5}$  g/ml. The B fibre elevation and part of the polyphasic C fibre elevation were rapidly blocked. This effect was reversible provided that the nerve was exposed to the drug for only a few minutes. Part of the C fibre elevation was, however, somewhat resistant to the toxin. The nature and origin of these toxin resistant C fibres remain to be established.

Because tetrodotoxin is able to block the response to nerve stimulation without affecting the response of the smooth muscle of the vas deferens to transmitter or its electrical excitability, tetrodotoxin is likely to prove a useful tool for further studies on the transmission

of excitation at this autonomic nerve smooth muscle junction.

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### Rapid Movement of Epinephrine and Norepinephrine into Human Erythrocytes

HUMAN erythrocytes are permeable to many substances such as catechol, *p*-phenylenediamine and sulphanilamide<sup>1</sup>. Knowledge about the entry of the catecholamines into erythrocytes was the objective of the present study. The technique used required incubation of freshly drawn heparinized human blood with a radioactive catecholamine for various periods of time. Ultrafiltration was used because of the poor recovery of unchanged epinephrine and norepinephrine during chemical deproteinization procedure in red cells.

Radioactive epinephrine or norepinephrine hydrochloride, with tritium attached to the proximal carbon on the side chain, was used. One-tenth ml., containing 0.4 mc. and 0.011  $\mu$ g of epinephrine, was added to 4 ml. of blood and 4.0 ml. of 0.1 molar sodium phosphate buffer of pH 7.4 at zero time. Typically, 4.1 ml. of blood containing the radioactive epinephrine was incubated in a water bath at 37° C. The blood was then centrifuged at approximately 2,500 r.p.m. for 1 min. The separated cells were haemo-

lysed in boats made of aluminium foil, which were placed into liquid nitrogen. An equal volume of water was added to the thawed cells to facilitate handling.

Ultrafiltration was performed (Fig. 1) in cellulose tubing 4 in. in length and 0.25 in. in diameter<sup>2</sup>. To 10 ml. of a scintillation solution was added 0.1 ml. of the ultrafiltrate plus either 0.1 ml. of water or 0.1 ml. of the epinephrine standard. Scintillations were counted for 10 min in a Packard 'Tri-Carb' liquid scintillation spectrometer. Duplicate counts were in agreement within 1 per cent in every instance.

The cellulose tubing was not equally permeable to epinephrine and to water. Regardless of whether the tubing contained a sodium phosphate solution, plasma or haemolysed erythrocytes, the concentration of the radioactivity within the sac was 1.91 times greater than that in the ultrafiltrate. No catecholamine could be separated from the cellulose membrane by soaking or vigorous washing during any phase of the study. Background counts in the absence of added radioactivity were negligible. Duplicate ultrafiltrations of different portions of the same aqueous plasma or cell specimen yielded agreement within 6 per cent in each instance.

The total radioactivity in the plasma or cell layer was determined by multiplication of the scintillation count in 0.1 ml. of the respective ultrafiltrate by 1.91, as well as the volume of the solution undergoing ultrafiltration. Subtraction of the contribution of the 11 per cent of the total plasma remaining in the erythrocyte layer yielded the radioactivity associated with the cells alone. The use of the conversion factor, 1.91, permitted agreement within essentially 100 per cent between the total amount of radioactivity added, as determined in aqueous solution, and the total amount recovered after the ultrafiltrations. The catecholamines were not adsorbed on the erythrocyte walls, because the same counts were found in the ultrafiltrates of haemolysed red cells before and after removal of the erythrocyte ghosts by centrifugation<sup>3</sup> at 12,000g for 30 min.

Incubation of epinephrine with 4 ml. of blood from 120 different human beings yielded ratios of distribution of radioactivity between cells and plasma which differed by no more than 20 per cent from each other, for each time interval tested. The distribution of epinephrine between the erythrocytes and plasma of a typical specimen of blood (haematocrit 45) for various periods of incubation at 37° C is given in Table 1. In the absence of incubation at 37° C, epinephrine could enter the cells during the 90 sec of centrifugation and manipulation of the blood at room temperature. The distribution of epinephrine was expressible as the ratio of its concentrations in the cell water ( $C_e$ ) and plasma ( $C_p$ ) (Table 1). The water content of red cells was found to be 72 per cent (w/v)<sup>4</sup>.

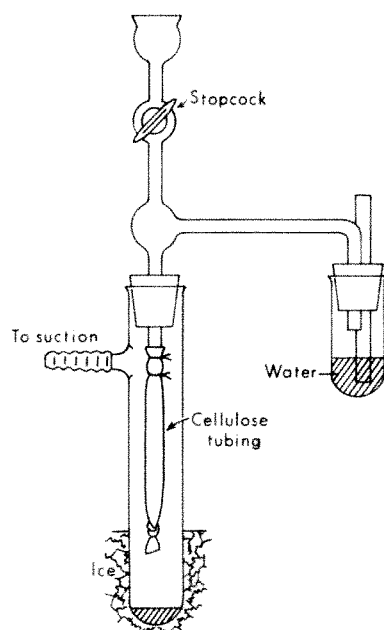


Fig. 1. Diagram of the ultrafiltration apparatus.

Table 1. DISTRIBUTION OF RADIOACTIVE EPINEPHRINE BETWEEN ERYTHROCYTES AND PLASMA OF HUMAN BLOOD

Minutes of incubation of blood at 37° C	Per cent epinephrine in plasma	Per cent epinephrine in cells	$C_e/C_p$
0	75	25	0.60
1	69	31	0.80
3	63	37	1.04
30	62	38	1.09

In each instance, the recovery of radioactivity after passage of the ultrafiltrate through an aluminium oxide column at pH 8.4, followed by elution at pH 3.5, exceeded 80 per cent. This behaviour was typical of epinephrine. In contrast metanephrine was not adsorbed on aluminium oxide<sup>5</sup> and adrenochrome was not eluted at pH 3.5. Oxygenation of the blood, defibrination by stirring, or anticoagulation by (ethylenedinitril) tetra-acetic acid, disodium salt, did not change the results. The findings for norepinephrine-<sup>3</sup>H were essentially the same as for radioactive epinephrine.

Entry of so relatively small a molecule as epinephrine into the ultrafiltrate more slowly than water was consistent with the behaviour of urea, glucose and sucrose<sup>6,7</sup>. No evidence existed for adsorption of the catecholamines on a blood protein<sup>8</sup>. The amount of epinephrine added to each ml. of plasma,  $3 \times 10^{-3} \mu\text{g}$ , was within the range of possible physiological concentrations<sup>9</sup>. In previous investigations<sup>4,10</sup>, the cells were thoroughly washed with Tyrode's solution, and large amounts of a catecholamine were added. The values for  $C_e/C_p$  found after 30 min of incubation were much lower than those in the present study. Active transport may therefore account for the rapid penetration of small amounts of the catecholamines into erythrocytes. Diffusion is the process for larger amounts<sup>10</sup>.

Rapid entry into erythrocytes was consistent with some known aspects of catecholamine behaviour *in vivo* and *in vitro*<sup>11</sup>. In the cat, 90 per cent of an aortic infusion of epinephrine disappeared without physiological effects before reaching the venous circulation<sup>12</sup>. Appreciable amounts of injected epinephrine remained unchanged in the whole mouse after 3 h<sup>13</sup>. In the past, the catecholamine levels within erythrocytes were not determined<sup>9,13,14</sup>. In future studies, this intracellular concentration might be an important variable for study under basal conditions and during stress.

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### Intravenous Infusion of Vasopressin to increase the Sensitivity of its Assay in the Water-Ethanol Loaded Rat

THE work of Heller and Stule<sup>1</sup> has been followed by several reports of methods designed to increase the sensitivity of the rat for the assay of vasopressin. In many of these reports the greater sensitivity has been attributed to a more careful operative technique, which may reduce the basal level of endogenous vasopressin secretion by the rat<sup>2,3</sup>. Czaczkes *et al.*<sup>2</sup>, confirming the earlier work<sup>1</sup>, suggested using pre-operated rats in order to avoid surgical trauma on the day of assay, but Jones and Lee<sup>4</sup> did not find the pre-operated rat to be any more sensitive. It seemed desirable therefore to establish whether a high basal level of vasopressin would, in fact, decrease the response of the rat preparation to vasopressin injections.

Female Wistar rats (140–200 g body weight) were water loaded and infused at 0.1 ml./min with hypotonic glucose-saline as previously described<sup>5</sup>. The volume and conductivity of the urine were recorded and the response

to three or four standard doses (2.5–20  $\mu\text{U}$ ) of arginine vasopressin (Sandoz) determined. Arginine vasopressin was added to the infusion in order to reduce the urine flow by about half (0.5  $\mu\text{U}/\text{min}$  was usually required). The response to vasopressin was determined and again after stopping the vasopressin infusion.

Table 1. DOSE OF ARGININE VASOPRESSIN (IN MICRO-UNITS) REQUIRED TO REDUCE THE URINE FLOW BY 25 PER CENT DURING A CONTROL PERIOD AND DURING AN INTRAVENOUS INFUSION CONTAINING VASOPRESSIN (0.5–3  $\mu\text{U}/\text{MIN}$ )

Control	Vasopressin infusion
20	10
20	10
18	6
15	3
11	5
9	4
8	4
8	3
8	2.5
7	5
7	4
7	3
7	2
5	0.5
4	1.5
2.5	0.5
Median 8 $\mu\text{U}$	Median 3.5 $\mu\text{U}$

Table 1 shows that in each of the sixteen experiments the response to a small dose of vasopressin was enhanced by the infusion although larger doses were usually unaffected (Fig. 1). The increased sensitivity permitted the detection of smaller doses of vasopressin; thus 0.5  $\mu\text{U}$  gave a definite response. We noticed that the effects of small doses became greatly prolonged and closely resembled the records published by Tata and Gauer<sup>6</sup>, who claim to have developed an improved assay. Thus the greater sensitivity in their experiments may have been due to a relatively high basal secretion of vasopressin by the neurohypophysis. The prolonged nature of the response and the tendency of the urine flow to become progressively smaller make a background infusion unsuitable for the routine assay of large doses of vasopressin. Such an infusion should, however, prove useful for the detection and assay of vasopressin at physiological levels in the plasma, but only at the cost of a corresponding reduction in specificity and reproducibility. We are in agreement with earlier workers who found the rat to be most sensitive immediately after completion of the surgical procedure, indicating that endogenous secretion of vasopressin is probably enhancing the response to exogenous hormone.

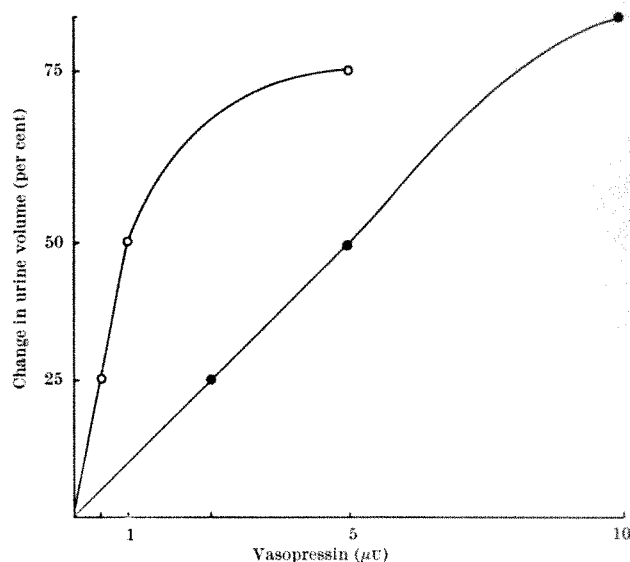


Fig. 1. Effect of an intravenous infusion of arginine vasopressin (1  $\mu\text{U}/\text{min}$ ) on the change of urine volume produced by the intravenous injection of vasopressin. The closed circles are from the control period and the open circles from the period of infusion.



It may seem surprising that the response to vasopressin is enhanced by a background infusion, whereas the effect of oxytocin is reduced<sup>7</sup>. This may be due to the difference in the site of action of the two hormones. Oxytocin acts at the protoplasmic surface membrane of the plain muscle cell<sup>8</sup> where a background infusion might be expected to block the oxytocin "receptors". On the other hand, vasopressin is believed to enter the renal cells at their serosal surface and to exert its action within the cell in which it is subsequently inactivated<sup>9</sup>. Consequently, a background infusion, by saturation of these inactivating mechanisms, would be expected to increase the effect of injections of the hormone.

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## MICROBIOLOGY

### Diaminopimelic Acid in the Mu Particles of *Paramecium aurelia*

Mu particles, found in the cytoplasm of *Paramecium aurelia*, stock 540, syngen 1, are responsible for the mate killing effect of this stock on other syngen 1 stocks after conjugation with them<sup>1</sup>. The particles are visible in the phase contrast microscope as rods 2–5  $\mu$   $\times$  10–5  $\mu$  in size, and several thousand may be present in the cytoplasm of a single animal. Beale and Jurand<sup>2</sup> carried out a cytochemical and electron microscope study of these particles and suggested that they were probably bacterial in nature. Van Wagtenonk, Clark and Godoy<sup>3</sup> succeeded in growing the lambda particle of stock 299 (syngen 4) in a complex medium outside the host cell, thus clearly showing that this particle was a symbiont inside the *Paramecium*. It will be shown here that the mu particle is a bacterium in a similar situation, because the analysis of isolated particles has shown the presence of  $\alpha$ - $\epsilon$ -diaminopimelic acid (DAP), and possibly of muramic acid; these are compounds typical of the bacterial cell wall<sup>4</sup>.

Mu particles were isolated from *P. aurelia* stock 540 by a method which involved fractionation of homogenates in a column of cellulose<sup>5</sup>, followed by density gradient centrifugation in a 10–40 per cent gradient of 'Ficoll'. The final preparation contained more than 95 per cent mu particles by phase contrast microscopy, and was contaminated with a small number of mitochondria and other small cell particulates. The number of viable *Aerobacter aerogenes*, the *Paramecium* food, was 0.1 per cent or less. The yield of mu particles was about 1 mg dry weight from 20 mg dry weight of cells. Control preparations from other syngen 1 stocks lacking mu particles gave preparations which contained no mu particles, although they did contain mitochondria, cell particulates and *A. aerogenes* in about the same quantities as the preparations of mu particles. Yield in control preparations was about 1 mg dry weight material from 500 mg dry weight cells. The preparation method will be described in detail later.

Preparations were analysed by paper chromatography and the 'Technicon' amino-acid analyser. Mu particles (2–4 mg) were hydrolysed in 1 ml. 5 normal hydrochloric acid in a sealed tube at 106° for 14 h. After opening the tube, any debris was centrifuged off, and the supernatant was dried *in vacuo* using an infra-red lamp. The residue was washed and dried three times with distilled water, and the final residue was dissolved in 0.05 ml. water (for paper chromatography) or 0.8 ml. water (for amino-acid analysis). Control preparations were treated in the same way, except that no more than 1 mg of material was hydrolysed, because more was not obtained in any preparation.

Paper chromatograms were on 'Whatman No. 3MM' paper, using phenol ammonia in hydrocyanic acid in the first dimension, and *N*-butanol-acetic acid-water (4 : 1 : 5) in the second. Load was equivalent to 1.5–2 mg dry weight of mu. Chromatograms were dipped in 0.25 per cent ninhydrin in 9 : 1 acetone water and heated at 105° for 10 min.

For analysis in the 'Technicon' amino-acid analyser, samples were made 0.5 normal in hydrochloric acid, 0.2  $\mu$ molar norleucine was added, and the final volume was made to 1 ml. The hydrolysate was kept at 100° for 30 min before application. Elution was with a gradient of pH 2.875–5.0, 0.02–0.6 molar sodium ions, as sodium citrate.

Fig. 1 is a photograph of a mu hydrolysate chromatogram, which shows that mu contains at least eighteen amino-acids; chromatograms of control material from *Paramecium* lacking mu showed only 8–10 spots, all feeble, and the number and appearance were variable, unlike those from mu. The small spot arrowed was found reproducibly in mu hydrolysates but never in control preparations; it has an  $R_F$  of 0.18 in phenol, and an  $R_{gy}$  in butanol-acetic acid-water of 0.22, values similar to those reported by Work and Dewey<sup>6</sup> for diaminopimelic acid. It was possible that this spot was caused by ethanolamine-*o*-phosphoric acid, or an oxidation product of cystine, but analysis in the amino-acid analyser showed that a small peak appeared immediately before isoleucine in the elution pattern; this position is that given by Hamilton<sup>7</sup> for diaminopimelic acid, and corresponded with the elution position of authentic diaminopimelic acid. This peak was not found in the elution patterns of control preparations, which showed only twelve amino-acid constituents compared with the nineteen found in mu preparations.

$\gamma$ -Aminobutyric acid was also present in mu preparations but not in control preparations; according to Work

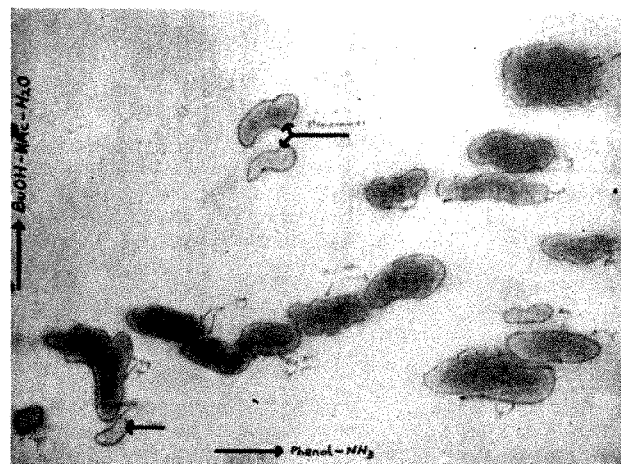


Fig. 1. Chromatogram of a hydrolysate of mu particles, stained with ninhydrin; the diaminopimelic acid spot is arrowed (lower left), and the two hexosamine spots are also arrowed (upper centre). Directions of solvents were as indicated, and the origin was at the lower left-hand corner.

and Dewey<sup>6</sup> this amino-acid is found in many bacteria but is rare in other organisms.

The amount of diaminopimelic acid present was 0.32  $\mu$ M/100 mg, or about 0.07 per cent of dry weight, a value in the range given by Work and Dewey<sup>6</sup>, who in an extensive survey of 118 micro-organisms found diaminopimelic acid in almost all bacteria, but not in yeasts or other fungi, protozoa or plant viruses. It was absent from *Tetrahymena pyriformis*, a close relative of *P. aurelia*. Salton<sup>4</sup> states that the presence of diaminopimelic acid is a distinguishing feature of bacteria and blue green algae, as it is found in the cell wall glycosaminopeptide but in no other cell components.

Attempts were also made to detect the other typical cell wall component of bacteria, muramic acid. While there were usually two hexosamine spots on chromatograms (Fig. 1), and one of these could be identified as glucosamine, the other could not be identified with any certainty as muramic acid, because the ninhydrin and Elson-Morgan reactions were weak and the position of the muramic acid peak in the amino-acid analyser was obscured by the much larger peak of glutamic acid, because of the gradient used for elution; however, there was a small shoulder present on this peak, so it is possible that muramic acid was present.

It may be concluded that the mu particle is a bacterium which has established itself symbiotically in the *Paramecium* cytoplasm, like the lambda particle of stock 299. Further work will aim at investigating biosynthetic capacity in the mu particle, to try to gain an insight into the relationship of protozoan and particle.

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### Orthorhombic Sulphur formed by Photosynthetic Sulphur Bacteria

THE formation of large mineral deposits of elementary sulphur, for example in Sicily, France, Texas and Louisiana, has often been attributed to microbial processes<sup>1-3</sup>. The similarity of stable sulphur isotope ratios in various sulphur bearing minerals<sup>4-8</sup> to fractionation rates obtained in laboratory bacterial cultures<sup>9-12</sup> gives strong support to the biogenic origin of sulphur deposits.

Elementary sulphur is continuously formed by bacterial action, for example in Cyrenaican lakes near El Agheila<sup>13</sup> and in the Wadi Natrûn, Egypt<sup>14</sup>, typical environments ecologically classified as "sulphureta". In a sulphuretum, primarily, sulphate is reduced to sulphide by sulphate-reducing bacteria of the genus *Desulfovibrio*. The sulphide is then oxidized to elementary sulphur either by photolithotrophic *Thiorhodaceae* and *Chlorobacteriaceae* or by chemolithotrophic *Thiobacteriaceae* or atmospheric oxygen. The elementary sulphur is formed as globules inside the cells of the *Thiorhodaceae* and the large colourless sulphur

bacteria (*Beggiatoaceae*, and in part, *Thiobacteriaceae*), but it is formed outside the cells of the *Chlorobacteriaceae* and *Thiobacillus thio-parus*. Whereas *Thiorhodaceae* and *Chlorobacteriaceae* use reduced sulphur compounds as the hydrogen donor for their anaerobic photosynthesis<sup>15</sup>, thiobacilli obtain all their energy from the oxidation of reduced sulphur compounds, in which molecular oxygen is involved<sup>16,17</sup>. The chemolithotrophic nature of *Beggiatoa* and related forms is doubtful according to recent findings<sup>18-20</sup>. The formation of sulphur globules from sulphide in these species could be just a removal reaction of poisonous hydrogen peroxide which is produced during cell metabolism<sup>21</sup>.

Microscopically, the accumulated sulphur seems to be almost identical among these different metabolic types<sup>22</sup>. The sulphur globules have a high index of refraction<sup>23</sup> and some authors have found them to be birefringent in polarized light<sup>24,25</sup>. As we have seen the globules inside the cells of *Thiocystis* (Fig. 1), we suggest that the interference colours observed in polarized light are not caused by the crystal structure of the sulphur but by the spherical form of the tiny globules in the same way that very small air bubbles exhibit interference colours along their boundaries.

In our investigation, by X-ray diffraction, of the nature of the sulphur formed by purple and green sulphur bacteria (*Thiorhodaceae* and *Chlorobacteriaceae*) we used *Thiocystis violacea* strain 8311, *Chromatium* strain 8111, and *Chlorobium* strain 8430. The strains were isolated from salt water environments using the method of Pfennig<sup>26</sup> and grown in screw-cap bottles using Pfennig's nutrient solution plus 3 per cent sodium chloride at 23° C in continuous illumination of 1,000 lux, provided by a 60 W tungsten lamp. To obtain the sulphur globules, the cells were supplied with freshly prepared and neutralized sterile Na<sub>2</sub>S·9H<sub>2</sub>O solution to a final concentration of 0.05 per cent. After illumination with 2,000 lux, that is, light saturation<sup>27</sup>, for about 30 min the cells had transformed the sulphide into sulphur, which was shown by sulphide analysis of the supernatant<sup>27</sup>. The extracellular sulphur globules of the green bacteria were obtained by low speed centrifugation at 800g. The intracellular globules of *Thiocystis* were recovered by washing the cells twice with sterile filtered seawater and resuspending them in double distilled water; this caused the disruption of the cells by osmotic shock<sup>25</sup>. The sulphur was separated from the other cell constituents by centrifugation at 800g. After washing the sulphur thus obtained from green and purple bacteria with double distilled water, we dried the samples over silica gel for 12 h. All steps were carried out at room temperature to avoid modification of the

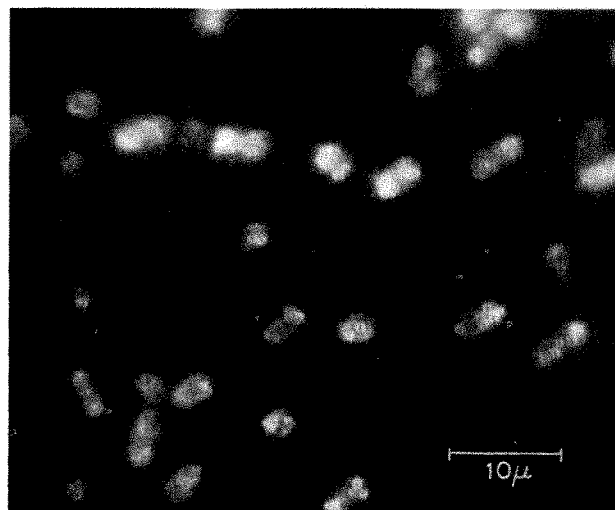


Fig. 1. *Thiocystis violacea*, living cells ( $\times c. 1,450$ ) crossed polars.

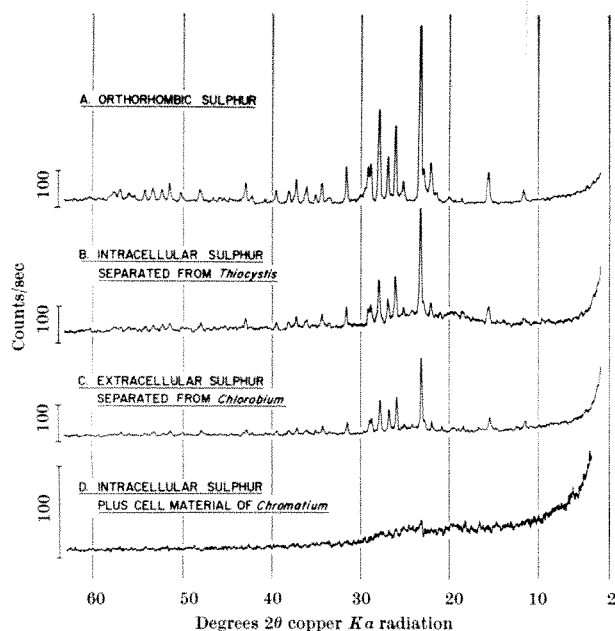


Fig. 2. X-ray diffractometer traces of sulphur samples. Nickel-filtered copper  $K\alpha$  radiation was used with  $\lambda = 1.5418 \text{ \AA}$ , 40 kVp., 40 m.amp.,  $1^\circ$  divergence slit, 0.006 in. receiving slit,  $1^\circ$  scatter slit,  $2^\circ/\text{min}$  scanning speed, time constant 2 sec, scale in c.p.s. as shown. (A) Reagent grade sulphur, orthorhombic structure, powder packed in aluminium holder, 33 mm sample length. (B) Intracellular sulphur from *Thiocystis* mounted on collodion film, 44 mm sample length. (C) Extracellular sulphur from *Chlorobium* mounted on collodion film, 33 mm sample length. (D) Intracellular sulphur and cell material of *Chromatium* freeze dried on collodion film, 33 mm sample length.

sulphur. The powdered samples were mounted on collodion films<sup>28</sup> and subjected to X-ray diffraction. Fig. 2 shows these diffraction patterns compared with that of reagent grade sulphur. All the patterns give diffraction lines that agree with those of orthorhombic sulphur given in the ASTM powder diffraction file<sup>29</sup>. Some additional weak diffraction maxima are probably the result of unidentified organic material associated with the preparations of sulphur bacteria.

Interference from the diffraction peaks of sodium chloride and other nutrient salts in the medium and from a broad diffraction maximum caused by water defeated several attempts to obtain a useful diffraction pattern of the sulphur still inside whole cells. To avoid the interference of the salinity, the *Chromatium* strain was trained to grow at least very slowly in non-saline medium. The measurement of these cells was also completely inhibited by the presence of water in the cell smear. Ultrasonic treatment of the suspension for 10 min, breaking up the cells and releasing the sulphur into the suspending water, did not have any effect on the diffraction pattern. Freeze drying of the same sample, mounted between collodion pellicles and containing the sulphur as well as the broken cell material, finally showed a small peak indicating the presence of orthorhombic sulphur (Fig. 2D). Considering that about 85 per cent of the dry weight was not sulphur but amorphous organic material, this result seems reasonable.

While purple sulphur bacteria were discovered in 1838 by Ehrenberg<sup>30</sup>, and *Beggiatoa* by Trevisan<sup>31</sup>, their globules were identified as sulphur about 30 yr later by Cramer and Cohn<sup>32</sup>, who showed that the spherical cell inclusions were soluble in carbon disulphide. Winogradsky<sup>33</sup> stated that living cells would always contain globules of amorphous sulphur, while in dead cells crystals would become visible. Sulphur droplets obtained by chemical decomposition of polysulphide solutions were found to appear identical with those inside the cells of *Beggiatoa*<sup>34</sup>. Von Deines postulated that the globules excreted by *Thiobacillus thioautotrophicus* were highly sulphurized polysulphides<sup>35</sup>, but Starkey<sup>22</sup> showed by a

careful analytical investigation that the evidence for this postulate was insufficient. He again confirmed that the globules were sulphur. La Rivière<sup>22</sup> was the first to approach the problem by use of X-ray diffraction, investigating the sulphur of *Thiovulum majus*, a large colourless sulphur bacterium. He obtained the sulphur by osmotic shock of the cells and subsequent low speed centrifugation and found an X-ray diffraction pattern closely resembling that of orthorhombic sulphur.

Despite the different metabolic roles of sulphur in colourless and in photosynthetic sulphur bacteria, we found that the sulphur formed by the latter is also of orthorhombic structure, thus confirming Starkey's<sup>22</sup> statement that "there is no evidence that the globules produced by the larger colourless sulphur bacteria, by the purple (and green) bacteria, or by *Thiobacillus thioautotrophicus* differ chemically".

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### Phenoloxidase of *Mycobacterium leprae*

INVESTIGATIONS of the metabolism of *Mycobacterium leprae*<sup>1</sup> have revealed a few of the metabolic characteristics of this micro-organism. Concentrates of *M. leprae* prepared from lepromatous material actively oxidized 3,4-dihydroxyphenylalanine (DOPA) to pigmented products, *in vitro*<sup>2</sup>.

Among several strains of mycobacteria tested (including *M. tuberculosis*, *M. lepraemurium*, and alleged *M. leprae* cultures), only the leprosy bacilli obtained from infected tissues oxidized DOPA; and this specific metabolic activity has been proposed as an identification test for *M. leprae*<sup>3</sup>.

All efforts, extending for nearly a century, to cultivate the causative agent of leprosy in inert culture media have so far ended in failure, none of the numerous claims of success having been confirmed by subsequent work. In consequence, any investigation on the metabolism of *M. leprae* is dependent on limited amounts of infected human tissues available, and only those techniques applicable to small quantities of material could be used. Concentrates of *M. leprae* used in the present investigation were directly separated from lepromatous material. The method used and the purity of the preparation obtained have been reported earlier<sup>2,4</sup>. Because melanocytes in the human skin contain phenolase<sup>5</sup>, it would be useful to investigate if the DOPA oxidase of *M. leprae* is in any way distinguishable from the mammalian enzyme. This enzyme is present in a highly concentrated form in mammalian melanomas. Mammalian phenolase has a limited substrate specificity, and oxidizes tyrosine and DOPA to melanin, L-DOPA being oxidized at a much faster rate than D-DOPA (ref. 6). Diphenols like catechol and catecholamines are not oxidized to any significant degree. The amino group of the tyrosine derivatives must be unsubstituted for activity of the mammalian enzyme<sup>7</sup>. Plant phenolase, on the other hand, has a wide substrate specificity<sup>8</sup>.

This communication reports results on oxidation of D- and L-DOPA and catechol by *M. leprae*. Oxidation of catecholamines by the leprosy bacilli has been reported already<sup>3</sup>. The organisms were separated from infected spleen of a case of lepromatous leprosy removed after death. Cultures of the spleen remained negative, precluding any contamination. Normal human spleen by itself did not contain phenolase<sup>3</sup>. The suspension of bacilli (2 mg protein) was incubated with the substrates at 37° C, pH 6.8, volume 3 ml. (see Table 1 for details). After incubation, the reaction mixture was centrifuged for 45 min at 15,000g and the spectrum of the supernatant was measured in a 'Beckman DU' spectrophotometer. This method was used because the oxidation products of DOPA are well characterized by their absorption spectra<sup>9</sup> and it is sensitive enough for the small amounts of material available. Indole-5,6-quinone was formed by oxidation of DOPA and absorbed maximally at 540 mμ, while o-benzoquinone, produced from catechol, showed a broad peak in the region 520–560 mμ. The results are presented in Table 1. Each substance was tested at least three times; the values given are for typical experiments, and have been corrected for any autooxidation of the substrates and absorbance caused by the bacilli. Heating at 100° C for 15–20 min inactivated the bacilli.

Table 1. PHENOLOXIDASE OF *Mycobacterium leprae*

Substrate	(molar)	Time (min)	Increase in absorbance (540 mμ)
L-DOPA	$3 \times 10^{-2}$	90	0.170
D-DOPA	$3 \times 10^{-2}$	90	0.187
Catechol	$6 \times 10^{-2}$	60	0.143

The results indicate that in the range of substrates oxidized, phenoloxidase of *M. leprae* resembles more closely the enzyme from plant sources than that of mammalian origin. *M. leprae* oxidizes D-DOPA at the same rate as L-DOPA, and also catechol and catecholamines. In the case of melanomas, utilization of D-DOPA is rather low and catechol or catecholamines are not oxidized. Because phenolase of *M. leprae* is different from the mammalian enzyme, the activity of the bacilli could not be caused by adsorption of host tissue enzymes. It is known that phenolase specificity becomes narrower with rise in the phylogenetic scale<sup>9</sup>. It is interesting to point

out that taxonomically *M. leprae* comes under class Schizomycetes, to which fungi also belong. Fungi are known to be rich in phenolase<sup>10</sup>. Because phenolase might serve as an alternative mechanism for the oxidation of various substrates by *M. leprae* through the quinones formed in the reaction<sup>2</sup>, this finding offers possibilities for a rational approach to cultivation of the bacillus (providing utilizable substrates) as well as chemotherapy of the disease (use of selective inhibitors).

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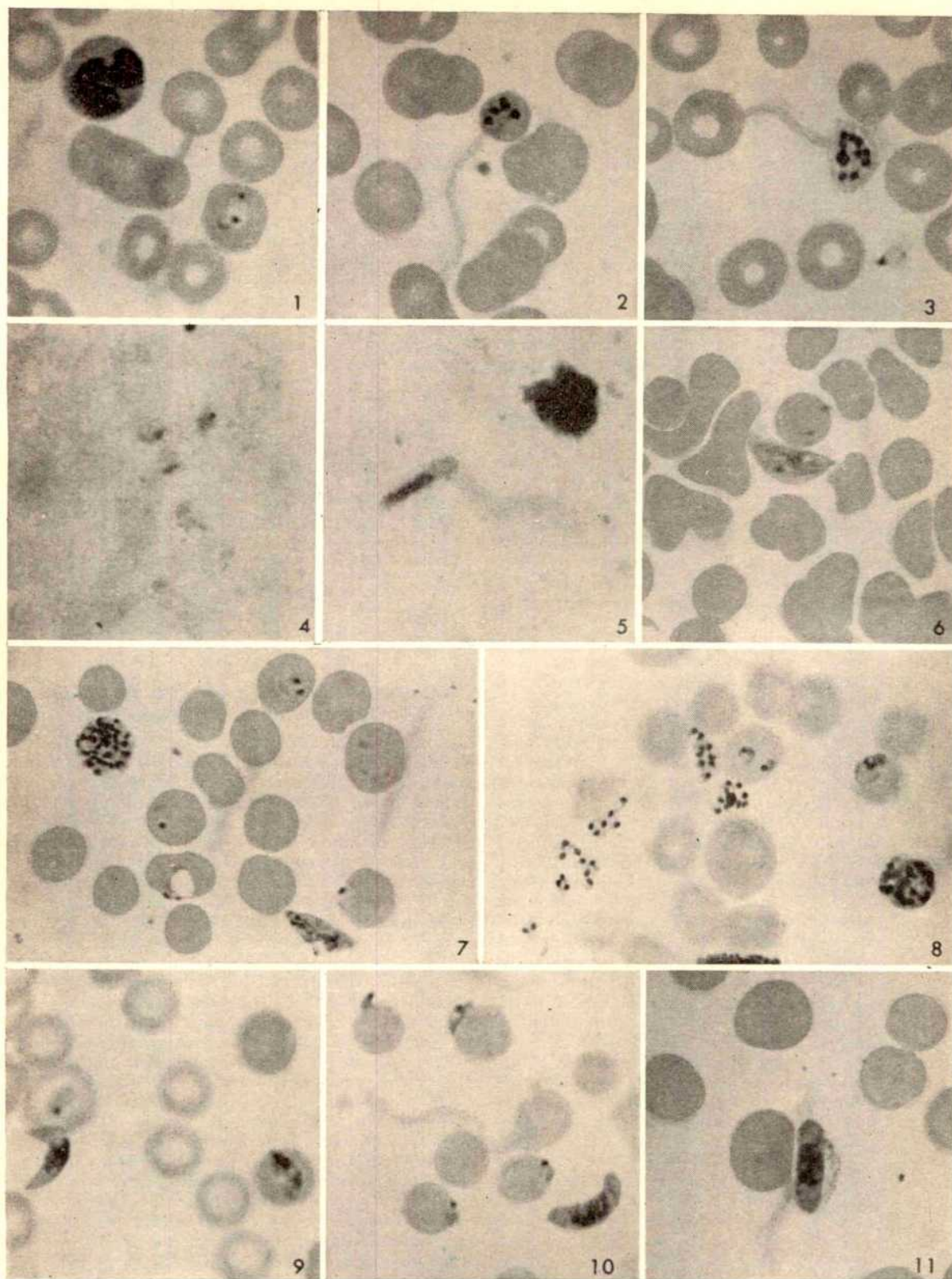
## Susceptibility of a New World Monkey to *Plasmodium falciparum* from Man

LABORATORY investigations of the biological and biochemical characteristics of malarial parasites from man have been hampered by the lack of infected patients and of susceptible laboratory animals. In order to prolong the cultivation of plasmodial species *in vitro*<sup>1</sup>, we sought a supply of blood infected with *Plasmodium falciparum* and *P. vivax*. Apart from nine cases of human malaria and two samples of frozen human infected blood, no steady supply for our experiments was available in the Bay area of California. Storing or freezing the blood causes the parasite to change before the experiments are begun.

The report by Porter and Young<sup>2</sup> on the susceptibility of the New World monkey, *Aotus trivirgatus*, to *Plasmodium vivax* offered a solution of this problem. Previous attempts to infect Old and New World non-human primates<sup>2-4</sup> showed that the splenectomized chimpanzee (*Pan satyrus*) was susceptible to *P. vivax* and *P. falciparum* and that the howler monkey, *Alouatta* spp., was susceptible to *P. falciparum*. More recently, splenectomized gibbons, *Hylobates lar*, have been shown to be susceptible to *P. falciparum*<sup>5,6</sup>. Chimpanzees and gibbons are costly and difficult to feed and house, so we obtained instead night monkeys, *Aotus trivirgatus*, from a local dealer. The animals were examined for spontaneous infections of blood parasites before and after splenectomy. One splenectomized *Aotus* was inoculated intraperitoneally with blood containing  $105 \times 10^6$  *P. vivax* taken from a soldier who had returned from Vietnam. After a 24 day pre-patent period, we confirmed the report of Porter and Young<sup>2</sup> about susceptibility. The maximum parasite count in this animal was 76,200/mm<sup>3</sup> on the seventeenth day of the patent period.

On October 25, 1966, a female patient, who had been admitted to the Palo Alto-Stanford Hospital five days before, was diagnosed as having an acute infection with *P. falciparum* (Fig. 1). The patient was semi-comatose when seen and the parasitaemia was 125,000 parasites/mm<sup>3</sup> of blood. The infection was contracted in Uganda. The severity of the infection was also shown





Figs. 1-3. Rings and schizonts of *Plasmodium falciparum* from the patient who was semicomatose. Leishman's stain, except for Figs. 4 and 5. Fig. 4. A ring stage of *P. falciparum* from a thick film made from a splenectomized *Aotus trivirgatus* 54 days after intraperitoneal inoculation. Giemsa stain.

Fig. 5. An immature gametocyte with pigment on the same film as Fig. 4.

Figs. 6-7. Rings, a segmenter and immature gametocytes on blood films from *A. trivirgatus* 7 days after appearance of infection.

Fig. 8. A schizont and a cluster of merozoites after rupture of host erythrocyte.

Fig. 9. An early immature and mature gametocyte from a second passage infection in *A. trivirgatus*.

Fig. 10. Rings and a mature gametocyte.

Fig. 11. A mature gametocyte showing membrane of host red cell.

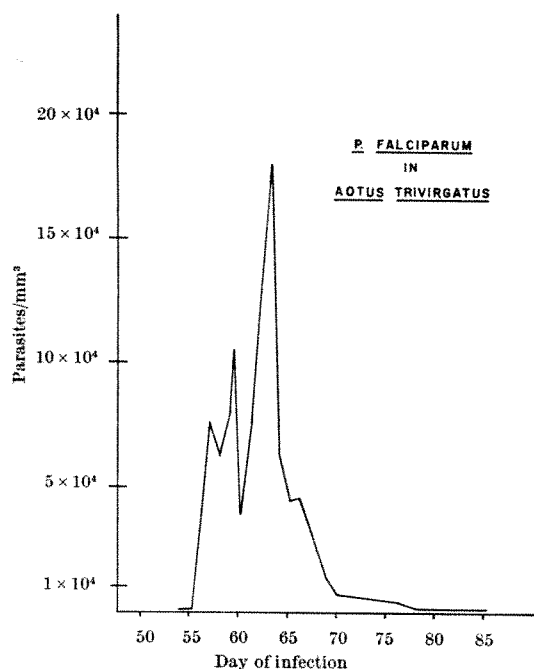


Fig. 12. The course of infection of *Plasmodium falciparum* in a New World monkey, *Aotus trivirgatus*.

by the presence of asexual stages of parasites in the circulating blood (Figs. 2 and 3).

Blood obtained from this patient was heparinized, and 10.2 ml. containing  $1.29 \times 10^9$  parasites was inoculated intraperitoneally into a splenectomized *Aotus*. After a pre-patent period of 54 days, a few rings and immature gametocytes were found on thick films. The later course of infection is shown in Fig. 12. Asexual stages, immature and eventually mature gametocytes (Figs. 6–11) appeared in the circulating blood, and the infection at its peak reached a parasitaemia of  $180,000/\text{mm}^3$  (5.25 per cent).

1.8 ml. of blood from this animal (containing  $7.02 \times 10^7$  parasites) was inoculated intraperitoneally into another *Aotus*. After a 19 day pre-patent period, this animal gave a positive reaction and 24 per cent of the erythrocytes were parasitized on the nineteenth day of the patent period. The parasites showed no preference for reticulo-cyte invasion. The animal died on the thirty-first day of the patent period because it was bled heavily for passage and for *in vitro* studies to be reported elsewhere.

The first infected animal and other animals later infected at no time showed detectable gross clinical symptoms. The strain of parasites in the donor patient was certainly highly virulent, but additional infections and autopsies are needed to determine pathogenesis for *Aotus*.

We are now able to maintain the infection by passage to splenectomized *Aotus*. An infection in an intact recipient animal has also become positive, indicating adaptation to this host. In the passage animals, asexual stages of the parasites continue to occur in the peripheral blood together with immature and mature gametocytes. We plan to study the gametocytes to determine their maturity and infectivity for anopheline mosquitoes.

In order to have different strains of *P. falciparum* available for *in vitro* culture and biochemical study, we are trying to isolate a chloroquine-resistant strain of *P. falciparum* in *Aotus trivirgatus*.

Finally, we should point out that infected animals must be kept in adequately screened cages to prevent access by anopheline mosquitoes to a blood meal containing a high percentage of gametocytes. Also, *Aotus* is scarce and apparently difficult to keep in captivity. Mature animals are relatively small, weighing about 0.5–0.8 kg; they must be housed at not less than 80° F; and they

must be adapted to available but acceptable food. Perhaps primate centres can be approached to study and propagate these animals for malaria experiments and for the study of other aetiological agents of disease in man, such as infectious hepatitis, because the susceptibility of *A. trivirgatus* to human malaria suggests that its erythrocytes and plasma are similar to the constituents of human blood.

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### Inhibition of Growth of Subtilis Phage SP-50 by Histones

BUKRINSKAYA *et al.*<sup>1</sup> demonstrated the inhibitory effect of histones on the growth of fowl plague virus—one of the viruses which contains RNA. Synthesis of RNA and viral haemagglutinin in infected chicken fibroblast cells were inhibited by calf thymus histones. Recently, it was found that when histones were added they reduced the multiplication of Semliki forest virus—another virus containing RNA, and vaccinia virus, which contains DNA<sup>2,3</sup>. In all these studies chicken fibroblast cells were used. The present investigation was carried out to examine the effect of histones on the growth of subtilis phage SP-50 in bacterial cells thought to be free of histones.

The bacterium used in the experiments was *B. subtilis*, r168. The phage was subtilis phage SP-50 which contains DNA. It was originally isolated by Trautner<sup>4</sup>. Both the bacteria and the phage were kindly provided by Dr A. Trautner. The histones used were total histones from calf thymus, prepared by acid extraction.

Bacteria were grown to a density of about  $2 \times 10^8$  bacteria/ml. in a minimal medium containing salts, yeast extract, asparagin and tryptophan. After centrifugation of the bacteria the adsorption of the phage was allowed to take place for 5 min at 37° C in a medium containing sodium chloride, yeast extract and tryptone. The bacteria were then centrifuged again, resuspended in a tenth of the original volume in a solution containing sodium chloride, sodium citrate and 200 g sucrose/l., and transformed into protoplasts by treatment with 100 µg/ml. of lysozyme for 30 min at 25° C. Finally, the suspension of infected protoplasts was diluted to a thousandth in the minimal medium containing 200 g sucrose/l. and raised in temperature to 28° C.

The method used to determine the incorporation of radioactive phosphorus into RNA during phage synthesis was as follows. About 1 mc. of radioactive phosphorus was added to 500 ml. of infected protoplasts. After incubation for 30 min at 28° C the suspension was passed through a 'Millipore' filter to retain the protoplasts, and treated first with 5 per cent perchloric acid, and then with ethanol. Afterwards the filter was dissolved in acetone. The precipitate was then centrifuged, the RNA extracted with 17 per cent perchloric acid overnight at 4° C and the radioactivity determined.

When histones were added to a culture of bacteria infected with SP-50, no decrease in the production of

phage was observed. The bacterial cell wall probably inhibited the entry of histones into the cell. Brenner *et al.*<sup>5</sup> had previously succeeded in growing phages in protoplasts, and therefore we tried this with subtilis phage SP-50. Fig. 1 is the growth curve of subtilis phage SP-50 in protoplasts. The infectious phage increased by a factor of about thirty. Intact bacteria gave somewhat higher yields than protoplasts.

Fig. 2 shows the effect of different concentrations of histones on the growth of subtilis phage. Small amounts of histones were able to suppress the growth of the phage, but more histones were needed to increase the concentration of protoplasts. Addition of histones was more effective if it was done early in the latent period (Fig. 3). Suppression of the growth of phage by dilution with sucroseless medium (causing disruption of the protoplasts) remained approximately the same throughout the latent period. It is therefore unlikely that the

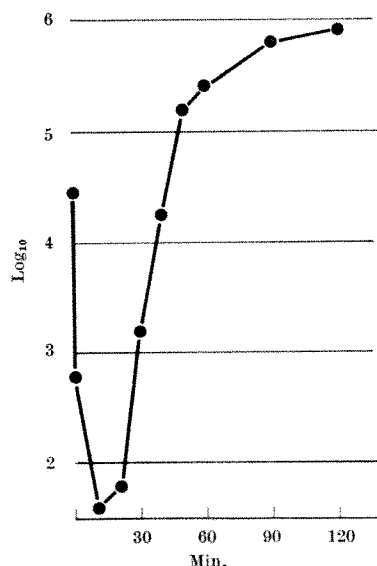


Fig. 1. Growth of subtilis phage SP-50 in protoplasts. Bacteria were infected at a multiplicity of about 0.1, transformed into protoplasts, incubated at 28° C and samples were removed at different times.

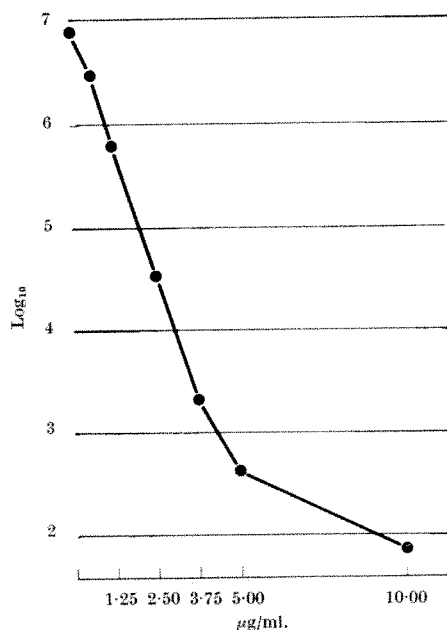


Fig. 2. Effect of different concentrations of histones on the growth of subtilis phage SP-50. Multiplicity of infection was about 1.5. The protoplasts were incubated for 120 min with different concentrations of histones.

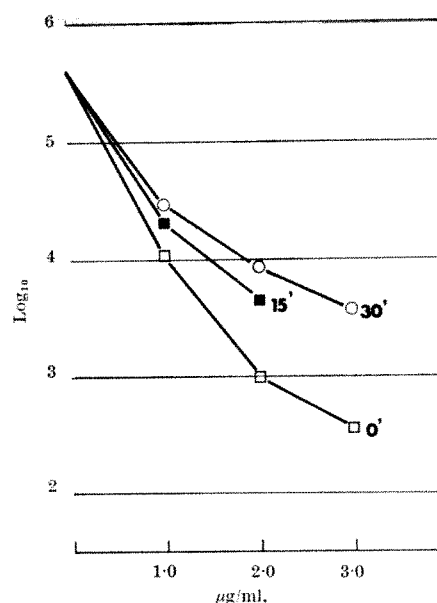


Fig. 3. Effect of histones at different times after infection. Multiplicity of infection was about 0.1. The protoplasts were incubated for 120 min. Histones were added at different times during the incubation.

increased resistance to histones was caused by partial repair of the protoplasts during incubation.

Direct observation under the microscope shows that small amounts of histones, which have an inhibiting effect on the multiplication of the phage, do not disrupt the protoplasts to any noticeable extent. It is probable that histones do not act simply by disrupting the protoplasts. Experiments by Huang *et al.*<sup>6</sup>, Allfrey *et al.*<sup>7</sup> and others have demonstrated that histones block RNA synthesis. To determine whether this is the mechanism by which histones inhibit the synthesis of the new phage, I studied the incorporation of radioactive phosphorus into RNA during phage growth both in the presence and in the absence of histones. The results are presented in Table 1. In all three experiments

Table 1. EFFECT OF HISTONES ON THE INCORPORATION OF RADIOACTIVE PHOSPHORUS INTO RNA DURING PHAGE SYNTHESIS

Experiment	Acid and ethanol soluble fraction 10 <sup>3</sup> counts/ml.	RNA 10 <sup>3</sup> c.p.s.	Histone µg/ml.
1	1,120	24	—
	844	6	1.25
2	32	4	—
	33	1	1.25
3	770	37	—
	721	17	1.25

Bacteria were infected at a multiplicity of about 2.0 and transformed into protoplasts. About 1 µc. of radioactive phosphorus was added to 500 ml. of the suspension. The protoplasts were then incubated for 30 min at 28° C with and without histones, the RNA was extracted and the radioactivity determined.

the radioactivity in the RNA extracted from samples incubated without histones was between three and four times greater than that in samples incubated with histones. This difference is not great enough to account for all the suppression of the phage growth caused by histones. Possibly the samples which contained histones showed excessively high radioactivity because of incomplete removal of contaminating radioactive phosphorus by the treatment with perchloric acid and ethanol. The results support the idea that histones inhibit the growth of subtilis phage SP-50 by preventing RNA synthesis. This would mean that extraneous histones act in bacterial cells in the same way as in animal cells. It is possible that the increase in the resistance to histones at the later stages of the latent period is because in that time most of the phage specific RNAs have already been synthesized.

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## PATHOLOGY

### Infectivity of *Trypanosoma rhodesiense* to Tsetse Flies fed through Animal Membranes

THE trypanosomes belonging to the *Trypanosoma brucei* group (agents of sleeping sickness and nagana) exist in the blood of the host in three main morphological forms—long thin, intermediate and short stumpy. When these are taken up by the tsetse fly they undergo developmental changes, namely trypanosome, crithridial and metacyclic forms, the last being infective to the vertebrate host. There is controversy about which forms are infective to the fly, the long thin, the short stumpy or both.

It is well established that the blood forms of the trypanosomes retain their viability as well as other biological characteristics after long periods of storage at  $-79^{\circ}\text{C}$  (refs. 1 and 2). Feeding through membranes has been used for the regular maintenance of tsetse flies in the laboratory<sup>3</sup>, and for the collection of metacyclic trypanosomes from infected flies<sup>4</sup>. An unsuccessful attempt to infect *Glossina pallidipes* with preserved *Trypanosoma brucei* group trypanosomes was made by Cunningham<sup>5</sup>, who suggested that the failure was caused by the absence of short stumpy forms from the suspension used.

During a study on the infectivity of the two main morphological forms of these trypanosomes to tsetse flies, a batch of twenty-three *G. palpalis* were given an infective blood meal which contained only the long thin forms of *T. rhodesiense*, through a fresh mouse skin membrane. The morphology of the trypanosomes was determined by the method described by Ormerod *et al.*<sup>6</sup>. One of the flies later extruded trypanosomes in its salivary secretion, and transmitted the infection to a guinea-pig before it died (42 days after). This suggests that, under certain conditions, both morphological forms of these trypanosomes may become established in the fly<sup>7-10</sup>, contrary to the results of several workers<sup>11-13</sup>. The blood of the guinea-pig, containing both long thin and short stumpy trypanosomes, was frozen at  $-79^{\circ}\text{C}$  in ampoules.

After 14 months the contents of one ampoule were fed to nine newly emerged *G. austeni* through a fresh mouse skin membrane. Three flies died within 10 days and were not examined. All other flies were dissected as soon as possible after death. One infected fly was detected on the twenty-sixth day; however, when the flagellates obtained were subsequently inoculated into a rat, no infection occurred.

After 16 months' storage at  $-79^{\circ}\text{C}$ , the contents of another ampoule were similarly fed to twenty-two *G. austeni*, of which eleven survived to the tenth day. Two flies which died on the fourteenth day contained active trypanosomes which produced an infection in a mouse. Five other flies dissected between the nineteenth and

twenty-eighth days contained active trypanosomes, but these did not infect test animals.

It is therefore concluded that tsetse flies may be infected with preserved *T. brucei* group trypanosomes by feeding through membranes. The successful infection of a fly from a suspension which contained only long thin trypanosomes indicates that negative results, such as those of Cunningham<sup>5</sup>, would result from some reason other than the absence of short stumpy forms in the blood meal. It is suggested that the technique of membrane feeding, as opposed to that of feeding flies on infected animals, offers more precise conditions for investigation of the factors which influence the infection rate of tsetse flies with these trypanosomes. These investigations should be based on the factors in the trypanosomes; the mammalian host; and the individual tsetse<sup>14</sup>. Other characteristics of the trypanosomes that may be investigated in this way include their infectivity, transmissibility by tsetse, antigenicity and drug resistance. The preservation of metacyclic trypanosomes derived from tsetse flies<sup>4</sup> adds considerably to the possibilities of such studies.

I thank Dr W. E. Ormerod for his guidance, and Dr T. A. M. Nash and Mr T. M. Leach for the supply of tsetse pupae. This work was supported by the Rockefeller Foundation.

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### Leaching of Constituents of Chrysotile Asbestos *in vivo*

IN recent years, Wagner<sup>1</sup> and Selikoff *et al.*<sup>2</sup> have shown that a rare tumour, the diffuse mesothelioma of the pleura and peritoneum, is associated with past exposure to asbestos. It appears that the amount of asbestos required to produce these tumours is small and that the latent period is very long. The connexion between exposure to asbestos and the production of mesotheliomas is being studied in a number of laboratories, and the possibility that trace metal constituents or contaminating oils may have a role has been suggested<sup>3</sup>. As yet, little is known about the fate of inhaled asbestos fibres and in particular about their movement out of the lung into other organs. The experiment described here was planned to assess the possibility of using radioactivity, induced in asbestos fibres by neutron irradiation, to trace their translocation in rats after administration by intrapleural injection.

A sample of Rhodesian chrysotile was irradiated in a high flux of thermal neutrons ( $1.5 \times 10^{14}/\text{cm}^2/\text{sec}$ ) for 10 h. After allowing some weeks for the short-lived activity to



decay, the principal  $\gamma$ -emitting constituents were found to be scandium-46, chromium-51, iron-59 and cobalt-60, all of which are produced in  $(n,\gamma)$  reactions on the stable elements. The half-lives and principal  $\gamma$ -ray energies of these nuclides are listed in Table 1, together with the concentrations of the corresponding stable elements in the chrysotile determined by activation analysis (Sandalls, F. J., personal communication).

Table 1. CHARACTERISTICS OF NUCLIDES INDUCED IN CHRYSOTILE

Element	Concentration in chrysotile (p.p.m.)	Radio-nuclide	Half-life	Principal $\gamma$ energies (MeV)	
Scandium	$5 \pm 1$	$^{46}\text{Sc}$	84 days	0.89	1.12
Chromium	$780 \pm 10$	$^{51}\text{Cr}$	28 days	0.323	
Iron	$38,000 \pm 1,000$	$^{59}\text{Fe}$	45 days	1.10	1.29
Cobalt	$77 \pm 2$	$^{60}\text{Co}$	5.27 yr	1.17	1.33

Before administration, the irradiated fibre was suspended in physiological saline and sterilized by  $\gamma$ -irradiation. Two 3 month old rats were anaesthetized and injected intrapleurally<sup>4</sup> with 0.4 ml. of this suspension (equivalent to about 3 mg of asbestos). The injection was in the right axilla at the level of the second nipple. After administration, the rats were housed in metabolic cages of the type described by Howells *et al.*<sup>5</sup> which permit the separate collection of urine and faeces. One animal was killed after 8 and the other after 50 days, after which they were dissected and the principal organs removed for analysis by  $\gamma$ -ray scintillation spectrometry. Care was taken to avoid cross contamination of tissue samples.

The  $\gamma$ -ray scintillation spectra of the asbestos and of samples of tissue and excreta arising during the experiment were measured with a 3 in. diameter  $\times$  3 in. deep thallium activated sodium iodide crystal detector, using a linear amplifying system and multi-channel pulse height analyser. The amounts of each nuclide in the various samples were calculated using a least squares analysis of the  $\gamma$ -ray spectra using standard spectra of the individual components. The analyses were performed by the GASP computer programme devised by Salmon<sup>6</sup> which produces a graphical presentation of the experimental points and the fitted curve. An example of this presentation showing the  $\gamma$ -ray spectrum of irradiated chrysotile and the fitted curve is shown in Fig. 1.

Analysis of the  $\gamma$ -ray spectra of urine samples showed them to contain only chromium-51 and cobalt-60. After 50 days, 19 per cent of the chromium-51 and 57 per cent of the cobalt-60 had been excreted by this route. This

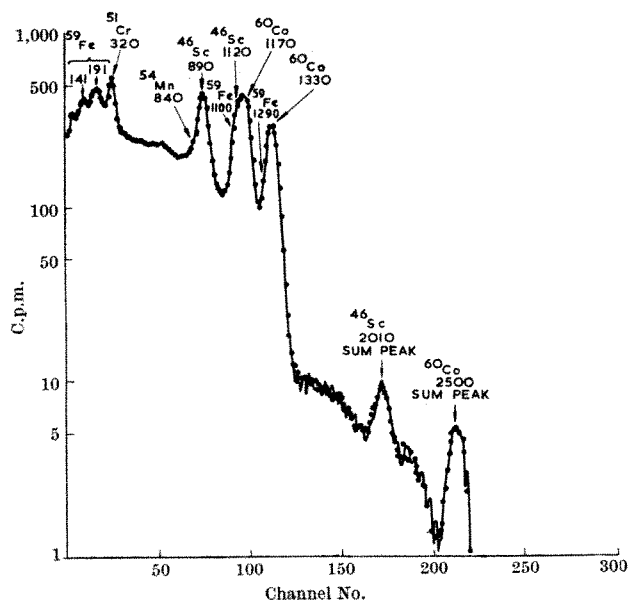


Fig. 1.  $\gamma$ -Ray scintillation spectrum of neutron irradiated chrysotile. —, Fitted curve; ●, experimental points. All energies in KeV.

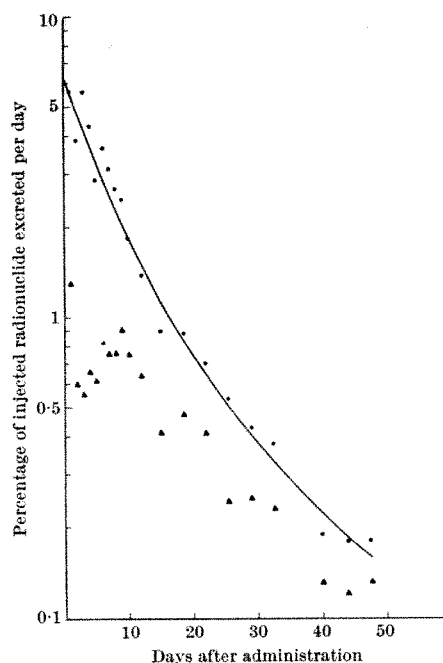


Fig. 2. Urinary excretion of chromium-51 (▲) and cobalt-60 (●).

shows conclusively that chromium and cobalt are both leached quite rapidly *in vivo* from chrysotile asbestos. The daily excretion rates of these two nuclides are shown in Fig. 2.

All four nuclides were detected in faeces but in comparatively small amounts. Their presence in faeces could be accounted for by small amounts of fibre entering the lung from the pleura and subsequently being transported by the normal ciliary clearance mechanism to the glottis and gastro-intestinal tract.

Small amounts of all four radionuclides were detected in tissue samples. Relatively high levels of iron-59 in both blood and liver indicate that iron is also leached from the asbestos, but to a much smaller extent than either chromium or cobalt. The evidence does not permit any conclusions to be drawn regarding the leaching of scandium.

In both animals, about 90 per cent of the amount of each radionuclide remaining at the time of death was found in the pleural cavity and lungs. Some of the activity was associated with visible plaques on the diaphragm and pleura. The distribution indicates that the translocation of fibres out of the pleural cavity into other organs is a comparatively slow process. The fact that small amounts of all four radionuclides were, however, found in organs outside the chest region suggests that some translocation of fibres does occur although additional evidence possibly supported by autoradiographic studies, will be required to prove this conclusively.

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# BOOK REVIEWS

## MEDIAEVAL MINERALOGY

### Book of Minerals

By Albertus Magnus. Translated by Dorothy Wyckoff. Pp. xlii + 309 + 2 plates. (Oxford: Clarendon Press; London: Oxford University Press, 1967.) 84s. net.

ALBERTUS MAGNUS, whose dates are usually given as 1206–1280, was the most learned man of his day, taking all knowledge for his province. He was a Dominican, and famous in his own time not only for his scholarship but for his piety. Even in his lifetime he became the centre of legend. In some quarters his knowledge of alchemy caused him to be regarded as a magician skilled in the black arts; by others he was looked upon as a saint who could perform miracles. As early as 1484, the date of his death (November 15) became officially recognized by the Pope as “Albert’s Day”, and in 1931 he was canonized.

Albert was a scholar through and through, and a devout follower, translator and commentator of the works of Aristotle, the shadow of whose doctrine extended over two millennia, effectively preventing true scientific advance based on observation and experiment. But yet in Albert’s works there are many signs that, almost alone among mediaeval thinkers and writers, he was a shrewd observer of animal and mineral nature. His written treatises cover an enormous range, and even their sheer bulk is sufficiently impressive. The new Cologne edition of his works, for example, is in forty volumes, of which nine comprise his works on natural science. Most of these are based on works of Aristotle, in which case the original text is paraphrased and interwoven with expositions of Albert’s own, or refutations of the opinions of earlier commentators. In the *Book of Minerals*, there was no basic text, which left more scope for development of his own ideas, though his model is still Aristotle, with his doctrine that concrete particulars obtained by direct observation of nature are often confused and difficult to understand and that science should apply itself to analysing these data in order to arrive at the underlying general principles or causes. And for Aristotle, and therefore for Albert, the four causes of minerals come under the headings “Material”, “Efficient”, “Formal” and “Final”.

For the material cause, the composition of minerals, Albert had to juggle with the accepted four “elements”, fire, air, water and earth. On this unpromising basis, Albert divides minerals into three groups—stones, which are mixtures of earth and water; metals, which are made up of sulphur and quicksilver, which are themselves mixtures; and intermediates, which have certain characteristics of both stones and metals.

For the efficient cause, or the processes by which minerals are made, Albert relies on the reactions of exhalations such as “dry smoke”, or sulphur, and “moist vapour” or quicksilver, which are converted by a mineralizing power acting through the instruments of heat and cold. The formal cause is that which determines the nature of the mineral, and this formative power comes to us from the heavens through the influence of the planets and stars. Lastly, the final cause, which is concerned with why a thing exists, is not stressed by Albert, because inanimate mineral matter was not considered by Aristotle or by Albert to have a significant purpose of its own.

With such a scheme as this, an author cannot be expected to produce a work on mineralogy which has a recognizable affinity with any tract written on the same subject in the nineteenth or twentieth century. The sort of qualities of a mineral which we consider to be valuable

indications of its nature and identity—texture, colour, hardness, cleavage, density—are here regarded as “accidental”, not essential, and thus unimportant. Even crude figures for density, for example (and certain Arabs had by this time arrived at quite fair values, using Archimedes’ principle), would have helped us greatly in identifying correctly the species with which Albert deals: but none at all is given.

The first book having dealt with the general properties of stones, the second concerns itself with precious stones and their powers, which, rather curiously, are considered in alphabetical order, from asbeston, admas, absinthus, agathes . . . to zemech (lapis lazuli) and zigrifis (probably fabulous). There is a section (“tractate”) on images and sigils in stones in which Albert shows a curious ignorance of the art of gem-cutting or cameo carving, and accepts so highly finished a work of art as the “Ptolemy” cameo as a product of nature.

The third book concerns “Metals in General”, the fourth book deals with “Individual Metals”, and the fifth book with “Minerals that seem to be intermediate between stones and metals”.

The translation of the *Book of Minerals* itself takes up 250 pages of the present volume, and incorporates ample notes on the text and cross references to, for example, Albert’s possible sources and other comparable works. In addition there are some twenty-five pages of introduction, sketching Albert’s life and the circumstances in which the *Book of Minerals* was written; the book ends with some valuable appendices and an index.

The translator and editor, Dr Dorothy Wyckoff, was until recently professor of geology at Bryn Mawr College, and had spent many years in her study of the *Book of Minerals*. She is to be congratulated on a fine piece of scholarship, which will not only reward and delight students of mediaeval science, but will serve for others who are not familiar with the period as an accurate yet very readable introduction to those strange times, when superstition and learning were intimately mixed, when astrology was indistinguishable from astronomy, alchemy from chemistry, and hearsay from hard fact.

B. W. ANDERSON

## FUNCTION THEORY APPLIED

### New Methods for Solving Elliptic Equations

By I. N. Vekua. Translated from the Russian by D. E. Brown. Translation edited by A. B. Tayler. (North-Holland Series in Applied Mathematics and Mechanics, Vol. 1.) Pp. xii + 385. (Amsterdam: North-Holland Publishing Company; New York: John Wiley and Sons, Inc., 1967.) 100s.

THE original Russian edition of this book appeared in 1948, so that since then a knowledge of the methods used has had time to spread. Vekua himself published a very large number of papers in this field during the 1940’s. Many of these papers related to plane problems in anisotropic elastic media, part of the notable contribution to the mathematical theory of elasticity made in recent years by Soviet scientists; much of Vekua’s work shows the influence of Muskhelishvili. Related material has also been made available in Vekua’s book on generalized analytic functions (1959; with an English translation, published by the Pergamon Press in 1962); this again gave many applications to elasticity.

The familiar simplification of many two-dimensional problems obtained by the use of a complex variable  $z$  can be widely generalized by a transformation  $z = x + iy$ ,  $\bar{z} = x - iy$ , where if  $x$  and  $y$  are restricted to be real,  $\bar{z}$  specializes to the conjugate of  $z$ . The differential operators associated with these variables permit an application of complex function-theory to the general representation of solutions of second order partial differ-

ential equations of elliptic type in two independent variables. The kernels of these operators can be expressed in terms of the Riemann function, dependent on the coefficients in the partial differential equation. As an illustration, the connexion of special cases with the Bessel and Legendre functions is shown. The imposition of boundary value conditions effects a reduction to integral equations: these may be of Fredholm type, but much use is made of singular integral equations involving principal value integrals, a field in which Muskhelishvili's 1946 book (with an English translation in 1953) is a basic reference. Applications are made to vibrations of a membrane, elastic spherical shells and other elasticity problems.

The reader should possess a sound knowledge of the classical theory of functions of a complex variable, and of the basic equations of theoretical elasticity. He will then appreciate the book as another example of the way in which Soviet mathematicians exploit the resources of high-powered analysis in the practical application of mathematical theories.

T. A. A. BROADBENT

## OPERATIONAL CALCULUS

### Introduction to the Operational Calculus

By L. Berg. English translation of the second edition. (North-Holland Series on Applied Mathematics and Mechanics, Vol. 2.) Pp. x+294. (Amsterdam: North-Holland Publishing Company, 1967.) 80s.

OPERATIONAL calculus was developed by Heaviside and used by many of his successors on an intuitive basis. The closest approach to a mathematical theory of the original Heaviside calculus is presented by J. P. Dalton in *Symbolic Operators* in 1954. About fifty years ago it was discovered that Laplace integrals could be used effectively as a substitute for operational calculus, and since then most investigators aiming at precision and mathematical rigour have used Laplace transforms.

Laplace transforms, however, have some shortcomings from the point of view of an applied mathematician. Laplace integrals do not have any obvious physical significance; in order to ensure their convergence, it is necessary to impose certain unnatural growth restrictions on the functions subjected to the transformation; and Laplace transforms involve the values of the functions concerned for all positive values of the variable, while the physical processes to which they are applied depend at any time  $t$  on the past of the system, but not on its future.

About 1950, Jan Mikusinski developed an operational calculus which is free from these blemishes and is mathematically rigorous. Mikusinski's operational calculus is based on the convolution integral

$$\int_0^t f(u)g(t-u)du$$

which is Duhamel's integral and can serve to introduce a species of product in the space of continuous functions of a positive variable. Continuous functions endowed with pointwise addition and convolution as multiplication form an algebraic system to which the usual processes of modern abstract algebra can be applied, and lead not only to an operational calculus but also to a concept of generalized functions including impulse functions. The function which is constant and equal to unity serves as the operator of integration, its reciprocal is Heaviside's operator of differentiation, the unit element of the algebraic system constructed by Mikusinski is the delta function, and so on.

The book being reviewed has grown out of courses of lectures delivered by Berg to students of mathematics, physics and electrical engineering. The author presents both the Laplace transform approach and Mikusinski's

operational calculus, and he describes more briefly a extension of Laplace transforms which relaxes considerably the global integrability conditions. The reader is assumed to be thoroughly familiar with advanced calculus and the elements of complex variable theory, but in other respects the book is self-contained, and includes, for example, a detailed exposition of the concepts and method of abstract algebra needed in the sequel. Before developing Mikusinski's operational calculus, Berg presents the corresponding operational calculus for functions of a discrete variable, that is, for sequences. This is in the nature of a preliminary exercise, and serves to introduce some of the concepts and techniques that will appear in later parts of the book, but the calculus so developed is of interest for its own sake. In the development of operational calculus for functions of a continuous variable the author departs from Mikusinski in that he introduces the modified convolution product

$$fg(t) = \frac{d}{dt} \int_0^t f(u)g(t-u)du$$

in order to achieve certain formal simplifications. The operational calculus so developed is then applied to the solution of ordinary differential equations with constant coefficients, systems of such equations, and to integral equations of the convolution type.

Laplace transforms are also developed and applied to the solution of ordinary linear differential equations with polynomial coefficients, partial differential equations of difference equations, and integral equations of convolution type. Additional material includes asymptotics; an extended concept of Laplace transforms; an operational calculus based on the finite Laplace transformation; operational calculus for functions of two variables; and an operational calculus based on Volterra's theory of compositions and applicable to ordinary linear differential equations with variable coefficients.

The first German edition of this book appeared in 1961 and sold so well that a second corrected and somewhat enlarged edition could be published by 1964. The translation reviewed here is based on this second edition. Problems with answers have been added. A. ERDÉLYI

## DIFFICULT FLUIDS

### Non-Newtonian Flow and Heat Transfer

By A. H. P. Skelland. Pp. xvi+469. (New York and London: John Wiley and Sons, Inc., 1967.) 140s.

THE time is undoubtedly ripe for a general book on non-Newtonian technology. A few years ago the subject was sufficiently new and specialized for original papers to be the appropriate source of basic information, but the body of knowledge and the range of users have grown so fast that a readable guide to the state of knowledge is very welcome. With the exception of Wilkinson's monograph *Non-Newtonian Fluids*, this field has been noticeably lacking in such surveys, and Professor Skelland has done a good service in undertaking the task. The size of the task is perhaps indicated by the length of the bibliographies attached to each chapter. More than four hundred books and papers are quoted, so that this book would provide an excellent start to a literature search.

The author has aimed to make his book useful to a wide range of readers, while keeping engineering applications, rather than analytical detail, to the forefront. The practising engineer who meets an isolated problem in non-Newtonian flow will find here all the information he needs, if it exists yet, although it is not always presented in the most convenient form for immediate use. The undergraduate will appreciate the down to earth approach and the worked and unworked examples, but may be

confused by occasionally woolly arguments and unnecessary changes in nomenclature, both of which, one hopes, will be corrected in a second edition. Finally, the more advanced worker will value the book as a background survey, going to more sophisticated texts in his own specialism. One hopes that the very least result of Skelland's effort will be that future writers of research papers on non-Newtonian flow will refer tyro readers to his work instead of swelling the literature volume with needless repetitions of elementary definitions.

Skelland develops his subject through a logical sequence of sections on fluid classification, determination of properties, laminar and turbulent flow, boundary layers, mixing and heat transfer. Each topic is covered by a well balanced combination of theory and empirical data, and the layout of the chapters has been carefully thought out so as to facilitate extraction of information. The only exception is the section on laminar flow, in which an artificial distinction between fluids with and without yield stress has led to the derivation of generalized correlations being split between three chapters.

A surprising, but welcome, inclusion is a chapter on optimization. This will be appreciated by the pipeline designer for reference, but the development is far more general than the book title would suggest and deserves a wider student readership than is expected for this work. The concept of costing is ill-appreciated by too many engineering students, and Skelland may have established a desirable precedent by emphasizing this point in a book not specifically devoted to design economics.

Although this book is rather expensive for many individual buyers, any organization which might encounter non-Newtonian fluids should regard it as an essential purchase for the library.

JEAN M. DRABBLE

## LOOKING INTO PETROL

### *Petroleum Microbiology*

By J. B. Davis. Pp. xiv + 604 with 208 illustrations and 160 tables. (Amsterdam, London and New York: Elsevier Publishing Company, 1967.) 240s.

THE exploding science of petroleum microbiology has been particularly devoid of good literature. Ernest Beerstecher's *Petroleum Microbiology* of 1954 has become the standard reference for most workers in the field and has done much to promote his original aim, which was the marriage of petroleum engineering and bacteriology. A revision or a new standard text has, however, been long awaited and Dr J. B. Davis has set out to produce the latter but in the Beerstecher mould.

The expansion of Beerstecher reflects Dr Davis's particular interests in the wealth of information on geological factors, prospecting and oil recovery and acknowledges the Russian literature in this field. It is perhaps a sign of the times that the disposal of petroleum waste, which occupies less than a page in Beerstecher, commands forty-two pages in the new text. Not only are conventional activated sludge processes covered in some detail but the account of the successful use of a refinery cooling tower for waste treatment must surely stimulate more venturesome methods. At a time when oil tanker wrecks are an international talking point, it is gratifying to find space devoted to the natural degradation of petroleum by micro-organisms in natural waterways, a fact of life which is sometimes missed by those responsible for beach and sea cleanliness.

Perhaps the greatest advances since Beerstecher have been in the field of biosynthesis from petroleum products, and Dr Davis draws on a variety of sources to indicate current trends in the production of high cost chemicals from such low cost substrates as methane.

In dealing with detrimental aspects of microbial activity he explodes the myth that conditions at the

bottom of fuel storage tanks are anaerobic and advocates a reappraisal of spoilage prevention by careful attention to formulation rather than the indiscriminate use of additives. Dr Davis exposes our rudimentary knowledge of degradation in complex oil formulations, but fails to indicate the wider implications of microbial spoilage in steel and aluminium rolling oils, hydraulic oils and water contaminated lubricating oils.

In assessing the merit of this book there can be little doubt that it will become as much a standard reference as Beerstecher, and probably more so. In a book aiming to bridge conventional subject frontiers, clear, adequate and relevant illustration is particularly important, and it is a pleasure to record that even the photomicrographs, so often hazy squiggles and dots, show recognizable micro-organisms. A few cases of inadequate legend (pages 505 and 535) are minor irritations. Engineers and chemists will find little to enable them to isolate, enumerate and identify micro-organisms. More guidance on this technology would be helpful, because it is fraught with pitfalls for the beginner. A level of knowledge of microbiology is assumed which is probably greater than that of even the interested chemist or engineer. A few pages of basic biology would therefore add to the completeness of the book, and a glossary would ease the task of initiates in this multi-subject discipline.

E. C. HILL

## MICROBES IN THE AIR

### *Airborne Microbes*

Edited by P. H. Gregory and J. L. Monteith. (Seventeenth Symposium of the Society for General Microbiology held at the Imperial College, London, April 1967.) Pp. xii + 385. (London: Cambridge University Press, 1967. Published for the Society for General Microbiology.) 75s. net; \$13.50.

*Airborne Microbes* comprises the contributions to a most successful symposium held by the Society for General Microbiology in London in April 1967. The editors in their preface point out that the sampling measurement and interpretation of airborne microbes have challenged the ingenuity of workers in several disciplines, and a symposium providing a common platform on which progress could be reviewed was both timely and apposite.

In the first section, entitled "Air Movement", F. H. Ludlam, in non-mathematical terms, describes the factors affecting the circulation of air, water and particles over trans-continental distances in the Earth's atmosphere. J. B. Tyldesley discusses the suspension and movement of particles in the "frictional layer of the atmosphere" and on the basis of a computer study presents an analysis of eddy diffusion over a short scale. The flow of illuminated tracer particles in air streams indoors is illustrated with a series of photographs by L. F. Daws, who shows the effects of, for example, external wind, heating and even the occupants of a room.

In the second section, entitled "Air Sampling", K. R. May deals with the physical aspects of sampling airborne microbes. The efficiencies of suction and impact systems, isokinetic and anisokinetic sampling, the effect of yaw and its avoidance and the new concept of stagnation point sampling are comprehensively examined. He reports on a limited number of sampling instruments only and it is noteworthy that the cascade impactor which he described in 1945 remains a standard reference instrument with which the efficiencies of others are compared, and the commonly used continuously recording spore trap of Hirst and size grading slit-sampler of Lidwell are based on it. Under the heading "Handling the Catch", W. C. Noble reviews the numerous techniques used to study airborne microbes ranging from algae to viruses.

In the section "Take Off and Landing of Particles", the "Liberation Mechanisms of Fungi" are naturally the



province of C. T. Ingold, who writes: "In the fungi the spores are essentially airborne". He gives details of the types of violent spore discharge, that brought about by the turgor of living cells, the not fully understood ballistospore discharge mechanism, the violent discharge of conidia, periodicity in spore release, and in the passive liberation of spores the effects of wind and rain. In "Take off of Bacteria and Viruses" O. M. Lidwell discusses the effects of talking, coughing and sneezing on the droplet discharge of *Streptococci*, desquamation in the dissemination of *Staphylococci* and dispersal into air from equipment, solid surfaces and by laboratory procedures. A. C. Chamberlain gives a detailed account of the physics of such processes as sedimentation, the impaction of particles on to the surfaces of vegetation, the resuspension of dust and the washout of particles from the atmosphere in rain. The final paper in this section, by H. A. Druett, is a most comprehensive account of the inhalation and retention of particles in the lung and nasal region in man.

In the section "Airborne Disease" D. Anderson and C. S. Cox consider the factors affecting the survival of bacteria in artificially generated aerosols. The survival of airborne bacteria appears to have received far more attention than that of the fungi, for among the 132 references cited by these workers, only one is concerned with fungi (*Aspergillus* and *Pestalotia*) and that published in the *Journal of Bacteriology*. J. E. Van der Plank, writing on the spread of plant pathogens in space and time, distinguishes between the spread of a pathogen and the dispersal of its propagules, with detailed reference to potato blight. J. M. Ogawa, D. H. Hall and P. A. Koepsell, writing about the spread of plant pathogens within crops as affected by life cycle and environment, state: "The least obvious, but very important phase of the life cycle of certain pathogens is the dispersal of their spores by air". In the light of this they discuss their investigations into control measures to prevent the dispersal of *Monilia laxa*, powdery mildew of plum and *Pseudoperonospora humuli*. The spread of airborne bacteria pathogenic for man is eruditely examined by R. E. O. Williams, who points out that they tend to be obligate pathogens and, unlike the fungal spores of plant pathogens, occur in the air indoors in small concentrations. Then he discusses airborne microbes in relation to pulmonary tuberculosis, inhalation anthrax, throat infections, pneumococcal pneumonia, pulmonary plague and the nasal carriage of *Staphylococci*. He stresses the difficulty in distinguishing between airborne infection and that acquired by other routes; that bacteria deposited in the upper respiratory tract induce infections at other sites, while the tubercle bacillus appears to be unique, for it cannot colonize the upper respiratory tract and infection begins at the periphery of the lung. He makes brief reference to sedimentation infection in operating rooms and concludes that the benefits of sanitary ventilation are too small to have a detectable effect on the incidence of infection. D. A. J. Tyrrell, concerned with the airborne spread of viruses of the respiratory tract, is confronted with the problem that air sampling for viruses is more complex than that for other microbes. His paper deals mainly with the epidemiology of respiratory disease in man, and possible routes of transmission. He describes experimental studies of the mechanisms of dispersion, such as the effect of coughing and sneezing in ejecting infected droplets, and stresses the need for a quantitative description of the transmission of infection.

In the last section, on microbial transport in the atmosphere and space, J. M. Hirst and A. W. Hurst are concerned with the long-distance transport of spores as inert particles, and have analysed spore catches obtained from aircraft flying downwind from a land source at various heights above the sea. They have traced a succession of diurnal spore clouds and have found that the clouds occurred where a meteorological analysis of air trajectories

suggested they should be. Vertical profiles and sections spore concentrations in relation to meteorological facts such as atmospheric turbulence and stability are discussed in detail and, after an examination of the importance of large pressure systems in the long-distance transport of spores, the authors conclude that distant transport is governed by the weather and not the climate or prevailing winds which just indicate the most frequent weather. C. W. Bruch describes the American Space Administration programme for determining the boundaries of the Earth's biosphere. He reports the response of microbes to some of the conditions in outer space, the effect of supersonic shock waves on microbes, the theories of panspermia in exobiology, and gives details of the techniques used for sampling microbes in the upper atmosphere with samples carried by balloons, and rocket-borne samplers for the collection of micrometeorites and microbes in space. It concludes with a note on the real-time (during flight) detection of atmospheric micro-organisms with techniques such as the fire-fly lantern enzyme reaction on adenine triphosphate.

This collection of papers, written independently by workers in disciplines ranging from physics to medical virology, has been edited to provide an up to date comprehensive and authoritative account, by two of the leading workers in the group which organized the symposium, and it is a tribute to their enthusiasm that such a coherent theme and exacting standard have been maintained throughout. The volume lists more than 800 references to airborne microbes and related topics and will be of value to workers in many fields who are concerned with the content of the air. R. R. DAVIES

## INTERACTION IN THE CELL

### The Cytology of the Protein Synthesis in an Animal Cell

By B. V. Kedrovskii. (Life Sciences: a Series of Monographs and Texts.) Pp. xi + 462. (New York and London: Gordon and Breach, 1965.) \$29.50.

This book was completed in October 1957, and the rapid progress in the field of protein biosynthesis during the ten years since then has resulted in the clarification of many of the problems which seemed obscure at that time. This is particularly true of such aspects as the role of nucleic acids and the mechanism of polypeptide synthesis which have been largely solved as a result of recent discoveries. Nevertheless, this study of the earlier literature serves as a timely reminder of the complexity of the interplay between the various parts of the animal cell, as well as between different cells, in the overall control of protein biosynthesis. These aspects of the synthesis of proteins in higher organisms are still not properly understood and this book provides a useful discussion of some of these problems.

The first part of the book consists of seven chapters dealing with investigations of protein synthesis during oögenesis of lepidoptera, mainly by cytochemical methods. These studies are essentially qualitative, and unfortunately the photographs are only in black-and-white and generally of poor quality. Furthermore, the figures and diagrams are often inadequately labelled and not clearly explained.

In the twelve chapters of the second part of the book protein synthesis in the animal cell is discussed with special reference to the function of the nucleus and cytoplasmic structures. The discussion of the biochemical mechanism of protein synthesis is, as already mentioned, necessarily incomplete and there are several more up to date reviews and books on these topics.

For these reasons, it is felt that this book is likely to be of only limited interest. The scanty bibliography and the absence of an index are further important drawbacks to its usefulness. H. R. V. ARNSTEIN

## ACID FOR METABOLIC CONJUGATION

### Glucuronic Acid, Free and Combined

Chemistry, Biochemistry, Pharmacology and Medicine. Edited by Geoffrey J. Dutton. Pp. xviii + 629. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1966.) 200s.

THE reader will agree that no defence is necessary for considering glucuronic acid to be a glucose metabolite of biochemical interest in its own right. Acceptance of this view will facilitate further progress in research of the role of this acid in living matter. Although historically glucuronic acid has been considered for a long time to be a conjugating radical made available by the body for detoxification purposes, this process is currently seen simply as one of the many recognized metabolic conjugations which have biochemical significance.

This book represents a valuable compilation of the published work relating to glucuronic acid, and some of the chapters are outstanding. One of these is the first chapter, by Marsh, discoverer of the saccharolactone pathway of glucuronic acid metabolism, which represents an authoritative, exhaustive but nevertheless readable treatment of the chemistry of D-glucuronic acid and its glycosides. There is an encyclopaedic list of all the glucosiduronic acids reported in the literature, and the degree of completeness of the evidence in support of their nature is indicated.

A central chapter written by the editor of the book is an honest, detailed survey of the development of our understanding of the mechanisms of glucuronic acid transfer reactions. That this understanding is far from complete is obvious when anyone searches for information about the nature of the enzyme UDP glucuronyl transferase and about the kinetics of the reaction it catalyses. In these circumstances resort to legal terms, such as "inadmissible evidence", seems out of place in the absence of all the facts regarding the nature of the transferase(s). Here, also, "multiplicity" of transferases seems to lack biochemical definition.

Levy and Conchie's noteworthy studies on aldono-lactone inhibition of glycosidases are included. Their statement that the lysosome theory begs the whole question of the *in vivo* action of acid hydrolases will fall on an increasing number of receptive ears today.

Jayle and Pasqualini's review of steroids and thyroxine, Silbert's of polysaccharides, Smith and Williams's of drugs and Schmidt and Lester's of bilirubin, are all written in a manner which is both informative and readable. A valuable feature of the book is the collection of analytical methods at the end of several chapters. I recommend the book for the serious student and investigator in biochemistry, pharmacology and medicine.

WILLIAM H. FISHMAN

## COMPOUNDS HOLD TOGETHER

### Structure and Bonding

Vol. 1. Edited by C. K. Jørgensen, J. B. Neilands, R. S. Nyholm, D. Reinen and R. J. P. Williams. Pp. 281. (Berlin and New York: Springer-Verlag, 1966.) 48 D.M.

THIS new review series is intended to appear at irregular intervals and to deal with problems in chemical structure and bonding in modern inorganic chemistry, chemical physics and biochemistry.

The first volume contains nine reviews received between January and May 1966. "Recent Progress in Ligand Field Theory", by C. K. Jørgensen, is based on lectures given at the Summer School on Ligand Field Theory held at Constance in September 1962. This gives a brief account of the interpretation of electronic spectra of

transition metal complexes and of refinements of electrostatic ligand field theory. "The Ambident Nature of Cyanide", by D. F. Shriver, is a clear review of structures containing the bifunctional cyanide ligand.

Three fully documented biochemical articles occupy half the volume. "Naturally Occurring Non-porphyrin Iron Compounds", by J. B. Neilands, deals mainly with iron(III) hydroxamates. These stable complexes seem to carry iron through metabolic channels and insert it into porphyrins, enzymes and proteins. "The Chemistry and Function of Ferredoxin" is by B. B. Buchanan, and describes the non-haem iron protein, which plays an important part in the action of anaerobic fermentative bacteria, in photosynthetic bacteria and in plant photosynthesis. "The Transferrins", by R. E. Feeney and S. K. Komatsu, concerns the homologous proteins found in various fluids of vertebrate animals; for example, blood serum, milk and egg-white (conalbumin).

Three of the final group of four articles are revised versions of lectures given at the Symposium on Soft and Hard Acids and Bases held at the Cynamide European Research Institute at Cologny in May 1965. "Factors Contributing to (b)-behaviour in Acceptors", by S. Ahrland, resembles another article by the same author (*Svensk Kemisk Tidskrift*, 77, 584; 1965) and is an outgrowth of Ahrland, Chatt and Davies's original division of metal ions into classes (a) and (b) on the basis of their affinity sequences for donor atoms. "The Classification of Acceptors and Donors in Inorganic Reactions", contributed by R. J. P. Williams and J. D. Hale, is a more critical account of the inter-relations of the (a) and (b) classification, Pearson's hard and soft terminology and polarizability. "Electronic Polarizability, Innocent Ligands and Spectroscopic Oxidation States", by C. K. Jørgensen, also discusses these concepts. Finally, there is "Displacement Reactions and the Concept of Soft and Hard Acids and Bases", by R. F. Hudson, which describes correlations of rate constants with various properties of nucleophiles.

The first volume has no index and the five editors have not quite achieved a common format. It is not yet clear whether this particular marriage of chemistry and biochemistry will be happy. The soft back is rather highly priced for the individual, but is an essential purchase for chemical and biochemical libraries. It contains much to interest, stimulate and provoke.

F. J. C. ROSSOTTI

## IMMUNOLOGICAL DANGER

### Delayed Hypersensitivity

By J. L. Turk. (North-Holland Research Monographs: Frontiers of Biology, Vol. 4.) Pp. ix + 252. (Amsterdam: North-Holland Publishing Company, 1967.) 80s.

SYMPOSIA on delayed hypersensitivity commonly begin with a discussion of the definition of the term. This usually ends inconclusively, and accurately indicates our confusion and lack of understanding of this phenomenon. Hypersensitivity, however, is commonly defined as an immunological reaction harmful to the body, and the term "delayed" refers to the interval that is observed between the injection of antigen into a sensitized animal and the development of an inflammatory reaction at this site. The phenomenon was first described as characteristic of hypersensitivity to bacteria and was initially termed "bacterial allergy". The phrase "delayed-type hypersensitivity" was introduced by Chase, who obtained such delayed, slowly developing reactions, not only with bacteria but also with purified antigens of various types. The next crucial finding was again made by Chase who, in collaboration with Landsteiner, discovered that passive transfer of delayed-type hypersensitivity could not be achieved with serum, that is circulating antibodies, but could be so transferred with lymphoid cells or cells from

peritoneal exudates. Our present concept of delayed hypersensitivity is based very largely on the experiments of Chase and his collaborators. Most workers agree that the passive transfer by lymphoid cells and the demonstrable unimportance of circulating antibody are the essential criteria which characterize delayed hypersensitivity. As such it seems to represent a separate aspect of the immune response in general, and is now sometimes termed cell-mediated immunity or hypersensitivity. By these criteria certain important biological phenomena can be considered examples of delayed hypersensitivity. Instances that can be cited are the rejection of skin or organ grafts by incompatible recipients, or the similar reactions to tumours, either implanted or arising *de novo* in response to viral or chemical stimulation. There is also the possibility, although it is not yet convincingly proved, that certain "auto-allergic" experimentally induced pathological lesions, for example encephalitis or thyroiditis, may be, at least in part, further examples. In its simplest form of an acute, transient, but slowly developing inflammatory reaction to the local presence of antigen there are many examples, bacterial or non-bacterial.

The most superficial study of delayed hypersensitivity immediately reveals a number of very important and unsolved problems. Examination of immunoglobulins is lending clarity to some aspects of immunology, but delayed hypersensitivity remains in a miasma of uncertainty that is all the more frustrating, because, as a phenomenon, it is likely to be of great importance both to immunology and to some of the most serious problems of contemporary clinical medicine.

Dr Turk has himself made distinguished contributions to our knowledge of delayed hypersensitivity and has had the courage to present and discuss the mass of experimental data, much of it conflicting, that has now accumulated. Doubts are expressed from time to time as to the wisdom of producing "hard cover" monographs on subjects that are the object of intensive current research and controversy and where views are likely to change rapidly. On the other hand, review articles seldom provide space for a full critical study of such a large field. The complexity and importance of the subject further justify the venture.

One of Dr Turk's principal problems has clearly been to decide what to include in his terms of reference or indeed whether to regard cell mediated hypersensitivity as an entity or as an immunological rag-bag. He has wisely steered a middle course and included practically every phenomenon that could be considered relevant without committing himself as to whether it will prove ultimately to be so. The exception is tumour immunity, which is not given a separate section although it might well have done so, especially in view of the high quality of some of the work now being carried out in this field, once regarded as the "lunatic fringe" of both cancer studies and immunology. In general, in his choice of topics, Dr Turk has inevitably been guided by the criteria quoted here and based on the work of Chase and his predecessors.

The first part of the book is devoted to "classical" experimental delayed hypersensitivity, that is, slowly developing but transient inflammatory reactions in skin provoked by local injection of antigen into a suitably sensitized host. This section is unexciting but definitive. The author then deals with homograft reactions in the light of the part played by delayed hypersensitivity, and next discusses unresponsiveness from the point of view of the light shed by various unresponsive states on the basic mechanism of delayed hypersensitivity. In this context, however, "light" is a relative term. In the next chapter Dr Turk deals with his own *forte*, the events in local lymph nodes during delayed hypersensitivity. The next topic is antigenic specificity in delayed hypersensitivity, especially in relation to the intriguing experiments of Benacerraf, Gell, Leskowitz and others. This induces the feeling, commonly experienced while reading about

delayed hypersensitivity, that the results are of basic importance even if not fully understood.

The most crucial section is left until last, and that is the role of antibody in delayed hypersensitivity and the role of delayed hypersensitivity in antibody production. After a succinct survey of the available evidence he comes down fairly firmly against antibody as we know it playing an important part in delayed hypersensitivity, including the so-called cytophilic antibody. He takes less than two pages to make a similar decision against delayed hypersensitivity as a stage (at which arrest may occur) in the production of classical antibody, preferring the view that it is a separate phenomenon.

Earlier in the book Dr Turk weighs the evidence linking delayed hypersensitivity and cellular immunity (for example in tuberculosis) and concludes that there is no justification for assuming a direct relationship. It may be that this hoary ghost has at last been exorcized or it may be that it will rise again to haunt us. Dr Turk has more trouble with his discussion of the respective roles of macrophages and lymphocytes in delayed hypersensitivity and does not entirely succeed in disentangling for the reader the conflicting experimental evidence, although he certainly cannot be blamed for this.

Nevertheless, the author deserves congratulations for his careful assessment of the results of others and for the reasoned conclusions he draws from them. Not everyone will agree with his evaluation of individual pieces of research, but many will be grateful where he makes order out of chaos. This is an instance where the reviewer's cliché that an author has performed a valuable service is more than justified.

W. G. SPECTOR

## BRAIN AND HORMONES

### Neuroendocrinology

Edited by Luciano Martini and William F. Ganong. Vol. 1. Pp. xx+774. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1966.) 260s.

In the past twenty years the study of the mutual interaction of the central nervous system and the glands of internal secretion has been one of the most active areas of experimental endocrinology. A major part of this work has been concerned with the details of the mechanism by which the hypothalamus, through the anterior pituitary, affects the activity of the gonads, adrenal cortex and the thyroid gland. During this period the use of a wide range of techniques, previously not often encountered in endocrine research laboratories, has become common. Some of the methods employed are sectioning of the pituitary stalk, transplantation of the pituitary, cultivation of pituitary fragments *in vitro*, the destruction of discrete areas of the hypothalamus by stereotaxic methods, electrical stimulation of the brain in the conscious animal, electrical recording from groups of neurones or single cells, cannulation of the pituitary, and the injection or implantation of hormones into the pituitary or hypothalamus. Employing these novel techniques and, more importantly, sharing a few basic concepts, research workers with a wide range of interests have found themselves grouped together and their common field of study, brain-hormone interactions, has been designated "neuroendocrinology". This book, published under that title, illustrates the range and volume of recent and current work that may be classified under this heading.

A concise introductory chapter by Ganong is followed by a detailed account of the anatomy, gross and microscopic, of the hypothalamus and pituitary by Daniel. Particular attention is rightly paid to the vascular connections, the hypothalamo-hypophysial portal vessels, that form the essential functional link between the brain

and the adenohypophysis. The neural connexions of the hypothalamus and the limbic system are reviewed by De Groot, but here, and elsewhere in the book, the fact that no agreed or precise function, in the control of endocrine activity, can be ascribed to extra hypothalamic structures indicates where limitations in present techniques leave gaps in our knowledge. A survey of lesion making techniques and a useful listing of stereotaxic brain atlases for different species follow, and bring us to an important chapter on neurosecretion. Bern and Knowles deal extensively with this difficult topic, drawing their examples and their excellent illustrations from many different species. Their definition of a neurosecretory neurone on a functional basis is stimulating and seems likely to provoke much discussion.

A series of reviews of specific topics follows. Two critical but balanced chapters by Sawyer and Mills, and by Cross, deal with the control of secretion of the vasopressin and oxytocin. The value of these is greatly increased by the fact that they have not confined themselves to purely neuroendocrinological topics but include discussions on such problems as assay methods and the physiological significance of the various actions of oxytocin. The chapter by McCann and Dhariwal on hypothalamic releasing factors is a good survey of the physiology of this sometimes contentious field, though not all workers would agree with their assessment of the physiological significance of the fact that vasopressin can act on the anterior pituitary to cause the secretion of adrenocorticotrophic hormone, that is as a corticotrophin releasing factor or CRF. Their conclusion that the releasing factors are polypeptides may need to be revised in the light of more recent findings that were not available to them at the time their contribution was written. The remainder of the book deals mainly with the control of the secretion of specific pituitary trophic hormones; an interesting and successful innovation is a separate discussion of the secretion of gonadotrophin in the male by Davidson. The general standard of these chapters is high, but three are of particular interest. Reichlin deals comprehensively with the pituitary-thyroid system, emphasizing the necessity for an integrative approach that considers the interaction of a "neural" stimulus from the hypothalamus and a "humoral" feedback control, taking into account the physico-chemical state of the target organ hormone in the blood and variations in the rate of disposal by the peripheral tissues. Mulrow discusses the control of aldosterone secretion, giving due attention to neural factors, established and postulated, but also to the renin-angiotensin system and other influences on the adrenal cortex. Meites, in a wide ranging review, gives a detailed account not only of the neuroendocrine but also of the many other factors that must be considered in the discussion of the physiological control of mammary gland growth and lactation. There is some danger that to concentrate exclusively on purely neural factors may lead to neglect of the other mechanisms that can affect the activity of the endocrine glands. These three articles show how the importance of neuroendocrine control is best seen in the context of a wider analysis of system function in the whole animal.

This volume has been produced and edited to a high standard, but a confusing transposition of lines was noted in the section on neurosecretion (page 146) and one *non requitur* (section VB, page 546 of Chapter 13) appears to have escaped notice. The other aspect of brain-hormone interaction, the study of the effects of hormones on the central nervous system, will be the main theme of a second volume from the same editors. Together the two volumes should provide a detailed and comprehensive survey of the literature of a rapidly developing but rather diffuse subject that will be of value to the research worker and to the senior student who has already acquired a sound basic knowledge of endocrinology.

K. BROWN-GRANT

## University News:

## Liverpool

MR F. SAWKO, reader in civil engineering in the University of Leeds, has been appointed to the new second chair in civil engineering, and Mr R. K. Penny, assistant director of research in the University of Cambridge, has been appointed to the new chair of engineering design and production.

## London

THE following titles have been conferred: professor of mechanical engineering, on Dr T. H. Lambert, in respect of his post at University College; professor of physics, on Dr D. H. Martin, in respect of his post at Queen Mary College; professor of rational mechanics, on Dr F. A. E. Pirani, in respect of his post at King's College; professor of psychology, on Dr P. H. Venable, in respect of his post at Birkbeck College; reader in electrical engineering, on Dr R. M. Redwood, in respect of his post at Queen Mary College. Professor D. V. Lindley, professor of statistics in the University College of Wales, Aberystwyth, has been appointed to the chair of statistics tenable at University College, and Dr J. H. E. Cohn, lecturer in mathematics at Bedford College, has been appointed to the readership in mathematics tenable at Royal Holloway College.

# CORRESPONDENCE

## What Place for Engineers?

SIR,—On June 17 you commented on Professor Thring's article advocating the establishment of an academy for engineers. Much of this discussion was based on the example of the recently created National Academy of Engineering in the United States which, as you rightly said, has a number of features which would not easily fit into the British scene. May I draw your attention to the experience of an earlier and well-tried example of engineering academy, namely, the Royal Swedish Academy of Engineering Sciences (Ingeniörsvetenskapsakademien, IVA)? This body, having developed in a European country, has many features worth considering in relation to present British engineering and industrial needs.

The Swedish Academy, established in 1919 and hence the first of its kind in the world, was a deliberate attempt on the part of an influential group of Swedish industrialists and engineers to correct a national situation. Sweden has for long enjoyed an eminence in fundamental research which is great in relation to the size of the population. By the end of the First World War, however, the draw of academic research was so strong that industry became concerned that only a small proportion of the best scientific minds of the country were willing to take up applied work. A conscious attempt was therefore made to create a centre for the applied sciences with status and scientific acceptance to provide a reasonable balance of attraction. The decision was not to create an engineering academy in the general sense, but a Royal Academy of Engineering Sciences. This was done with the personal interest and participation of the King, who became its patron. The director of the Academy was given the title of Professor to provide the necessary academic respectability and the Crown Prince attended a number of early meetings, thus symbolizing the importance of the new institution in the eyes of the State.

The result has been outstandingly successful and, although it would be extravagant to claim that the success of Sweden during the past few decades in creating highly competitive science based industries is due to the Academy, nevertheless its part has been very great. IVA has not only succeeded in achieving for the engineer



a high status in society and public interest in his work, but has greatly encouraged the penetration of the scientific approach within engineering itself.

The Academy has been particularly fortunate in having at its head three outstanding men: Axel Enström, Edy Velander and now Sven Brökhult, very different personalities but each uniquely appropriate for the phase of development which he has led.

An important feature of IVA's activity is the annual commemoration meeting held each October on the anniversary of the granting of its charter. This is a splendid occasion, attended generally by the king and the élite of Sweden in addition to the members of the Academy, at which the director is bound by constitution to deliver a survey of the development of science and technology during the past year. This has provided a series of brilliantly staged demonstrations and lectures on new technological developments which have brought to the attention of the informed public many of the important applications of science, often long before their significance has been generally recognized.

This is not the place to describe the work of IVA in detail. But it should be mentioned that it consists of a maximum of 200 persons under 65 years of age. There are ten sections—mechanical, electrotechnical, building, chemical, mining and metallurgical science, computer engineering, basic science, forest and wood technology, economics and biotechnics. Each section is allotted a statutory number of seats. Once a member reaches the age of 65, his seat is no longer counted (although his membership continues) and a new member can be elected. This arrangement enables a steady rejuvenation of the body. Members are engineering scientists of prominence from industry, higher education and research institutes. IVA has done much to promote the establishment of new research institutes, particularly for borderline subjects, and is frequently called on to advise the government.

Far from exacerbating the difference between scientists and engineers which you fear, IVA has built a bridge between them and has the respect and support of the academic scientists.

Yours faithfully,

ALEXANDER KING

Director for Scientific Affairs,  
OECD,  
Paris.

### Applying Research

SIR,—While one would agree that some of the points made by Professor Temperley (*Nature*, 214, 1378; 1967) are valid, I would like to take issue with one of them.

He says that he "once interviewed a distinguished PhD of several years' standing whose job was to devise schedules of cutting up steel rod and plate in order to fill orders with minimum wastage!". Professor Temperley hoped this was a very extreme case of inefficient use of scientists.

In fact this particular problem is very important. In the special case of minimizing waste in cutting orders out of steel plate it is mathematically intricate and poses great difficulties. This, however, is not the point at issue, which is the value of this sort of work to the steel industry.

At present about 4 per cent of steel production is waste, due, amongst other things, to the problem of cutting up orders out of batches of steel. This represents an annual loss of about £6m a year. Hence, although at first sight this seems a trivial problem, it is, on a national scale, most important, and any PhD scientist who could save a few per cent of this £6m would be making a sizable contribution to his employers' economic wellbeing. In addition, of course, general solutions to this problem could be applied not only throughout the steel industry but also to other industries, including glass and paper

manufacture where similar problems arise and where important economies remain to be effected.

Yours faithfully,

B. H. P. RIVETT

University of Lancaster,  
Skein House,  
Queen Square, Lancaster.

### Titles

SIR,—I have read the letter from Mr Eugene Munroe in *Nature* (214, 1064; 1967) concerning titles. I trust that you will not be swayed by his arguments.

Surely the function of the titles of articles in *Nature* about general scientific policy is that they should be "catch-reader". Far too few scientists, I fancy, concern themselves with such matters and anything which can trap them into broadening their outlook and thinking is to be welcomed. To set against this socially desirable end the convenience of people who want to have "a card file on scientific policy" is to be irresponsible to the scientific community as a whole.

Yours faithfully,

D. W. EWER

Department of Zoology,  
University of Ghana,  
P.O. Box 67,  
Legon, Accra,  
Ghana.

### Pharmacology

SIR,—A drug has been defined as "any chemical substance that, administered to a living organism (or its part), gives rise to a scientific paper". Although drugs may be administered for other purposes than scientific investigation, this purpose concerns us now.

Reading through the classified list of Letters to the Editor in this week's *Nature* (July 1, 1967), I notice six letters on the effects of drugs, but no heading of Pharmacology, which is the science of drugs. One of these letters is placed under Physiology ("Inhibition of Gastric Acid Secretion by a Purified Bacterial Lipopolysaccharide"). Two are under Pathology ("Effect of Cyclophosphamide, 6-Mercaptopurine or Methotrexate on the Furth Rat Leukaemia" and "Demonstration of Copper and Acid Phosphatase Activity in Hepatocyte Lysosomes in Experimental Copper Toxicity"). Another pharmacological letter is placed under Biochemistry ("Effect of Actinomycin on Protein Synthesis by Lymphocytes"). The fifth letter is under Microbiology ("Action of Ethidium Bromide on Growth of Herpes Virus in Cell Cultures") and the sixth under Biology ("Toxicity of Tobacco Smoke to the Spotted Alfalfa Aphid *Therioaphis maculata* (Buckton)").

Concerned as it is with the use of chemical substances to explore the nature of life, pharmacology is a meeting point of chemistry and biology and therefore a subject that is growing fast. To deny it a place among the headings of your letters distorts the picture of how work is distributed in the various fields of research. It also forces pharmacologists to search harder for what they ought to read. Does this forced searching not deny a simple extension of your own thesis that "the professional reader is the one most concerned to know precisely what claim on his attention an article sets out to make" (*Nature*, June 10, 1967, Vol. 214, p. 1078)?

The removal of the subject head Pharmacology from the classification of Letters to the Editor is not a pleasing change.

Yours faithfully,

H. O. J. COLLIER

Department of Pharmacological Research,  
Parke, Davis and Company,  
Hounslow, Middlesex.

## APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

**LABORATORY TECHNICIAN** in the DEPARTMENT OF BIOLOGY AND CELL BIOLOGY to assist (a) with the preparation of classwork and (b) with histochemical and biochemical research—The Clerk to the Governing Body, Colwich Polytechnic, Wellington Street, London, S.E.18 (July 28).

**LECTURER IN PHYSICS**—The Secretary, St. Mary's Hospital Medical School (University of London), Paddington, London, W.2 (July 29).

**COMPUTER PROGRAMMER** (with a good honours degree in mathematics or pure or applied science, and experience of computer programming and numerical analysis) in the DEPARTMENT OF ELECTRICAL AND ELECTRONIC ENGINEERING—The Registrar and Secretary, The University, Leeds, 2 (July 31).

**LECTURER** (preferably with experience in immunochemistry) in the SENIOR RESEARCH UNIT, DEPARTMENT OF CLINICAL BIOCHEMISTRY—The Registrar, The University, Newcastle upon Tyne (July 31).

**RESEARCH ASSISTANT** (with previous programming experience, and preferably some statistical training) to develop methods of using computers analysis of results of medical examinations—Dr. W. W. Holland, Department of Clinical Epidemiology and Social Medicine, St. Thomas's Hospital Medical School, London, S.E.1 (July 31).

**RESEARCH FELLOW** (with a Ph.D. or the equivalent and at least some experience in biochemical research) in BIOCHEMISTRY or CELLULAR PHYSIOLOGY to take part in a research project on the mode of action of the thyroid hormones—Prof. J. R. Bronk, Department of Biology, University of York, Heslington, York (July 31).

**ASSISTANT EXPERIMENTAL OFFICER** (with a degree in botany or agricultural botany) to work on the physiology of growth and yield of field crops, particularly on root growth and on effects of growth regulators—The Secretary, Rothamsted Experimental Station, Harpenden, Herts (August 4).

**LECTURER IN GEOPHYSICS** in the DEPARTMENT OF GEOLOGY—The Registrar and Secretary, Old Shire Hall, University of Durham, Durham (August 4).

**NERC SENIOR RESEARCH ASSISTANT or RESEARCH ASSISTANT** (preferably with qualifications in physics or electronic engineering) in MARINE GEOPHYSICS to take part in a programme of geophysical investigations of the crust and mantle in the North-Eastern Atlantic and on the British Continental shelf—The Registrar and Secretary, Old Shire Hall, University of Durham, Durham (August 4).

**ASSISTANT LECTURER or LECTURER** (with special qualifications in moral philosophy) in PHILOSOPHY—The Registrar (Room 39, O.R.B.), The University, Reading, Berkshire (August 5).

**ASSISTANT LECTURER and a LECTURER** (experienced in the use of high speed digital computers and an interest in numerical analysis, statistics or operational research) in COMPUTATION in the DEPARTMENT OF MATHEMATICS—The Registrar, University of Manchester Institute of Science and Technology, Sackville Street, Manchester, 1 (August 7).

**LECTURER or ASSISTANT LECTURER** (preferably with research interests in operational research or the computational aspects of statistics) in the ELECTRONIC COMPUTING LABORATORY—The Registrar, The University, Leeds, 2 (August 7).

**SENIOR LECTURER** in the DEPARTMENT OF PSYCHIATRY—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh (August 7).

**CHAIR OF PHARMACOLOGY**—The Secretary of the University Court, The University, Glasgow (August 11).

**LECTURER or ASSISTANT LECTURER** (with a good honours degree or equivalent qualification) in SOCIOLOGY—The Academic Registrar, The City University, St. John Street, London, E.C.1, quoting Ref. 41/SS (August 11).

**RESEARCH ASSISTANT** (science graduate, either in chemistry or in biochemistry) in DERMATOLOGY to undertake work on the histochemistry of the skin with special reference to steroid synthesis—The Secretary of the University Court, The University, Glasgow (August 11).

**LECTURER IN ENGINEERING** (Materials Science); a **LECTURER IN ENGINEERING** (Mechanical/Electrical); and a **LECTURER IN CIVIL ENGINEERING** at the University College of Townsville, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, W.1 (Australia and London, August 12).

**DEMONSTRATOR** (graduate or about to graduate) in BIOLOGY—The Registrar, The University, Keele, Staffordshire (August 14).

**TECHNICAL OFFICER/RESEARCH ASSISTANT** (experienced in conventional analytical techniques and preferably experienced in radio-chemistry) in the DEPARTMENT OF GEOPHYSICS AND GEOCHEMISTRY, Research School of Physical Sciences, to use and develop neutron activation methods in a research programme concerned with elemental abundances in meteorites, minerals and rocks—The Bursar, Australian National University, Box 4 P.O., Canberra, A.C.T., Australia (August 14).

**LECTURER** (with a special interest in any systematic aspect of geography and a wish to obtain a regional interest in Africa) in GEOGRAPHY—The Assistant Registrar (Establishment), The University of Sussex, Essex House, Falmer, Brighton, Sussex, quoting Ref. 911/2 (August 15).

**RESEARCH ASSISTANT** (with a good honours degree in psychology or a related subject) in the DEPARTMENT OF PSYCHOLOGY—The Registrar, The University, Newcastle upon Tyne (August 15).

**SENIOR LECTURERS (2) IN SURGERY** at the University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 18).

**CHEMIST, BIOCHEMIST or MICROBIOLOGIST** (with an honours degree or equivalent) in the ANIMAL SCIENCES DIVISION of An Foras Taluntais (The Agricultural Institute) to carry out research on the problems associated with extending the shelf life of packaged fresh meat—The Director, An Foras Taluntais, 33 Merrion Road, Dublin 4, Republic of Ireland (August 21).

**PROFESSOR OF CHEMICAL PATHOLOGY**; **PROFESSOR OF OPHTHALMOLOGY**; and a **PROFESSOR OF RADIOLOGY** at the University of Ibadan, Nigeria—The Inter-University Council, 33 Bedford Place, London, W.C.1 (August 21).

**LECTURER and an ASSISTANT LECTURER** (with a good honours degree in a relevant subject) in MATERIALS SCIENCE in the DEPARTMENT OF MECHANICAL ENGINEERING—The Secretary of the University Court, The University, Glasgow (August 26).

**ASSISTANT LECTURER** in SOCIAL AND PREVENTIVE MEDICINE (statistical aspects) (a medical qualification is not necessary)—The Secretary, The Queen's University, Belfast, Northern Ireland (August 31).

**CHAIR OF DENTAL PROSTHETICS**—The Registrar, The University, Newcastle upon Tyne (August 31).

**CHAIR OF MICROBIOLOGY** at the University of Hong Kong—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Hong Kong and London, August 31).

**LECTURER or SENIOR LECTURER** (with a qualification in photogrammetry and photogrammetric engineering from a University or other recognized

institution, and preferably a degree or other qualification in surveying or civil engineering, and some teaching and professional experience) in PHOTOGRAMMETRY at the University of Otago, Dunedin, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, August 31).

**LECTURER** (with a good honours degree in engineering or an equivalent qualification and preferably industrial experience in mechanical engineering design) in MECHANICAL ENGINEERING DESIGN—The Registrar, University of Strathclyde, George Street, Glasgow, C.1 (August 31).

**SENIOR LECTURER or LECTURER** (suitably qualified and interested in solid state studies or atmospheric physics) in PHYSICS at the University of Canterbury, Christchurch, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (August 31).

**LECTURER IN PHYSICS** at the University of Western Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 2).

**READER** (with a degree in mechanical engineering at least at Master's level) in MECHANICAL ENGINEERING; and a **SENIOR LECTURER or LECTURER** (with a degree in mechanical engineering, preferably at honours level, of a recognized Commonwealth university, together with either experience in industry, or research and teaching experience at university level) in MECHANICAL ENGINEERING at the University of Canterbury, Christchurch, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (September 15).

**CHAIR OF COMPUTER SCIENCE**—The Registrar, University of Warwick, Coventry, Warwickshire (September 30).

**CHAIR OF BOTANY**—The Secretary, The Queen's University, Belfast, Northern Ireland (October 14).

**FORBES CHAIR OF ORGANIC CHEMISTRY**—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh (October 31).

**ASSISTANT BIOCHEMIST** (preferably with previous experience in protein chemistry) to assist in the supervision of the production and testing of blood plasma protein fractions for clinical use in hospitals and to participate in associated research and development work—The Secretary, The Lister Institute of Preventive Medicine, Elstree, Herts.

**DEMONSTRATOR** (graduate in biochemistry, microbiology or chemistry); and a **RESEARCH ASSISTANT** (graduate in biochemistry, microbiology or chemistry) in the DEPARTMENT OF BIOCHEMISTRY AND AGRICULTURAL BIOCHEMISTRY—The Registrar, University of Wales, Aberystwyth.

**LIBRARIAN and INFORMATION OFFICER** (preferably with a scientific qualification) to take charge of the library and archives, information and public relations and to be editor of the Institution's publications—The Royal Institution, 21 Albemarle Street, London, W.1.

**PROFESSOR OF COMPUTER SCIENCE**—The Registrar (Room 39, O.R.B.), The University, Reading, Berkshire.

**SENIOR RESEARCH TECHNICIAN** for interesting and varied work on immunity in reticuloses and other malignant diseases—Clerk to the Governors, St. Bartholomew's Hospital, London, E.C.1, quoting Project No. 863, Ref.: ASCE1089.

## REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

### Great Britain and Ireland

- Bulletin of the British Museum (Natural History). Geology. Vol. 14, No. 5: Further Notes on Palaeoniscoid Fishes with a Classification of the Chondrostei. By B. G. Gardiner. Pp. 143-206 + 3 plates. (London: British Museum (Natural History), 1967.) 32s. [245]
- The Work of the Cruse Clubs, 1965/66. (Counselling Service for Widows and their Children.) Pp. 14. (Richmond, Surrey: Cruse Clubs, 1967.) [245]
- Smith Kline and French Foundation. Fourth Annual Report 1966. Pp. 8. (Welwyn Garden City: Smith Kline and French Foundation, 1967.) [245]
- Statistical Review of the World Oil Industry 1966: Reserves, Production, Consumption, Trade, Refining, Tankers, Energy. Pp. 24. (London: The British Petroleum Co., Ltd., 1967.) [255]
- National Lending Library for Science and Technology—Descriptive Brochure. Pp. 16. (Walton, Boston Spa: National Lending Library for Science and Technology, 1967.) [255]
- Quest, No. 1 (May 1967). (The Journal of The City University, London.) Pp. 32. (London: The City University, 1967.) 4s. 6d. [255]
- The Wellcome Trust 1964-1966—Sixth Report. Pp. 99. (London: The Wellcome Trust, 1967.) [265]
- Bulletin of the British Museum (Natural History). Geology. Vol. 15, No. 1: The Palaeontology and Stratigraphy of the Lower Part of the Upper Kimmeridge Clay of Dorset. By J. C. W. Cope. Pp. 1-79 + 33 plates. (London: British Museum (Natural History), 1967.) 95s. [265]
- National Physical Laboratory. Report for the year 1966. Pp. xiv + 230 + 18 plates. (London: H.M. Stationery Office, 1967.) 21s. net. [265]
- Northern Ireland: Ministry of Agriculture. Leaflet No. 124: Weed Control in Agriculture. Pp. 16. Leaflet No. 145: Principles of Chemical Weed Control in Horticulture. Pp. 15. (Belfast: Ministry of Agriculture, 1967.) [265]
- Journal of Materials Science, Vol. 1, No. 1 (February, 1966). Pp. 1-116. Published quarterly. Subscription rates: (surface mail) £8; \$23; single copies 50s.; \$7. Air mail rates: £10 10s.; \$30; single copies 64s.; \$9. (London: Chapman and Hall, Ltd., 1966.) [265]
- Cotton Research Corporation. Progress Reports from Experiment Stations, Season 1964-65. Malawi. Pp. 15. 2s. 6d. Progress Reports from Experiment Stations, Season 1965-66. Northern Nigeria. Pp. 28. 2s. 6d. South Arabia. Pp. 16. 2s. 6d. Thailand. Pp. 19. 2s. 6d. (London: Cotton Research Corporation, 1966 and 1967.) [305]
- Report of the Rugby School Natural History Society for the year 1966. (Ninety-ninth Issue.) Pp. 23. (Rugby: Rugby School Natural History Society, 1966.) [305]
- Report of the Astronomer Royal for Scotland for the year ending 31st March 1967. Pp. 19. (Edinburgh: The Royal Observatory, 1967.) [305]
- British Flame Research Committee. 1966 Annual Report. Pp. 20. (London: British Flame Research Committee, 1967.) [305]
- Council for the Preservation of Rural England: Sheffield and Peak District Branch. Annual Report, April 1967. Pp. 26. (Sheffield: Council for the Preservation of Rural England, Sheffield and Peak District Branch, 1967.) [315]
- Research Using Transplanted Tumours of Laboratory Animals: a Cross-Referenced Bibliography—III. By D. C. Roberts. Pp. 164. (London: Imperial Cancer Research Fund, 1967.) [315]
- The Sterling Problems and The Six. By Susan Strange. (European Series, No. 4.) Pp. 70. (London: Political and Economic Planning, 1967.) 7s. 6d. [316]

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Paper 1674: *Magnitude and Frequency of Floods in the United States Part 2-B: South Atlantic Slope and Eastern Gulf of Mexico Basins, Ogeechee River to Pearl River*. By Harry H. Barnes, Jr., and Harold G. Gole. Pp. xvi + 409 + plate 1. Water-Supply Paper 1743: *Quality of Sur*

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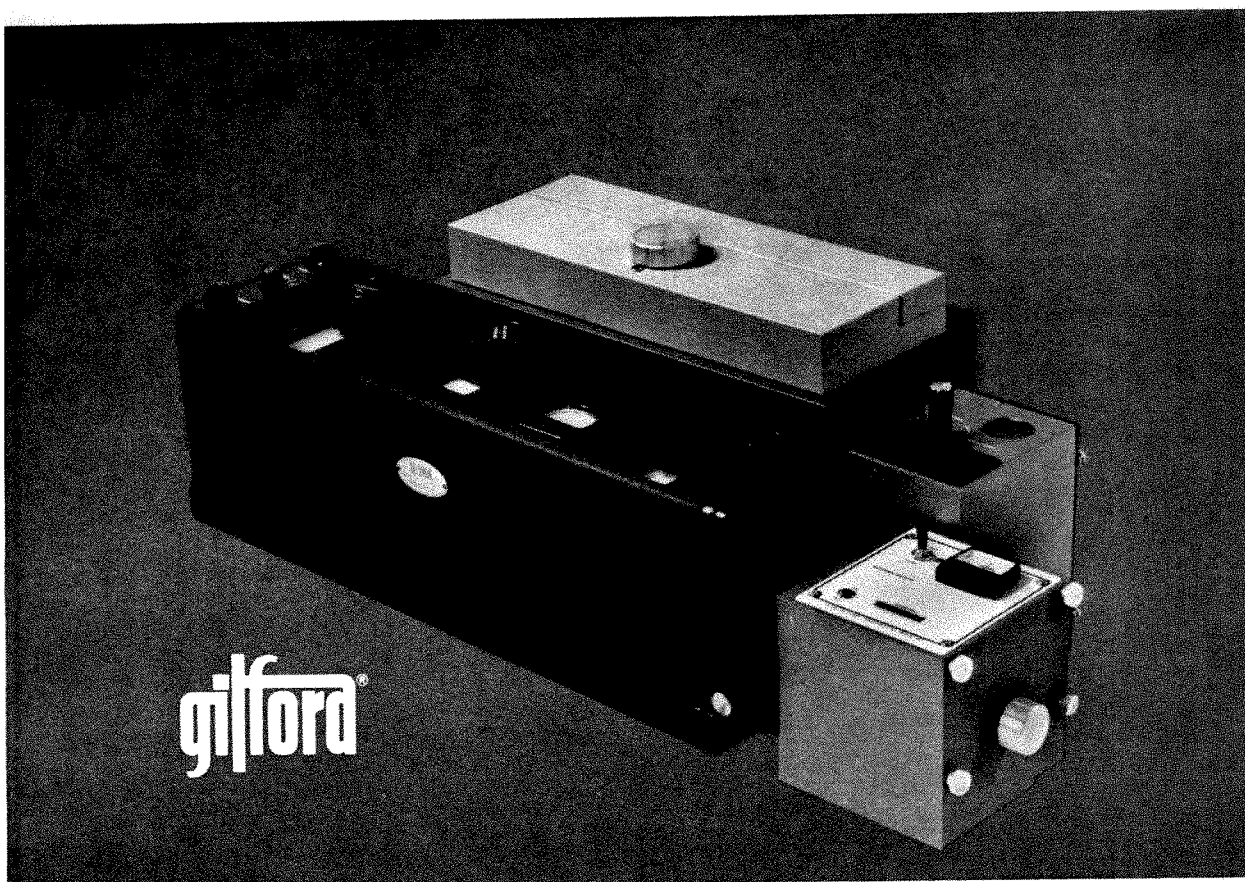
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Further information and application forms from Secretary, Commonwealth Agricultural Bureaux, Farnham House, Farnham Royal, Bucks, England. Applications must be submitted not later than October 15, 1967. Government employees must apply through official channels. (X120)

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## Scientific Civil Service

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Further particulars should be obtained from The Secretary, The University, Aberdeen, with whom applications (two copies) should be lodged by September 30, 1967. (313)

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A **HEAD TECHNICIAN** is required for the Laboratory of Cytopathology at the National Institute for Medical Research, to be responsible for supervision and training of technical staff providing an Institute histological service. The department also includes electron microscopical and photographic facilities. Applicants should have wide experience in the techniques of normal or pathological histology, and should preferably possess F.I.M.L.T. or equivalent qualifications. Grade and salary on appointment will be according to age, experience and qualifications on the Senior Technician II scale, range £1,208 to £1,802, with good prospects of promotion to higher grades with a maximum of £2,109.

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Staff Manager,

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(304)

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Further particulars and information as to the method of application should be obtained from the Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1. (320)

## UNIVERSITY COLLEGE CARDIFF

DEPARTMENT OF APPLIED MATHEMATICS  
AND MATHEMATICAL PHYSICS

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Applications (three copies), including the names of two referees, should be submitted as soon as possible to Dr. Alan R. Beattie, Department of Applied Mathematics and Mathematical Physics, University College, Cardiff, from whom further particulars may be obtained. (344)



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ROBT. T. HUTCHESON,  
Secretary of the University Court.  
(260)

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(UNIVERSITY OF LONDON), JUDD STREET  
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NEWCASTLE UPON TYNE  
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Further particulars may be obtained from the Registrar, the University, Newcastle upon Tyne 2, with whom applications (three copies), together with the names and addresses of two persons to whom reference may be made, should be lodged not later than August 15, 1967. (291)

City of Liverpool  
Education CommitteeLIVERPOOL REGIONAL  
COLLEGE OF TECHNOLOGY

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THOMAS ALKER,  
Town Clerk.  
(360)

## UNIVERSITY OF BRISTOL

Applications are invited for the post of LECTURER or ASSISTANT LECTURER in APPLIED MATHEMATICS within the Department of Mathematics tenable from October 1, 1967, or as soon after this date as possible. Salary scales: Lecturer, £1,470 to £2,630; Assistant Lecturer, £1,105 to £1,340 per annum with superannuation benefits.

Further particulars may be obtained from the Registrar, the University, Senate House, Bristol, 2, to whom applications should be sent by August 31, 1967. (316)

UNIVERSITY COLLEGE LONDON requires **Research Assistant** in Department of Botany (Professor D. Lewis) from October 1, 1967. Salary, according to qualifications and experience, not less than £850 per annum. Candidates should have degree in Botany, Biochemistry, Microbiology or Genetics, and special interest in biochemical genetics, particularly enzymology.—Applications by August 11 to Secretary, University College London, Gower Street, W.C.1, from whom further particulars may be obtained (ref. 14). (302)

**TECHNICIAN REQUIRED SEPTEMBER** for teaching unit. Sound knowledge of volumetric analysis essential; experience in biochemical techniques an advantage. Salary on scale £577 to £973 according to age and qualifications.—Written applications with names of two referees to Administrator, Department of Biochemistry, South Parks Road, Oxford. (286)

**RESEARCH ASSISTANTS. RECENT B.S. or M.S. or equivalent.** Organic Separation Chemistry, Basic Chemistry, Immunology or Electronics. Salary commensurate with experience and ability.—Apply, J. R. Mitchell, New England Institute for Medical Research, P.O. Box 308, Ridgefield, Connecticut, U.S.A. (352)

## Technicians

I.M.L.T. qualifications or H.N.C. (Applied Biology) for Bio-Chemical, Bacteriological, Haematological, Histological, virological posts in hospitals, industry, and research.—Miss J. R. Horton, G.K. Bureau Ltd., 196 Oxford Street, W.1. MUS 6858. (351)

## UNIVERSITY OF BELFAST

DEPARTMENT OF PURE AND APPLIED  
PHYSICS(SCHOOL OF PHYSICS AND APPLIED  
MATHEMATICS)S.R.C. POST DOCTORAL  
RESEARCH FELLOWSHIP

Applications are invited for the post of Research Fellow which will be tenable from October 1, 1967, or a mutually agreeable later date until September 30, 1971. The Fellow will take an active and responsible part in a new research programme, supported by substantial S.R.C. grants, to measure heavy particle collision cross sections in the thermal energy range from 0.5 eV to 10 eV using crossed beams and pulsed nozzle sources. Candidates should have considerable experimental experience in either physical gas dynamics including shock tube research, atom and molecular beams, measurements of collision cross-sections, or ion detection and analysis systems. Salary will be in the range £1,470 to £2,010 with F.S.S.U.

Applications, including a curriculum vitae and the names of two referees, should be forwarded to Dr. R. M. Hobson, Department of Pure and Applied Physics, before August 14, 1967. (365)

PORTSMOUTH COLLEGE OF  
TECHNOLOGY

## DEPARTMENT OF BIOLOGICAL SCIENCES

Applications are invited for the post of **RESEARCH ASSISTANT in PLANT PHYSIOLOGY/BIOCHEMISTRY** tenable from September, 1967. The Assistant will be required to investigate the effect of leaf maturity on photosynthetic activity. Applicants should have an honours degree and will have the opportunity to register for a higher degree. Salary scale £950 to £1,050.

Further particulars may be obtained from the Registrar, Staff Office, Portsmouth College of Technology, Hampshire Terrace, Portsmouth, to whom application forms should be forwarded by Friday, August 11, 1967. (364)

## ROWETT RESEARCH INSTITUTE

## BUCKSBURN, ABERDEEN

## MICROBIOLOGY DEPARTMENT

Applications are invited for a post on the **Scientific Officer/Senior Scientific Officer** scale. The post is concerned with extension of work on the nitrogen metabolism of micro-organisms in rumen contents using stable isotopes of N and <sup>14</sup>C and <sup>3</sup>H. Applicants should have a degree in microbiology or in biochemistry or chemistry with experience in microbiology. Salary according to experience within the range S.O. £926 to £1,574 or S.S.O. £1,744 to £2,155. Superannuation under F.S.S.U. Further particulars are available.

Applications, including the names of three referees, should be lodged with the Secretary of the Institute within one month. (329)

**MANCHESTER REGIONAL HOSPITAL BOARD** have a vacancy for a male or female **Scientific Officer** in the laboratories of the **BLOOD TRANSFUSION SERVICE**. An Honours degree in science, pharmacy or biology is essential. Salary £963 (first- or second-class Honours) rising to £1,658, though candidates with postgraduate qualifications or experience may be given a higher starting salary.—Applications should be sent to the Director, Regional Blood Transfusion Centre, Roby Street, Manchester, 1, from whom further particulars may be obtained. (357)

**BIOCHEMIST, PREFERABLY POST-doctoral**, wanted to work on physical and chemical properties of the proteins of the lens, with reference to formation of lens opacities. Salary on scale £1,100 to £1,550 per annum, plus F.S.S.U.—Further information from and applications to Dr. A. Pirie, Nuffield Laboratory of Ophthalmology, Walton Street, Oxford. (356)

**TECHNICIAN REQUIRED TO ASSIST** with research programme in steroid unit. Laboratories in West London Hospital. Whitley Council salary and conditions of service.—Apply: Professor N. F. Morris, Charing Cross Hospital Medical School, London, W.C.2. (295)

## THE QUIET SUN

THE programme of international collaboration in geophysics under the general rubric of the International Years of the Quiet Sun (IQSY, pronounced *iksee* by common tacit consent) has been something of a success, at least if the round-up assembly in London last week is anything to go by. Not merely were the proceedings attended by more people than the organizers had expected—that is not by itself a proof of quality—the assembly itself seems to have been stimulating, and therefore worthwhile in its own right. At the same time it is clear that the machinery of collaboration has been a spur to many people. To make the least of it, a great many people have been stimulated to make useful observations by the knowledge that others are likely to be doing the same things at the same time. And, of course, there are few who receive a telegram saying SOLACTIVITY EXISTS and yet decline at least to look up into the sky. In other words, the IQSY has turned out to be much more real than the pale imitation of the IGY which many people feared would emerge from the proposals first designed in the early sixties.

The IQSY assembly was particularly valuable in showing how new techniques have made possible exceedingly penetrating programmes of research. The studies which have been made of the Sun during the last minimum of the sunspot cycle (in 1964) and afterwards are striking examples of what has been done. There is, for example, the development of birefringent but tunable filters which make it possible to photograph the Sun in chosen spectral ranges no wider than  $0.25 \text{ \AA}$  within several Angstroms of the centre of the Lyman-alpha radiation. The same period has also seen the development of instruments for recording solar X-rays, and enough is now known about the pattern and even the mechanism of microwave radiation from the Sun for the details of particular outbursts of radio waves to be interpreted in terms of the movement of plasma upwards through the atmosphere of the Sun. The fact that sunspots themselves are still more easily described than understood is not a black mark against the IQSY; everybody knows, now at any rate, that it will be several years before the intricacies of sunspots are properly understood.

The abundance of these and other questions is by itself a sufficient proof that the IQSY should somehow continue even though the Sun is no longer quiet. But in what way? And what should be the objectives? Issues like these were given a lively airing in London last week, but perhaps the first thing to say is that there is already something like a firm assurance that a good many of the institutions which have become familiar in recent years are already certain to continue. The data centres, perhaps the most valuable parts of the machinery of the IGY and the IQSY, are likely

to continue without change—except possibly for the better. Moscow and Boulder (Colorado) will become central repositories for a vast quarry of information, and several of the less dominant centres are also likely to carry on. In passing, it is significant that this chimes in well with one common pattern of international collaboration in scientific matters in recent years, for it turns out to be easier to persuade governments to match each other's efforts in some common cause than to put money into a common pool. But what is a data centre? The name itself is possibly unfortunate, and suggests the kind of place which absorbs information more easily than it can surrender it. There was some welcome talk in London last week of how the data centres might be fitted out with facilities for research, so that they could digest as well as store, but it is hard to think that the promoters of the IQSY and its potential successors are thinking ambitiously enough in this respect. The real need is that the data centres should somehow help to simplify the management of the literature which has been created by the spurt of activity in geophysics in the past decade. Quite often, a good deal of what is published in the open literature could be consigned directly to the data centres if only there were some recognizable way in which those responsible in the first place for the collection of the data could be given proper recognition for their work. It is also important that the centres should be places equipped to receive visitors as of right for weeks or even months on end, and be able to provide facilities for research and analysis. The ideal would be if the data centres could become so intellectually attractive that they command the respect and the attendance of a great community of geophysicists.

This raises the issue of what kind of organization should continue to superintend international collaboration in geophysics, which in turn hangs on the answer to the question of what kinds of collaboration seem to be profitable. One of the virtues of the assembly in London last week is that the management provided an outline of the kind of programme it would like to pursue in the years between now and the next solar maximum. Although a great many of the practical suggestions which were made have no particular bearing either on the opportunities which exist for collaboration or on the ways in which the impending solar maximum lends urgency to common problems—everybody knows, for example, that the time has now come for attempts to study the solar wind away from the plane of the Ecliptic—there is enough substance in them to show how the Inter-Union Commission on Solar Terrestrial Physics could occupy itself valuably in the next five years in a continuation and an extension of the work of the past decade. It is exceedingly unlikely that the executive committee of ICSU will fail to recognize this case when it comes to consider final



proposals for the solar maximum at its meeting in October. But here, again, the time has probably come to start thinking of institutions, not just of programmes. In particular, there is a great need for some means of keeping the ball rolling from one sunspot maximum to the next. It is also, of course, important that the management should get itself into a position in which it can tactfully discriminate between efficient research proposals and those likely only to repeat earlier investigations—a need which, to be fair, seemed to have been fully recognized in London last week. On these counts as well, it would be prudent to think of devices by means of which beneficent centralization could be encouraged, perhaps by money.

## BRITISH ECONOMY

MR STANLEY BALDWIN helped to win a place for himself in the history books by saying that wherever six economists were gathered together, there would be seven erudite opinions from which to choose. Without attributing to him one iota of clairvoyance more than he deserves, it is a fair guess that he would have been downcast to see how meagre was the harvest of economic innovation in the debate on the British economy on July 24. There was no shortage of economists, but nothing like a convincing remedy for what seems by common consent to be an unpleasant situation. In the circumstances, it is naturally tempting to wonder whether the traditional arguments about the economic condition of countries like the United Kingdom do not leave entirely out of account essential components of the problem. To say this is not to decry the economists or even to suggest that there is some mystical quality in the construction of an economic system which overrides the doctrines of Keynes and his successors. On the contrary, it is entirely possible that some simple economic stratagem might work like magic—a touch of devaluation, perhaps, or import controls. But which of the many possible remedies would it be best in practice to apply? Which diagnoses of the economic discontents which afflict the British economy are the most accurate? As the British economy is managed, it is uncommonly difficult to answer questions like these. The complaint that would have earned Mr Baldwin gratitude as well as renown is that the trouble with economists is that they have too few tests for testing alternative theories. This is what lends a sense of unreality to debates like that in the House of Commons earlier this week. It is like being compelled to stake a fortune on the outcome of a horse race.

The way things are done in Britain, even diagnosis is difficult. Although the bare bones of the present economic troubles are all too plain to see—too great a disparity between imports and exports, too great a labour force with no work to do, and too small a rate of economic growth—the fine details are surprisingly obscure. Why, for example, has the British economy been flying in the face of economic logic in recent

months by sustaining a rapid growth of the import bill at a time at which credit is being restricted and industrial production held down precisely so as to achieve the opposite effect? What kinds of imports are they, anyway? And why are importers so attached to their potential imports that they go to all this trouble to surmount the several potential barriers which have been constructed to keep imports out? Just as people guess at remedies which might be applied, so they guess at reasons why things like this are happening. In reality, of course, it would be much safer to take steps to find out. No manufacturer of washing powder worth his salt would let his sales decline without seeking to discover in exhaustive detail just what is wrong with what he is trying to sell. By now, in any case, the necessary techniques of market research are quite well understood. In the circumstances, it is entirely anomalous that governments should be shy to do the same things in the complicated and therefore treacherous fields in which they must operate. The most urgent need in the management of the British economy may well be the huge battery of social analysis which could help everybody to understand why this great formless system responds in the way it does to the pressures which afflict it constantly. It would be valuable, for example, to know just how and to what extent people's economic habits are affected by taxes, or by shortages of credit at the bank, or even—at times of economic wellbeing—to encouragements to spend.

In terms borrowed from the kinetic theory, this suggests that the management of the British economy would be a good deal easier if there were a microscopic as well as a macroscopic theory of how it functions. At the same time a better understanding of how the behaviour of the whole is determined by the behaviour of the parts of which it is composed would most probably suggest that the parameters which are supposed to characterize the working of the economic system may not be the most suitable or the most illuminating. As things are, it is easy enough to construct examples of how the fashionable statistics do not serve the purposes which are usually attributed to them. The numbers of people unemployed at any time may be, for example, a good measure of the inability of employers to keep unwanted men and women on their books, but they can only be the roughest measure of how efficiently the manpower of the country is deployed. The 17 per cent or so of the GNP being spent on industrial investment is low enough by comparison with what is spent in other countries comparable with Britain, but who is to answer the crucial question of how much of this investment will add to the real productive capacity of the country as a whole? It is just possible, of course, that these resources are being managed wisely, but it is unfortunately equally possible that they are being used merely to replace assets long since worn out. In this and a host of other ways, there are exceedingly fruitful problems which should be tackled by social scientists or people very much like them.

In the long run, a more intimate involvement of social scientists in the foundations of the economy could serve not merely to reach more accurate decisions about the management of the economy but also to suggest a greater wealth of actions which might be taken. It may be something of a triumph that in the debate this week the Chancellor of the Exchequer was able to mention the word "devaluation" in public without pretending that all holders of sterling would turn their money into any other currency on offer, but this is only a modest relaxation. Why does he not also take up in public the arguments one way and the other for the maintenance of sterling as an international currency? And why does not his colleague, Mr Patrick Gordon Walker, take the edge off his fears that people on the margin between poverty and prosperity might be tempted not to work if welfare benefits were too generous by considering the potential advantages of a statutory minimum wage? Indeed, there is a good case for going even further and asking whether the British Government could not create the mobile labour force for which it is always crying out by making payments of actual money to people who change their jobs. Another possibility is that removal expenses might qualify for deduction of income tax, and there is of course a host of other possibilities all equally at odds with the conventions now followed. The difficulty in all this is that, in the absence of a detailed understanding of how the economy functions, the conventions are indispensable. They have become not safeguards but crutches.

## MORE COLLABORATORS

In all the present rash of international projects looking for members and finance, from Cern and its ambitions to build a 300 GeV proton accelerator to the several offshoots of the international unions, it is important that EMBO should not be forgotten. (Given the name, indifference is a more real danger than forgetfulness.) Formally, the organization is now nearly four years old, and for much of that time it has leaned on the Volkswagen Foundation for support. Constitutionally it is a private company registered in Switzerland, and its aims are to foster research in molecular biology in whatever ways seem appropriate and potentially rewarding. There has been a small but imaginative programme of exchange visits between laboratories, and EMBO (which stands for European Molecular Biology Organization) reckons to have been able to act more quickly and informally in assisting research people to spend short periods in other laboratories than their own. But this is only a beginning. The organization is anxious quickly to increase its activities and to enlarge their scope. It would like to be able to finance—or at least to catalyse—long-term appointments; it wants to provide advanced courses, to be able to make research grants and finally to establish a laboratory of its own, with an independent source of funds. But ambitious plans like these are

not easily accommodated within the framework of a private organization. Everybody seems to agree that funds would have to come from governments, and that governments would often be unable to hand over money unconditionally. Yet, as a meeting in Geneva some months ago (see *Nature*, 214, 445; 1967) seems to have determined, this is not a serious stumbling block. Some kind of agreement between European governments and EMBO seems fortunately to be inevitable. The question remaining to be determined is what the agreement shall consist of.

The first thing to be said is that there was no accident in the choice of Geneva as a site for the first confrontation between the organizers and the governments which may eventually support them. (The Government of Israel has been helping for some time.) Obviously the example of Cern is intended as a model and, if it comes to that, Geneva would make a splendid site for the laboratory which EMBO would like to build. It is right to add, however, that EMBO's plans are comparatively modest. Not so long ago, the organizers had calculated that the cost of their ideal programme would come to hardly more than £250,000 a year—roughly three times its rate of spending now.

The laboratory is a bigger undertaking, costing perhaps £2.5 million to build and to equip, and something in excess of £1 million a year to operate. It is no-surprising that governments have been more sympathetic towards the kind of work which EMBO has been doing already, and sceptical about the laboratory and the delegation to EMBO of the duty to make grants for scientific research. The cost is not entirely negligible even when shared out among a dozen governments or more. But there are also fears, mostly unreasonable, that the creation of a centre of excellence on a European scale would simply serve to drain away from existing laboratories people who are scarce to begin with. The fallacy in this is that the laboratories like that which EMBO has in mind quite quickly increase the stock of people working in the field concerned. A more serious problem is that a central laboratory might take too many people away from teaching; some attention should be paid to this problem in the few months which remain before the next confrontation with the governments. On balance, it would make sense if the European governments—those which adhere to Cern and possibly some others as well—could agree to build the laboratory as well as to finance on a continuing basis the kind of work which EMBO has been doing so far.

Providing money for the financing of research projects on a European basis is a more tricky proposition, cheaper though it might well turn out to be. The trouble here, of course, is that a private organization must necessarily be less able than a government to make compromises between such conflicting pressures as the need to finance excellence and the need to help backwardness. In the long run, there would be great benefits in research councils operating on a continental scale, but nobody should be surprised if, for the time being, this particular dream is unfulfilled.

## NEWS AND VIEWS

### Airbus Launched

NOBODY can claim that the negotiations about the European Airbus have been easy. Sometimes they have seemed endless, but when they were finally concluded on July 25 after a further eight hours of talks in Lancaster House, agreement was apparently secure. Mr John Stonehouse, for the Ministry of Technology, announced that the project will go ahead in the autumn. It will be a two engined 300 seater, using the Rolls-Royce RB 207 engine (a condition of British agreement), and will cost £190 million to develop, £130 million for the airframe and £60 million for the engines.

The aeroplane should make its first flight in 1971 and be in service in 1973. Airframe costs will be shared 37.5 per cent each by Britain and France and 25 per cent by Germany, and engine development will be shared 75 per cent by Britain with the remainder equally divided between the other two countries. In concrete terms, Britain will pay £91.25 million, France £53.75 million and Germany £45 million. Clearly Mr Stonehouse was willing to concede a good deal to get the project off the ground, including design leadership for the airframe, which goes to France. To make the project viable, British European Airways, Air France and Lufthansa will have to buy 75 aircraft, so that unit costs are likely to be about £2.5 million. Mr Stonehouse hopes to sell 300 models of the airbus by 1990, and expects at least some of these to go to American airlines. This may be unduly optimistic; airlines in the US are much more interested in three engined aeroplanes with greater range, and several of these are already on the drawing boards of American companies.

### Cern Goes On

UNDETERRED by uncertainties about the 300 GeV proton accelerator, the management of Cern at Geneva is pushing on with schemes for the further development of high energy physics. For one thing, Cern has now signed an agreement with the Soviet Union for a joint programme of research on the 70 GeV proton accelerator which will be completed later this year at the Serpukhov Institute of High Energy Physics, 100 km south of Moscow. The organization has also signed a contract with the French and German governments for the development and construction of a bubble chamber filled with no less than 20 m<sup>3</sup> of liquid hydrogen and intended for use with the existing 28 GeV proton accelerator. It will cost £7 million.

The Russian agreement, valuable in itself, will no doubt be followed by a similar agreement operating in the opposite direction if ever the 300 GeV machine is built in Europe. Negotiations have been under way for 18 months, and the agreement now signed will be valid for five years. Under the terms of the agreement, teams from Cern will be entitled to bid for experimental time on the Serpukhov machine, competing for the privilege before the appropriate laboratory committee with teams of Russian experimentalists. A programme

of counter experiments has already been designed. In return for access to the machine, Cern will provide for the Serpukhov accelerator pieces of equipment concerned with beam manipulation. A device based on a rapidly increasing magnetic field for extracting fast protons from the machine, of a type which has been operating successfully at Geneva for several years, will be a permanent gift. Cern will also supply a beam separator designed to prepare a beam of high-energy kaons, and this will remain on loan to Serpukhov for ten years.

### Less Defence Science

RESEARCH and development financed by the British defence budget is likely to remain at a standstill for the next three years at least. As part of the cost cutting operation announced in a government White Paper on July 18, research and development costs will be reduced by £30 million from the level planned for the year 1970-71. This represents a cut of just over 10 per cent of the intended budget, which can therefore be assumed to have been in the range £290 to £300 million. The budget for 1967-68 is £260 million, so Mr Healey will be holding expenditure at a constant level at least until 1971.

The ministry is not saying where the cuts will be made—indeed it is doubtful whether that has yet been decided. One problem may be that a large slice of the budget is not directly under Mr Healey's control. A sum of almost £245 million, £188 million of it directly concerned with research and development, is paid through the Ministry of Technology vote, although it is included in the global totals for defence expenditure. Most of this sum—£171 million—is for research and development carried out by industry, and only £17.2 million covers work done in the ministry establishments. Thus, although the establishments cost a grand total of £56 million to run, the defence figure includes only £17 million of it. On this basis, the work of the establishments—including the Royal Radar Establishment, the Explosives Research and Development Establishment, and the Royal Aircraft Establishment—is 70 per cent civil and only 30 per cent military. Even this £17 million does not come under Mr Healey's control. Unless Mr Benn himself starts to wield the axe (which is not impossible) the establishments look relatively safe.

The industrial research and development work looks a more likely target, although again it is Mr Benn and not Mr Healey who will have to make the decisions. Of the total research and development figure for the defence budget, aircraft take the lion's share—£107 million, or 41 per cent. Great savings could clearly be made by dropping aircraft projects, or simply by admitting that the swing wing aircraft which Mr Healey was hoping to build with France is now a lost cause. Other projects in this section of the budget include development work on the Spey engined Phantom aircraft, the vertical take off Harrier (P 1127), the Sea King, a helicopter being developed for the Royal Navy, and two experimental hovercraft designed for military purposes. A limited amount of work on the F 111 and the Anglo-French helicopter agreement have also been approved. Guided weapon research this year will cost £53 million and electronics research £27 million.

It is in the scientific work which falls directly under the Ministry of Defence that cuts seem most likely. The Royal Navy, for example, spends a total of £34.6 million on activities classified as research and development. About one-third of this goes on weapon systems and radio and navigation equipment. The Army spends about £21 million, £5 million of it in industry. It supports several research establishments, including the Chemical Defence Experimental Establishment and the Microbiological Research Establishment. Finally, there is the Meteorological Office, costing around £6 million per year, supported by the Royal Air Force.

## Teaching Adults

THE demand for adult education is a "growing and not a diminishing problem", according to Mr Goronwy Roberts, Minister of State at the Department of Education and Science. Mr Roberts was opening a conference at Birkbeck College, London, convened to discuss the problems of the mature student. Mr Roberts encouraged those involved in part-time education in Britain; not only did it help occupational mobility, but it could perhaps do more, and he visualized tired middle-aged executives being revived by the study of Plato and Aristotle. On a more down to earth note, he had some sharp words to say to Colleges of Advanced Technology which had abandoned sandwich courses since becoming universities. "We are not, I hope, going to lose the sandwich courses," he said, making a hope sound more like a challenge.

The address at the opening ceremony was given by Dr F. Cyril James, Principal Emeritus of McGill University, Montreal. Why, asked Dr James, was there still a need for part-time education, when government grants were available for most able students to take full-time courses? He gave four reasons; the need for higher degrees, supplying deeper and more specialized knowledge; the need for regular retraining; the changing pattern of employment—"something like half the working population are today working at jobs that did not exist at the opening of the twentieth century"—and finally, the fact that more than half the children in Britain give up full-time education as soon as they can, at the age of 15. Part-time students did have different problems from regular ones, he thought; they needed libraries and laboratories open until a late hour every night of the week, and on Saturdays and Sundays. Correspondence courses and a proper use of television as an educational medium could also help.

During the conference itself, Mr G. O. Arlt, President of the Council of Graduate Schools in the United States, gave details of the numbers of graduate part-time students in America. Despite difficulties of definition, which make it possible for the Ivy League universities to deny that they have any part-time students at all, while the California State Colleges, anxious to document their services to the community, claim 28,738 part-time and only 5,536 full-time students, the US Office of Education reported in 1965 a total of 477,535 graduate students, of whom 280,714 (59 per cent) were part-time. The wastage among part-time students is worse than that among full-timers—"There appears to be a staggering attrition rate, probably as high as 60 per cent . . . but no matter how you look at

it, part-time graduate study is with us to stay, and universities may as well abandon their futile efforts to exorcise the phenomenon." Other delegates might have put the case for part-time study in more enthusiastic terms, but probably agreed with the conclusion.

## Nice Work if You Can Get It

RARELY can a House of Commons committee have produced a more scathing report than that published last week by the Committee of Public Accounts. It was investigating the case in which Bristol Siddeley Engines had overcharged the Ministry of Aviation (later Technology) for work done on the overhaul of Sapphire and Viper engines between 1959 and 1963, costing a total of £16.5 million. In March this year the company finally agreed to repay to the ministry £3.96 million, which includes an unspecified figure (about £400,000) for accidental double charging.

The committee's description of how the situation came about makes fascinating reading. In the end, nobody emerges with reputation unscathed—even the final settlement is criticized. A separate contract was placed each year, on a "price to be agreed" basis, for the stripping, inspection, reassembly and testing of each engine. In many cases the final price was not settled until a major part of the work had already been done, so that the company must have had a very firm idea of costs. Under the contract system, however, it was not obliged to reveal these to the ministry. After the Ferranti affair, in which Ferranti, Ltd., agreed to repay £4.25 million excess profits made under the contract to produce the Bloodhound missile, the ministry seems to have smelt a rat. It asked BSE for costs incurred on past contracts, which were refused; BSE did admit, however, that reductions in prices for 1963-64 and 1964-65 might be necessary, and submitted new quotations which seem merely to have encouraged the ministry to probe further.

Much of the ethical weight of argument in the report hangs on what is meant by the "fair and reasonable" prices to which both the ministry and the company were contractually committed. The company argued that a total view of its government contracts should be taken, and that on this view it was not overcharging. The ministry, and the committee, did not accept this view. "Government support . . . can be properly given only by way of conscious decision . . . and not by submitting to overcharging, of which the government is unaware, on quite different contracts." On some contracts the profit on costs amounted to more than 150 per cent, and the average figure over the four years was 74 per cent.

The ministry is also blamed by the committee. Its performance "fell far short of an acceptable standard". Even after the discovery by the ministry of the size of the overcharging, things seem to have moved at a leisurely pace, with gaps of up to a month between letters from the ministry and replies from the company. The final settlement was reached quite quickly after a letter in which is recorded Mr John Stonehouse's view that it was "the most disturbing case he had come across in the whole of his public life". Even the final settlement gets its share of criticism; in the Ferranti case, the final settlement left the company with profits of 21.4 per cent on costs, although the contract had involved a new project with consequent risks. The



BSE repair contracts involved virtually no risk at all, and yet the company has been left with a retained profit of 32 per cent on costs. Not surprisingly, the committee was dissatisfied with this. It also recommended a review of the spare parts contracts entered into in the same period. Mr Wedgwood Benn, Minister of Technology, who could hardly do nothing about a report so critical of his ministry, agreed on July 25 to undertake this review, but declined to reopen the profits inquiry.

## No Redundant Geophysicists

THE future for international geophysical co-operation seems likely to be one of consistent, if more modest, progress than in the past. This has emerged from the meeting of the Committee on Space Research which has been going on in London over the past week. Dr Herbert Friedman, President of the Inter-Union Commission on Solar Terrestrial Physics—which is likely to be charged with the responsibility of maintaining the international programmes—said last week, “The IGY and the IQSY were special efforts—there is no need to generate special efforts now. The problem is to keep programmes up on a continuing basis; we must keep momentum up as long as possible.” The inter-union commission consists of Dr Friedman, twelve representatives from different scientific disciplines and representatives from various organizations, including the World Meteorological Organization, the Scientific Committee on Antarctic Research (SCAR), the Scientific Committee on Oceanic Research (SCOR) and the Federation of Astronomical and Geophysical Services (FAGS). “This is a continuing field, and therefore the commission will continue indefinitely,” Dr Friedman added. It would not, he said, be a stop-go operation.

At least two of the data centres set up for the IGY and IQSY will remain in existence. These are the *A* and *B* centres in Moscow and Boulder, Colorado. There is also a good chance that a number of the *C* centres will be kept in operation, although this presumably depends on the attitude of the governments supporting them. The *C* centre at the Radio and Space Research Station at Slough will be one that will keep going. Both Dr Friedman and Professor W. J. G. Beynon, chairman of the organizing committee of the IQSY, were optimistic about the international reaction to collaborative projects. In developed countries there is no objection, and underdeveloped countries see the projects as an entry ticket to big science. “The motivation is strong to see the programmes continue,” Dr Friedman observed.

There is likely, though, to be a change in the emphasis of the programmes. More discriminating experiments are likely to take over from what Dr Friedman described as the “shot-gun” approach adopted so far. He suggested that simultaneous measurements at different points in space—one either side of an auroral curtain, for instance—could provide much useful information. Although synoptic measurements would be continued, they might well start to concentrate on the inhomogeneities in the atmosphere and the fine scale detail of the ionosphere. The data centres themselves, Dr Friedman hoped, could initiate research on data handling, rather than being merely passive recipients of data.

## Going to University

THE regulations which determine whether young people can get into a British university in 1968 differ most obviously from those now current in their greater bulk. But the *Compendium of University Entrance Requirements* (published by the Association of Commonwealth Universities for the Committee of Vice-Chancellors and to be had from Percy Lund, Humphries and Co., 14s. 6d.) is also a slightly more liberal document in that there is now a more obvious sprinkling of university departments which do not rigorously specify in advance the qualifications which potential entrants must somehow attain. In a foreword to the document, however, Sir Robert Aitken, Chairman of the Standing Conference on University Entrance, and Sir Charles Wilson, Chairman of the Committee of Vice-Chancellors, promise better things for the succeeding year 1969–70. For by now it is an open secret that the vice-chancellors have been working hard on a document intended to clothe with flesh the bare bones of the policy enunciated several years ago which would have the effect of allowing intending entrants to universities to do so without having spent virtually all their time as seniors at school working on the courses they will later follow at university.

## New Zealand Brain Drain

NEW ZEALAND'S loss of about 40 per cent of a year's output of first class honours graduates each year to the brain drain makes Britain's loss of about 15 to 20 per cent of all PhD graduates look small. In the New Zealand National Research Advisory Council publication, *Some Aspects of Technical Manpower in New Zealand*, it is estimated that the permanent loss amounts to 67 per cent of first class honours physicists, 48 per cent of first class honours mathematicians, and 35 per cent of first class honours chemists and geologists. New Zealand also loses about 25 per cent of those with a lower level of honours degree. New Zealand's total output of masters and bachelors graduates from 1960 to 1964 was 663, of whom 242 were first class graduates. In these years there was a total output of 199 chemists, 63 first class honours; 131 mathematicians, 48 first class honours; 102 physicists, 36 first class honours; 69 zoologists, 34 first class honours; 55 geologists, 15 first class honours; 42 agricultural scientists, 16 first class honours; and 35 botanists, 15 first class honours.

The report says that according to the Government Statistician, New Zealand gains more scientists than it loses, though it in no way confirms the figure. The main problem is the quality and research interests of those gained and lost rather than their total number. A striking feature of the figures is that there is a comparatively small loss in botanists (4 per cent), agricultural scientists (19 per cent) and zoologists (24 per cent), which confirms that those sciences with strong local links more easily retain good graduates than those which are more independent of the environment.

The report suggests that there is a need to arouse interest in all students in all disciplines in research problems peculiar to New Zealand. While this may require more conscious effort in disciplines such as mathematics and physics than in zoology, for example, it is suggested that this is not impossible. This is, in

fact, one of the declared purposes of the postgraduate scholarship scheme of the New Zealand University Grants Committee.

## In the Steps of Rutherford

THE New Zealand National Research Advisory Council's estimates for expenditure on scientific research slowly increase, both absolutely and as a percentage of the gross national product. The estimate for 1966-67 is just over £10 million or 0.5 per cent of the gross national product. When this is compared with the British figure of about 2.6 per cent of the gross national product or the American figure of about 3 per cent, it is perhaps not surprising that New Zealand loses such a large percentage of first class graduates in the brain drain. The Council recommends that the Government should return to its support of the policy the Council originally set out in its first annual report, that of a steady and deliberate growth in the research effort with growth rates concentrated in those areas of research most likely to prove beneficial to the country's economy.

The Council recommends the expansion of the laboratory building programme if present urgent needs are to be met and if expansion of certain research is to be carried out. It points out that accommodation is already overcrowded and that, although it is estimated that the building requirements for the Department of Scientific and Industrial Research would involve £5 million over the next five years (excluding the cost of land), the annual expenditure over the past two years for land and building for scientific purposes for all Government Departments has amounted to only £0.5 million.

The chief new recommendation of the Council is that a programme of ecological research should be implemented by the Government. Less than one-third of New Zealand is intensively farmed and a large proportion of the remainder is composed of forest and country tussock grassland, forming important water catchment areas. Problems of soil erosion which might lead to flooding have pointed the way to urgent changes in the basis of land usage. Recently there has been a realization that water resources, long regarded as unlimited, need prudent management.

## Discussing Proteins

THE Chemical Society has found enthusiasm for its proposal to form a Protein Group. The idea is to bring together scientists from different disciplines who share an interest in proteins and peptides. Discussion would cover the synthesis, structure, physical properties and biological activity of proteins, and organization of the meetings would be provided by the Chemical Society. Dr G. T. Young, of the Dyson Perrins Laboratory at Oxford, to whom interested scientists have been asked to write, reports an encouraging response—about 50 have replied approving the idea. Membership of the group would not be confined to members of the Chemical Society, but in order to get it started it is necessary to provide evidence that at least 25 fellows are interested. The idea can then be put forward to the Council of the Chemical Society.

The next step will be to organize an inaugural meeting, some time before the end of the year. This would probably be a scientific meeting, to encourage as many

as possible to come. It would also be necessary to elect a committee, but Dr Young modestly declares that he has no ambitions in this direction. He is hoping that the group will attract the younger workers, and that they will be prepared to run it. "We don't want the same old people giving the same old papers," he says.

## Extending Photography

THE year 1967 could be a vintage year for the Loch Ness monster. Earlier this year it was announced that the Highland and Islands Development Board had given a grant of £1,000 to a "Monster" study group, headed by Mr David James, and on July 20 Kodak Ltd. announced that it was giving £1,230 to an insurance underwriter, Mr T. K. Dinsdale, to carry out his own studies into the phenomenon. This sudden largesse in the direction of Mr Dinsdale is all part of the Kodak Awards for 1967-68. The purpose of these awards is to stimulate original thought on problems relating to the photographic process and to extend the use of photography into other fields. Kodak has made seventeen awards this year totalling £9,433. The research supported ranges from Mr Dinsdale's project to medical photography, and includes a large number of studies of audio-visual aids in teaching.

Another project is that of Dr W. Hartson, who is studying techniques for producing photomicrographs of the smallest blood vessels in normal and diseased skin using intense infra-red light and infra-red sensitive film. Mr C. C. Gilson, a medical photographer, will investigate various methods of making contour maps of the human cornea using interferometry. His purpose is to detect damage to the cornea caused by wearing contact lenses; the established method, called keratography, is apparently not sufficiently accurate.

The awards are given to individuals rather than institutes. They are given not only to professional researchers but also to people such as schoolteachers who wish to study the uses of photography in their vocation.

## Radiation and the Immune Response

from a Correspondent

RADIATION and the control of the immune response was the topic discussed at a meeting organized by the International Atomic Energy Agency and the French Atomic Energy Commission, which was held in June this year at the Fondation Curie in Paris.

The nineteen delegates heard persuasive evidence for a radiosensitive antigen-processing step inside macrophages which might play a significant part in the immune response. Immunological tolerance can be induced in newborn rats by extremely low doses of salmonella flagellin antigen which, when labelled, cannot be detected by electron microscopy. Thus, the determination of a tolerance-inducing threshold for purified histo-compatibility antigens in man may be a practical proposition.

A suggestion that in the evolution of the immune response the antibodies appear in the sequence IgM, IgA, IgG contrasted with the evidence for the affinity of antibodies for monovalent haptens, which is in the reverse order. Memory was excellent for IgG, but less impressive for IgM and IgA. Evidence seems to sug-

gest that different cells produce antibodies of different affinities but that the immunoglobulins produced by one cell are homogeneous. No immunoglobulin class disappears completely after whole body irradiation of mice; amino-acid incorporation has shown that one day after 950 rads all classes are produced. A primary immune response has been induced in an entirely *in vitro* system, which seems to favour the view that lymphoid cells are multipotent and can form antibody without division when forced to do so.

In thymectomized X-irradiated mice, grafted with a thymus and with restored bone-marrow, two populations of cells have undergone mitosis after antigenic stimulation; these were cells derived from thymus and bone-marrow respectively. In animals thymectomized, but not grafted with a thymus, no detectable antibody has been found nor has antigenic stimulation caused an initial mitotic increase, which is further evidence that cells of thymic origin must divide before a population of cells from bone-marrow can proliferate and go on to form antibody. It has been confirmed that cells from bone-marrow can effectively re-equip the irradiated recipient's immune system only as long as the thymus is present.

Foetal liver is believed to be relatively poor in immuno-competent cells and has been used to restore lethally irradiated rhesus monkeys. Although up to  $10^9$  cells/kg have been injected as therapy, restoration of haematopoietic function has been achieved very rarely. Furthermore, the animals receiving allogeneic foetal cells developed the "secondary radiation syndrome" probably caused by a graft versus host reaction. When bone-marrow was transplanted into supralethally irradiated dogs, the rapid appearance of hyperbasophilic cells in the peripheral blood within a few days of bone-marrow therapy was indicative of a strong graft versus host response. This type of cytological transformation seems to be restricted to the dog. To render bone-marrow cells less likely to cause graft versus host attack, pre-incubation of immuno-competent cells at 37° C has been employed. This reduced the graft versus host response and indicated that the immunological potential of incubated lymph node cells had been reduced.

A substantial proportion of irradiated rats receiving allogeneic bone-marrow cells have developed myelofibrosis, occurring simultaneously with marked haemopoietic activity in the marrow. Part of the aetiology of this syndrome might be a delayed graft versus host rejection phenomenon. Heavily irradiated (10,000–30,000 r.) tissue undergoes a change, as indicated by the second-set like rejection of irradiated isografts.

## Dangerous Life

THE hazards of modern life were discussed last week by the British Academy of Forensic Sciences at its annual scientific meeting held at the Institute of Psychiatry at the Maudsley Hospital. Mr. R. Kell explained how prolonged exposure to noise, which he defined as "sound unwanted by the recipient", can cause deafness. A survey of female jute weavers in Dundee has shown that after twenty years working with the looms, which may produce 100 dB and more—a very loud noise—there is considerable loss of hearing for sounds which have a frequency of around

4,000 cycles/sec. This loss of hearing is not usually noticed until it extends into the frequency range of speech, when the inability to hear sounds of particular frequencies can make speech almost or wholly unintelligible. This is at the moment very difficult to mitigate, because hearing aids which are merely amplifiers of sound cannot help when the ear is unable to respond to certain frequencies. The deafness develops because noise of an intensity greater than about 110 dB can eventually destroy the nerve cells on the basilar membrane in the inner ear, which produce electrical potentials when stimulated by sound of a particular frequency. The ear drum rarely bursts and when it does usually heals up again. Once the nerve cells have been destroyed their particular frequency of sound cannot be heard again.

Loss of hearing is not only an occupational hazard; noisy hobbies such as rifle shooting can have the same effect, and some deafness has been found in pop singers and musicians who play loud instruments such as the bagpipes. Traffic is now the most common source of noise outside factories and the engine rooms of ships.

Discussing the hazards of breathing in the modern world, Dr A. E. J. Eggleton of the AERE, Harwell, described how air pollution by carbon monoxide is increasing as more cars produce more exhaust fumes. The concentration of this gas in Fleet Street on a normal summer day has been found to show two peaks, one at 9.00 a.m. and the other at 6.00 p.m., when the density of traffic is greatest. This is the pollutant the concentration of which on the air comes nearest to the maximum permitted industrial concentration of 100 p.p.m., and 200–300 p.p.m. have been recorded in busy streets. Other dangerous pollutants include smoke, sulphur dioxide, oxides of nitrogen and carcinogenic hydrocarbons, although in most cases it is very difficult to correlate their concentrations in the air with mortality and morbidity.

## Molecules Constrained

from our Correspondent in Thermodynamics

AN exceedingly elegant demonstration of how to calculate the thermodynamic properties of an assembly of polymer molecules each of which is subjected to some topological constraint has been provided by Professor S. F. Edwards of the University of Manchester in the current issue of the *Proceedings of the Physical Society* (91, 513; 1967). Elegance apart, the calculation will be of great interest to those concerned to account for the properties of materials such as rubber or glass, usually in terms of chemical linkages between different molecules. Edwards's analysis suggests that the properties of polymer molecules lumped together in bulk may be at least in part explicable by the fact that molecules in such assemblages are inevitably subjected to topological constraints of various kinds. It remains to be seen whether the simple calculations which he has now provided will prove capable of generalization to more realistic models, but there is at least some room for improvement.

In the simplest model of a constrained assemblage, identical molecules are supposed free to move in two dimensions and each of them is fixed at the ends to two fixed points. A constraint is provided by supposing that the end of an impenetrable rod is placed on

the plane among the loosely looped molecules. The effect of this constraint is permanently to separate the molecules into several different categories—there are those which lie entirely on one side of the rod, those which lie entirely on the other, and those which are looped about the rod once, twice and several times. But each molecule is a kind of random walk with fixed end points and a definite number of paces. For the simple two-dimensional problem, Edwards shows how it is possible to calculate the probability that a random walk will generate analogues of the molecules in the several distinct topological classes, which is something of a triumph in itself. Each probability, of course, is a function of the end points of the molecules and of the point at which the rod-like constraint is made to intervene. It is no surprise that the entropy of the system as a whole turns out to differ from that of an unconstrained system by the addition of terms of the form  $NkP \log P$ , where  $P$  is the probability that a random walk will yield molecules in a certain topological class and where there is one term for each class.

The great interest of the calculation is that it provides a general expression for the entropy of a constrained system in which the parameters of the constraint enter explicitly. On paper, at least, it is possible to calculate mechanical properties such as elastic constants. Edwards himself points out that the two-dimensional problem is unrealistic, that the three-dimensional analogue is that of an assemblage of molecules threaded in various ways through a closed loop and that this is more directly analogous to real situations. The next instalment in his calculation will be worth waiting for.

## Neuropathology at Wye

Professor C. W. M. Adams writes :

THE second symposium on neuropathology, organized by N. T. Blau and Helen Payling Wright, took place at Wye College from July 10 to 14. The chief purpose of these symposia is to highlight growing points in the neurological sciences. The topics on this occasion were Schwann cells, neuroviruses, immuno-neurology, neurochemistry, developmental neurology and cerebrovascular disease.

Peripheral nerve ultrastructure was lucidly illustrated by D. N. Landon and P. K. Thomas. We learnt that Schwann cell cytoplasm extends into the Schmitt-Lanterman cleft. Discussion also centred on the role of the Schwannian paranodal apparatus and on possible cation-binding by acid mucopolysaccharide in the internodal gap. This last theme was continued by C. J. Duncan, who suggested that the initial component in nerve conduction is mediated by  $\text{Ca}^{++}$ -activated ATPase, whereas the axon potential is propagated by an ion-sensitive protein. J. B. Cavanagh discussed Schwann cell injury: diphtheria toxin inhibits certain of the cell's metabolic activities, whereas X-irradiation impairs its proliferative activity.

The concept of "slow virus" in neurological disease was reviewed by A. P. Waterson, while J. A. Dudgeon showed that viruses persist in tissues for long periods after initial infection. Transmission of virus along Schwann cells appeared, from F. Kingsley Saunders' experimental work, to be a likely route by which an

encephalitis virus enters the brain. Within the brain H. E. Webb concluded that Langkat virus directly attacks neurones, while hypersensitivity is responsible for perivascular inflammation and oedema. The scrapie agent remains as enigmatic as ever; I. H. Pattison wondered whether it might be a relatively simple peptide quite unrelated to virus.

In experimental allergic encephalomyelitis S. Leibowitz reported that grossly increased vascular permeability is associated with perivascular inflammation, even though slight leakage occurs in the absence of local cellular infiltration. He showed that simultaneous injection of anti-lymphocytic serum slows down the immunological reaction, so that true demyelinating lesions appear even in the guinea-pig. D. Hughes illustrated the demyelinating and gliotoxic effect of multiple sclerosis serum. Nevertheless, similar results were obtained in motor neurone disease and in a quarter of control subjects. Continuing the immunological theme, the presence of anti-brain antibody was demonstrated by P. C. Wilkinson in the serum of patients with carcinomatous sensory neuropathy. Antibody to sympathetic nerve growth factor was the subject of E. Zaimis's paper; such immuno-sympathectomy is clearly a valuable tool for studying autonomic functions.

Several fruitful lines of neurochemical research were surveyed. A. N. Davison demonstrated differential incorporation of components into developing myelin and considered that movement of lipoprotein units within the sheath might explain such findings. After this examination of the brain's myelin fraction, V. P. Whittaker surveyed the synaptosome fraction: he showed that acetylcholine is synthesized in synaptic vesicles, whereas cholinesterase activity is sited on the synaptosome's external membrane. Biochemical lesions were next discussed. W. N. Aldridge outlined an elegant method for elucidating the reaction of organophosphorus compounds with certain brain protein fractions. The neurological manifestations of hydroxocobalamin deficiency, Leber's disease and tropical malnutrition were attributed by J. Wilson to different forms of impaired cyanide detoxication.

In the developing tadpole's cord, A. Hughes showed that some 85 per cent of motor cells atrophy; he inferred that such cells fail to make functional peripheral connexions. Turning to the nutritional aspects of brain development, J. Dobbing established that myelin and possibly other elements fail to "catch up" if the grain is undernourished during the early vulnerable period of its development. Concluding this subject, M. Berry and J. T. Eayrs portrayed morphological and behavioural changes after irradiation and hormonal injury to the developing brain.

## Phage Coat Protein

from a Correspondent in Cell Biology

THE entire genome of the closely related RNA bacteriophages R17, f2, MS2 and fr consists of a single stranded RNA molecule with sufficient information to code for about 1,100 amino-acids. This RNA molecule has the dual function of providing a store for the genetic information and also acting as a messenger. Zinder and his co-workers (1966) and Gussin (1966) have shown that the genomes of f2 and R17 have at



least three, and probably only three, cistrons specifying the phage coat protein, an enzyme RNA synthetase to replicate the phage RNA and the so-called "A" protein or maturation factor. Since each phage particle consists of one RNA molecule enclosed in 180 coat protein molecules and one or two molecules of "A" protein, some mechanism must regulate the translation of the genome to ensure that the coat protein cistron is translated much more often than the two other cistrons. Lodish and Zinder (1966) suggested that the coat protein itself acted as a 'repressor'. Sugiyama and Nakada (*Proc. US Nat. Acad. Sci.*, **57**, 1744; 1967), who recently achieved the *in vitro* assembly of phage-like particles from coat protein and phage RNA (*J. Mol. Biol.*, **25**, 455; 1967), have obtained some direct evidence suggesting that coat protein does in fact play a part in this regulation by complexing with the phage RNA.

They compared the *in vitro* incorporation of histidine, phenylalanine and leucine in an *E. coli* cell-free system programmed with MS2 RNA or MS2 RNA that had, by prior incubation with coat protein, been made into Complex I, that is a few coat protein molecules attached to an RNA molecule. Since MS2 coat protein does not contain histidine the incorporation of histidine is taken as a measure of the synthesis of RNA synthetase and "A" protein. Sugiyama and Nakada find that with Complex I as messenger incorporation of histidine is 71-77 per cent less than with MS2 RNA as messenger, whereas phenylalanine incorporation is only inhibited by 19-36 per cent. The incorporation of histidine was also always more strongly inhibited than the incorporation of leucine or alanine. It appears that the coat protein selectively reduces synthesis of non-coat protein. This inhibitory effect is specific; MS2 coat protein does not inhibit protein synthesis directed by TMV RNA or PolyU. Eggen and Nathans (*Fed. Proc.*, **26**, 449; 1967) have briefly reported similar observations to those of Sugiyama and Nakada.

Does the coat protein regulate translation *in vivo* as well as *in vitro*? Two pieces of evidence suggest that it may. First, in MS2 infected *E. coli* the ratio of coat protein to non-coat protein synthesized increases progressively during the infective cycle. Second, as Lodish and Zinder *et al.* (1964) found, amber mutants of f2 that are unable to make coat protein produce large quantities of RNA synthetase; this suggests that coat protein molecules are involved in the normal regulation of RNA synthetase synthesis.

The existence of polar coat amber mutants of f2 and R17 (Zinder *et al.*, 1966; Gussin *et al.*, 1966) indicates that the coat protein cistron precedes the synthetase cistron. Furthermore, the failure to synthesize "A" protein *in vitro* can be interpreted as showing that the "A" cistron is the last of the three. This would make the order of the cistrons coat protein first at the 5' terminus, followed by the RNA synthetase cistron and finally the "A" cistron at the 3' terminus. It may well be that the coat protein when regulating translation attaches to the 3' terminal end of the RNA first and then progressively towards the 5' terminus. If this is so, the "A" cistron will be translated least often and the coat protein cistron most often. This is an attractive hypothesis because we know that although each phage requires 180 coat protein molecules, it needs only a few (and certainly fewer than 10) molecules of "A" protein.

## Parliament in Britain

### Dragon Reactor

To avoid the premature shut-down of the Dragon reactor project at Winfrith Heath, the British Government is prepared to bear most of the cost of the project through 1968. This was announced by Mr Wedgwood Benn, Minister of Technology. The Dragon high temperature reactor is supported by the OECD, and doubts about the future of the project—which is due to run until 1970—have been spreading since the Euratom countries have been unable to agree about the support costs. To finish the project would need another £4.5 million, according to evidence given to the Select Committee on Science and Technology by Sir William Penney. Costs for 1968 may therefore be about £1.5 million. Unless Euratom stops dragging its feet on the project, it may well have to close down, as the British Government is unlikely to do more than a temporary holding operation. (Written reply, July 25.)

### Sonic Bangs

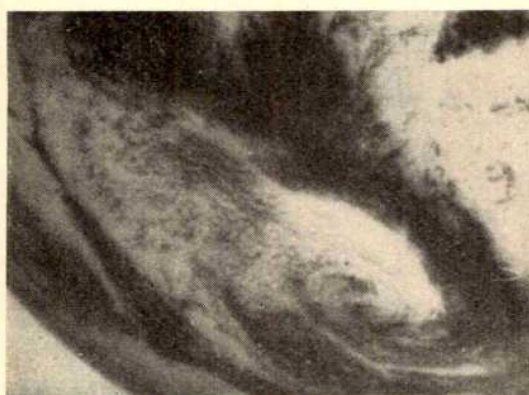
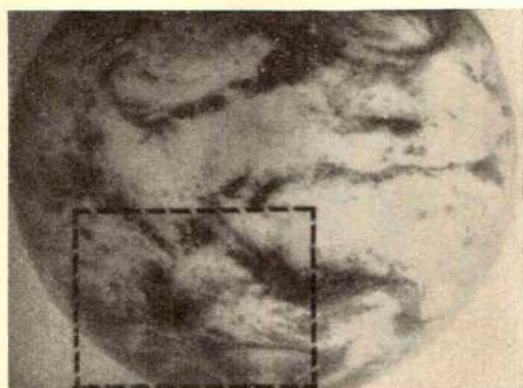
QUESTIONS on sonic bangs were prominent in both Houses. On July 17 in the House of Commons the Minister of Technology, Mr Wedgwood Benn, stated that his Department and others concerned would investigate any reports of adverse effects of sonic booms. His Department also had a programme of scientific research into the nature and effects of sonic bangs. Mr Benn preferred not to anticipate in detail the information which this series would provide about higher intensity tests, but valuable lessons should be learned. In reply to further questions on July 18, Mr Benn said he was aware that there was some concern at the disturbance being caused, but he did not think it justified stopping the exercise, which was planned to end that week. The place and time of the final flight—noon on July 21—would be announced in advance, and all the remaining flights in the series would be designed to hold the bangs to within the same degree of intensity as those that had already been made. Whether or not we built a supersonic airliner, at some stage this country would have to decide whether it was prepared to allow supersonic flying. One of the reasons for the tests, he said, was to see whether people complained about tests that do not take place. Possibly, he suggested, the high and prolonged intensity of an aircraft taking off and landing was more irritating and more likely to affect hospitals and others than the single impact of a supersonic plane.

In the House of Lords, Lord Shackleton said that two isolated instances of momentary fluctuation in the calibration of equipment at Frenchay Hospital had been reported but that the equipment immediately returned to normal and no damage of any kind occurred.

### Veterinary Medicine

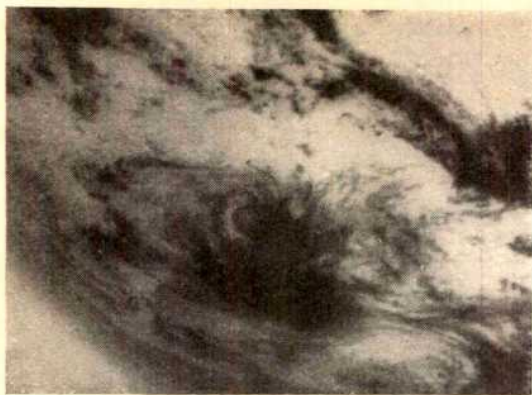
MR A. BOTTOMLEY, Minister of Overseas Development, reported that after discussions with Edinburgh University, he intended to provide a grant of up to £210,000 in the five year period up to March 1972 for erecting, furnishing and equipping a building next to the University's existing Veterinary Field Station to provide a Centre for Tropical Veterinary Medicine. An agreement had recently been concluded with the University on the conditions under which the centre would be established and managed.

## Growth and Decay of a Hurricane



*For explanation, see overleaf*





This sequence of pictures (reading down each column in turn) was taken from the stationary weather satellite ATS 1 launched from the United States earlier this year. It shows various stages in the development and decay of a hurricane system over the South Pacific between April 6-15, 1967.

Photographs are taken by the satellite at intervals of roughly 23 minutes.

These pictures are taken from a film prepared by V. E. Suomi, A. F. Hasler and J. Kornfield of the Department of Meteorology, University of Wisconsin.

## Stellar Accretion and X-ray Emission

by

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Shklovsky has suggested that the Scorpius X-ray source is a binary pair and that the X-radiation arises from the collision of material from a giant star with a neutron star. The theoretical complications of such a model are now found not to match actual measurements and it is suggested that the bremsstrahlung effect occurs between a giant star and a white dwarf.

THE blue stellar object which has been identified with the strong Scorpius X-ray source<sup>1</sup> emits about a thousand times as much energy in the X-ray region as in the visible region. It has been suggested<sup>2</sup> that the vibrations of a compact star, with a massive electron degenerate core, may be able to shock-heat a surrounding coronal region which could have these properties. This idea has been tested through a variety of hydrodynamic model calculations<sup>3</sup>, and it has been found that even with over-optimistic assumptions about the parameters, the resulting X-ray emission falls at least two orders of magnitude short of the  $4 \times 10^{36}$  ergs sec<sup>-1</sup> which appears to characterize the Scorpius source.

Shklovsky<sup>4</sup> has suggested that the Scorpius X-ray source is a member of a close binary pair of stars. He proposed that one of the stars is a neutron star and that it is rapidly accreting mass from its companion. Gravitational potential energy released then creates a hot plasma which radiates thin-source bremsstrahlung (collision radiation) in the X-ray region.

In this article we consider some general properties of mass accretion on to a compact star. We then point out some difficulties with Shklovsky's suggestion and suggest an alternative model.

It should first be noted that there is an upper limit to the rate at which mass can accrete on to a star, which results when the outgoing radiation stress on the infalling

material becomes equal to the gravitational stress<sup>5</sup>. This condition is

$$\frac{N_0 \sigma_T}{\mu_e c} \frac{L}{4\pi R^2} = \frac{GM}{R^2} \quad (1)$$

where  $\sigma_T$  is the Thomson scattering cross-section,  $L$  is the luminosity generated by the infalling material,  $N_0$  is the Avogadro number, and  $\mu_e$  is the mean mass per electron. For a star of one solar mass, with  $\mu_e = 4/3$ , the resulting maximal luminosity is  $1.7 \times 10^{38}$  ergs sec<sup>-1</sup>, which is considerably larger than needed to account for the luminosities of X-ray sources. For reasons which will become obvious, we take the total luminosity of the Scorpius source to be at least four times the apparent X-ray luminosity, or about  $1.6 \times 10^{37}$  ergs sec<sup>-1</sup>.

Here we shall investigate the following pattern of events. The infalling material is optically thin and the time required to cool by radiation is long compared with the infall time. When the infall is halted the gravitational potential energy released is converted to gas kinetic energy; the plasma remains optically thin. The temperature characteristic of the resulting thin source bremsstrahlung is estimated. The absorption and re-emission of radiation by the underlying stellar surface are also considered.

When the infalling material is halted, the gravitational potential energy released is converted into gas kinetic

energy. The maximum coronal temperature which thus results is

$$T_c = \frac{2}{3} \frac{GM\mu}{N_0 k R} \quad (2)$$

where  $\mu$  is the mean molecular weight,  $G$  is the gravitational constant,  $M$  is the stellar mass, and we have assumed that the conversion takes place at the stellar radius  $R$ . For a star of one solar mass, with  $\mu = 2/3$

$$T_c = \frac{7 \times 10^{17}}{R}$$

For a neutron star  $R \approx 10^6$  cm and  $T_c \approx 7 \times 10^{11}$  °K. This high temperature indicates that the gas contains relativistic electrons, but corrections for relativistic effects need not be made at the present crude level of our considerations. For a white dwarf we take  $R \approx 2 \times 10^8$  cm, and thus  $T_c \approx 3 \times 10^9$  °K.

It is also possible to estimate the pressure at the coronal level where these high temperatures are produced. The rate of momentum transport for freely falling material will be equal to this pressure. The free-fall velocity is

$$v_f = \left( \frac{2GM}{R} \right)^{1/2} \quad (3)$$

The mass flux necessary to produce the total stellar luminosity is

$$\rho v = \frac{R}{GM} \frac{L}{4\pi R^2} \quad (4)$$

Hence

$$\rho_f v_f^2 = \frac{N_0}{\mu} k T_c \rho_c \quad (5)$$

from which we find

$$\rho_c = \frac{3L}{2^{5/2} \pi G^{3/2} M^{3/2} R^{1/2}} \quad (6)$$

The coronal scale height in this region is

$$H_c = \frac{N_0 k T_c R^2}{GM\mu} \quad (7)$$

Thus for one solar mass the amount of material overlying this point in the corona is

$$\rho_c H_c = 1.17 \times 10^{-3} R^{\frac{1}{2}}$$

For a neutron star  $\rho_c H_c = 1.17$  g cm<sup>-2</sup>. For the  $\gamma$ -rays which will be radiated in this case the effective cross-section is less than the Thomson cross-section. Thus the optical depth is not too large and the incoming material can radiate without much absorption. For a white dwarf,  $\rho_c H_c = 16.5$  g cm<sup>-2</sup>, however, which indicates that the foregoing crude picture is not quite correct; the infalling material will stop close to the photosphere. It is necessary to determine where the radiation will occur in this case.

Of course, the infalling material is not completely stopped, because the incoming mass flux must be constant. Let us consider the fractional energy radiation,  $\Delta E/E$ , of the material as it moves through a coronal scale height with the properties estimated here. The rate of energy radiation by thin-source bremsstrahlung<sup>6</sup> is  $\sim 2 \times 10^{-27} T^{\frac{1}{2}} n_e n_i$  ergs cm<sup>-3</sup> sec<sup>-1</sup>, where  $n_e$  and  $n_i$  are the number densities of electrons and ions. We write the energy loss rate as  $2 \times 10^{-27} T^{\frac{1}{2}} N_0^2 \rho / 4\mu^2$  ergs g<sup>-1</sup> sec<sup>-1</sup>. The time required to move through a scale height is  $H_c/v$ , and the energy available for radiation is  $E = GM/R$  g<sup>-1</sup>. Hence

$$\frac{\Delta E}{E} = 2 \times 10^{-27} \frac{T_c^{\frac{1}{2}} N_0^2 \rho_c}{4\mu^2} \frac{H_c}{v} \frac{R}{GM} \quad (8)$$

From equations (2), (4), (6) and (7) we have

$$\frac{\Delta E}{E} = \frac{3^{\frac{1}{2}} \times 10^{-27}}{2^{5/2} \pi} \frac{N_0^{3/2} L R^{3/2}}{k^{\frac{1}{2}} G^{5/2} M^{5/2} \mu^{3/2}} \quad (9)$$

For one solar mass this becomes

$$\frac{\Delta E}{E} = 5.5 \times 10^{-13} R^{3/2}$$

For a neutron star this is  $5.5 \times 10^{-4}$ , showing that negligible radiation occurs before the infalling material is slowed and attains its high temperature. For a white dwarf star  $\Delta E/E \approx 1.5$ . This appears to indicate that the gas energy will be radiated away as the material slows down, but it must be remembered that a full scale height of gas is optically thick, and the value of  $\Delta E/E$  by the time the material has penetrated to a depth of  $\sim 1$  g cm<sup>-2</sup> is  $\sim 1.5/16.5 \approx 0.09$ . Thus in this case also the material arrives in the vicinity of the photosphere with most of its energy intact.

If all the released energy were to be radiated as black-body radiation from the photosphere, the photospheric temperature would be

$$T_e = \left[ \frac{L}{4\pi\sigma R^2} \right]^{\frac{1}{4}} \quad (10)$$

With  $L = 1.6 \times 10^{37}$  ergs sec<sup>-1</sup>

$$T_e = \frac{1.22 \times 10^{10}}{R^{\frac{1}{2}}}$$

For a neutron star the photospheric temperature will be  $\sim 1.2 \times 10^7$  °K, and for a white dwarf it will be  $\sim 8.6 \times 10^5$  °K. These temperatures are very much lower than the coronal temperatures. Thus it is evident that a steep temperature gradient must be set up near the photosphere.

We must consider thermal conduction of energy in the presence of such a temperature gradient. The conducted energy flux is  $\lambda dT/dz$ , where  $\lambda$  is the coefficient of thermal conductivity and  $z$  is the vertical distance in the atmosphere. For an ionized gas we may take<sup>7</sup>

$$\lambda = 5 \times 10^{-7} T^{5/2} \text{ ergs cm}^{-1} \text{ sec}^{-1} \text{ °K}^{-1}$$

For the purpose of a crude estimate let us write for the ratio of the energy conducted out of the photospheric scale height, during the time of passage of material through the scale height, to the energy content of the scale height as

$$\frac{1}{EH_c \rho_c} \lambda \frac{T_c H_c}{H_c} \frac{H_c}{v} = 3\pi \times 10^{-6} \left( \frac{2}{3} \frac{GM\mu}{N_0 k} \right)^{7/2} \frac{1}{R^{5/2} L} \quad (11)$$

For one solar mass this is  $1.8 \times 10^{20}/R^{5/2}$ . For a neutron star the result is  $1.8 \times 10^5$ , indicating that conduction can most easily remove the thermal content of the gas during its motion toward the photosphere. This conclusion would not be altered if a proper relativistic treatment had been used. For a white dwarf star the ratio is 0.32. For this case, however, a better estimate would be  $0.32 \times 16.5 = 5.3$ , and again conduction can remove much of the thermal content of the gas during its motion toward the photosphere.

It is evident that with a neutron star a very small temperature gradient is sufficient to conduct away the energy flux from the coronal region. Thus the temperature gradient will not become steep until very near the photosphere. In both kinds of star the steep part of the temperature gradient thus occurs within a photospheric pressure scale height and both temperature and density will change rapidly at almost constant pressure.

It is further evident that the conductive transport of energy will no longer be able to exceed the mass transport when the temperature is lower, because the thermal conductivity varies as a fairly high power of the temperature. We may roughly estimate that the two transport rates are equal at the radiation temperature of the gas, and thus this radiation temperature will be the coronal temperature reduced by the expression in equation (11) raised to the power 2/7. Thus, in the foregoing example the neutron star would radiate thin-source bremsstrahlung at



a temperature near  $2 \times 10^{10}$  °K, and the white dwarf would radiate at a temperature near  $2 \times 10^9$  °K.

It is evident that we do not find support in these results for Shklovsky's suggestion that the Scorpius X-ray source results from mass accretion on to a neutron star. For neutron stars the radius is a rather insensitive function of the mass<sup>8</sup>, and thus any reasonable adjustment of the parameters in the expressions given previously will not significantly lower the temperature at which the infalling material will radiate. Mass accretion on to neutron stars should give combination X-ray and  $\gamma$ -ray sources. The X-radiation results from the absorption of the thin-source bremsstrahlung by the photosphere and its re-radiation in a blackbody spectrum. Half the stellar luminosity will arise from this process, so that equation (10) should be modified to become

$$T_e = \left[ \frac{L}{8\pi\sigma R^2} \right] \quad (12)$$

Thus the blackbody spectrum of a neutron star in our example should have a temperature of  $10^7$  °K.

It is also evident that we have a much better chance of fitting the parameters of the Scorpius source if we assume a model with mass accretion on to a white dwarf. The masses and radii of white dwarfs are inversely correlated, and thus a much wider variation in parameters is possible. Because in our previous example the radiation temperature has come out too high, we must choose a lower mass which will have a larger radius. It is immediately evident from equation (11) that the conductive transport of energy will then become much less efficient, and the star will radiate at more nearly its coronal temperature. From the tabulated models of white dwarf stars<sup>9</sup>, it can readily be found that a white dwarf with a mass near 0.2 solar masses will have a coronal temperature near  $10^8$  °K. Its radius will be about  $1.4 \times 10^9$  cm. For this case the upper limit on the luminosity from the radiation stress condition is  $3 \times 10^{37}$  erg sec<sup>-1</sup>, which is somewhat higher than the apparent Scorpius luminosity. The total rate of mass infall needed to produce this luminosity is about one solar mass per  $10^6$  years.

These numbers are suggestive of the results obtained for the evolution of a close binary pair of stars by Kippenhahn, Weigert, and Kohl<sup>10,11</sup>. In their model the stellar primary transfers mass to the secondary after reaching the red giant phase; the transfer time is less than  $10^6$  years. The remnant settles down as a white dwarf star of 0.26 solar masses; it has an envelope which is rich in hydrogen and thus a somewhat larger radius than a pure helium white dwarf. If such an object exists in the Scorpius X-ray source system, we would therefore lower the estimate of coronal temperature somewhat. Of course, we would see the system at a later stage than indicated by the final models of Kippenhahn and Weigert, when the secondary had also evolved to the red giant stage and is transferring mass back to the original primary.

For  $M = 0.2$  solar masses and  $T_e = 10^8$  °K, the ratio of coronal pressure to photospheric pressure would be increased over the previous estimate by a factor of  $\sim 30$ . From equation (9) we see that  $\Delta E/E$  would be increased by a factor  $\sim 10^3$ , so that the infalling material will radiate thin-source bremsstrahlung before the bulk kinetic energy has been fully converted to thermal energy. From these results it is evident that an effective photosphere will develop in the infalling material, and thus conductive effects will not be important. The estimate for the coronal temperature is too high also for this reason. A steep temperature gradient will be set up near the effective photosphere. As the velocity of the infalling material will rapidly decrease along this temperature gradient according to the continuity equation (4), the bulk of the thin-source bremsstrahlung will be produced in a narrow shell near the photosphere.

From equation (12) we see that the photospheric temperature required to re-radiate the absorbed X-ray energy

will be  $3.3 \times 10^8$  °K. The spectrum will therefore show a peak near the 44–60 Å band in which Byram, Chubb and Friedman<sup>12</sup> have observed an additional component of X-irradiation from the Scorpius source. Their estimate of the interstellar opacity at 44 Å is based on an assumed ratio of hydrogen : helium of 7, but we prefer to assume double this number, which would be more characteristic of solar abundances. At an assumed distance of 200 parsec, and with an interstellar gas density of one hydrogen atom per cubic centimetre, 44 Å radiation is attenuated by one order of magnitude. Thus, in the absence of attenuation, Byram *et al.* should have seen about  $10^{-6}$  erg cm<sup>-2</sup> sec<sup>-1</sup> in the 44–60 Å band, corresponding to a luminosity of  $\sim 4 \times 10^{36}$  erg sec<sup>-1</sup> in this band from the Scorpius source. This is an order of magnitude higher than the previous discussion of our model would indicate.

We do not believe, however, that this is necessarily a difficulty with our model. We have seen that there is a rapid transfer of mass through the photosphere. If the total luminosity of the Scorpius source corresponds to the radiation stress limit, with much of the gravitational energy release occurring inside the photosphere, then the photospheric luminosity could be higher than the X-ray luminosity by a substantial factor. The white dwarf mass could also be substantially higher than 0.2 solar masses.

It is unlikely that the rapid rate of mass transfer to the white dwarf star can continue for a very long period without producing significant effects in the white dwarf itself. When a thick surface layer rich in hydrogen has been added, the source of the hydrogen-burning shell will be activated, and there may be an instability which will lead to nova explosions which will prevent the total mass from increasing too rapidly.

Because the binary companion will fill its Lagrangian surface in order to carry out rapid mass transfer, it will subtend a solid angle at the white dwarf of about  $\pi$ . Thus about a quarter of the white dwarf luminosity will be absorbed and re-radiated by the companion. There will be a large variation in the radiating temperature, but its general range will be of the order  $T \sim (L/4\pi R_c^2 \sigma)^{1/4}$ , where  $R_c$  is the radius of the companion. If we take  $L \sim 8 \times 10^{37}$  erg sec<sup>-1</sup> and  $R_c \sim 2 \times 10^{11}$  cm, then  $T \sim 4 \times 10^4$  °K.

The thin-source bremsstrahlung radiation will have a low-energy tail which will make a substantial contribution to the optical emission, as observed. The re-radiation by the white dwarf is peaked at a sufficiently high energy that there will be no substantial contribution in the visible. On the other hand, the re-radiation by the companion is peaked at a sufficiently low temperature that a substantial visible contribution is likely. We would therefore expect a variable visible luminosity, with a regular component due to orbital motion and an irregular component due to variable gas streams between the stars. The emission lines could well vary with respect to the continuum in this model.

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# Conifer Distributions and Continental Drift

by

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The distribution of fifty-eight genera of conifers over the world has been analysed statistically in order to group corresponding areas of the world's surface together. The results tend to confirm the existence of a Gondwanaland.

At a recent symposium at the University of Leicester, there was renewed discussion on how fossil faunal and floral distributions can contribute to information on continental drift. This is a topic that has attracted attention lately and is part of the wider problem of reconstructing the geography of the past<sup>2-12</sup>. The underlying logic is that if two areas have very similar faunas or floras, they were probably close together geographically; but it has always been clear that many factors interfere with simple interpretations of this principle.

It is therefore interesting to see how far floristic and faunistic difference does in fact reflect geographical distance, using known distributions of the present day, and to have methods for constructing the best fitting maps from biological data. This is needed to allow the interpretation of geological data, and to give some guide to the amount of disturbance produced by oceans, deserts, climatic zones, migratory or relict populations and so on.

The first step is clearly to construct a measure of difference in faunistic or floristic composition which may be termed "biotal distance". Many such measures are used in ecology<sup>13</sup>. They should be based on taxa recorded from a well spread series of sites for one time horizon. There are great problems of sampling and taxonomic

reliability<sup>5,6,8</sup>, which will not be dealt with in detail here. Even for living organisms, data of the quality and quantity needed may be difficult to find.

The next step is to arrange the sites into a map that reflects biotal distance. Relationships between the sites will be multidimensional, so that some way of reducing the dimensionality is needed. Factor analysis is applicable here, and is more rigorous than earlier ordination methods<sup>13,14</sup>. Although it is not difficult to get a good idea of the relations between a few sites (or organisms) by simple inspection, a systematic method is needed to deal impartially with all the data. The agreement between geographic and biotal maps can be measured by a recently described method if desired<sup>15</sup>. For restricted areas that can be regarded as a plane, the reduced dimensions will be two, and from the factor loadings the distorted biotal map can be drawn. For a roughly linear transect even simpler methods may suffice, and Fig. 1 shows an example based on Valentine's data on marine molluscs of the west coast of North America<sup>16</sup>.

For distributions covering the whole world, the required result will be a fitting to a sphere. This is in effect to allow the sampled sites to slide about the globe until their configuration fits best the biotal distances (expressed

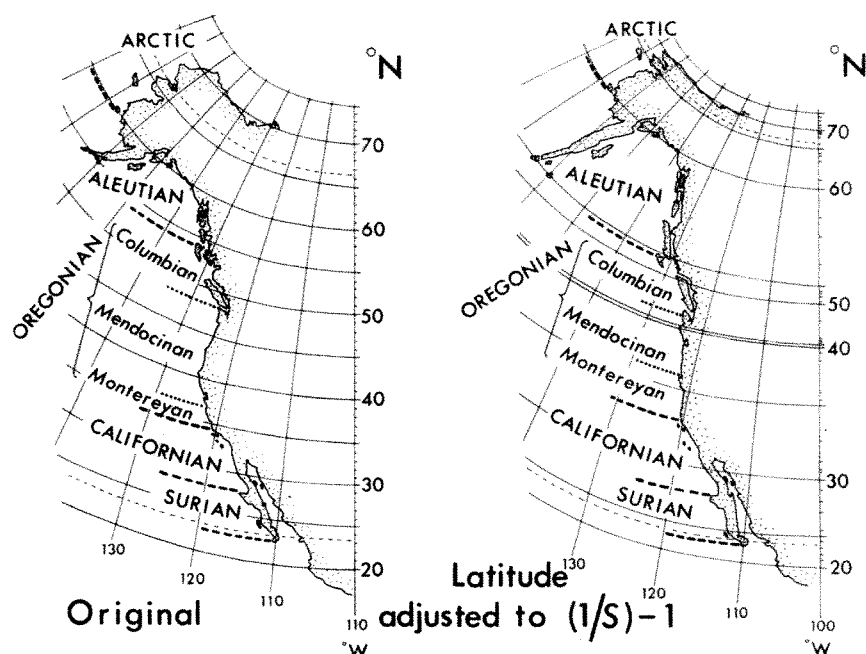


Fig. 1. The Pacific Coast of North America showing the marine faunal provinces and subprovinces of Valentine (1966). The map on the right has been obtained by using as a measure of biotal distance  $D = (1/S) - 1$ , which was found empirically to fit Valentine's  $S$  coefficients, and by displacing the shore line in latitude (though not in longitude).

as arcs). By extracting three factors, the positions of the sites are obtained in three dimensional space. Projection from the centroid on to a surrounding sphere will then give a fitting of the kind required. A point very close to the centroid will perhaps have an indeterminate position (small changes in biological composition due to sampling errors, for example, will alter the projection considerably) but this is unavoidable. Such a point represents a country that is almost equidistant biotically from all other countries, and it cannot be given a unique position on a globe. The closeness to the centroid will, however, indicate which points are most liable to this uncertainty.

This method has been applied to the present day distribution of conifers (including taxads). They were chosen because they are well documented, well classified, relatively non-mobile, and have a long evolutionary history. The fifty-eight living genera have been mapped by Florin<sup>17</sup> for the whole world. Only present day distributions (excluding human introductions) were used from those data, because the fossil records are not available for a world-wide series of sites at a single time period. Minor extensions of range<sup>18</sup> were not considered, and sections of genera, being of uncertain taxonomic validity, were not distinguished. The land masses of the globe—or all that possess conifers—were divided into fifty-eight “quadrats” of approximately equal area (some difficulty was found with archipelagoes, etc.), as shown in Fig. 2. Florin’s maps were overlaid by these quadrats on a transparent sheet, and the presence of the genera (in any part of a quadrat) was scored. The resulting table was treated as 1,0 data and the four point correlation coefficient  $\phi$  was computed between all pairs of quadrats, and used for principal component analysis. The first three vectors accounted for 29.27, 23.67 and 7.65 per cent of the variation respectively. No points were very close to the centroid; the closest were the points representing quadrats 26, 27 and 29 (between 0.24 and 0.29 from the centroid) and the most distant were 45 and 47 (both 0.80 from the centroid).

The loadings on the three vectors ( $x$ ,  $y$  and  $z$ ) give the best three-dimensional representation of the data. The points were fitted to a globe by projection from the centroid of the three vectors; first the vector means were subtracted from the loadings (the means were 0.4497, -0.2230 and 0.0348 respectively) and then the following formulae were used:  $\sin(\text{latitude}) = y/\sqrt{x^2 + y^2 + z^2}$  and  $\tan(\text{longitude}) = z/x$ . Although this projection will show

the points in their correct relative positions, it will not show what axis corresponds to geographical north and south. To find this, the projection was rotated by a three-dimensional method<sup>15</sup> to give the best fit between the points and the centres of the present-day quadrats in Fig. 2.

The resulting fitted projection is shown in Fig. 3. The confidence limits to be placed on these positions are not readily estimated, but a rough estimate was made as follows. The standard error of sampling of a correlation coefficient based on  $n$  items is about  $1/\sqrt{n-3}$ , in this case 0.135, and, treating  $\phi$  as a cosine, this represents about 7.7°. The shift of origin may increase this by a few degrees, but it is probably little changed by the principal component analysis. Cones on the globe of radius 10 and 20 degrees of arc will be about 1 and 2 standard errors respectively. The larger cone will therefore indicate roughly the 95 per cent confidence limits for the position of a point. The standard error may be somewhat unrealistic (because all living conifers were used) but it is an indication of the error one might expect if one had taken fifty-eight genera from a larger sample with the same distributional pattern as the conifers. To this extent it is a guide to reliability in such studies.

It is not clear whether, for studies of this kind, one should make the variances for the three vectors equal. This would have the effect of making the cluster of points roughly spherical instead of ellipsoidal before projection onto the globe, and can be achieved by dividing the loadings by the square roots of the latent roots. In the present case this would make little difference to the main features of Fig. 3, as the square roots for the vectors were respectively 4.12, 3.71 and 2.11. This would only lead to a somewhat more even spreading of points with respect to longitude.

It is seen by comparing Figs. 2 and 3 that the biotal distances differ a good deal from the present geographical distances. Nevertheless the index of misfit<sup>15</sup> between the figures,  $d_h^2$ , is 0.792 with standard error 0.148. The expected value of  $d_h^2$  for random points is 2.0, so there is better agreement between present day geography and the biotal distances than one would expect by chance, and this is highly significant statistically. It may perhaps be permissible to regard  $d_h^2$  as a measure of the variance that is “unexplained” by geography, out of the 2.0 expected, so that in this case 60.4 per cent of the variation in the first three vectors is “explained” by the present day

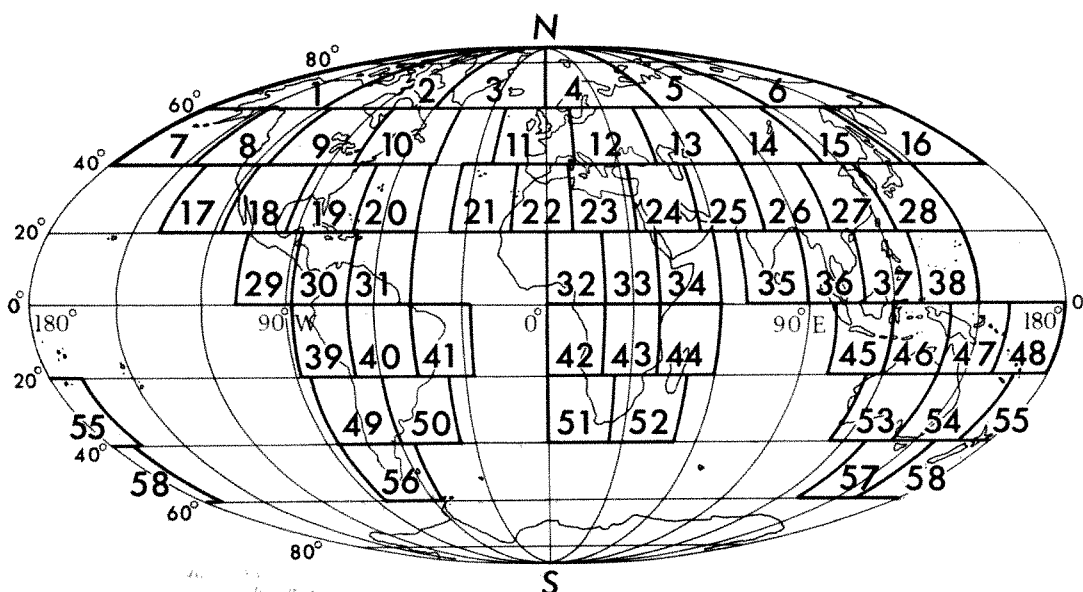


Fig. 2. The fifty-eight land areas possessing conifers that were used for analysis (Mollweide's projection).

geography. The percentage of total variation thus explained is rather lower, because these vectors themselves account for only about 60 per cent of the total variation.

Conifer distributions have often been cited as evidence for the theory of continental drift, and in particular for the southern continent of Gondwanaland. The points in Fig. 3 do bear some resemblance to the postulated positions of the continents in the later Palaeozoic on this theory, especially for the southern continents<sup>7,9,12</sup>. Numerical evidence for this is not easy to obtain: no reconstructions of the position of all the continents for the Carboniferous or Permian seem available; partial reconstructions raise serious statistical problems. The most complete reconstruction available at present is that of Irving (in ref. 1) for the Permian (based mainly on palaeomagnetic evidence), and  $d_h^2$  between that and Fig. 3 was found to be 0.692, with standard error 0.157. Though the fit is better than with present day geography this is not statistically significant. Clearly, more detailed geological evidence is needed to explore this further, but a few very tentative comments on the resemblances between Fig. 3 and the Palaeozoic land-masses may be added. The southern points approximate to the concept of Gondwanaland before it broke up in the Permian and Triassic<sup>9</sup>. Points for South America, southern Africa and southern India are grouped fairly closely, and those for Australia are relatively isolated; this is in keeping with reconstructions of Gondwanaland<sup>7,9,12</sup>. The difference in floras on the east and west of the Kalahari desert (indicated by the separation of points 32, 42 and points 33, 34, 43, 44) may have no significance. The holarctic points are closely grouped (as the uniformity of the conifers in this region would lead one to expect), and there is nothing to suggest the pattern of the northern Palaeozoic floras discussed by Just<sup>4</sup>.

It should be made plain that the points in Fig. 3 represent roughly the centres of the quadrats in Fig. 2, and that finer spacing of quadrats would presumably show many of the existing land links as a distorted geographical map. It was not feasible to draw such a map in this instance because of the complexity of the relationships between the points. Also, a number of quadrats are placed together because they possessed just one genus of

conifer. Thus quadrats 20 and 21 both possess only *Juniperus*, and southern India (No. 35) is placed with western Africa and central South America (Nos. 32, 39, 40, 42) because they all have only the genus *Podocarpus*. The weight to be placed on such slender evidence depends on how significant it is that they do not have any of the other genera. There seems to be relatively little influence of deserts and ice-caps compared with oceans, as is generally recognized<sup>10</sup> (except for the Sahara, which may be involved in continental drift).

Conifers would seem to be reasonably good for the kind of study made here. Though mostly monoecious, they probably do not migrate very readily. The genera listed by Florin are reasonably uniform phenetically, though the position of some southern quadrats might be rather different if *Podocarpus* (which is more diverse than most) had been divided into several genera. Ideally one should first make a numerical taxonomic study to obtain taxa of equal phenetic rank; this would be important with data such as labyrinthodont fossils<sup>10</sup>. Although fossil conifers were not considered (and many genera had a much wider distribution in the past<sup>17</sup>) this does provide a realistic example for studies where fossil evidence is not available. The positions of southern points in Fig. 3 can be interpreted in terms other than continental drift. Thus Darlington<sup>11</sup> considers it more probable that the present distributions are the result of spread by way of the tropics (though the present day southern genera do not seem to have moved into the northern hemisphere) and he believes that such distributions may reflect ancient climates rather than past land connexions.

One might suppose that for non-mobile organisms the reconstructions would reflect the position of land masses at the time when the main radiative evolution took place. The conifers appear to have arisen in the late- or mid-Carboniferous, and radiated soon after<sup>17</sup>, but there may have been several radiations, which would obscure the interpretations<sup>10</sup>. Presumably there would be little trace of periods before the evolutionary origin of the group.

It is clear from Fig. 1 that there are also biological distributions that reflect rather well the present geographical relationships. In that example, as Valentine pointed out, the biggest influence is clearly that of tem-

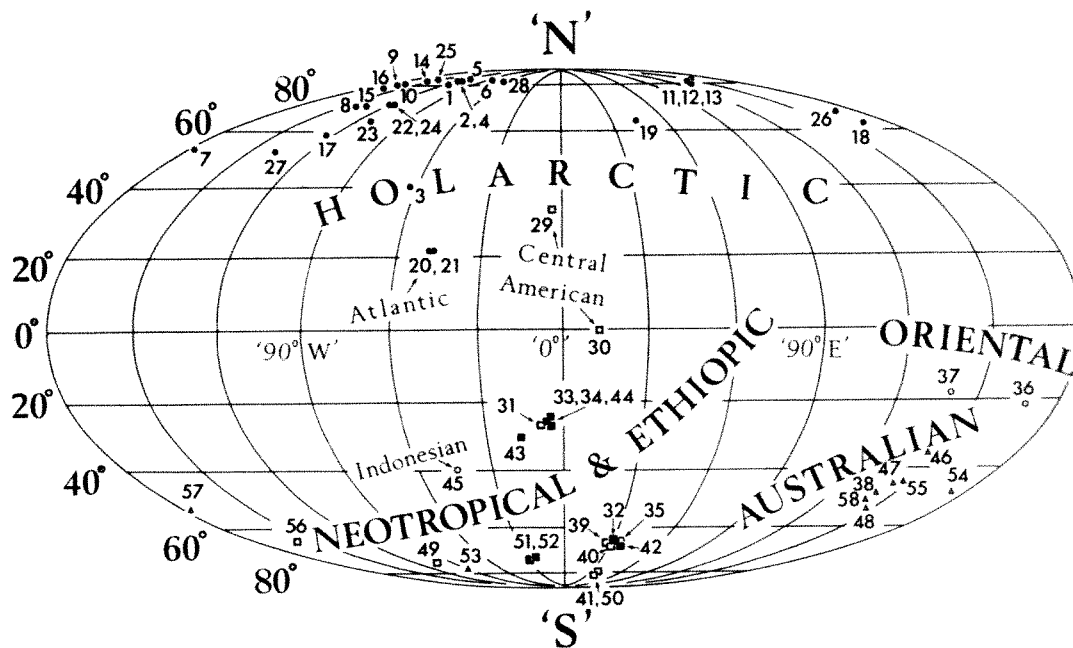


Fig. 3. The positions of the points representing the fifty-eight quadrats as given by the analysis of conifer distributions. The quadrat numbering is the same as in Fig. 2. The conventional biogeographical provinces are indicated. ●, Holarctic; ○, Oriental; ■, Ethiopian; □, Neotropical; △, Australian.



perature, largely due to ocean currents. There are also no important barriers to migration (other than climate) and the area is believed to have been a single geographical entity for a considerable time.

On more general points it should be noted that we need to know the probabilities of dispersal and migration (little information is usually available on this) if we are to assess the evidence for land connexions<sup>3,6</sup>. One must also guard against selecting simply those distributions that support some hypothesis<sup>10</sup>. These requirements may be conflicting, and one may have to make judgments on slender grounds. The most satisfactory approach seems to be to choose a large taxon of non-mobile organisms and to consider all the subtaxa within it, and then to use a statistical method such as factor analysis. In particular, one should note that the reconstructions may relate, not to the time for which the distributions are mapped, but to an unspecified time earlier to this.

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## Fallout Caesium-137 and Potassium in New-born Infants

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New techniques of low background counting have made possible the measurement of intake and retention of natural potassium and caesium-137 in infants. The biological half-lives of both potassium and caesium-137 are from 5 to 10 times lower than the corresponding values in adults.

Low background counting techniques have recently been developed to such an extent that both the natural potassium and the caesium-137 contents of infants can be measured with an overall accuracy (see below) of about  $\pm 7$  and  $\pm 10$  per cent respectively<sup>1</sup>. The uptake and retention of the isotope caesium-137 in the body is somewhat similar to that of potassium and it is known, from a number of investigations, that the biological half-life for caesium-137 in the adult averages about 100 days for the principal component, comprising about 90 per cent of the intake by ingestion<sup>2</sup>. The other, smaller, component is excreted with a short biological half-life of a little over one day<sup>3,4</sup>. Little corresponding information is available for children; values of the biological half-life of caesium-137 in children have been reported as about 35 days in the age range 6–12 yr<sup>4,5</sup> and three values of about 21–25 days have been given for infants<sup>6</sup>. Two values of 7 and 10 days have also been reported for infants by Rundo (personal communication). There are general grounds for expecting the turnover of caesium-137 to be more rapid during the early years of growth than in adult life. The presence of fall-out caesium-137 in all milk products in recent years has made it possible to investigate the uptake of caesium-137 in the new-born infant, if both the intake of caesium-137 in food and the retention of the isotope in the body are measured. We have been able to make the necessary measurements of intake and retention, over the period from birth to 6 months in the case of four infants who were bottle-fed. The

feeding regime, which is described below, could only be carried out with very careful co-operation on the part of the mothers and the period of investigation was, perforce, limited to about 6 months, because a mixed diet was usually introduced at that time and it became technically difficult to make the measurements on the more active and restless child of that age.

The following scheme has been used to determine the caesium-137 and potassium intakes and the retained whole body burden of infants during the first six months of life: the infant was measured once every week for the first 2 or 3 months and once every fortnight from then onwards; after each measurement the mother was given a known amount of dried milk (sufficient to last until the next measurement); at each feed, all the wasted dried milk (for example, rinses from mixing bowls and bottles, together with any vomit or dried milk spilled) was collected in a large container; the mother returned for the next measurement with the remaining dried milk and the container of residues. The milk was weighed and the residues reduced in volume and measured for caesium-137 and potassium content. Knowing the caesium-137 and potassium content of the milk, the intake of the infant since the last measurement could then be calculated.

### Whole Body Counting of Infants and Mothers

Whole body burdens were measured using a whole body counter with sodium iodide crystals doped with thallium. It was housed in a steel cubicle which had walls 5 in. thick, in the sub-basement of a five storey

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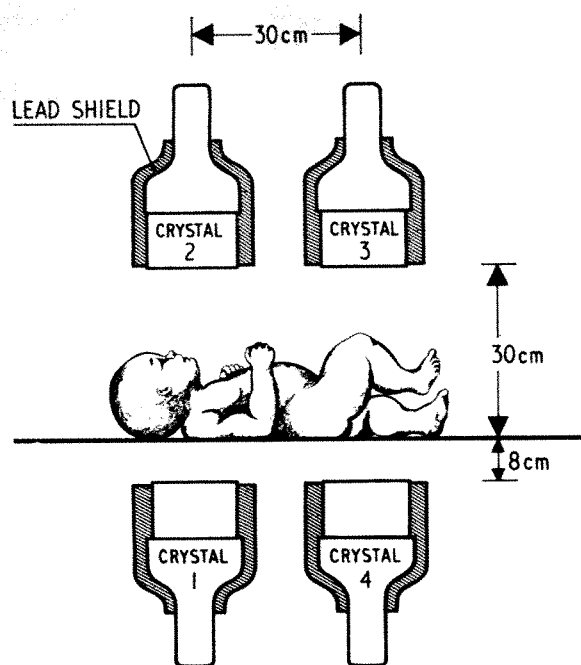


Fig. 1. Whole body counting geometry for infants.

building. The subject lay on a couch with two 6 in. diameter  $\times$  4 in. thick crystals fixed above the couch and another two fixed below.

Measurements of very young infants involved several difficulties not experienced in the measurement of adults and the following techniques were employed: in order to avoid changes in geometry during counting, measurement was only attempted with the infant asleep—usually and conveniently after the infant had been fed; the position and shape of the infant could not be defined exactly and it was necessary to use a crystal geometry which was as insensitive as possible to variations in these factors. This was achieved by placing the infant on the couch with the crystals arranged as in Fig. 1.

Calibrations were made with small polythene containers, filled with potassium and caesium-137 solutions, which approximated the mass and shape of the infant. The effect of a small distilled water (that is, inactive) phantom on the background count rate was negligible. In Table 1 the background count rate, caesium-137 and potassium sensitivities (c.p.m./nc. caesium-137 and c.p.m./g potassium) are recorded for a phantom, 6 kg in weight and 40 cm long, and for a phantom 10 kg in weight and 60 cm long. The sensitivity is not strongly dependent on the size of the infant nor on the exact position and shape of the infant—as was shown by measuring the sensitivity of phantoms simulating the possible positions and orientations of an infant on the couch.

The gamma ray spectrum of a new-born infant, after background subtraction, is shown in Fig. 2; the energy ranges chosen to cover the caesium-137 photopeak (B in Fig. 2), part of the potassium-40 Compton continuum (C) and the potassium-40 photopeak (D) are also shown. The whole body potassium was calculated from both of the

higher energy ranges corresponding to the photopeak and Compton continuum of the potassium-40 gamma ray spectrum; in all cases, good agreement between the two values was obtained. This indicated no additional gamma ray contaminant but also good matching of the spectra from infant and phantom. The whole body potassium contents reported in this paper were finally calculated from the summed count rates in the two high energy ranges. The whole body caesium-137 contents were calculated from the count-rate in the second energy range covering the caesium-137 photopeak corrected for the potassium-40 contribution. The count-rate in the lowest energy range (A), corrected for potassium-40 and caesium-137 contributions, showed that no significant amounts of low energy gamma emission and/or bremsstrahlung were present, and that any differences in scatter and absorption between infant and phantom were very small.

When possible, the infant and background were measured for at least 30 min and with a background counting time of 60 min the standard error in the counting statistics for the measurement of 1 nc. of caesium-137 and 8 g of potassium in an infant were approximately  $\pm 5$  per cent and  $\pm 8$  per cent respectively. Allowing for the further uncertainty, associated with differences between phantom and infant, the overall uncertainties are estimated to be  $\pm 7$  per cent and  $\pm 10$  per cent respectively.

The caesium-137 and potassium contents of the mothers were measured with the same whole body counter as the infants but with a different crystal geometry. The crystals were the same distance above and below the couch; one of the crystals was positioned over the mid-point of the subject while the other was displaced 40 cm towards the head. One of the lower crystals was displaced 70 cm from the mid-point towards the head, while the other was displaced 50 cm towards the feet. Calibrations were made with caesium-137 and potassium phantoms. The potassium contents were calculated from the same energy range (C + D) as that used for the infants.

### Measurement of Potassium and Caesium-137 in Dried Milk and Milk Residues

Samples containing 100 g of dried milk, made up with water to a standard volume of approximately 1 litre and contained in a 'Perspex' cylinder, were counted between two of the sodium iodide crystals. The residues were reduced to the same volume and measured in the same way.

Sufficient (homogenized) quantities of several brands of dried milk were measured and stored; from these a

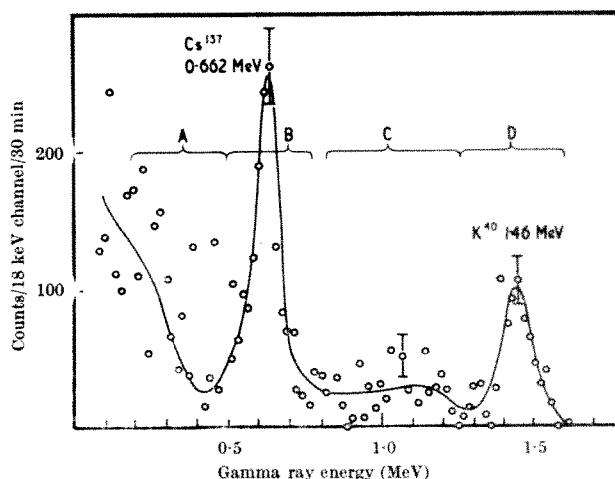


Fig. 2. Gamma ray spectrum of new-born infant: the infant was 2 days old, weighed 3.9 kg, and was counted for 30 min; the standard errors shown are calculated for a background counting time of 100 min. Whole body contents calculated from this measurement were  $0.62 \pm 0.06$  nc. caesium-137 and  $7.7 \pm 0.7$  g potassium.

Table 1. DATA ON SENSITIVITY OF WHOLE BODY COUNTER FOR THE DETECTION OF CAESIUM-137 AND POTASSIUM IN AN INFANT

Radiation source	C.p.m. in specified energy ranges			
	A 0.205- 0.535 MeV	B 0.535- 0.780 MeV	C 0.835- 1.270 MeV	D 1.270- 1.610 MeV
Background	489.4	189.2	166.6	80.8
1 g potassium				
In 6 kg 40 cm phantom	3.98	1.59	2.32	2.95
In 10 kg 60 cm phantom	3.79	1.49	2.21	3.58
1 nc. $^{137}\text{Cs}$				
In 6 kg 40 cm phantom	60.6	62.9		
In 10 kg 60 cm phantom	56.4	57.7		

milk suited to an individual infant could be selected. These milks are listed in Table 2 together with their

Table 2. CAESIUM-137 AND POTASSIUM CONTENTS OF VARIOUS DRIED MILKS

Sample	Contents/100 g dried milk <sup>137</sup> Cs (nc.)	Potassium (g)
Brand A (full cream)		
Batch 1	0.148 ± 0.011	1.43 ± 0.007
Batch 2	0.169 ± 0.010	1.53 ± 0.008
Brand B (half cream)	0.190 ± 0.016	1.57 ± 0.008
Brand C (full cream)	0.206 ± 0.016	1.34 ± 0.007
Brand D (full cream)	0.190 ± 0.016	1.31 ± 0.007
Brand E (full cream)	0.211 ± 0.017	1.45 ± 0.007

Brands A, B, C and D derived from English milk, brand E from Finnish milk.

measured potassium and caesium-137 contents. In the metabolic investigations on the four bottle fed infants, three infants were fed on milk A and one on milk C. It is probable that the different brands are derived from different parts of the country and it is perhaps surprising that the caesium-137 contents are so similar.

### Intake and Retention of Caesium-137 and Potassium

Values of the intakes and whole body contents of caesium-137 for one infant (infant C) are given in Table 3 and shown graphically in Fig. 3. The caesium-137 body content of the mother, measured several days after birth, is also listed in Table 3. Very similar data were obtained for the three other infants and mothers.

Table 3. MEASURED AND CALCULATED CAESIUM-137 CONTENTS OF AN INFANT FROM BIRTH TO 6 MONTHS

Age (days)	Weight (kg)	Measured caesium-137 content (nc.)	Intake of caesium-137 In interval (nc.)	Average per day (nc./day)	Calculated caesium-137 content (nc.)
0	3.06				0.74*
9	2.92	0.77	0.63	0.070	0.93
17	3.50	1.11	0.63	0.079	1.09
24	3.65	1.49	0.70	0.100	1.31
31	3.66	1.63	0.89	0.127	1.60
38	3.90	2.00	0.77	0.110	1.70
45	4.00	1.89	0.91	0.130	1.89
52	4.14	1.88	0.85	0.121	1.96
59	4.40	2.15	0.89	0.127	2.04
66	4.54	2.05	0.91	0.130	2.11
80	5.00	2.41	1.91	0.136	2.25
94	5.45	2.41	1.99	0.142	2.37
108	5.85	2.40	2.04	0.146	2.45
122	6.00	2.41	1.95	0.139	2.43
155	6.75	2.45	5.02	0.152	2.60
169	7.26	2.53	2.37	0.169	2.79
183	7.45	2.65	2.30	0.164	2.82
Mother	64.1	15.6			

\* Calculated assuming maternal and infantile concentrations of caesium-137 are equal at birth.

If, in the absence of evidence concerning the metabolic compartments for caesium-137 in infants, it is assumed

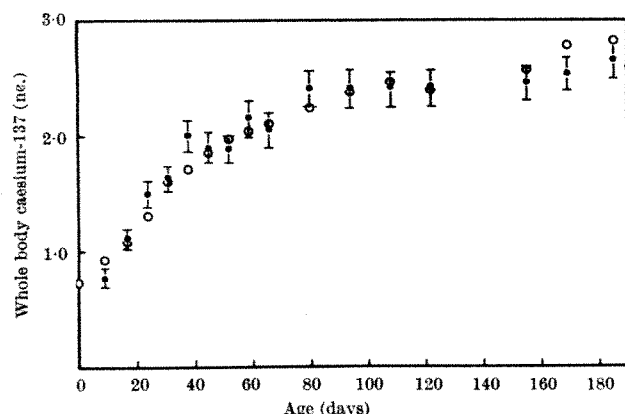


Fig. 3. Measured and calculated caesium-137 contents of an infant from birth to age 6 months. ●, Measured content with standard error; ○, calculated content.  $T = 12$  days.

that the metabolism can be represented by one compartment with a biological half-life of  $T$  days, values of the expected whole body retention can be calculated from the intake data by suitable choice of  $T$ . If  $P$  is the average intake per day over an interval  $t$  days, the compartment content  $Q$  at the end of the interval is given by

$$Q = Q'e^{kt} + \frac{P}{k}(1 - e^{kt})$$

where  $k = 0.693/T$  and  $Q'$  is the compartment content at the beginning of the interval. The value of  $Q'$  at birth can be deduced approximately from the concentration of caesium-137/kg in the mother because, as will be shown later, the calcium-137 concentrations in mothers and infants are almost the same. Calculated values of the caesium-137 content of an infant can then be derived step-wise from the successive values of the intake on the basis of this equation. The contents of the infant C, calculated in this way, are listed in the last column in Table 3, and are compared with the measured whole body retentions in Fig. 3. It can be seen that good agreement with the measured values is obtained by using a value of  $T$  of 12 days. Similar treatment of the data for two other infants gave values of 11 and 14 days for the biological half-life, extending over periods of about 140 days. In the case of the fourth infant, the measured caesium-137 content could be represented by a value of  $T$  of 11 days up to age 81 days and followed by a value of 13 days to age 154 days.

The assumption of a more elaborate model of caesium-137 metabolism in the infant was hardly justified by the results available by this method of investigation, for the existence of a small short-lived component in infants, as in adults<sup>3,4</sup>, would only increase the values of  $T$  derived here by about 10 per cent. To establish the presence of such a component would necessitate giving a small (but unjustifiable) amount of caesium-137 as a single dose.

The intake of potassium and the body content of potassium are given for infant C in Table 4 and also shown graphically in Fig. 4. Although the concept of a biological half-life is difficult to apply in the context of an essential physical ion like potassium, we have nevertheless derived values of the biological half-life  $T$  by treating the potas-

Table 4. MEASURED AND CALCULATED POTASSIUM CONTENT OF AN INFANT FROM BIRTH TO 6 MONTHS

Age (days)	Weight (kg)	Measured potassium content (g)	Potassium concentration (g/kg)	Intake of potassium In interval (g)	Average per day (g/day)	Calculated potassium content (g)
0	3.06			$T = 6$ days		5.2*
9	2.92	5.3	1.82	5.9	0.65	5.5
17	3.50	6.8	1.94	5.9	0.73	6.0
24	3.65	7.1	1.95	6.5	0.92	7.1
31	3.66	7.7	2.10	8.3	1.18	8.8
38	3.90	7.6	1.95	7.2	1.03	8.9
45	4.00	9.8	2.45	8.5	1.21	9.8
52	4.14	9.9	2.39	7.9	1.13	9.8
59	4.40	10.1	2.30	8.3	1.18	10.0
66	4.54	9.7	2.14	8.5	1.21	10.3
80	5.00	10.4	2.08	17.7	1.27	10.8
94	5.45	11.4	2.09	18.5	1.32	11.3
108	5.85	10.7	1.83	19.0	1.35	11.6
122	6.00	12.3	2.05	18.1	1.29	12.0
155	6.75	13.6	2.01	46.3	1.40	13.1
169	7.26	14.1	1.94	22.0	1.57	14.4
183	7.45	15.4	2.07	21.4	1.53	14.3
Mother	64.1	108.4	1.69			

\* Calculated assuming maternal and infantile concentrations of potassium are equal at birth.

sium data in the manner described for caesium-137. It can be seen that a good fit, between the measured potassium contents and those calculated for the one compartment model, is obtained with a value of  $T$  of 6 days up to age 100 days, beyond which a better fit is obtained with a value of  $T$  of 6.5 days. A similar increase in the value of  $T$  was found to be necessary for two of the

other three infants; for one,  $T$  increased from 5 to 7 days at 81 days, and for the other infant,  $T$  increased from 5 to 6 days at age 93 days. For the fourth infant, a good fit over the whole duration of the measurements (150 days) was obtained with a value of  $T$  of 4.5 days.

The values of the biological half-life  $T$  for both caesium-137 and potassium for the four infants investigated are assembled in Table 5. All the values of  $T$  for caesium-137 are less than those reported for older children<sup>4,5</sup> and very much less than those for adults<sup>2</sup>. The average value of about 12 days for  $T$  (caesium-137) is also less than values of 21–25 days reported by Bengtsson *et al.*<sup>6</sup> for three infants born to mothers who had considerable caesium-137 and caesium-132 burdens but in these cases the derived values of  $T$  depend on corrections which have to be made to allow for the intake of breast milk.

Table 5. COMPARISON OF CAESIUM-137 AND POTASSIUM DATA

Infant	Caesium		Potassium		Ratio $T(^{137}\text{Cs})$ $T(\text{K})$
	Period (days)	$T$ (days)	Period (days)	$T$ (days)	
A	0–81	11	0–81	5	2.2
	81–154	13	81–154	7	1.9
B	0–149	14	0–72	5	2.8
			72–149	6	2.3
C	0–183	12	0–108	6	2.0
			108–183	6.5	1.9
D	0–145	11	0–145	4.5	2.5

The values of  $T$  (potassium) in Table 5, like those for caesium-137, are also very much less than the biological half-life taken for potassium in adults<sup>7</sup>. The ratios of  $T$  (caesium-137) to  $T$  (potassium) given in the last column average about 2.2, and this is consistent with a discrimination factor of 2 for the caesium-137 to potassium ratio in adults with respect to that in diet, reported by Langham and Anderson<sup>8</sup>.

The caesium-137 and potassium contents of nine mothers and their infants were measured within 3 days of parturition. The results are listed in Table 6, together with values for the concentrations of caesium-137 and potassium, in nc. and g respectively per kg of body weight. The concentrations in the infants are plotted against those in the mothers in Fig. 5 for potassium and in Fig. 6 for caesium-137.

If there is no placental discrimination it is to be expected that the infant contents of caesium-137 and potassium will reflect the plasma concentrations of these elements during foetal life. If the relative proportions of different tissues and organs were the same in mother and infant, the concentrations of caesium-137 and potassium/kg in

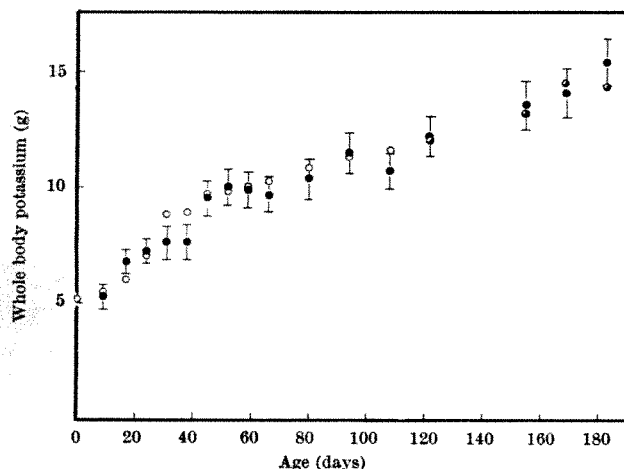


Fig. 4. Measured and calculated potassium contents of an infant from birth to age 6 months. ●, Measured content with standard error; ○, calculated content.  $T = 6$  days; ○, calculated content.  $T = 6.5$  days.

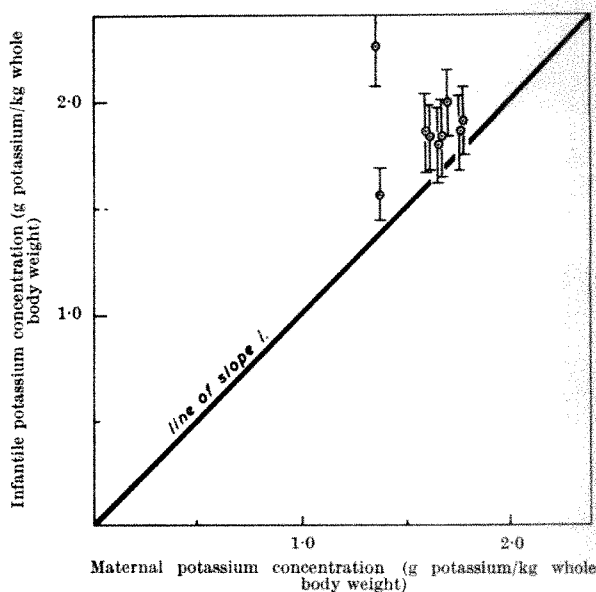


Fig. 5. Potassium concentrations in mothers and new-born infants.

the infant should be close to those in the mother. The results for potassium in Fig. 5 show that the infant potassium concentration is usually about 10 per cent above that of the mother, reflecting some difference in body composition, such as possibly less fatty tissue (which contains little potassium) in the infant than in the adult.

Table 6. POTASSIUM AND CAESIUM-137 CONTENTS OF MOTHERS AND THEIR NEW-BORN INFANTS

Subject + infants' age	Weight (kg)	$^{137}\text{Cs}$ content (nc.)	Potassium content (g)	$^{137}\text{Cs}$ concentration (nc./kg)	Potassium concentration (g/kg)
Mother 1	65.2	10.0	105.7	0.154	1.62
Infant 1, F (1 day)	3.35	0.51	6.2	0.152	1.84
Mother 2	54.7	11.0	97.0	0.202	1.77
Infant 2, F (1 day)	3.35	0.48	6.4	0.143	1.91
Mother 3	67.3	6.6	113.0	0.098	1.68
Infant 3, M (2 days)	3.66	0.27	6.7	0.074	1.84
Mother 4	57.3	7.6	96.0	0.133	1.67
Infant 4, M (2 days)	3.60	0.44	6.7	0.119	1.80
Mother 5	56.7	10.4	96.3	0.183	1.70
Infant 5, M (2 days)	3.86	0.62	7.7	0.161	2.00
Mother 6	69.6	9.3	94.1	0.134	1.85
Infant 6, F (2 days)	3.01	0.47	6.8	0.156	2.26
Mother 7	57.6	8.7	92.3	0.151	1.60
Infant 7, M (3 days)	3.06	0.60	5.7	0.196	1.86
Mother 8	49.5	5.2	86.9	0.106	1.76
Infant 8, F (2 days)	3.91	0.45	7.3	0.115	1.87
Mother 9	68.7	6.8	94.1	0.099	1.37
Infant 9, F (2 days)	5.0	0.31	7.8	0.062	1.57

The caesium-137 concentrations in Table 6 and Fig. 6 are very similar in mother and infant. Departure from this approximate equality might well occur, however, in view of the differences in the biological half-life of caesium-137 of adults and infants and the variability of the dietary intake of the isotope. The value of  $T$  of 70–100 days in the adult means that the caesium-137 content of the mother depends on the dietary intake over a period of the order of 6 months or more. The caesium-137 content of the infant, with a value of  $T$  of only 12 days, reflects the plasma concentration in the mother over the last month of pregnancy and thus on the dietary level during this shorter and later period. The quite considerable differences between mother and child in some of the caesium-137 concentrations in Table 6 are compatible with this hypothesis.

The measured concentrations of caesium-137/kg can be used to derive the consequent internal radiation dose to the body tissues. For the caesium-137 levels at the



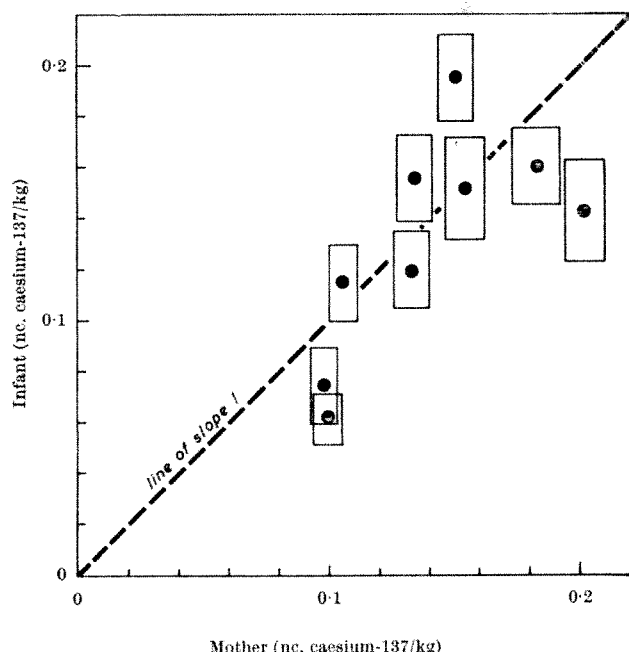


Fig. 6. Caesium-137 concentrations in mothers and new-born infants.

time of the measurements (1964) the dose rate to the infant was about 1 mrad/yr at birth and about 2 mrad/yr at 6 months. These values correspond approximately to 1 and 2 per cent respectively of the dose rate from natural background radiation.

We thank the Medical Research Council for support. We also thank Professor J. S. Scott and Dr E. C. Allibone for their help in obtaining the collaboration of the mothers of the infants measured. We are particularly indebted to the four mothers who took part in the metabolic investigation and followed the feeding regime so carefully. We also thank Mr Brian Oldroyd for assistance in making the measurements and Dr F. B. Ellis for help in obtaining some of the samples of dried milk.

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## Weather and Discharge Data used to compute Dose Rates from Coastal Discharges of Radioactivity

by

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Some of the waste material discharged into the sea from the Dounreay reactor establishment settles on the nets used by fishermen in the area. The dose rate to their hands is a function of the discharge rate and the local conditions around the fishermen's nets. Equations derived on this basis show a close agreement with findings in the field.

LIMITED amounts of dilute aqueous radioactivity from the Dounreay Experimental Reactor Establishment are discharged by pipeline into the coastal waters of Caithness<sup>1</sup>. Almost 2 miles away from the outlet fishermen work large fixed-net installations between May and the end of August each year. During those months the water along that coast is remarkably clear, but its continual movement through the nets leads to the deposition on the meshes of fine particles of inorganic and organic matter, mostly below 5 $\mu$  in diameter, but including some clusters, mostly below 50 $\mu$  in diameter; these particles may carry varying amounts of adsorbed radioactivity as a result of the discharges to sea. One of the restrictions on the rate of discharge is based on the beta radiation exposure to the hands of fishermen working the nets.

The physical features of the coastal area, and the relation of Dounreay to Sandside Bay in which the nets are worked, are shown in Fig. 1. There are prominent reefs along the coast, backed up by low rocky cliffs as far as the bay, and high cliffs beyond.

During the course of making regular measurements—up to twice a week during the summer season—of the beta radiation dose rate received on contact with such nets, an overall pattern of higher dose rates during calm weather and lower dose rates during rough weather emerged. For purposes of measurement a stretch of net—2 fathoms deep, suspended from floats and weighted with rope along the bottom edge—was set within a short

distance of the commercial nets. After lifting the net, measurements of dose rate were taken using GM tube and ionization chamber instruments. A short account of part of the work carried out since 1962 is given here.

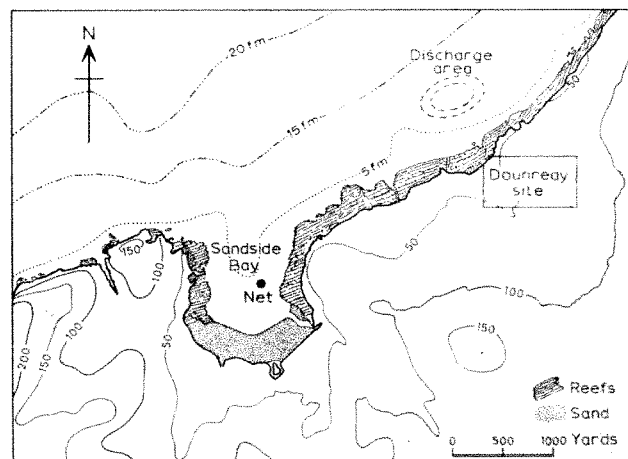


Fig. 1. Coastal features between the Dounreay Experimental Reactor Establishment and Sandside Bay, showing depths, reefs, contours and position of net.

It was supposed that the dose rates from the nets would be determined by (1) the rate of discharge from DERE and (2) local conditions round the nets. It was further supposed that wind strength determined the conditions around the nets, both as regards (2.1) the state of the sea—which decided the amount of sediment in equilibrium on the meshes of the nets—and (2.2) the quantity of sediment in suspension in the top 2 fathoms of water. These two wind effects might be expected to act in opposition on the dose rate, for example, increasing wind strength reducing the stability of sediment on the nets, but increasing the amount of sediment available in the upper layers of water.

For the first analyses a simple power relation was assumed

$$r = c (d_d)^{b_d} (u_{ns})^{b_w} \quad (1)$$

in which  $r$  is the dose rate on the net;  $c$ , a constant;  $d_d$ , the mean daily discharge of activity, taken over a period of  $t_d$  days before dose measurement, either with or without correction for decay;  $u_{ns}$ , the mean wind speed, taken over  $t_{ns}$  days before dose measurement; and  $b_d$ ,  $b_w$ , are exponents relating to discharge and wind.

Values of  $b_d$  and  $b_w$  were found by partial regression analysis of log transformed discharge and wind data. Chemical Services, DERE, provided discharge data, based on total beta and individual gamma determinations; wind data were provided by Health Physics, DERE.

**Analysis of results of first season.** For each observation of dose rate the following data were prepared. (1) Mean daily discharge rates over periods of 15, 30, . . . 75 days before dose measurement, using (1.1) total beta data, and (1.2) gamma measurements relating to the 0.13, 0.5 and 0.75 MeV peaks, that is to  $^{141}\text{Ce}/^{144}\text{Ce}$ ,  $^{103}\text{Ru}/^{106}\text{Ru}$  and  $^{95}\text{Zr}/^{95}\text{Nb}$  pair totals. (2) Mean scalar wind speeds, that is, taken over bearings  $0^\circ$ – $360^\circ$ , over periods of 1, 2, . . . 21 days before dose measurement.

Referring to equation (1), all combinations of  $t_d$  (relating to discharge, between 15 and 75 days), and  $t_{ns}$  (relating to wind, between 1 and 21 days) were analysed to show the variations of  $b_d$  and  $b_w$  with times of averaging. For discharge periods of 45 and 60 days the values of  $b_d$  bracketed the value  $b_d = 1.0$ , that is, a linear relation between discharge rate and dose rate obtained for discharges averaged over periods of 45–60 days. Taking this linear relationship as a reasonable foundation, the (45 and 60 day discharge) (1–21 day wind) combinations were examined for variation of  $b_w$ , the wind exponent over the 1–21 day wind period;  $b_w$  showed plateau characteristics over the interval of 3–9 days' wind, taking values in the range  $(-1.6 \pm 0.3)$ . This preliminary analysis showed features that appeared in all subsequent analyses: (1) an indicated discharge summation period approaching 2 months; (2) plateaux relating to  $b_w$ , and  $b_d$ , when winds were averaged over periods of 5–9 days before dose measurement. The two periods of time under (1) and (2) gave the first indications of the time characteristics of the DERE/Sandside Bay system.

Figs. 2 and 3 illustrate results obtained using a (1 discharge/1 scalar wind) combination. The simplest estimate of dose rate was based on 60 days' discharge, total beta corrected for decay,  $b_d = 1.066$ , with 7 days' scalar wind,  $b_w = -1.776$ ; see Fig. 2. An improved fit was obtained using weighted gamma data: the weighting factors 1.0, 0.5 and 0 were fitted to the discharges assayed under the  $^{141}\text{Ce}/^{144}\text{Ce}$ ,  $^{103}\text{Ru}/^{106}\text{Ru}$  and  $^{95}\text{Zr}/^{95}\text{Nb}$  peaks respectively; Fig. 3 shows dose rates estimated from 60 days (Ce + 0.5 Ru) and 11 days' wind. For this combination,  $b_d = 0.986$  and  $b_w = 2.052$ , the  $b_w$  value falling just below the plateau range. The fit was satisfactory over the weighting ranges (0.3–0.7) for ruthenium and (0–0.1) for zirconium–niobium.

In further analyses subdivided periods of discharge and scalar wind were used: also vector winds, air temperatures and air/sea temperature ratios. All these addi-

tional steps were developed in the analysis of the results of three consecutive seasons, except the use of vector wind, which was dispensed with over the wind averaging period used, namely 10 days.

**Analysis of results of three seasons.** At Sandside Bay winds were low in 1962, mixed in 1963 and higher in 1964: dose rates measured through 1964 were lower than those of the previous years. It was supposed that this was due to the effect of sustained winds on the turnover of activity and sediment. To deal with this variation between seasons—in addition to that within a single season—a further wind term was used. Wind speeds were averaged over periods of 28, 42, 56 and 70 days and used in conjunction with discharge, and short period winds: best fits were obtained with the 42 day period—approaching the time range already indicated for the treatment of discharge data. These long period mean winds were used in two ways: (1) directly, as with the short period winds, to examine whether the between seasons effect was directly related to wind power; and (2) exponentially, in the form  $\exp(b_w u^n)$  in which  $n$  was varied between 3 and 5, on the supposition that reduction of dose rate could be due to loss of activity to sea-bed solids raised by wave breaking.

In the analysis of the results of the first season the estimates of dose rate were related to the individual days of dose measurement, whereas it is usual to operate the restriction on discharges from DERE on a monthly basis. To bridge this time gap it was decided to average dose rate measurements over two weekly periods when processing the results obtained over three seasons. Times given below for discharge relate to mid-fortnight as zero, and for the other parameters to 3 days later—a displacement indicated by fitting.

Fig. 4 shows a typical fit based on (1) 56 days' discharge gamma (Ce + 0.7 Ru + 0.1 Zr):  $b_d = 0.955$ ; (2) 10 days'

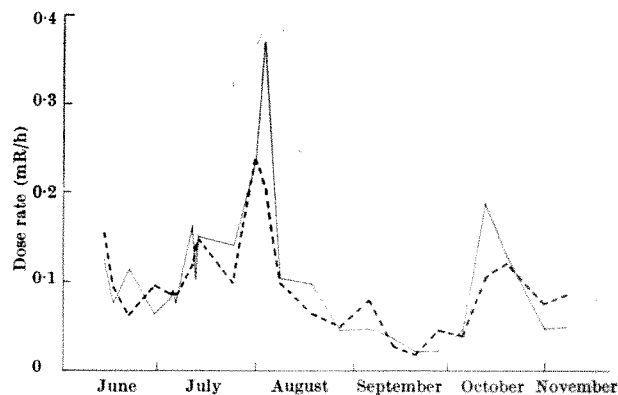


Fig. 2. Observed dose rates against dose rates computed from 60 days discharge (total beta) and 7 days scalar wind. —, Observed; ----, estimated.

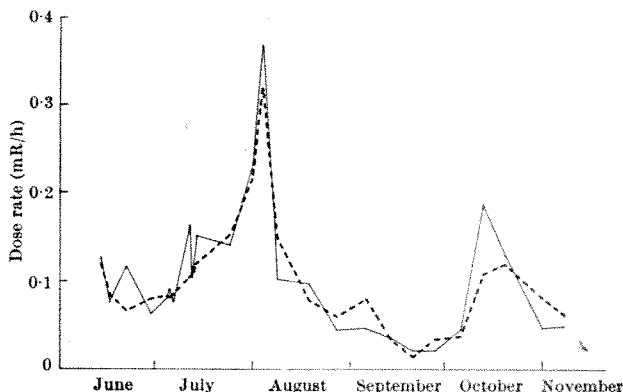


Fig. 3. Observed dose rates against dose rates computed from 60 days (Ce + 0.5 Ru) discharge and 11 days scalar wind. —, Observed; ----, estimated.

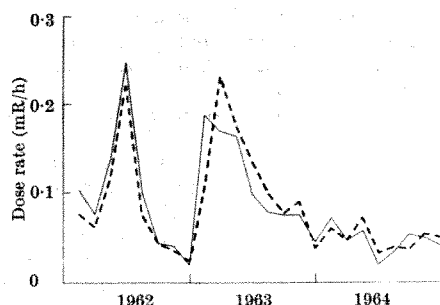


Fig. 4. Two weekly average dose rates observed 1962-3-4, against dose rates computed from 56 days ( $Ce + 0.7 Ru + 0.1 Zr/Nb$ ), 10 days scalar wind, 7 days air/10 days sea temperature ratio, 42 days wind (direct method). —, Observed; ----, estimated.

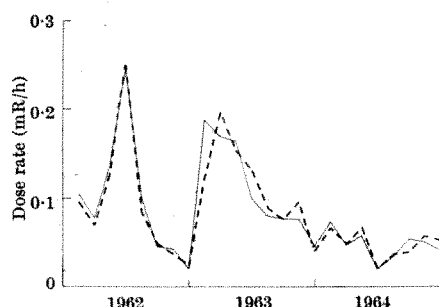


Fig. 5. Comparison with Fig. 4, using 42 days wind (exponential method). —, Observed; ----, estimated.

short wind:  $b_{sw} = -1.547$ ; (3) (7 days air/10 days sea) temperature ratio:  $b_{a/s} = 0.936$ ; (4) 42 days' long wind:  $b_{lw} = -3.188$ .

The air/sea temperature ratio was best based on maximum air temperatures, and was taken over a time range (3-15 days) similar to that used for short winds. The value of  $b_{lw} = -3.188$  is a typical value from a range of  $-2.9 \pm 0.3$  indicated by many analyses. The fit shown in Fig. 4 was improved by subdividing the 56 day period into four periods of 14 days.

The alternative, exponentially based method, is illustrated in Fig. 5 using (1) 56 days' discharge gamma ( $Ce + 0.7 Ru + 0.1 Zr$ ):  $b_d = 0.956$ ; (2) 10 days' short wind:  $b_{sw} = -1.462$ ; (3) (7 days air/10 days sea) temperature ratio:  $b_{a/s} = 1.265$ ; (4) 42 days' long wind: using  $\exp(u_{42})^5$ :  $b_{ew} = -2.520$ .

Closer fits than those shown in Figs. 4 and 5 were obtained by taking decay into account, that is, by applying sliding weighting factors to the four separate 14 day periods of discharge.

Since these analyses were completed, tests of the two methods of using long wind have been made on measurements taken in the summer of 1965, and during the winter 1966-67. It appears that: (1) the use of direct long wind, that is, in the form  $b_{lw} = -3$ , is indicated; (2) the accuracy of dose rate estimation meets the discharge control requirement.

The overall range of averaging times used in these analyses—from the shortest wind to the longest discharge—runs from 1 day to 75 days. But when plateau characteristics are taken into account, two well-marked periods emerge: (1) about 5-8 weeks, relating both to discharges and the long-term winds; (2) 1-2 weeks relating to the local conditions around the nets, as determined by wind and temperature.

The exponent relating to the long-term wind ( $b_{lw} = -2.9 \pm 0.3$ ), that is, cube, is that normally used to describe wind power, or rate of transfer of energy from wind. If the short wind effect is described in terms of wind power, the results of the analysis to date can be written as

$$r = \text{constant } d_{td} \cdot P_{td}^{-1} P_{tns}^f \cdot s_{tns} \quad (2)$$

in which  $r$  is the dose rate;  $d_{td}$ , the mean discharge rate, over time  $t_d$  fixed by turnover of activity and sediment;  $P_{td}$ , the mean wind power, over similar time  $t_d$ ;  $P_{tns}$ , mean wind power, over time  $t_{ns}$  fixed by local conditions relating to net and sediment;  $f$  is an exponent in the range 0.4-0.7; and  $s_{tns}$  is a measure of air stability, over similar time  $t_{ns}$ .

The indicated time periods are being studied in other experiments in order to separate the parts played by discharge, net and sediment.

Meanwhile at DERE during the past year environmental dose rates have been computed in the laboratory, from Chemical Services and meteorological data, using a slide rule constructed from the three seasons' analysis. The longer term interest of this work lies in the opportunity offered for the study of physical processes in coastal waters.

I thank the staff of DERE for their help in this work; the Ministry of Agriculture, Fisheries and Food and the Scottish Development Department for their support; and Mr J. Preece for his invaluable contributions and computations.

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<sup>1</sup> Environmental Monitoring Associated with Discharges of Radioactive Waste during 1964 from UKAEA Establishments, AHSB(RP) R66 (1965).

## Pleistocene Deposits and New Fossil Localities in Kenya

Bedded rocks near Lake Baringo have yielded a variety of vertebrate fossils, including a right temporal bone with features which suggest that it is an australopithecine or early hominine.

I HAVE been concerned with geological mapping of 400 square miles in the Northern Rift Valley of Kenya. In the process, I have found fossil localities in sediments to the west of Lake Baringo. The sediments, located at  $36^\circ 5' E$ ,  $0^\circ 47' N$ , were first described by Gregory<sup>1</sup>, and later became known as the Kamasia sediments, for they outcrop close to the foothills of the Kamasia Range. Until 1950, however, no fossils useful for dating had been obtained from these sediments.

In 1950, Fuchs<sup>2</sup> described vertebrate remains from a horizon which seems to lie about 60 ft. from the local top of the sediments. These were dated as Pleistocene by Hopwood (in Fuchs<sup>2</sup>), but beneath this faunal horizon is a maximum of 1,000-1,100 ft. of sediments which, until October 1965, had yielded only fossil fish remains (species of *Tilapia* and *Clarias*)<sup>3</sup>. Only recently has it been appreciated that the sediments do not comprise a straightforward, continuous sequence. McCall, Baker and Walsh<sup>3</sup>

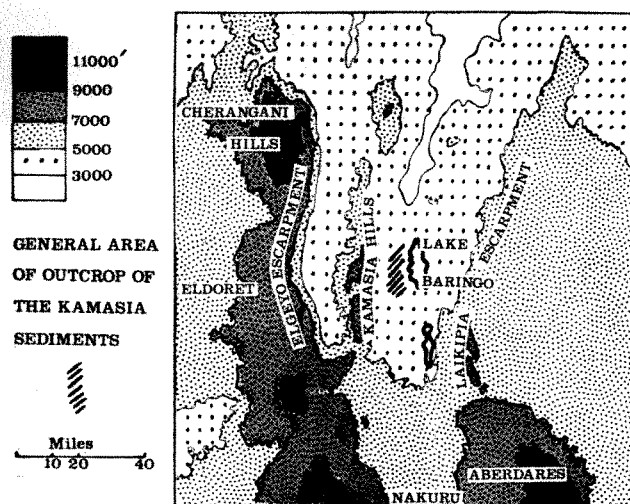


Fig. 1. Position of the area investigated in West Central Kenya.

made it clear that two sedimentary sequences are represented, and that these are separated by a period of lava outpouring, followed by the faulting of these lavas and the underlying sediments. The lower sequence was named the Chemeron Beds<sup>3</sup>, from the Chemeron River section. The upper sediments, which rest unconformably on the lower ones, and their capping lavas, were called the Kapthurin Beds after the nearby Kapthurin River. In October 1965, I recognized the two separate formations, and began to investigate them in detail.

Although this communication is primarily concerned with the lower or Chemeron Beds sequence, the succession of the Kapthurin Beds is summarized here for the sake of completeness. The Kapthurin Beds were deposited largely in a fluvial basin, although outliers of plateau and piedmont gravel occur well outside the main area of sedimentation. The thicknesses given below are the maximum ones, and all the sub-divisions listed die out completely against the lava plateau edges of the basin.

### The Kapthurin Beds

#### *Upper torrent wash* (80')

Unstratified brown earthy silts with gravelly horizons.

#### *Upper tuffs* (up to 40')

Buff coloured fine-grained tuffs well bedded in thick units.

#### *Middle torrent wash* (130')

Brown earthy silts with calcareous conglomerates locally near top. This sub-division has yielded vertebrate remains.

#### *Pumice tuffs* (up to 40' or more)

Coarse terrestrial pumice tuffs with washouts and a variable admixture of torrent wash silts.

#### *Lower torrent wash* (base not often seen, may exceed 150' locally)

Coarse bouldery gravels with brown earthy silts; rests on very uneven surface of lava fault blocks.

### The Lake Hannington Phonolites

Lavas of several different types are seen to overlie the Chemeron beds. Some are true phonolites, though many are trachytes with trachybasalt affinities. Most are doubtless equivalent in the broad sense to the Lake Hannington phonolites of the type area.

### The Chemeron Beds

The succession of the Chemeron beds is given below. The sub-divisions are lithological ones established as a

result of field work carried out by me in October and November 1965.

Upper Tuffs (20–100 ft.)  
Upper Fish Beds (up to 250 ft.)  
Lower Tuffs (9–16 ft.)  
Lower Fish Beds (up to 320 ft.)  
Basal Beds (0–150 ft. or more)

The Basal Beds rest on basalts wherever they are exposed, and they seem to have derived much of their material from the breakdown of these rocks. Much of the basalt was highly decomposed when the Basal Beds began to accumulate. The lavas weather to a soft earthy, gritty product, or in some places to deep red lateritic earths. This weathered material was washed and reworked, and deposited along with accumulations of sand, grit and gravel. Much of the coarser clastic material has its origin in fresher basalt, or occasionally from phonolite of a type now outcropping abundantly in the Kamasia Range to the west.

The Basal Beds accumulated in a depression not more than 5 miles from north to south; thus they are overlapped by the younger Chemeron Beds which extend farther. Although water was involved in deposition, no permanent lake existed in the depression. Impure diatomites and fish-bearing silts are restricted both vertically and horizontally and usually occur high in the Basal Beds. Proboscidean-bearing lithic grits and gravels are characteristic, and represent a fluvial environment. Important proboscidean-yielding sites are at J.M. 91, very close to the absolute base of the beds; J.M. 511, about 35 ft. from the base of the beds; J.M. 513, almost at the base; J.M. 514, close to the top of the Basal Beds. J.M. 511 also yielded hippopotamus remains.

Dr L. S. B. Leakey of the National Museum Centre for Prehistory and Palaeontology, Nairobi, Kenya, has commented as follows on the mammalian fossils from the Basal Beds.

"The proboscidean fauna of the Basal Beds viewed as a whole is strongly suggestive of that from strata attributed to the 'early Villafranchian'. It consists of *Elephas cf. subplanifrons* (J.M. 511), *Elephas cf. africanus* (J.M. 511), *Anancus kenyensis* (J.M. 513) and *Deinotherium cf. bozasi* (J.M. 511 and a juvenile from J.M. 514). Locality J.M. 514 has also yielded the almost complete skeleton of a proboscidean.

"At locality J.M. 91, there is a more highly evolved elephant, probably a derivative of *Elephas africanus* and approaching the early form of *Elephas recki*. Locality J.M. 91 is near the local base of the Basal Beds and has yielded also excellent specimens of a fossil species of *Papio* comprising a skull and also a nearly complete skull and skeleton.

"Remains of a large rhinoceros have been found at two localities, J.M. 91 (Basal Beds) and J.M. 507 (Lower Fish Beds). They resemble *Serengeticerus*, but exact determination is not yet possible and must await the revision of all East African Pleistocene Rhinocerotidae which will be carried out soon.

"Hippopotamus remains have been found in the Basal Beds (Localities J.M. 511, 512) and the Lower Fish Beds (Localities J.M. 106, 507). Both hexaprotodont and tetraprotodont forms are represented. This group is undergoing a major revision, after which specific identification of some of the material should be possible."

The Fish Beds comprise the bulk of the Chemeron Beds succession. They seem to be almost entirely lacustrine and fluvial; fish remains are abundant. A prominent tuff horizon has been used to divide the succession into an upper and a lower group, and this horizon has proved to be persistent over most of the area of outcrop examined.

The transition at the top of the Basal Beds into the lithology of the Lower Fish Beds marks the oncoming of a wholly lacustrine environment. The basin of deposition manifest in the distribution and thickness of the group shows a gradual expansion through the period of deposition of the group. A maximum thickness in the region of



320 ft. was measured in a tributary of the Kapthurin River, although this figure may be amplified further in a southerly direction because of the presence of grits and gravels locally.

Although lavas and tuffs were the parents of much of the sediment, the beds represent washed and sorted materials of fine texture. Diatomite units as much as 40 ft. thick illustrate slow or negligible deposition of clastic fragments. Tuffaceous grits and lava derived gravels are not common except in the Ndaui River area. Northward and southward thinning of the sub-division is evident, although there are no important changes in lithology. The observed north-south extent of the basin had increased to about 10 miles.

Fossil remains are quite common in the Lower Fish Beds; fish remains are particularly abundant, especially forms resembling species of *Tilapia* and *Clarias*. Thin ironshot sands and grits often contain abundant fish remains as well as occasional teeth of crocodiles. While such horizons indicate slow accumulation in shallow water, well-defined breaks in the sequence are rare. One particularly interesting exposure (J.M. 106) displays a thin grey clay resting on the eroded surface of a tuffaceous sandstone. The clay yielded the mandible of a hippopotamus associated with parts of the base of the skull. This particular horizon is believed to lie not more than 30 or 40 ft. from the base of the Lower Fish Beds. Another hippopotamus locality, J.M. 507, is at a similar position in the sequence.

The Lower Tuffs form a useful marker horizon separating the silty Lower Fish Beds from the more gravelly Upper Fish Beds. It is a primary tuff, and so this unit is a time plane. The light pumiceous ash was deposited under water, and the fine bands show the delicate current structures often seen in silts.

The Lower Tuffs came at a time when the southern part of the lake basin was about to become inundated with coarse clastics; thus in many places they emphasize a realistic dividing plane from the Upper Fish Beds. Evidence of cross bedding and general distribution of coarse clastics suggests that material was carried into the basin from the west in the southern part of the area. The successions in the Barsemoi, Ndaui and Chemeron Rivers thus contain gravels and sands deposited on a fluvial plain, as well as lacustrine silts containing fish remains. While an important pebble bed persists through the Kapthurin River section, and into unexposed country to the north, fine silts and diatomites become more important northwards. Probably the thin diatomaceous silts and diatomites persisting in the extreme north of the outcrop belong with the Upper Fish Beds, so that the lake basin extended to a maximum during this period.

Fish remains are well distributed and reasonably abundant, except in the gravels. Molluscs also lived in the lake, and locally their remains accumulated to form shelly limestones. It is the mammalian fossils which are most interesting, however, and these include a hominid temporal discovered in October 1965 by John Kimengich. The fossil was found weathered out on the surface of an exposure of the Upper Fish Beds at J.M. 85 in a small tributary of the Kapthurin River (Fig. 2). The specimen was initially identified as a hominid fragment by Mr Jonathan Leakey. There is little doubt that the fossil came from the Upper Fish Beds and not from a higher horizon in the Chemeron Beds or from the unconformable veneer of gravel capping the surrounding plateau. The surface of the exposure where the hominid was found is almost a bedding plane slope, and follows the strike very closely for short distances upstream and downstream. The specimen had fragments of lithic grit clinging to it when found, and this sediment matches the lithology of some lenses of calcareous lithic grit distributed through the lowest 16 ft. of the Upper Fish Beds. The most likely source of the hominid fragment is in a very thin grit band about 8 ft. from the base of the Upper Fish Beds.

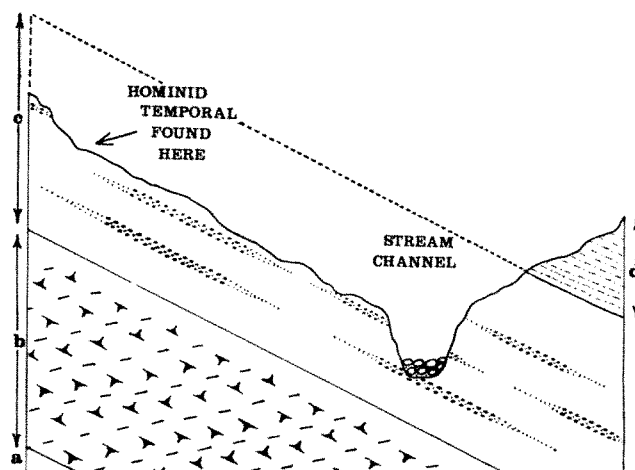


Fig. 2. Cross section of the hominid site, looking northwards. (a) Silts of Lower Fish Beds. (b) Lower Tuffs—coarse and fine waterlain pumice tuffs at a depth of 16 ft. (c) Buff coloured silts with bands and lenses of calcareous, lithic grit, some of which was found adhering to the hominid fragment. Fish remains are abundant in the silts at a depth of 16 ft. (d) Finer silts and light coloured tuffs. Both (c) and (d) belong to the Upper Fish Beds.

Dr Leakey has commented on the age suggested for the beds yielding the hominid fragment by the mammalian fauna obtained so far from the Chemeron Beds. "The lack of good mammalian fauna from the Upper Fish Beds, on the surface of which the fossil hominid temporal was found, precludes direct faunal dating of this deposit at present. It clearly overlies the lower members of the Chemeron Beds which yielded the mammalian fauna of 'early Villafranchian' aspect. It is unconformably overlain by the Kapthurin Beds which contain an advanced Acheulian industry in their upper levels."

Professor P. V. Tobias of the Department of Anatomy, University of the Witwatersrand, Johannesburg, has examined this temporal bone, and has commented on it (succeeding communication).

The Upper Tuffs are widespread, although so far they have yielded no fossils. Stratified and waterlain, they herald the extrusion of the Lake Hannington Phonolites, which terminated lacustrine conditions in the area in question. Earth movements after the Lake Hannington Phonolites had the effect of breaking the rocks up into tilted blocks bounded by closely spaced faults. This has complicated the picture somewhat and has made interpretation of the deposits difficult, because nowhere does one find a straightforward sequence unaffected by faulting.

The earth movements renewed the movements along faults bounding the Kamasia Range, and this led to rejuvenation of erosion followed by the submergence of large tracts of country beneath plateau gravels. The Kapthurin Beds accumulated in a basin, and in doing so buried a large part of the outcrop of the Chemeron Beds. The latter have now been only partly re-exposed by present day rivers.

Examination of the Chemeron Beds, and their possible equivalents elsewhere in the area, has yet to be completed. It is certain that a great deal of fossil material has yet to be discovered from these and other deposits in the Baringo area.

I hold a studentship from the Natural Environment Research Council and am engaged in a research project under the direction of Professor B. C. King. The project is primarily concerned with the geological mapping of an area of some 6,000 square miles in the northern Rift Valley of Kenya and is being undertaken with the co-operation of the Geological Survey of Kenya and in association with the Geology Department of University College, Nairobi. The project is financed jointly by the Ministry of Overseas Development and the Government of the Republic of Kenya. I thank Dr and Mrs L. S. B. Leakey

and Mr R. E. Leakey of Nairobi; Professor P. V. Tobias of the University of Witwatersrand; Professor B. C. King and Dr W. W. Bishop of the Geology Department, Bedford College, for advice, and Mr and Mrs J. H. E. Leakey of Kampi-Ya-Samaki, Kenya; Mr J. Walsh of the Geological Survey of Kenya; Mr L. A. J. Williams of the Geology Department, University College, Nairobi; and Messrs John Kimengich and Malakwen Cheptoo, who located many fossils.

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<sup>1</sup> Gregory, J. W., *The Rift Valleys and Geology of East Africa* (Seeley Service and Co., London, 1921).

<sup>2</sup> Fuchs, V. E., *Geol. Mag.*, **87**, 149 (1950).

<sup>3</sup> McCall, G. J. H., Baker, B. H., and Walsh, J., *Background to Evolution in Africa* (edit. by Bishop, W. W., and Clark, J. D.) (University of Chicago Press, 1967).

THE specimen found by John Martyn comprises most of the right temporal bone and includes a number of diagnostically useful regions, notably the mandibular (or glenoid) fossa, the tympanic bone and external acoustic meatus, part of the mastoid process and the supramastoid crest, the root of the zygoma, and most of the petrous pyramid. Close examination suggests that the specimen should be assigned either to the genus *Australopithecus* or to a lowly hominine taxon, but the exact genus and species cannot be identified on this temporal bone alone.

The mandibular fossa is very long and wide, but absolutely and relatively shallow. The dimensions have been determined using landmarks and definitions given by Weidenreich<sup>1</sup>. The values obtained and the derived indices are given in Table 1, where they are compared with values for other hominoids. I obtained the values for australopithecines and for Old.H13 on the original specimens; the values for hominines and pongids are quoted from Weidenreich (op. cit., Table X, page 46). In absolute length (26.7), the Chemeron mandibular fossa fits within the sample ranges for *Australopithecus* and for *Homo erectus*. The absolute breadth of the Chemeron fossa is somewhat greater than in the australopithecine sample, with the exception of one of the Old.H5 (*Zinjanthropus*) fossae (Fig. 2), and exceeds the upper limit of the *H. erectus* sample by 1.8 mm. The difficulty of determining absolute landmarks for these measurements means that the length and breadth of the Chemeron mandibular fossa cannot be differentiated from those of the available samples of either *Australopithecus* or *H. erectus*; this holds as well for the L/B index.

Absolute depth can be determined with more precision. The sample ranges for *Australopithecus* (7.5–10.0) and for

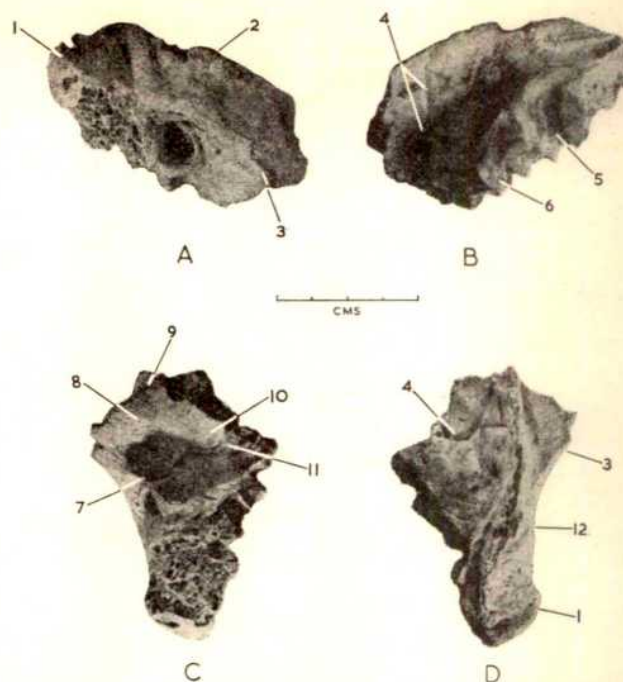


Fig. 1. The Chemeron hominid temporal bone. (A) Lateral aspect; (B) medial aspect; (C) inferior aspect; (D) superior aspect. (1) Mastoid crest; (2) temporal squame; (3) root of zygoma; (4) grooves for middle meningeal artery; (5) sulcus for sigmoid sinus; (6) petrous pyramid; (7) postglenoid process; (8) anterior wall of mandibular fossa; (9) pre-glenoid plane; (10) entoglenoid lip; (11) downturned edge of tegment tympani; (12) auriculare.

*H. erectus* (11.5–18) do not overlap; the fossa in the former hominid is absolutely and relatively shallower<sup>2,3</sup>. The value in Chemeron (10.6) lies between the two quoted sample ranges, but nearer to that for *Australopithecus*. The same is true of the D/L index which distinguishes fairly sharply between the australopithecine and *H. erectus* samples, while the Chemeron D/B index falls within the sample range for *Australopithecus*. The fossae of *H. erectus* and *H. sapiens* differ from those of the australopithecines and pongids principally in their greater absolute and relative depth. Ashton and Zuckerman<sup>4</sup> have shown that the hominine mandibular fossa is usually anteroposteriorly "compressed", that is, deeper and shorter than in pongids. This "compression" of the fossa is not apparent in australopithecines, although in a number of other respects the fossa in this group departs from the pongid pattern<sup>5</sup>. The Chemeron mandibular fossa agrees with those of the australopithecines in not showing such "compression"; but so does that of the Olduvai Bed II hominine represented by the specimen

Table 1. DIMENSIONS AND INDICES OF THE MANDIBULAR FOSSA IN THE CHERMERON TEMPORAL COMPARED WITH THOSE OF OTHER HOMINIDS

	Length	Breadth	Depth	L/B%	D/L%	D/B%
Chemeron (R)	26.7	35.7	10.6	74.8	39.7	29.7
Olduvai Hom 13	18.0	About 22.5	About 8.0	About 80.0	About 44.4	About 35.5
<i>A. africanus</i> (MLD 37/38)	22.3–23.1	29.3–31.6	7.5–8.0	73.1–76.1	32.5–35.9	23.7–27.3
<i>A. robustus</i> (2)	25.2–28.1	31.4–32.4	9.0–10.0	77.8–89.2	35.6–36.0	27.8–31.7
<i>A. boisei</i> (Old.H5)	27.6–27.9	32.9–35.9	8.5–8.8	76.9–84.8	30.8–31.5	23.7–26.7
Total <i>Australopithecus</i> (7 fossae, 4 skulls)	22.3–28.1	29.3–35.9	7.5–10.0	73.1–89.2	30.8–36.0	23.7–31.7
<i>H. erectus pekinensis</i> (5 fossae, 4 skulls)	16–21	23–27	11.5–15.0	72.0–78.3	63.8–83.2	46.0–65.2
<i>H. erectus erectus</i> (2 skulls)	28	23–28	13–18	100–123	46.4–64.3	56.5–64.3
Old.H9	22	34.1	13	64.5	59.1	38.1
Total <i>H. erectus</i> (7 skulls)	16–28	23–34.1	11.5–18	64.5–123	46.4–83.2	38.1–65.2
<i>H. sapiens</i> *	23.0–27.0	21.5–26.0	12.5–16.5	88.4–109.5	53.2–69.5	58.2–63.4
Gorilla (male)†	27	46	10	58.7	37.1	22.1
Chimpanzee (male)†	25	29	7	86.3	27.9	24.1
Orang-utan (male)†	18	40	9	45.0	50.0	22.5

\* Ranges of population means.

† Sample means.



officially catalogued as Old.H13 (ref. 4), and included originally as a paratype of *H. habilis*<sup>5</sup>. The teeth and jaws of Old.H13 were recognized later to be more hominine in character than those of the Bed I type specimen of *H. habilis*<sup>6</sup> and some people have "allocated" Old.H13 as a whole to *H. erectus*. While not yet committing myself on the final taxonomic designation of the Old.H13, it seems to be commonly accepted that it is a hominine and not an australopithecine. But it does not show as marked a degree of anteroposterior "compression" of the fossa as in *H. erectus* and other hominines (Table 1). Thus its D/L and D/B indices (44.4 and 35.5) fall in between the sample ranges for *Australopithecus* and *H. erectus* and exceed those of Chemeron (39.7 and 29.7) by only 4.7 and 5.8 respectively. Thus although in absolute size the fossa of the adolescent female Old.H13 is smaller, it indicates that—on the depth of the fossa—we cannot exclude the possibility that the Chemeron temporal belonged to a lowly hominine.

The anterior wall of the mandibular fossa (Fig. 1C) has a threefold curvature typical of australopithecines and some early hominines: it is strongly concave mediolaterally, it slopes downwards and forwards at an angle of about 45° to the estimated plane of the Frankfurt horizontal, and it slopes from anterolateral to posteromedial. The postglenoid process is small, compressed and uninflated, and it furnishes a very small part of the posterior articular surface of the glenoid fossa (Fig. 2). Most of the posterior articular surface is formed by the nearly vertical anterior face of the tympanic, as in most australopithecines, Old.H13 and hominines, but unlike the arrangement in pongids. The medial boundary (Fig. 1C) is not a distinct entoglenoid process as in *A. boisei*, but simply the downturned medial margin of the squamosal, as in some australopithecines, and Old.H13. The sphenosquamosal suture is not preserved and it is therefore impossible to see whether the alisphenoid contributes to the entoglenoid wall as in *A. africanus* and Old.H13. Thus the overall features of the mandibular fossa have much in common with those of the australopithecines, as well as those of some lowly hominines, and do not therefore help in the determination of the exact taxonomic affinities of the specimen.

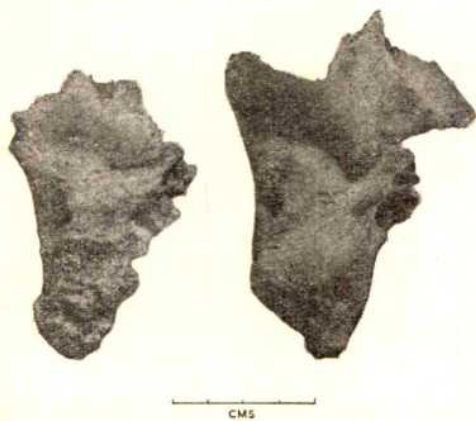


Fig. 2. The Chemeron temporal compared with that of *Australopithecus (Zinjanthropus) boisei*. Note the delicate entoglenoid lip of Chemeron, contrasting with the bulbous entoglenoid process of *A. boisei*; contrast, too, the compressed postglenoid process of Chemeron with the somewhat inflated process of *A. boisei*.

The external acoustic porus is large (12.5 × 10.0 mm), circular to oval with its long axis almost vertical, a thin tympanic rim thickened below and behind with a small notch between the two thickenings (Fig. 1A). There is a distinct suprimeatal spine. These features of the porus are clearly hominid rather than pongid, and closely resemble those of the original Kromdraai *A. robustus* specimen<sup>7</sup>. The plane of the porus is set almost flush

with the auricular point, as in Kromdraai, skull 8 from Sterkfontein<sup>7,8</sup>, and Old.H13, and there is little projection of the posterior root of the zygoma above the ear-hole. In this respect, Chemeron contrasts strongly with *A. boisei* and *H. erectus* with their massive shelf-like projections<sup>2</sup>, and resembles more closely *A. africanus*.

Although it was partly broken, enough of the mastoid process was preserved to indicate that it projected strongly on the side of the base of the skull (Fig. 1C and D), as in australopithecines, far exceeding in degree the projection on *H. erectus* crania, even including the massive cranium from LLK Olduvai (Old.H9, formerly called "Chellean Man").

The petrous part (Fig. 1D) has a nearly vertical posterior face and detailed morphology as in hominids<sup>9</sup>. The superior margin is a sharp edge which partly overlaps the posterior surface, as in Old.H5 (*A. boisei*), Sts 5 (*A. africanus*) and modern man; it contrasts with the blunt, rounded border in *H. erectus* including Old.H9.

The root of the zygomatic process (Fig. 1D) is very broad (about 34 mm long), slopes fairly steeply downwards towards the preglenoid plane and has a faint hollow, the sulcus processus zygomatici, on its antero-superior surface. It resembles the corresponding feature in both *Australopithecus* and *H. erectus*.

Although the supramastoid crest is interrupted by small areas of damage and by marked vascular grooves (presumably for the middle temporal artery and perhaps the deep temporal arteries), it is clearly a poorly developed feature, resulting in a marked hollow on the side of the cranium between the mastoid process behind the zygoma in front (Fig. 1D). This feature reproduces the structure found in *A. africanus* and in modern man.

The broken mastoid process and upper edge of the squame indicate a considerable degree of pneumatization, as in all early hominid crania, but not as marked as in the very heavily pneumatized cranium of *A. boisei*, nor probably as in the crania of *A. robustus* and *H. erectus*<sup>3</sup>.

Most of the features of the Chemeron temporal suggest that it belonged to an australopithecine. The only feature suggesting an affinity with *A. boisei* is the large size of the mandibular fossa; in other respects, the Chemeron specimen resembles more closely *A. robustus* and *A. africanus*. A good proportion of its traits, however, are compatible with lowly hominine status, some suggesting a closer affinity with an evolved *H. habilis* (Old.H13), others with *H. erectus*. On the evidence of this temporal bone alone, it is impossible to assign the specimen firmly to either of the most widely recognized Pleistocene genera of the Hominidae, *Australopithecus* or *Homo*. Until more material is recovered, the form represented by the temporal is identified as follows:

Family: Hominidae  
Gen. et spec. indet.

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# Mechanism of Biological Memory

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General mechanism for brain memory and immunological memory is proposed. Immunological memory could involve the transfer of information for the synthesis of the active site of an antibody, by an antigen-RNA complex, to a potentially immune cell. This transfer would involve the reversal of the path of information from DNA to RNA to protein which occurs during protein synthesis.

THERE are known to be three types of biological memory: (a) genetic memory, the discovery and unravelling of which has been due to molecular biology; (b) conventional memory, which is a function of the brain; and (c) immunological memory. In spite of the apparent differences between these types of memory they probably have much in common, and presumably a single mechanism is responsible for the working of all three. Any signal received by the nervous system from external media for a time sufficient for the brain to memorize it is thought to be converted to structural change<sup>1-7</sup>. This means that the brain memory, like genetic and immunological memory, is dependent on the formation of tangible structures which are responsible for the existence and content of the memory.

There is disagreement when an attempt is made to determine the position, chemical nature, and initiating and fixing mechanism of the suggested structural changes<sup>1-7</sup>. It is interesting that, as with immunological memory, two contrasting points of view have been voiced to explain the essence of brain memory. The first suggests that the sum of information which is finally fixed by the brain is contained in the body itself, for example in DNA, and that the signal only serves to select the information<sup>6,7</sup>. This is in principle analogous to the theories of immunological memory of Burnet<sup>8</sup> and Szilard<sup>9</sup>. The second point of view is based on the assumption that the role of the signal is not selection but formation of a particular structure<sup>5,10</sup>, which coincides in principle with the theories of immunological memory put forward by Haurowitz<sup>11,12</sup> and Pauling<sup>13</sup>. But there may be a third point of view which is to a certain extent a product of the two previous ones<sup>6-13</sup>, although they appear to be mutually exclusive. This third approach permits an explanation of the general nature of all types of biological memory.

A signal is fixed by the appropriate receptor and is transmitted from neurone to neurone by means of a certain chemical mediator. This substance reacts with the neuronal membrane, that is, with a certain protein causing a chain of reactions which lead to a certain action. It is, however, established that the reaction of the small molecule with the protein can cause a change in the conformation of the protein. This takes place, for example, during the interaction of oxygen or carbon monoxide with haemoglobin, when the change in conformation is so evident that it is recorded both by changes in the crystal formation of the protein and by X-ray photography<sup>14</sup>. I assume that the structural change obtaining while an event is being memorized is the result of a change in protein conformation. Although the number of proteins which the body can synthesize as a result of the genetic information contained in its DNA is large, it is, nevertheless, limited and is smaller than the enormous stream of information which the organism is capable of receiving and memorizing. Accepting this assumption I conclude that one of the brain's memory links is the emergence of a protein with a new conformation, formerly not peculiar

to the organism in question. In immunological terminology this newly emerged information can be described as a new antigenic structure of the particular protein's surface. This new antigenic structure has obviously emerged as a result of a certain signal to which the organism reacted in a definite manner. This means that the presence of the particular protein molecule with a particular item of information predetermines the possibility of a specific response if the information is preserved in time, that is, memorized by the organism. It is therefore essential to determine how the organism retains the information of a particular protein conformation for a prolonged period of time. It is well known that proteins, including the structural proteins of the organism, disintegrate rather rapidly within the organism. The shortest half-life of the total brain protein is 2.2 days<sup>15</sup>, and the appearance of the new protein conformation in response to the signal is not reflected in the genetic code of phylogenetic origin. It is necessary to determine how the organism can memorize the information of the new antigenic structure, a task which is analogous to the problem of immunological memory.

The subject of immunological memory has been elaborated much more theoretically and experimentally than has that of memory, which is a function of the brain. It is, however, far from fully understood. The mechanism of immunological memory as it is known consists of the biosynthesis of antibodies and the formation of immune lymphocytes. It is impossible here to discuss all aspects of the classical theories<sup>16</sup>, but I must point out that the principal difficulty of the instructive theory is its inability to explain the complexity of immunological phenomena which result from the functions of the immune lymphocytes; the theory also involves a biosynthesis of antibodies which runs counter to all modern conceptions of the biosynthesis of proteins. A weakness of the clonal selection and mutation-genetic theories is their inability to explain the organism's ability to produce antibodies for the synthetic antigenic determinants, and the inability of haptens, which bind with the active centre of the antibody like the substrate with the enzyme, to induce biosynthesis of antibodies.

I hope that my hypothesis will make it possible to solve the difficulties referred to here in connexion with the immunological memory theory, and serve to describe a process which explains the mechanism of biological memory as a whole. For the sake of simplicity I first examine how this hypothesis explains the formation of an antibody active site in response to an antigen introduced into the body. If a foreign protein is introduced into the body, the response is a quantity of antibodies, all of which react with their specific antigenic determinants. The antigenic determinant is either a section of polypeptide consisting of six to ten amino-acid residues, or a section of polysaccharide consisting of approximately six monose residues, or of a similar but more complex structure consisting of amino-acid residues, sugars and lipids<sup>17</sup>.



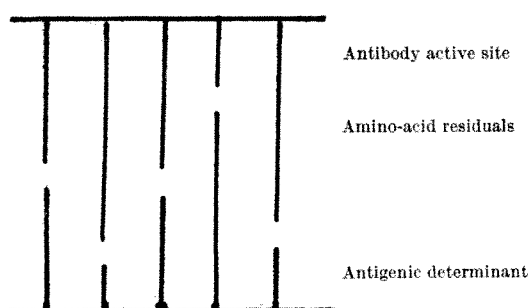


Fig. 1. A schematic representation of the antigenic determinant and the antibody active site, and the dislocation of their functional groups in the antigen-antibody complex.

The antigenic determinant can thus be schematically conceived as a chain of linearly arranged recurring bonds, such as peptide bonds, perpendicular to which are the side groups of, for example, carbon chains of amino-acid residues, which as a whole determine the specificity of the particular antigenic determinant (Fig. 1).

An analogous scheme may describe the structure of the antibody active site, which is also a section of a polypeptide chain. The link between the antigen and its respective antibody and its unusual strength is explained by their conformity to one another, that is, their complementarity to one another. This is shown schematically in Fig. 1, where the residues of amino-acids of the antigenic determinant peptide chain complement the amino-acid residues of the peptide chain of the antibody active site. This conformity probably permits the binding of a given number of chemical groups of the antigenic determinant (the van der Waals bonds, the hydrogen bonds, the hydrophobic and heteropolar bonds) which Pauling considers to be the basis of the exceptional strength of the bond which results from the antigen-antibody reaction. This is known to be a purely physico-chemical process and is subordinated to the law of mass action. The latter is confirmed experimentally by hapten inhibition of the antigen-antibody reaction<sup>17</sup>.

Consider a situation in which the biological mechanism of protein synthesis is unknown, and the antibody active site to a given antigenic determinant is to be synthesized by physico-chemical means. The active site of the antibody is known to consist of amino-acid residues, but the identity of the amino-acids which should be taken to build a peptide chain with the properties of the antibody active site to the given antigenic determinant is not known. There is, however, a single set of amino-acids which is determined by the structure of the antigenic determinant itself, which can therefore make the selection itself. Thus a single antigenic determinant can be introduced into the solution; this adds to the solution the twenty amino-acids which constitute all proteins. Like the antigen-antibody and the hapten-antibody reactions, this particular synthetic system should obey the law of mass action<sup>18</sup>, which means that some of the twenty amino-acids added may spontaneously orientate themselves around the antigenic determinant. These will be those amino-acids which can ensure a sum bond of maximum firmness, that is, those which store the most energy (Fig. 2). The rest of the amino-acids will remain in solution.

This unfortunately is as far as an attempt to synthesize the active site of the antibody can be taken, for it is well known that peptide bonds are not formed spontaneously. Nevertheless, in the normal conditions of life, during the biosynthesis of the protein, it is not free amino-acids which are employed in the cell but their complexes with the appropriate transport RNA. It will not therefore be amino-acids, complementary to the antigenic determinant, which orientate themselves around the latter, but it will be the amino-acids complexed with their respective

tRNA, similar to the situation in Fig. 2. The chief significance of this scheme is the fact that the structure of the antigenic determinant is thus translated into the language of a nucleotide sequence, based on the inherent ability of every tRNA to link up only with a definite amino-acid. But this kind of structure, although it bears the information recorded in the nucleotide code, necessary for the synthesis of the active site of the antibody, is unstable and can hardly serve as a basis for a consecutive synthesis of the active site of the antibody. There must be a process which stabilizes the emerging sequence of nucleotides.

The second part of my hypothesis of the mechanism of the biological memory is the assumption that this process consists of the synthesis of mRNA on a template of orientated tRNA from which appears a stabilized polynucleotide structure. This means that in nature information is transmitted not only in the direction of DNA to RNA to protein, but also in the opposite direction, that is, protein to RNA to DNA. It has been shown experimentally that half of the postulated information transmission is in the direction from protein to DNA<sup>19,20</sup>.

The process described here is a hypothetical scheme for the translation of information contained in the protein into the language of the nucleotide sequence, but it does not describe the manner in which the organism synthesizes antibodies from the various fragments of peptide chains<sup>21</sup>, nor the manner of the formation of immune lymphocytes. I have discussed here only the synthesis of the active site of the antibody the structural dependence of which on DNA has not yet been shown<sup>21</sup>, unlike other peptide fragments from which antibodies are synthesized by some unknown mechanism. The particular mechanism behind this process constitutes the third part of the hypothesis of biological memory, the substance of which is as follows.

The three layered structure shown in Fig. 2, consisting of an antigenic determinant, the antibody active site, and a corresponding mRNA, has a kind of lineal dimension. For clarity and simplicity of discussion this ribbon-like arrangement can be wound around the position of the mRNA. The result will be a ball, as shown in Fig. 3. It is easy to see that this ball is designed like a virus, with mRNA in the centre bearing the genetic information necessary for the synthesis of one of its membranes, in this case analogous to the complex of antibody active sites.

When such a "virus" penetrates a sensitive cell, as was suggested<sup>22</sup>, and later confirmed experimentally<sup>23</sup>, the viruses of the second and fourth groups of the classified types of virus-cell interaction<sup>22,23</sup> are capable of trans-

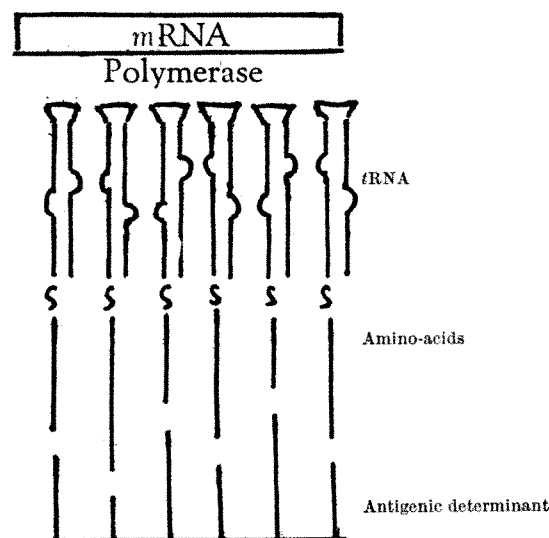


Fig. 2. A schematic representation of the orientation of amino-acid and tRNA complexes, and mRNA synthesis on tRNA-template.

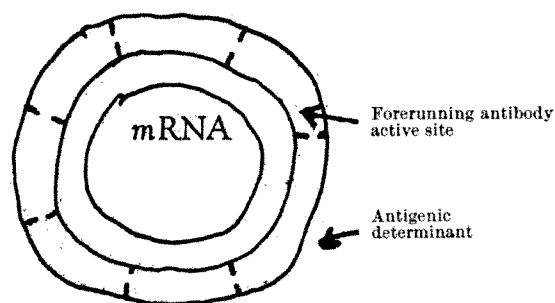


Fig. 3. A schematic representation of the virus-like particle carrying information (*mRNA*) for the synthesis of the antibody active site and transmitting it from the macrophage to the immuno-competent cell.

forming the cell membrane into an immunological structure identical to that of the virus, that is, genetically determined by the virus RNA. In this case if the "virus" described here penetrates the cell which will eventually become a lymphocyte, it can, in accordance with the second type of virus-cell interaction, convert the lymphocyte cell membrane into a structure, genetically determined by the *mRNA* of our "virus", that is, into a structure equal to the active site of the antibody. This signifies that the lymphocyte will become immune with respect to the antigen, which evoked the formation of the *mRNA* of the "virus". The lymphocyte thus transformed will simultaneously be able to synthesize a certain amount of humoral antibodies consisting of "virus" and fragments determined by cell DNA, just as when, for example, during the replication of the influenza virus, capsides of the virus membrane synthesized on the cell ribosomes are determined by the influenza virus RNA as well as by the cell DNA<sup>22,24</sup>. Should such a virus, on the other hand, enter a cell which will later become plasmatic with a ramified ribosome network, then the process will be a synthesis of humoral antibodies, like the synthesis of the influenza virus membrane capsid on the cell ribosomes.

It should be emphasized here that according to my hypothesis the antibody consists of the principal part of the molecule, the structure of which is determined by the DNA of the organism and the active site, the structure of which is determined by the antigen. It is because of this structural and genetic heterogeneity, and in spite of the suggested mechanism of reversed transmission of information, that the immunization of, say, mice with rabbit antibodies to albumin does not evoke the formation of a whole molecule of albumin. It evokes instead only the biosynthesis of a composite group of mice antibodies, each one of which may be the bearer of a locus peculiar to the initial albumin. Conversely, when the reversal of the transmission of information in the neurone is initiated by the new antigenic structure, a product of the mediator, the formation of an active site of the "antibody" should be followed by the formation of a structure complementary to the "active site", resulting in the formation of a double membrane. These double membranes of the cell are therefore by my hypothesis complementary structures, as if constituting continuous rows of antigenic determinants and antibody active sites, forming an internal and external surface of the cell membrane respectively.

It is, however, necessary to explain the conditions in which the entry of the protein into the cell will evoke a reversal of transmission of information and when it will not. The fourth part of my hypothesis of the mechanism of biological memory is the assumption that the transmission of information is reversed when a protein is produced in or penetrates into the cell without the appropriate *mRNA*. The entry of protein is blocked if the *mRNA* is present. If it is a foreign protein and there is therefore no appropriate *mRNA* in the cell, reversal commences.

It must be pointed out that the mechanism of immunological memory described here agrees with the latest

experimental data concerning antibody biosynthesis. This has been shown to occur in two stages. After the macrophage has consumed the antigen a complex of antigen and RNA (according to this scheme corresponding to stage 1 in Fig. 4) appears in the macrophage. This complex is eventually ejected from the macrophage and bears the information necessary for the synthesis of the active site of the antibody in another cell, say, one which is turning plasmatic, and in which the antibody will then be synthesized (the second stage in this scheme, Fig. 4) (refs. 25-30). It has been proved further that the surface of the immune lymphocyte actually reacts to a corresponding antigen just as the cell membrane of a cell infected with the influenza virus undergoes a virus-like transformation of its structure, and begins to react with the red blood cells in the haemo-absorption reaction in a manner similar to the reaction in which the virus agglutinates the red blood cells.

This mechanism of biological memory also agrees with available experimental data, scarce as it is, concerning the processes taking place in the brain during memorization. Part of my hypothesis supports the conceptions of Hyden<sup>5,10</sup> which are based on the accumulation of RNA in the functioning neurones<sup>5</sup>, which he discovered. My mechanism of biological memory also facilitates understanding of why the injection of actinomycin *D* interferes with the formation of a long-time memory, which is, according to my hypothesis, the consequence of a synthesis of appropriate *mRNA*. This synthesis makes permanent the reversal changes of the membrane structure, and does not interfere with the formation of a short-term memory, which from the point of view of my hypothesis is equal to a reversal of conformation of the protein structures of the neurone membrane<sup>7</sup>.

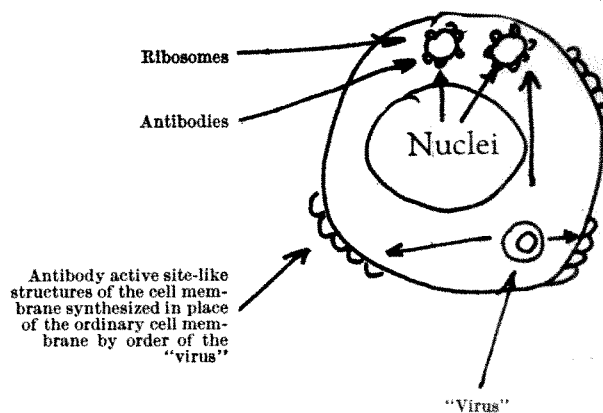


Fig. 4. A schematic representation of the transformation of the intact immuno-competent cell in the immuno-lymphocyte.

It is also made clear why the anatomy of the brain, unlike that of any other organ, precludes the entry of brain antigens into the blood and further into the lymphoid system<sup>31</sup>. This anatomy and functional property of the brain is essential to my hypothesis, for otherwise the newly formed antigens on the surface of the neurone would pass into the blood during the disintegration of the membrane and would cause autoimmune diseases. Evidence of the presence of DNA in the cytoplasmatic membrane, and of the autonomous synthesis of the cytoplasmatic membrane from the DNA nucleus<sup>32</sup>, is essential to my hypothesis. It makes possible an understanding of the dual nature of all biological membranes, and emphasizes the universality of the complementary principle, that is, it confirms its validity not only with regard to nucleic acid but to the protein structures of the membrane type as well. The complementary nature of the antibody active site to the antigenic determinant is only an individual case, a local reproduction of a pheno-

menon which is characteristic of the double membrane in its entirety.

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## Spermadsorption and Spermagglutination by Mycoplasmas

by

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Adsorption of bovine and human spermatozoa to colonies of mycoplasmas has been demonstrated, as well as spermagglutination by an avian mycoplasma. These phenomena show some similarity to haemadsorption and haemagglutination by mycoplasmas.

CHU<sup>1</sup> observed that bovine and rooster spermatozoa were adsorbed to tissue culture cells infected with influenza and Newcastle disease viruses and, furthermore, that the viruses agglutinated the spermatozoa. Peleg and Ianculescu have made similar observations<sup>2</sup>: adsorption and agglutination of spermatozoa closely paralleled the phenomenon observed with erythrocytes and spermagglutination and haemagglutination titres were similar. The adsorption and adherence of erythrocytes to the surface of *Mycoplasma pneumoniae* colonies on solid agar medium were first observed by Del Guidice and Pavia<sup>3</sup>. Haemagglutination by a mycoplasma was first demonstrated by Van Herick and Eaton<sup>4</sup> and is a prominent feature of mycoplasmas isolated from birds, although it and haemadsorption both occur with certain strains of mycoplasmas isolated from several animal species<sup>5</sup>. It seemed likely that spermatozoa could adsorb to mycoplasma colonies or be agglutinated by mycoplasmas, and we have shown that this is so in experiments reported here.

Our experimental details for spermadsorption are outlined in Fig. 1. Samples of semen from one bull and two humans were used. Motility was assessed after diluting semen in phosphate buffer-saline (PBS) at 37° C so that individual spermatozoa could be observed. The proportion of motile spermatozoa was obtained by comparing the count of motile spermatozoa with the total count of non-motile spermatozoa after the addition of a drop of mercuric chloride. Viability was assessed by mixing one part of semen with ten parts of nigrosine-eosin stain, holding the suspension at room temperature for 5 min and then making a slide smear. The proportion of viable spermatozoa was obtained by comparing the number of unstained spermatozoa with the total of unstained and stained spermatozoa. The preparation and storage of erythrocyte suspensions have been described in detail previously<sup>6</sup>. For spermagglutination and haemagglutination tests, *M.*

*gallisepticum* (A514) adherent to the surface of 'Pyrex' bottles was used as antigen. The adherent sheet was washed three times with PBS, pH 7.2, scraped into a small volume of PBS, dispersed by pipetting and frozen at -20° C until used.

Haemadsorption is a striking property of *M. pneumoniae* and *M. gallisepticum* colonies on agar, and so we carried out preliminary spermadsorption experiments with these mycoplasmas. Suspensions of human and bovine spermatozoa were added in 2.0 ml. quantities to colonies which had recently developed and these cultures were incubated at 37° C for up to 30 min. After this time the heads of many spermatozoa had adhered to the surface and edge of the colonies (Fig. 2). The tails of spermatozoa whose heads were adherent to the edge of a colony were the most easily observed because they lay radially to the colony and moved rhythmically and rapidly over translucent agar. The adherence of both bovine and human spermatozoa was easily seen, although the use of bovine spermatozoa was an advantage in this respect because of their larger size. Spermatozoa suspended in PBS, pH 7.2, adsorbed just as well as those in phosphate buffer, pH 6.0, and the former diluent was usually used. Unwashed human spermatozoa adsorbed just as well as washed spermatozoa, and this was also true for bovine spermatozoa with semen diluted twenty-fold, except in one instance when washed spermatozoa adsorbed more rapidly and extensively.

In a previous study<sup>6</sup> adsorption of erythrocytes to colonies of various mycoplasmas did not occur when colonies were crowded on the agar surface. The possible occurrence of this phenomenon with spermatozoa was investigated. Ten-fold serial dilutions of a suspension of *M. pneumoniae* were inoculated on agar so that a decreasing number of colonies from approximately 10<sup>6</sup> to 10 developed on incubation. Human spermatozoa were added to the series of agar cultures and, for comparison, human group

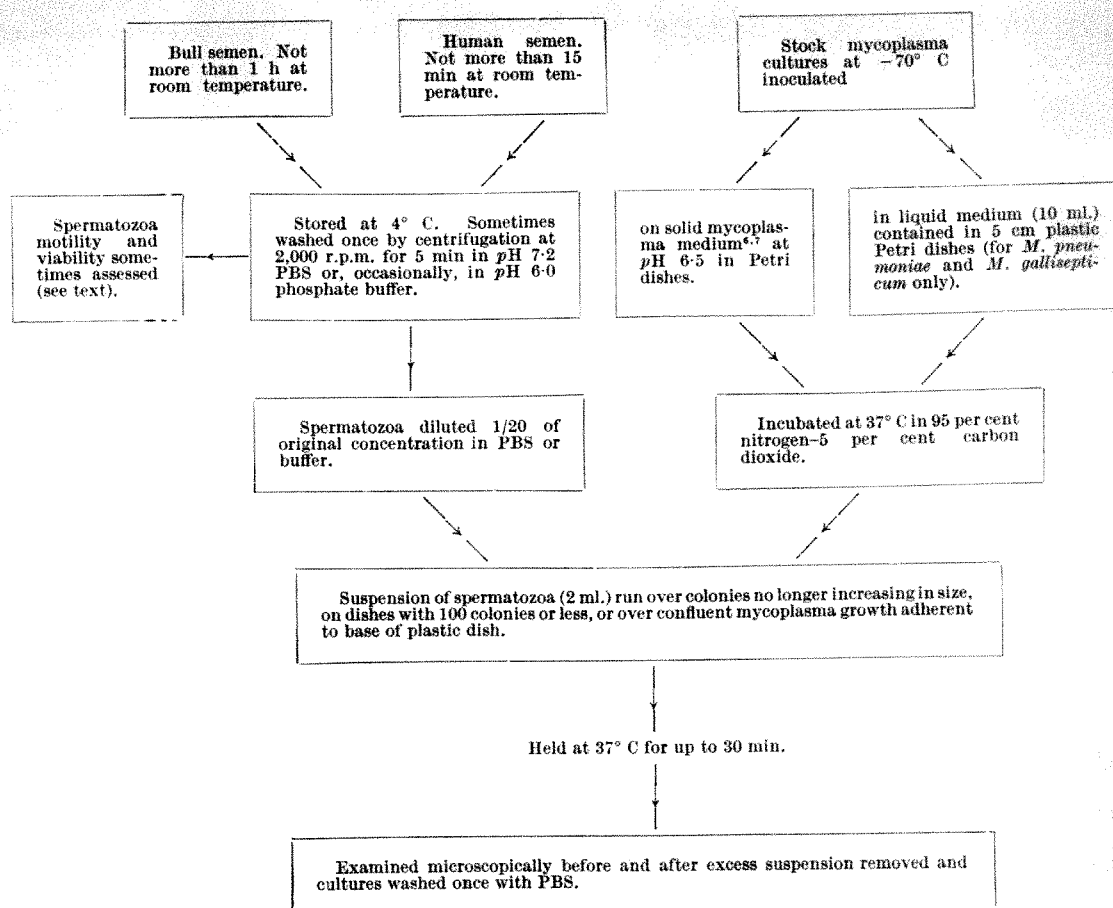


Fig. 1.

O erythrocytes to duplicate cultures. When ten colonies only were present, spermatozoa covered them completely; with increasing numbers of colonies, the extent to which the colony surfaces were covered with adherent spermatozoa diminished, so that no spermadsorption was observed with  $10^6$  colonies. A similar pattern was observed for haemadsorption. In further experiments, care was taken to use agar cultures on which there were no more than 100 colonies.

The effect of temperature on the speed of spermadsorption was then investigated. Dishes of agar medium, each with not more than 100 colonies of *M. pneumoniae* (FH strain), were incubated at 37° C, 22° C and 4° C with a suspension of bovine spermatozoa collected 4 h before. At various time intervals thereafter the extent of spermadsorption was recorded and the results are shown in Table 1. At 37° C adsorption was more rapid and colonies were more completely covered with spermatozoa than at 22° C. Thus adsorption after 50 min at 37° C was more extensive than after 5.5 h at 22° C. Furthermore, at 4° C spermadsorption was extremely poor, for colonies observed after 5.5 h had only one or two spermatozoa adherent to some of them; spermadsorption occurred, however, when the temperature of culture was changed to 37° C. In a similar experiment with colonies of *M. gallisepticum* (S6 strain), and human spermatozoa used within 30 min of collection,

spermadsorption occurred most rapidly and extensively at 37° C, although in this case extensive spermadsorption occurred also at 4° C after 2.5 h. In all further experiments colonies of mycoplasma and spermatozoa were incubated at 37° C.

The relationship between motility and viability of spermatozoa and their adsorption to colonies was investigated. Human semen was diluted twenty-fold in PBS, pH 7.2, and samples were held at 56° C, 36° C and 4° C. At various times the proportions of motile and viable spermatozoa in the suspensions were estimated and adsorption to colonies of *M. pneumoniae* (FH) and *M. gallisepticum* (S6) were observed after incubation at 37° C for 30 min. The results are given in Table 2. Twenty minutes after collection 90 per cent of the spermatozoa were actively motile and viable and spermadsorption was rapid and complete. Spermatozoa in suspensions incubated at 37° C for 9.5 h or at 56° C for 5 min were non-motile, but about half the spermatozoa remained viable and spermadsorption occurred, although less rapidly and less extensively than with an untreated spermatozoa suspension. Spermatozoa in suspensions subjected to a temperature of 56° C for 5.5 h were non-viable and adsorbed poorly. Subsequent experiments were successfully performed with semen which had been kept at 4° C after arrival at the laboratory and then used within 6 h.

Bovine spermatozoa after storage in liquid nitrogen also adsorbed, and the rate of attachment was only a little slower than that observed with a suspension of unstored spermatozoa and could be accounted for by the greater proportion of non-viable spermatozoa in the suspension that had been frozen (20 per cent compared with 10 per cent in the unfrozen suspension).

Adsorption could be demonstrated best with fresh spermatozoa at 37° C, and so further experiments were performed to determine how rapidly the phenomenon

Table 1. EFFECT OF TIME AND TEMPERATURE ON THE ADSORPTION OF BOVINE SPERMATOZOA TO COLONIES OF *M. pneumoniae*

Time after addition of spermatozoa	Spermadsorption at temperature indicated		
	37° C	22° C	4° C
30 min	+	±	—
50 min	++	±	—
1 h	++	+	—
2.5 h	++	+	—
5.5 h	++	+	±

±, 1 or 2 spermatozoa attached to edge of colony; ±, 5-25 per cent of colony surface covered by spermatozoa; +, 25-50 per cent; ++, 50-75 per cent.



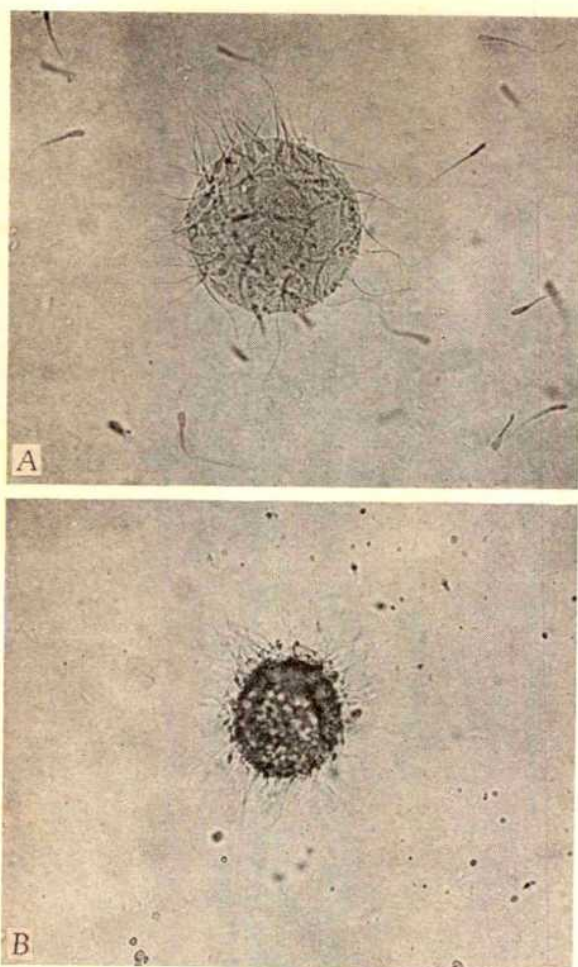


Fig. 2. Adsorption of spermatozoa to colonies of the FH strain of *M. pneumoniae* ( $\times c. 175$ ). (A) Bovine spermatozoa after incubation at 37°C for 15 min. (B) Human spermatozoa after incubation at 37°C for 30 min.

occurred. Adsorption of human spermatozoa to colonies of *M. pneumoniae* and *M. gallisepticum* commenced as soon as the suspension, pre-warmed to 37°C, was added to the agar, also at 37°C. Likewise, guinea-pig erythrocytes adsorbed to colonies almost immediately.

Spermatozoa adsorbed strongly to mycoplasma colonies, even at 22°C and 4°C. Those which adsorbed to colonies of *M. pneumoniae* and *M. gallisepticum* at 37°C did not detach spontaneously even after 2 or 3 days at this temperature. It appeared that the spermatozoa merged with the colony surface as though autolysis had occurred, although this has not been examined in detail. It was sometimes possible, by repeated agitation of the agar culture, to detach the tails of spermatozoa while leaving the heads firmly attached to the colonies. Complete removal of spermatozoa could be achieved by extremely vigorous washing, that is by forcibly pipetting 10 ml. of

Table 2. EFFECT OF THERMAL TREATMENT OF HUMAN SPERMATOZOA ON THEIR ABILITY TO ADSORB TO COLONIES OF *M. pneumoniae* AND *M. gallisepticum*

Thermal treatment Time (h and min)	Temp. (°C)	Condition of spermatozoa after thermal treatment		Spermadsorption* to colonies of	
		Motility (per cent)	Viability (per cent)	<i>M. pneu- moniae</i> (FH)	<i>M. gallisepti- cum</i> (S6)
—	20	4	90	100†	100†
9	30	37	0	57	NT
—	5	56	0	46	50
46	—	4	1	30	25-50
5	30	56	0	10	1‡
46	—	37	0	1‡	NT

\* Area (per cent) of colonies covered by adherent spermatozoa after 30 min at 37°C.

† Spermadsorption after 10 min at 37°C.

‡ One or two spermatozoa attached to edge of colonies.

PBS directly at a colony. This procedure, however, sometimes removed the surface of the colony from the agar before detaching the spermatozoa. If detachment was successful, spermadsorption occurred again when more spermatozoa were added.

Colonies of several haemadsorbing and non-haemadsorbing mycoplasmas were examined for spermadsorption. The results are shown in Table 3. Five mycoplasmas which exhibited haemadsorption were examined. Both human and bovine spermatozoa adsorbed to colonies of *M. pneumoniae* and *M. gallisepticum* (Fig. 3), as described previously, and also to colonies of *M. pulmonis*; only bovine spermatozoa adsorbed to colonies of *M. bovis genitalium*, while neither human nor bovine spermatozoa adsorbed to colonies of *M. agalactiae*. Colonies of *M. fermentans* and *M. hominis* type 1, mycoplasmas found in the human genital tract, neither spermadsorbed nor haemadsorbed.

In addition to adsorption of spermatozoa to colonies on agar medium, the adsorption of human spermatozoa to sheets of confluent growth of *M. pneumoniae* (FH) and *M. gallisepticum* (A514) adherent to plastic Petri dishes was demonstrated. Haemadsorption was shown also. Adsorption in these circumstances was surprising, for crowding of colonies on agar medium inhibited adsorption.

Table 3. SPERMADSORPTION AND HAEMADSORPTION WITH MYCOPLASMAS BELONGING TO DIFFERENT SEROTYPES

Mycoplasma Designation	Source	Spermadsorption with spermatozoa from		Haem- adsorption*
		Man	Bull	
<i>M. pneumoniae</i> (FH)	Man	+	+	+
<i>M. gallisepticum</i> (S6)	Fowl	+	+	+
(A514)				
<i>M. pulmonis</i> (PG22)	Mouse	+	+	+
<i>M. bovis genitalium</i> (PG11)	Cattle	—	+	+
<i>M. agalactiae</i> (PG2)	Goat	—	—	+
<i>M. fermentans</i> (PG18)	Man	—	—	—
<i>M. hominis</i> 1 (PG21)	Man	—	—	—

+, Adsorption demonstrated.

\* Adsorption with erythrocytes from man, fowl, mouse, cattle, goat and guinea-pig demonstrated with each mycoplasma.

Haemadsorption can be inhibited by specific antiserum, and therefore inhibition of adsorption of human spermatozoa to *M. pneumoniae* colonies was attempted with goat antiserum. Two-fold antiserum dilutions in 20 per cent unheated guinea-pig serum were added to the colonies, and these were kept at room temperature for 30 min. Then a suspension of spermatozoa was added and the colonies were incubated at 37°C for 1 h. Pre-inoculation goat serum at a dilution of 1:5 did not inhibit spermadsorption, but a 1:40 dilution of antiserum completely inhibited spermadsorption. The same antiserum inhibited haemadsorption also at a dilution of 1:40 and inhibited the colour change in a metabolic-inhibition test<sup>8</sup> at a dilution of 1:320. Neither haemadsorption nor spermadsorption was inhibited in the absence of unheated guinea-pig serum.

It was difficult to demonstrate spermagglutination with bovine spermatozoa even when washed spermatozoa were used. Several successful experiments were, however, performed with both washed and unwashed human spermatozoa and *M. gallisepticum* (A514) antigens. Human semen diluted twenty-fold in PBS produced an approximate 1 per cent suspension of spermatozoa. Erythrocytes were used at the same concentration. An equal volume of the antigen dilution was mixed with an equal volume of spermatozoa or erythrocyte suspension, the mixture agitated and then incubated at 37°C until the spermatozoa or erythrocytes had settled. Agglutinated spermatozoa produced a sheet covering the well, whereas unagglutinated spermatozoa formed a tight "button", similar to that observed with erythrocytes. This pattern was observed with both motile and non-motile spermatozoa and spermagglutination titres were similar with motile and non-motile spermatozoa. Spermagglutination titres were the same or only twice as small as haemagglutination titres obtained with either chicken or human erythrocytes. The results of comparative spermagglutina-



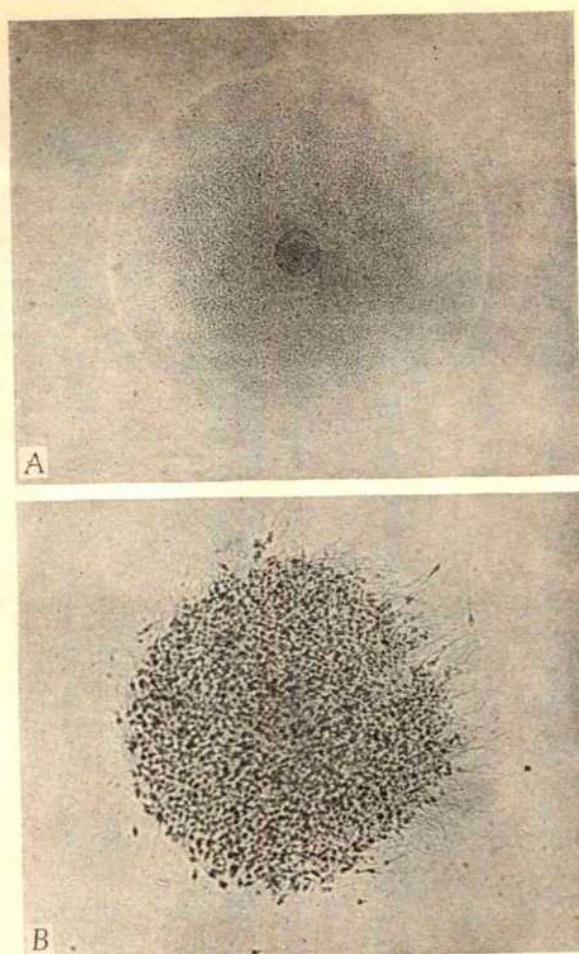


Fig. 3. Adsorption of human spermatozoa to a colony of the A514 strain of *M. gallisepticum*. (A) A colony before addition of spermatozoa. (B) A colony after incubation at 37° C for 10 min with spermatozoa ( $\times$  c.175).

tion and haemagglutination tests with *M. gallisepticum* are shown in Fig. 4.

In tests with 4–8  $\mu$  of antigen, an *M. gallisepticum* rabbit antiserum inhibited spermagglutination at an initial dilution of 1 : 16 and inhibited haemagglutination at a dilution of 1 : 64. Pre-immune rabbit serum did not inhibit at a dilution of 1 : 2.

Spermadsorption and haemadsorption by mycoplasmas have several common features which are summarized in Table 4. Adsorption of both spermatozoa and erythrocytes to colonies of *M. pneumoniae* may be inhibited by high concentrations of specific antiserum. The occurrence of inhibitors in bovine semen plasma<sup>1,2</sup> has not been a problem in spermadsorption experiments with the semen from the one bull used in the present investigation. On one occasion, however, spermatozoa from this bull adsorbed more rapidly after washing, suggesting the

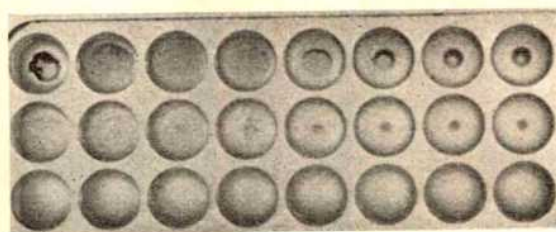


Fig. 4. Spermagglutination and haemagglutination by *M. gallisepticum* (A514). Top row, titration of A514 antigen with 1 per cent suspension of human erythrocytes. Middle row, titration of A514 antigen with 1 per cent suspension of human spermatozoa.

Table 4. SIMILARITIES BETWEEN SPERMADSORPTION AND HAEMADSORPTION

Condition	Spermadsorption	Haemadsorption
Rapid and extensive at 37° C	+	+
Colonies on agar and growth on plastic	+	+
Adhesion tenacious	+	+
Crowding of colonies inhibits	+	+
Inhibited by antiserum	+	+

occurrence and possible fluctuation in concentration of an inhibitor in various ejaculates from one animal. This and the possible occurrence of inhibitors in samples of semen from other bulls need further investigation. Spermadsorption may be inhibited also by the crowding of colonies on agar medium. This is in contrast to the adsorption which occurs to confluent growth of mycoplasmas on plastic. The reason for this anomaly is not understood. It is possible, however, that some unknown factors are required at the colony surface for spermadsorption to occur and that these are readily supplied to growth on plastic by the overlying liquid medium and less easily to colonies on the surface of agar medium where many colonies could exhaust the required factors.

Spermadsorption experiments have been performed chiefly on those mycoplasma colonies which haemadsorb, and spermadsorption has usually been found to occur also. With *M. agalactiae*, however, haemadsorption but not spermadsorption was observed. It will be necessary to examine many more mycoplasma serotypes, particularly those that do not haemadsorb, before it is possible to determine how good a correlation exists between spermadsorption and haemadsorption. In addition, it is known<sup>5</sup> that not all strains within a given mycoplasma serotype haemadsorb, so it will also be necessary to examine several strains of a serotype.

The possible role of sialic acid in spermadsorption and haemadsorption should be kept in mind when considering the mechanism of these phenomena. It has been indicated that in the haemagglutination reaction, a sialic acid at the surface of turkey erythrocytes provides binding sites or receptors for *M. gallisepticum*<sup>9</sup>, although in other experiments removal of receptors on chicken erythrocytes by neuraminidase was not achieved<sup>10</sup>. If such a receptor mechanism is involved in spermadsorption it is difficult to understand why loss of spermatozoal viability should result in less rapid and quantitatively less spermatozoa adherence, unless loss of receptors on such spermatozoa also occurs. Some adsorption occurs when a suspension containing a complete population of non-viable spermatozoa is used. It is possible that in these circumstances the adhesion is similar to that observed when mycoplasmas adsorb on to inert substances such as polystyrene latex particles<sup>11</sup> or glass<sup>12</sup>.

We cannot yet assess the significance of our observations. Whether they have any relevance to the aetiology of infertility and foetal abnormalities and to the mechanism of transfer of infection within the female genital tract remains to be seen.

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# Antigenicity in Mice of Antilymphocyte Gamma Globulin

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Antigenicity of  $\gamma$ G serum protein from rabbits immunized with mouse lymphocytes has been compared with that of  $\gamma$ G from normal rabbit serum. Results indicate that this property is not involved in the prolongation of homograft survival by antilymphocyte serum.

ANTISERA raised in rabbits against mouse lymphocytes exhibit potent immunosuppressive qualities. Investigation of rabbit serum fractions has shown that the component responsible for prolongation of skin homografts can only be demonstrated in the  $\gamma$ G fraction<sup>1,2</sup>. It is not known how antilymphocyte serum  $\gamma$ G exerts its effect. One possible mechanism is that of antigen competition, and so we have studied the antigenicity of antilymphocyte serum  $\gamma$ G by means of the antigen-elimination test.

In the experiments described here antilymphocyte serum was prepared in rabbits by immunizing them with the thymocytes of *CBA* mice, according to the procedure of Levey and Medawar<sup>3</sup>. We have investigated the response of *CBA* mice to gamma G globulin from both antilymphocyte serum and normal rabbit serum.

We used male mice, 10–20 weeks old. Gamma G globulin fractions were prepared from normal rabbit serum, and rabbit antilymphocyte serum by gel-filtration using 'Sephadex G-150' with a 0.2 molar phosphate saline buffer containing 0.002 molar ethyldiaminetetraacetic acid. The '7S' fraction was concentrated by pressure dialysis against 0.15 molar buffered saline and the concentration of protein was measured spectrophotometrically. Rabbit gamma globulin, fraction II by ethanol fractionation, was purchased from Pentex. The antigen-elimination assay was carried out by a standard method<sup>4</sup> using gamma globulins labelled with iodine-131 (ref. 5). The different immune responses of *CBA* mice to rabbit gamma globulin as defined by the rate of elimination of the labelled gamma globulin have been divided into categories 0–10. Each successive category (2–8) represents a two-fold increase in rate of antibody release into the circulation. Category 0 represents an undetectable antibody response and category 10 the maximum response. The categories were calibrated by passive immunization of mice injected with 2 mg of labelled rabbit gamma globulin; for the same absolute response the observed category is inversely related to the amount of test antigen used<sup>4</sup>. Fig. 1 illustrates these categories and this family of curves can be used as a "slide-rule" to quantify the experimental response. Protein solutions were centrifuged at 4,500*g* for 60 min before being injected intraperitoneally for both the antigen-elimination assay and the induction of immunological paralysis. The mice were immunized against rabbit gamma globulins by intraperitoneal injections of 0.1 mg of alum-precipitated gamma globulin<sup>6</sup> mixed with  $2 \times 10^8$  *Bordetella pertussis* organisms (RAP)<sup>4</sup>.

The experiments and results are summarized in Table 1. A single injection of 500  $\mu$ g of normal rabbit serum  $\gamma$ G (group A) is non-immunogenic and is eliminated from the circulation of previously untreated mice with a half-life of 5.4 days (Fig. 2). Using the categories given in Fig. 1 the geometric mean category was 0.2. In contradistinction, the same amount of antilymphocyte serum  $\gamma$ G is immunogenic (group B; geometric mean category 5.2, and Fig. 3). In groups C and D both normal rabbit serum  $\gamma$ G and antilymphocyte serum  $\gamma$ G are rapidly

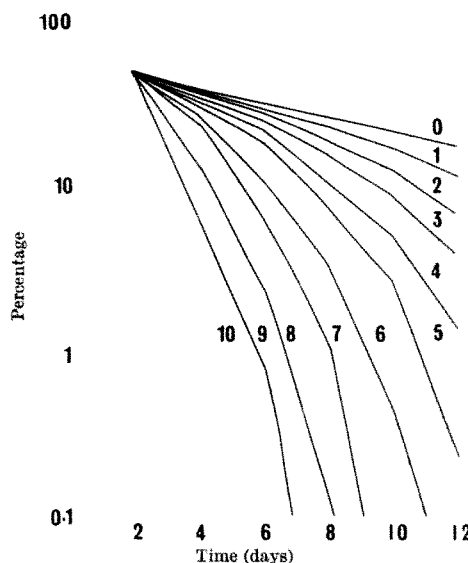


Fig. 1. Categories of antigen-elimination responses. Based on passive immunization of *CBA* mice injected intraperitoneally on day 0 with 2 mg of labelled rabbit gamma globulin. In the range 2–8 the categories accurately reflect two-fold dilutions in the amount of passive antibody injected intraperitoneally immediately after test bleedings on days 2, 4, 6, 8 and 10 after injection of labelled antigen. Category 4 represents the injection every 2 days of sufficient antiserum to precipitate half ( $P_{50}$  per cent) of 28  $\mu$ g of labelled rabbit gamma globulin *in vitro*; category 5 is therefore the elimination response after passive immunization with twice this amount. The ordinate represents the percentage of the 2 mg of labelled rabbit gamma globulin left in the circulation.

Table 1

Group	No. of mice	Pretreatment (first injection)	Interval (weeks)	Challenge (second injection)	Interval (weeks)	Test protein label-iodine-131	Geometric mean score
A	7	—	—	—	—	NRS $\gamma$ (500 $\mu$ g)	0.2
B	7	—	—	—	—	ALS $\gamma$ (500 $\mu$ g)	5.2
C	7	—	—	RAP (100 $\mu$ g RGG)	4	NRS $\gamma$ (500 $\mu$ g)	10.0
D	7	—	—	RAP (100 $\mu$ g RGG)	4	ALS $\gamma$ (500 $\mu$ g)	10.0
E	7	NRS $\gamma$ (200 $\mu$ g)	2	RAP (100 $\mu$ g RGG)	4	NRS $\gamma$ (500 $\mu$ g)	6.7
F	7	ALS $\gamma$ (200 $\mu$ g)	2	RAP (100 $\mu$ g RGG)	4	NRS $\gamma$ (500 $\mu$ g)	10.0
G	7	NRS $\gamma$ (500 $\mu$ g)	3	NRS $\gamma$ (500 $\mu$ g)	4	NRS $\gamma$ (500 $\mu$ g)	0.7
H	7	NRS $\gamma$ (500 $\mu$ g)	3	ALS $\gamma$ (500 $\mu$ g)	4	NRS $\gamma$ (500 $\mu$ g)	2.1
I	6	ALS (1 ml.)*	12	RAP (100 $\mu$ g RGG)	2	NRS $\gamma$ (500 $\mu$ g)	10.0
J	7	—	—	ALS (1 ml.)*	12	NRS $\gamma$ (500 $\mu$ g)	7.5
K	10	RAP (1 mg RGG)	3	RAP (100 $\mu$ g RGG)	3	NRS $\gamma$ (500 $\mu$ g)	9.0
L	6	—	—	ALS (0.25–0.5 ml./week) for 14 weeks*	14	NRS $\gamma$ (500 $\mu$ g) + ALS 1 ml.*	1.1

NRS, normal rabbit serum; ALS, antilymphocyte serum; RGG, rabbit gamma globulin.

\*All injections were intraperitoneal, except for antilymphocyte serum, which was administered subcutaneously.



cleared in previously immunized animals. Comparison of groups *E* and *F* shows that mice injected with 200  $\mu$ g of normal rabbit serum  $\gamma$ G are at least eight times less responsive than mice injected with the same amount of antilymphocyte serum  $\gamma$ G (Figs. 4 and 5). Comparison of groups *C* and *E* shows that this suppressed response is the result of the induction of a degree of immunological paralysis. It is clear from Fig. 4 that mice receiving 200  $\mu$ g of normal rabbit serum  $\gamma$ G fall naturally into two groups, one of which shows a high degree of paralysis; this detail is lost in the geometric mean category of 6.7 (Table

1). Groups *G* and *H* demonstrate that paralysis induced by previous injection of 500  $\mu$ g of normal rabbit serum  $\gamma$ G also extends to a subsequent exposure to the other  $\gamma$ G.

In groups *I* and *J* (Table 1) the response to normal rabbit serum  $\gamma$ G was measured in mice previously exposed to "therapeutic doses" of antilymphocyte serum. These mice had received a total of 1 ml. of whole antilymphocyte serum subcutaneously, shortly after skin homografting. Survival of the skin homografts (*A*→*CBA*) was two to three times longer than on untreated mice. Twelve weeks later half the animals (group *I*) were challenged with

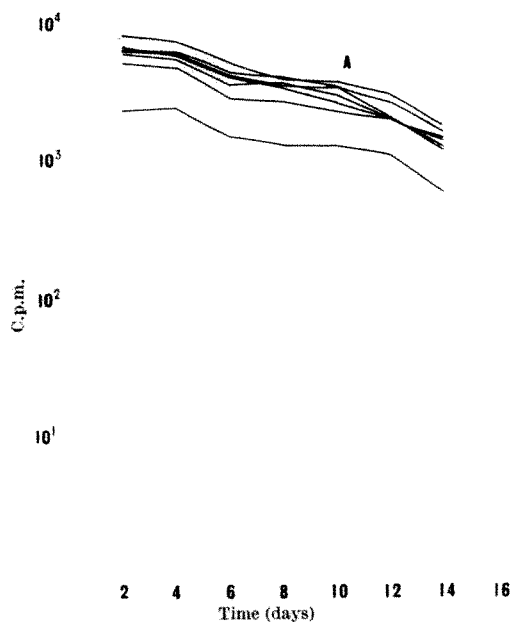


Fig. 2. Individual elimination curves in seven mice injected intraperitoneally with labelled normal rabbit serum  $\gamma$ G (group *A*). The half-life is 5.4 days.

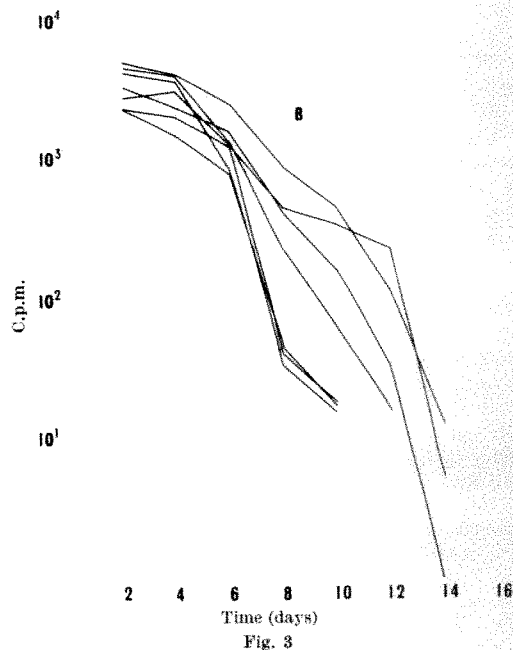


Fig. 3. Similar to Fig. 2 but with labelled antilymphocyte serum  $\gamma$ G (group *B*). This figure shows a primary immune response.

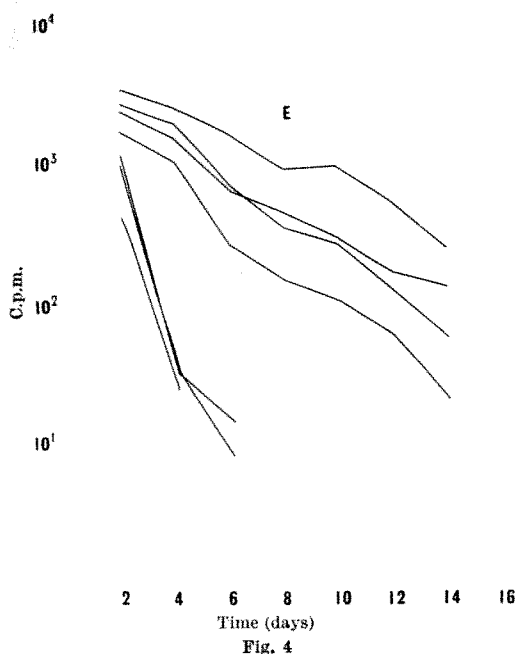


Fig. 4. The elimination response in seven mice immunologically paralysed by 200  $\mu$ g normal rabbit serum  $\gamma$ G 2 weeks before challenge intraperitoneally with 100  $\mu$ g of alum-precipitated rabbit gamma globulin plus  $2 \times 10^6$  pertussis (RAP) followed by test 4 weeks later. Four of the mice show a considerable degree of paralysis (group *E*).

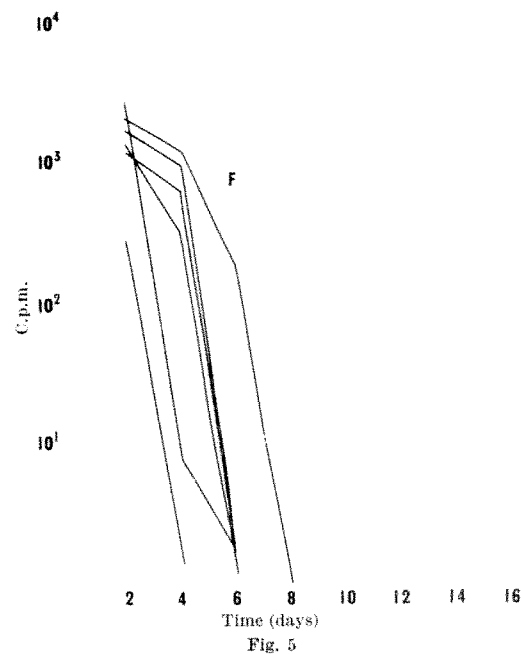


Fig. 5. Similar to Fig. 4, but the first injection was with 200  $\mu$ g of antilymphocyte serum  $\gamma$ G. It can be seen that all mice showed a considerable degree of immunity (group *F*).



RAP and subsequently both groups were tested with labelled normal rabbit serum  $\gamma$ G. Both groups eliminated the test antigen extremely rapidly, indicating that antilymphocyte serum is very immunogenic in doses producing potent immunosuppression. Group K shows that the inclusion of 1 ml. of antilymphocyte serum in the test dose of normal rabbit serum  $\gamma$ G injected into previously immunized mice induces only a minor suppression or delay of the immune response, which could be the result of what is in effect a large increase in the dose of test antigen<sup>4</sup>.

The administration for prolonged periods of time of weekly doses of antilymphocyte serum induces a state of unresponsiveness to normal rabbit serum  $\gamma$ G (group L). Although this state could be ascribed to non-specific immunosuppressive features of antilymphocyte serum, it is thought that a more likely explanation is that immunological paralysis has been induced by the large amount of  $\gamma$ G antigen present<sup>7</sup>.

These results show that normal rabbit serum  $\gamma$ G is a poor immunogen and readily induces a state of immunological paralysis, whereas antilymphocyte serum  $\gamma$ G is very immunogenic. On the other hand, a state of non-reactivity to antilymphocyte serum  $\gamma$ G can be induced either by previous paralysis with normal rabbit serum  $\gamma$ G or by chronic administration of closely spaced doses of antilymphocyte serum. The immunogenicity of this  $\gamma$ G may be explained in one of two ways. (a) It could act as a non-specific adjuvant stimulating immunization by the "normal" gamma globulins in the antilymphocyte serum preparation, or (b) the adherence of the molecules of antilymphocyte serum  $\gamma$ G to either macrophages or lymphocytes could be the decisive factor stimulating immunity as opposed to the paralysis induced by normal (non-adherent)  $\gamma$ G. The first possibility seems unlikely, for the antilymphocyte serum used in these experiments

usually caused immunosuppression in doses which still induced immunity to normal rabbit serum  $\gamma$ G. On the other hand, there is no question that antilymphocyte serum coats lymphocytes *in vivo*<sup>3</sup>, and one of us (E. M. L.) in unpublished preliminary experiments has tentatively shown that such coated cells are filtered from the circulation by the spleen.

Adler<sup>8</sup> has reviewed the evidence which shows that antigen competition may operate to produce decreased immunological responsiveness. Our results demonstrate that antilymphocyte serum is immunogenic, and so such a hypothesis must be considered. If antilymphocyte serum were to work by preoccupying the attention of all immunologically competent cells as obligative immunogen, then in conditions which prohibit immunity antilymphocyte serum should be ineffective. It has been found that the previous induction of paralysis using normal rabbit serum  $\gamma$ G did not diminish the potency of a subsequent "therapeutic" dose of antilymphocyte serum in prolonging skin homograft survival<sup>1</sup>. It therefore seems that the immunogenicity of antilymphocyte serum is irrelevant to its mode of action in the promotion of homograft survival.

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## A GTP Requirement for Binding Initiator tRNA to Ribosomes

by

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Formylmethionyl-tRNA (F-Met-tRNA<sub>F</sub>) is bound by particular codons to ribosomes in the initiation of protein synthesis. When a certain small concentration of magnesium ions is present, initiation factors and GTP are also required for this binding to take place. An analogue can be substituted for GTP, which suggests that this role of the nucleotide is uncoupled from its later hydrolysis.

FORMYLMETHIONYL-tRNA (F-Met-tRNA<sub>F</sub>)<sup>1,2</sup> has been demonstrated to be the initiator of protein synthesis in bacterial cell-free systems<sup>3-6</sup>. Of the two species of methionine-accepting tRNA present in *Escherichia coli* only the formylatable species, whether formylated or not, contributes methionine residues to N-terminal positions of polypeptide products<sup>3</sup>. Whereas both species of Met-tRNA respond to the codon AUG, F-Met-tRNA<sub>F</sub> also responds to GUG (refs. 3 and 7) and further differs from Met-tRNA<sub>M</sub> in that the formylatable species can directly enter the peptidyl-tRNA binding site of the ribosome<sup>8</sup>.

Two protein factors, F<sub>1</sub> and F<sub>2</sub>, which stimulate the translation of natural messenger RNA by washed ribosomes and supernatant enzymes have been isolated either from ribosomal washings<sup>9,10</sup> or from a DNA complex<sup>11</sup>. These factors are thought to be involved in the initiation step of protein synthesis, for F<sub>1</sub> and F<sub>2</sub> stimulate the bind-

ing of F-Met-tRNA<sub>F</sub> to ribosomes in the presence of AUG (ref. 12).

Since the initial demonstration of the requirement for GTP in protein synthesis<sup>13</sup>, a stoichiometric relationship has been found between the amount of GTP hydrolysed to GDP and *ortho*-phosphate on the one hand and the number of peptide bonds formed on the other<sup>14</sup>. The  $\beta$ , $\gamma$ -methylene analogue of GTP, 5'-guanylyl diphosphonate (GMP-PCP), has been shown to be a competitive inhibitor of the action of GTP in protein synthesis<sup>15</sup>. GTP has been reported to be needed in the formation of F-Met-puromycin from bound F-Met-tRNA<sub>F</sub> and puromycin<sup>16</sup>, and also for the formation of formylmethionyl-phenylalanine in a system directed by the synthetic messenger AUGUUU (ref. 17). These authors<sup>16,17</sup> concluded that GTP does not have its effect at the level of binding the F-Met-tRNA<sub>F</sub>, but in some way activates

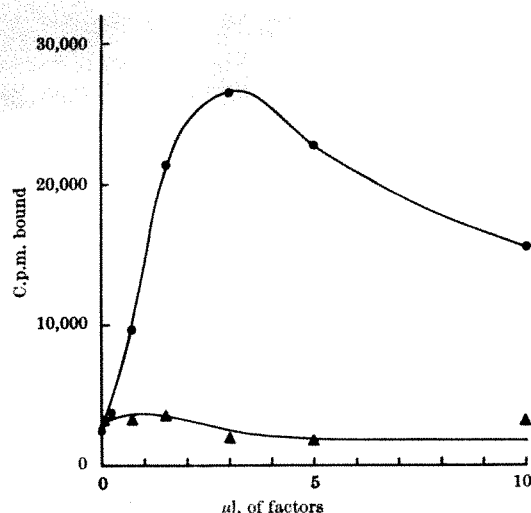


Fig. 1. Each reaction mixture (50  $\mu$ l.) contained 0.10 molar *tris* acetate, pH 7.2, 0.05 molar potassium chloride, 0.01 molar ammonium chloride, 0.005 molar magnesium acetate, 0.94  $A_{260}$  units of ribosomes, 0.096  $A_{260}$  units of AUG, and 0.18  $A_{260}$  units of  $^{35}$ S-F-Met-tRNA ( $1.0 \times 10^6$  c.p.m. counted at 70 per cent efficiency in a Packard liquid scintillation spectrometer; 70 per cent as F-Met). Crude initiation factors (10  $\mu$ g/ $\mu$ l.) were added in the amounts indicated. When present, GTP was 0.0002 molar. After incubation at 25°C for 10 min, the reaction mixtures were diluted sixty-fold in 0.10 molar *tris* acetate, pH 7.2, 0.05 molar potassium chloride, 0.005 molar magnesium acetate (buffer B) and filtered on 'Millipore' filters by the procedure of Nirenberg and Leder<sup>21</sup>.

●, Plus factors and GTP; ▲, plus factors only.

it once bound. GTP stimulates the binding of *N*-acetylphenylalanyl-tRNA to ribosomes in the presence of polyuridylylate and initiation factors<sup>18</sup>. The stimulation of *N*-acetylphenylalanyl-tRNA binding by GTP occurred in the presence of a small concentration of magnesium.

We report here that GTP and initiation factors are required for the binding of F-Met-tRNA<sub>F</sub> to ribosomes with AUG at a 5 mmolar concentration of magnesium ions, and that in these conditions GMP-PCP is an effective substitute for GTP. Non-formylated Met-tRNA<sub>F</sub> does not bind in identical conditions. The stimulation of F-Met-tRNA<sub>F</sub> binding to ribosomes by either GMP-PCP or GTP indicates that the initial effect of GTP in protein synthesis has been uncoupled from a subsequent step in which hydrolysis of GTP occurs.

Ribosomes were prepared from cells of *E. coli* MRE600, after grinding with alumina. The broken-cell paste, suspended in 0.01 molar *tris* chloride, pH 7.4, 0.01 molar magnesium chloride, 0.06 molar ammonium chloride and 0.006 molar  $\beta$ -mercaptoethanol (buffer A), was freed of DNA by digestion with 3  $\mu$ g/ml. of DNase. After the removal of alumina and cellular debris from the cell extract by centrifugation at 30,000*g*, the ribosomes were pelleted at 230,000*g*, resuspended in buffer A and repelleted. Initiation factors were removed from the ribosomes by washing in a salt solution containing 0.01 molar *tris* chloride, pH 7.4, 0.01 molar magnesium chloride, 2.0 molar ammonium chloride, and 0.006 molar  $\beta$ -mercaptoethanol. The ribosomes were sedimented at 270,000*g* and the supernatant was retained for the initiation factors. The ribosomes were suspended in buffer A and sedimented again at 270,000*g*. After resuspension in buffer A, the ribosomes were stored in liquid nitrogen. The initiation factors were recovered from the high-salt ribosomal supernatant by ammonium sulphate precipitation (52 g/100 ml.). The precipitate was dissolved in a solution containing 0.02 molar *tris* chloride, pH 7.8, 0.002 molar magnesium acetate and 0.18 molar ammonium chloride, dialysed against the same buffer and stored in liquid nitrogen. F<sub>1</sub> and F<sub>2</sub> were fractionated by a procedure essentially the same as that described by Stanley and co-workers<sup>9</sup>.

tRNA from *E. coli* CA244 was charged with  $^{35}$ S-L-methionine (7,000 mc./mmole) and nineteen unlabelled

amino-acids in the presence of *N*<sub>10</sub>-formyl tetrahydrofolate with a crude *E. coli* supernatant fraction to prepare fully formylated  $^{35}$ S-F-Met-tRNA. To obtain non-formylated  $^{35}$ S-Met-tRNA, the formyl donor was omitted and the enzyme used was an *E. coli* supernatant fraction freed of the transformylase by passage through a phosphocellulose column. After treatment with phenol and two precipitations with ethanol, the charged tRNA preparations were passed through 'Sephadex G-25'. The extent of formylation was determined by treatment of aliquots with pancreatic ribonuclease followed by paper electrophoresis at pH 3.5. Preparations charged in the presence of the formyl donor usually yielded 70 per cent of the charged radioactivity as formylmethionyladenosine. In the absence of formyl donor, less than 2 per cent of the charged radioactivity corresponded to formylmethionyladenosine.

Triribonucleoside diphosphates permit the binding of amino-acyl-tRNA to ribosomes at concentrations of magnesium of 10 mmolar and more, but only poorly at 5 mmolar. F-Met-tRNA<sub>F</sub> binds exceptionally well with either AUG or GUG at 10 mmolar<sup>3,7</sup>, but at 5 mmolar almost none is bound. In contrast, when the RNA from the f2 bacteriophage is used in place of AUG, the maximum binding of F-Met-tRNA occurs in the presence of 5 mmolar magnesium ions<sup>19</sup>. In this context there are reports that only natural initiation occurs at a small concentration of magnesium<sup>20</sup>. If ribosomes are supplemented with GTP and initiation factors, a significant amount of F-Met-tRNA can be bound with the triplet AUG at a concentration of magnesium ions of 5 mmolar. GTP, the initiation factors and formylated Met-tRNA are required for this binding.

Fig. 1 shows the quantity of initiation factors required to give maximum stimulation of F-Met-tRNA binding to ribosomes with AUG and GTP with 5 mmolar magnesium ions. When the GTP is omitted the initiation factors cause only relatively small effects at any of the concentrations tested. With the preparation of ribosomes and initiation factors used, maximum stimulation was obtained with 1  $\mu$ g of initiation factors for each  $\mu$ g of ribosomes.

The specificity of this system is shown in Fig. 2. In the complete system containing ribosomes, AUG, initiation factors, GTP and F-Met-tRNA with 5 mmolar magnesium ions, the fully formylated F-Met-tRNA is bound rapidly, and the plateau is reached after about 10 min at 25°C. Both F<sub>1</sub> and F<sub>2</sub> are required. When either GTP or initiation factors are omitted, no binding occurs. Although not shown in Fig. 2, if AUG is omitted but both initiation factors and GTP are present, a blank

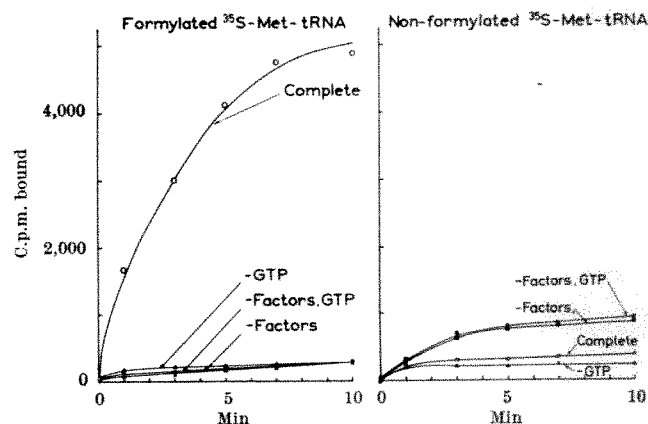


Fig. 2. The complete reaction mixture (250  $\mu$ l.) contained 0.10 molar *tris* acetate, pH 7.2, 0.05 molar potassium chloride, 0.01 molar ammonium chloride, 0.005 molar magnesium acetate, 4.7  $A_{260}$  units of ribosomes, 0.50  $A_{260}$  units of AUG, 75  $\mu$ g of initiation factors, 0.0002 molar GTP and either 0.53  $A_{260}$  units of  $^{35}$ S-F-Met-tRNA ( $3.2 \times 10^6$  c.p.m.; 70 per cent as F-Met) or 0.46  $A_{260}$  units of  $^{35}$ S-Met-tRNA ( $3.1 \times 10^6$  c.p.m.; less than 2 per cent as F-Met). Incubation was at 25°C. At the intervals indicated 25  $\mu$ l. samples were withdrawn, diluted 100-fold in buffer B, and filtered immediately on a 'Millipore' filter.

value of similar size is obtained. When non-formylated Met-tRNA is substituted for F-Met-tRNA, very little binding occurs with any combination of initiation factors and GTP. The failure of Met-tRNA to bind to ribosomes with 5 mmolar magnesium ions was shown not to be caused by any damage to the Met-tRNA, for significant amounts could be bound in the presence of 10 mmolar magnesium ions with or without initiation factors and GTP. Similar results were obtained with purified fractions of F-Met-tRNA<sub>F</sub> and Met-tRNA<sub>F</sub>. Met-tRNA<sub>M</sub> was not bound to ribosomes with AUG in the presence of 5 mmolar magnesium ions even in the presence of initiation factors and GTP.

Table 1. INITIATION FACTOR-STIMULATED BINDING OF <sup>35</sup>S-F-Met-tRNA TO RIBOSOMES

Triplet	Conditions Initiation factors	c.p.m. bound in the presence of GTP	GMP-PCP
None	+	2,400	2,000
None	-	250	280
AUG	+	21,800	20,700
AUG	-	1,420	820
GUG	+	8,570	16,300
GUG	-	530	560

Reaction mixtures (50  $\mu$ l.) contained 0.10 molar *tris* acetate, pH 7.2, 0.05 molar potassium acetate, 0.01 molar ammonium chloride, 0.005 molar magnesium acetate, 1.1 A<sub>260</sub> units of ribosomes, 0.096 A<sub>260</sub> units of AUG or 0.091 A<sub>260</sub> units of GUG, 30  $\mu$ g of initiation factors when indicated, 0.21 mmolar GTP or 0.18 mmolar GMP-PCP, and 0.10 A<sub>260</sub> units of <sup>35</sup>S-F-Met-tRNA (0.58  $\times 10^5$  c.p.m.; 70 per cent as F-Met). After incubation for 20 min at 25° C, the reaction mixtures were diluted 100-fold in buffer B and filtered on a 'Millipore' filter.

The specificity of the binding of F-Met-tRNA for GTP was also examined. Fig. 3 shows that GMP-PCP, the  $\beta,\gamma$ -methylene analogue of GTP, can substitute for GTP although the rate of binding is less. The stimulation observed with GMP-PCP sometimes approached the level of that observed with an equivalent amount of GTP (see Table 1). ATP and ITP can substitute for GTP to a lesser extent. It is possible that the effect seen with ATP results from the generation of GTP from endogenous GDP or GMP. The results obtained when UTP, CTP or GDP were substituted for GTP were indistinguishable from the blank. The stimulation by GMP-PCP, ATP and ITP did not result from contamination of these nucleotides with GTP, because the GMP-PCP had been treated extensively with alkaline phosphatase and the ATP and ITP had been chromatographed on Whatman No. 3 MM paper in the solvent *n*-propanol-ammonia-water (55/10/35 v/v) which separates GTP from other ribonucleoside triphosphates.

Evidence from an investigation of the synthesis of polypeptides *in vitro* directed by poly (UG)<sub>n</sub> and poly (AUG)<sub>n</sub> confirmed that both AUG and GUG are codons for F-Met-tRNA<sub>F</sub> (ref. 7). When the triplets AUG and GUG were compared in the initiation factor- and GTP-dependent binding reaction with F-Met-tRNA, the results shown in Table 1 were obtained. Both AUG and GUG significantly stimulated binding to a degree greater than that observed in the absence of triplet. When GMP-PCP was substituted for GTP, the binding observed with GUG more nearly approached that observed with AUG. The reason for the greater binding of F-Met-tRNA with GUG in the presence of GMP-PCP relative to GTP is not clear. In all cases the initiation factors were required.

We conclude that for F-Met-tRNA to be bound to ribosomes by the codons AUG or GUG with 5 mmolar magnesium ions, the formyl group, initiation factors and GTP are all obligatorily required. The observation that an analogue of GTP (which cannot be degraded in the manner in which GTP is normally hydrolysed) can effectively substitute for GTP implies that this role of GTP has been uncoupled from the subsequent step in which it is hydrolysed.

Finally, we must emphasize that the effects observed here only describe the binding of F-Met-tRNA<sub>F</sub> with 5 mmolar magnesium ion. As the concentration of mag-

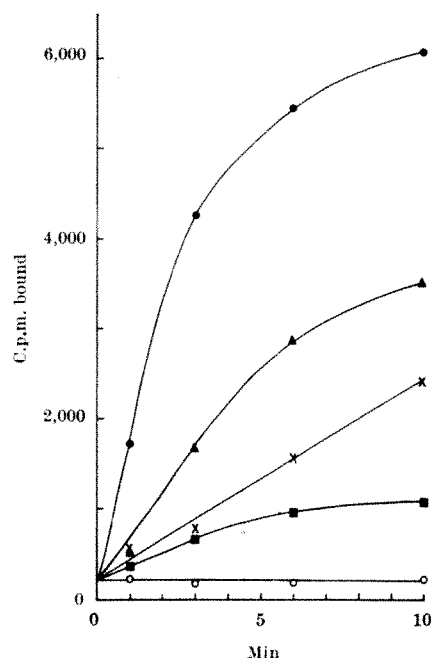


Fig. 3. Each reaction mixture (125  $\mu$ l.) contained 0.10 molar *tris* acetate, pH 7.2, 0.05 molar potassium chloride, 0.01 molar ammonium chloride, 0.005 molar magnesium acetate, 2.4 A<sub>260</sub> units of ribosomes, 0.31 A<sub>260</sub> units of AUG, 38  $\mu$ g of initiation factors and 0.90 A<sub>260</sub> units of <sup>35</sup>S-F-Met-tRNA which had been pyrophosphorylated to remove all amino-acids except the formylated-methionine ( $1.76 \times 10^5$  c.p.m.). As indicated below, GTP was 0.26 mmolar, GMP-PCP was 0.26 mmolar, ATP was 0.24 mmolar and ITP was 0.21 mmolar. Incubation was at 25° C. At the intervals indicated 25  $\mu$ l. samples were withdrawn, diluted forty-fold in buffer B and filtered on 'Millipore' filters. ●, GTP; ▲, GMP-PCP; ×, ATP; ■, ITP; ○, blank.

nesium ions is increased to 10 mmolar, the requirements for the formyl group, initiation factors and GTP become less accentuated, or disappear altogether. We believe that our previous conclusions<sup>3,8</sup> about the special structure of tRNA<sub>F</sub> and its relation to ribosomal binding sites at 10 mmolar magnesium ion are correct.

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## LETTERS TO THE EDITOR

## ASTRONOMY

## An Upper Limit to Circularly Polarized Radiation from the Crab Nebula

RECENTLY a small intense source of low frequency radio emission has been detected in the Crab Nebula<sup>1,2</sup>. Subsequent investigations have revealed that this source has a flux spectral index of  $\alpha \sim 1.2$  (refs. 2 and 3), an angular diameter of  $\sim 0.2$  sec arc (refs. 3 and 4), and a brightness temperature of  $\sim 10^{11}$  °K (ref. 4). Neither the thermal nor the synchrotron emission mechanisms can satisfactorily account for these characteristics<sup>3-5</sup>. Accordingly, a plasma mechanism, possibly similar to that of a solar flare, has been proposed<sup>4,5</sup>. It seems likely that such a mechanism would be associated with the stellar remnant of the supposed supernova which gave rise to the Crab Nebula, and a recent measurement of the position of the compact source indicates that it does indeed coincide with the position of the stellar remnant<sup>6</sup>.

Ginzburg and Ozernoy<sup>5</sup> stated that radiation from a plasma mechanism would probably be strongly circularly or elliptically polarized. The present communication reports a search for such polarized radiation from the Crab Nebula (Tau A) at a frequency of 40 Mc/s using the 1,000 ft. diameter radio telescope of the Arecibo Ionospheric Observatory. At this frequency the compact source accounts for  $\sim 20$  per cent of the integrated radiation from the nebula<sup>1,4</sup>.

The telescope has been described previously<sup>7</sup>. The reflector was illuminated by two orthogonal pairs of Yagi antennae. The signals from each pair were combined in a hybrid network in such a way that two output signals were obtained, corresponding to right and left circular polarization respectively. These outputs, shown as channels A and B in Fig. 1, were amplified and detected independently. Bandwidths of 25 kc/s and 125 kc/s were used simultaneously on each channel; if the radiation were elliptically rather than circularly polarized the percentage polarization would be less at the wider bandwidth because of Faraday rotation in the Nebula. The final outputs were recorded by both a magnetic tape unit, for computer processing, and a pen recorder.

The receiving system, which is illustrated schematically in Fig. 1, could be operated in two switching modes. There was a half-wave switch preceding one input of the hybrid, and a Dicke switch following the hybrid in each of channels A and B. In the first mode of operation, which was used to detect the circularly polarized component of the radiation, the half-wave switch was operated at 400 c/s and the Dicke switches held in the positions connecting the receivers to the antenna. After synchronous detection, the outputs from channels A and B corresponded to circularly polarized signals in the senses left minus right, and right minus left, respectively. The sense of the output was determined by transmitting a circularly polarized signal from a small antenna erected in the centre of the reflecting surface. In the second mode of operation, which was used to detect the total emission from the nebula in one polarization, the half-wave switch was held in position and the Dicke switch was operated, using a diode noise source as a reference load. The effective temperature of the reference load was maintained at a level approximately equal to the sky

brightness temperature in order to reduce effects arising from non-linearity in the receiving system. A second diode noise source connected to each channel through a directional coupler was used in this switching mode to monitor the gain stability of the receiving system. By using these two switching modes successively the circularly polarized component of the radiation could be expressed directly as a fraction of the total emission from the nebula.

The half-power beamwidth of the antenna at 40 Mc/s was  $2.0^\circ$ . Each source observed remained within the tracking range of the telescope for  $\sim 2$  h. An observation consisted of tracking the source for 5 min, and establishing a baseline by tracking, for three minutes each, the positions 15 min of R.A. earlier and later than the source. The sequence was off-source earlier, on source, then off-source later, so that the zenith angle was approximately constant throughout each observation. This method reduced to a minimum any spurious effects which arose from illuminating different portions of the fixed reflector surface. This method of observation was used for both switching modes. A receiver time constant of 0.1 sec was used during the observations to allow analysis of the circularly polarized component of the radiation for evidence of the interplanetary scintillations which would be expected for radiation from the compact source during the time of the observations (March 1967)<sup>3,4,8</sup>. Sample records showing observations of the Crab Nebula are reproduced in Fig. 2.

Before reduction of the observations, the non-linearity of the receivers was measured by periodically injecting a constant noise signal through the directional couplers during a drift scan observation of Virgo A. The dependence of the antenna gain on zenith angle was determined using observations of the Crab Nebula. Coupling between the orthogonal pairs of Yagi antennas was  $\sim -27$  dB. Measurements of the phase error of the system indicated that it was  $\sim 10^\circ$ , which has a negligible effect on the measured amplitude of the circularly

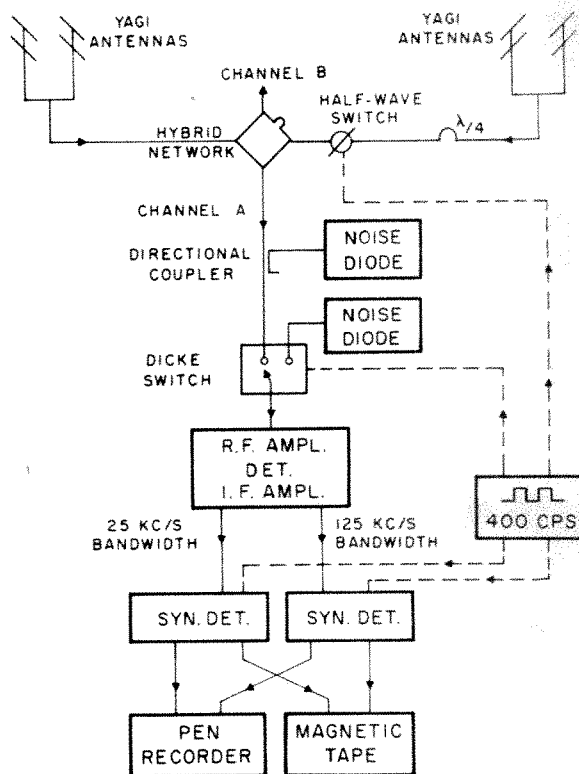


Fig. 1. The receiving system.



polarized signal. After correcting for non-linearity and zenith angle effects, the output representing the apparent circularly polarized signal was expressed as a fraction of the total emission for each channel and at each bandwidth independently. For each bandwidth the channel *B* values were subtracted from the channel *A* values; these channels represented opposite senses of the polarization. This procedure eliminated the systematic error arising from the difference in attenuation between the two positions of the half-wave switch.

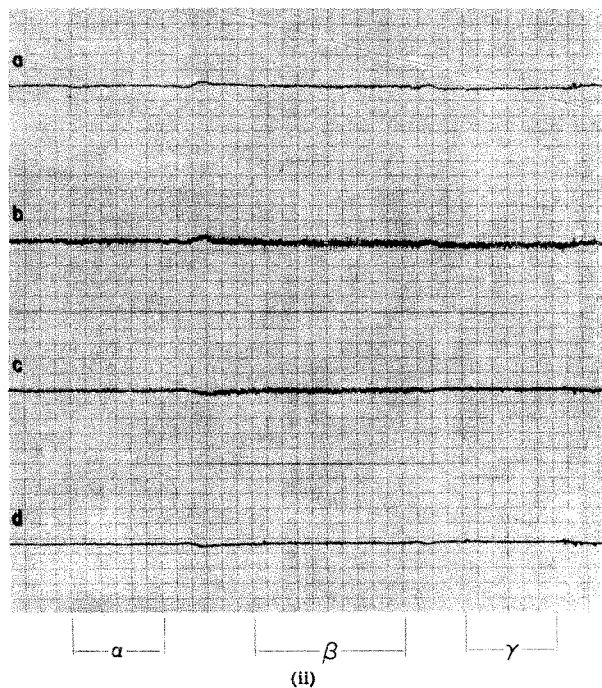
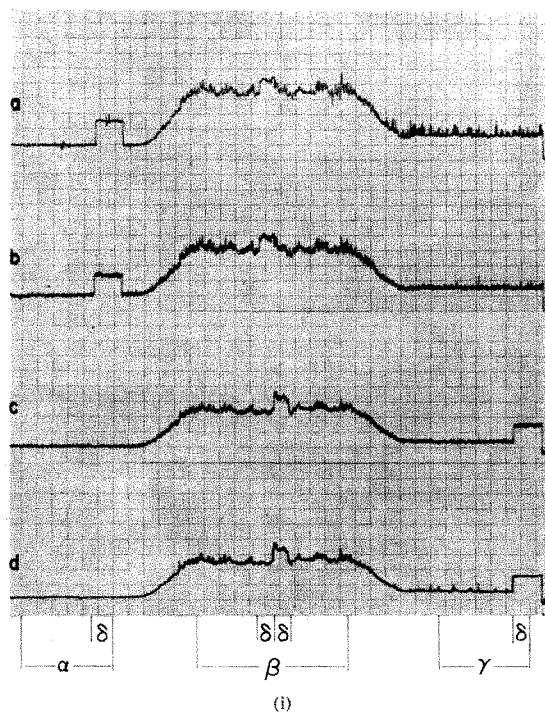


Fig. 2. Sample records of Taurus *A* observations: (i) Dicke switching; (ii) half-wave switching. The four traces are (a) channel *A*, 125 kc/s; (b) channel *A*, 25 kc/s; (c) channel *B*, 25 kc/s; (d) channel *B*, 125 kc/s. The indicated sections of the records represent (a) off-source earlier; (b) on-source; (c) off-source later; (d) noise injection from diode noise source, one channel only.

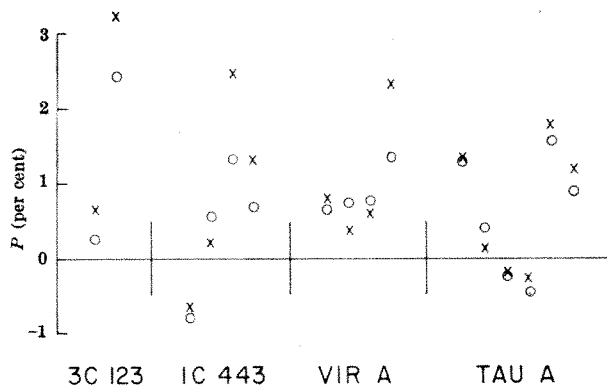


Fig. 3. Apparent circular polarization at 40 Mc/s. *P* is defined as  $100 \times (\text{left circ.} - \text{right circ.}) / (\text{left circ.} + \text{right circ.})$ . The measurements with 125 kc/s bandwidth are represented by circles and those with 25 kc/s by crosses. The plotted points for each source are arranged in chronological order of observation.

Observations were made of the Crab Nebula and of three reference sources, Virgo *A*, 3C 123 and IC 443. There is no reason to expect a circularly polarized signal from any of the reference sources. Fig. 3 shows the polarized signal measured for each bandwidth during each independent observation of the four sources. It is clear that a small systematic effect was present, characteristic of absolute measurements. The circular polarization of the radiation from the Crab Nebula, however, was determined relative to that from the three reference sources, thus eliminating this systematic error. A weighted mean of the data illustrated in Fig. 3 was calculated for the three reference sources together and for the Crab Nebula. The difference between the two means, which expresses the circularly polarized signal from the Crab Nebula, gave a value of  $(0.4 \pm 0.5)$  per cent right circular polarization. There was no significant difference between the results obtained from the 25 kc/s bandwidth observations and those using the 125 kc/s bandwidth.

Assuming that the compact source contributes about 20 per cent of the total radiation from the Crab Nebula<sup>1,4</sup>, the above answer corresponds to  $(2.0 \pm 2.5)$  per cent right circular polarization of the compact source. The absence of significant circular polarization does not rule out a plasma mechanism, but indicates that if a plasma mechanism is responsible for the emission from the compact source, then the magnetic field in the source does not play an important part in the process.

We are indebted to Mr F. S. Harris for his generous and valuable assistance in this work. Two of us (B. H. A. and C. R. P.) thank Dr F. D. Drake for making available to us the many facilities of the Arecibo Ionospheric Observatory.

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### Profile of the Unidentified Interstellar Absorption Band at 6180 Å at Different Galactic Longitudes

THERE is evidence<sup>1</sup> that the profile of the unidentified interstellar absorption band at 4430 Å is constant over a range of galactic longitudes. This band is the strongest of a number of apparently similar bands centred on wavelengths of 6180 Å, 4890 Å and 4760 Å. The band at 6180 Å was first noticed by Wilson<sup>2</sup>, who speculated as to an interstellar origin. The second band, at 4890 Å, was discovered independently by Wilson<sup>3</sup> and by Butler and Seddon<sup>4</sup>, while the third was tentatively identified as interstellar by Merrill in private correspondence with Beals<sup>5</sup>. The only evidence presented so far about the interstellar nature of these bands, apart from studies of correlation of strengths with those of known interstellar absorptions, comes from Merrill and Humason<sup>6</sup>, who have shown that the position of the 4430 Å band is stationary with respect to stellar absorption lines in the spectrum of an early type binary star. Strength correlation is difficult for all these bands because of their weakness and width, and has only been attempted for the band at 4430 Å. For the same reasons profile determination is difficult, but may well be crucial in the identification of their origin.

The band at 6180 Å is, apart from that at 4430 Å, the most suitable for such investigations because it is not appreciably affected by stellar or atmospheric line absorption. Measurements in this wavelength region made in the course of the spectrophotometric investigation of early-type stars carried out at this observatory<sup>7</sup> are available for the stars used in the determination of the 4430 Å profile<sup>1</sup>. Because the central depth of the feature at 6180 Å is only about one-third that of the 4430 Å band, it has not been thought worthwhile to reproduce the mean spectra in each of the three regions of galactic longitude used in this earlier work<sup>1</sup>, but simply to show the differences between them. The three groups are defined as: group 1 (Cygnus),  $020^\circ < l < 120^\circ$ ; group 2 (Perseus),  $120^\circ < l < 145^\circ$ ; and group 3 (Auriga-Gemini),

$145^\circ < l < 190^\circ$ ; and the stars used are those given in Table 1 of ref. 1. The ordinates in Fig. 1 are all on a magnitude scale; the abscissa is approximately linear in wave-number. The top two traces are of magnitude differences between group 1 and group 2 and between group 1 and group 3. It can be seen that almost all stellar and interstellar features are cancelled out. Those features that have not been cancelled out are (a) the general slow curvature over the whole of  $m_1 - m_2$ , (b) a peak in  $m_1 - m_2$  at about 6285 Å, and (c) an apparent absorption line at 6401 Å in region 1 which is missing in regions 2 and 3. Effect (a) almost certainly arises from the use of two different photographic emulsions at different times in the original programme of measurements. Group 2 contains more measures from Kodak '103a-F' emulsions than the average, and groups 1 and 3 more from Ilford 'Astra VIII'. Curvature of the wavelength/sensitivity characteristics is much more pronounced in the red and yellow than in the blue, and is more difficult to balance out. This curvature will not significantly affect the measures, except as to be noted later. Effect (b) corresponds to a greater absorption in region 2 than in region 1 at about 6285 Å. It does not appear to be related to the interstellar absorption at 6284 Å, because its centre is well separated, and for the same reason it does not appear to arise from greater atmospheric absorption by the  $\alpha$ -band of oxygen. It does appear, however, to be real, as also does the absorption at 6401 Å in group 1. No explanation is offered for either of these two lines, except to acknowledge that the feature (b) may result from a difference between the profiles of the interstellar line 6284 in Cygnus and Perseus. This difference, if it exists, is not present in the comparison of Cygnus with Auriga-Gemini.

It can be seen that the interstellar features at 6284 Å, 6203 Å and 6180 Å have been cancelled out, which indicates that the profiles and strengths of these features are intercorrelated, as they are<sup>1</sup> with the band at 4430 Å and the colour excess, because the same groupings of the same stars have been used throughout. This leads one to suspect that the causes of the continuous interstellar absorption

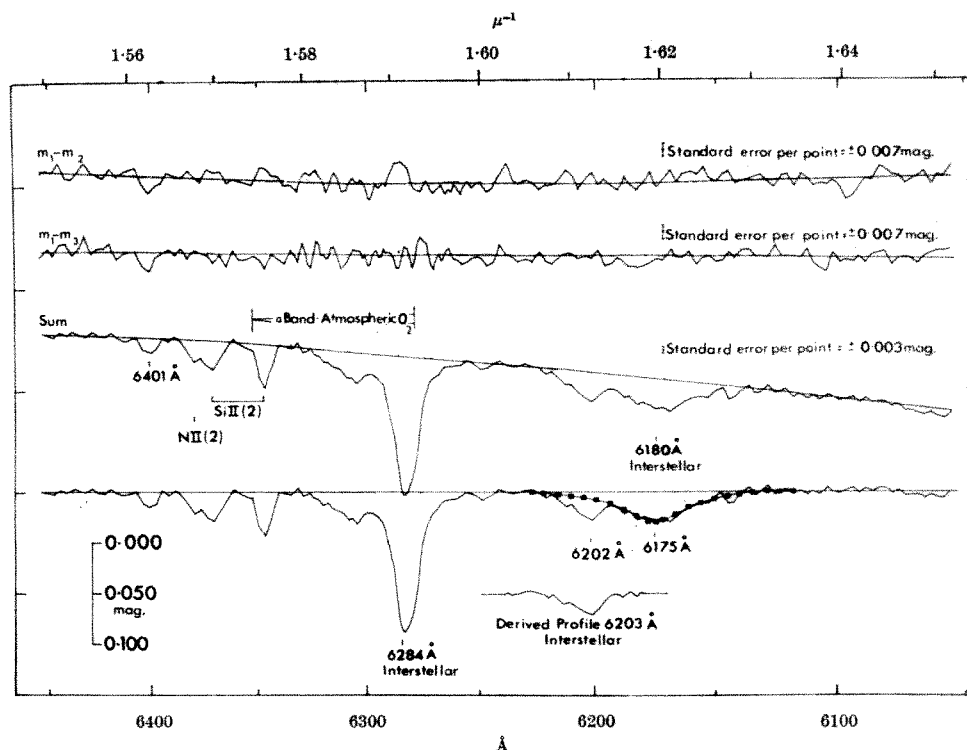


Fig. 1.

and of the diffuse lines are closely linked in the same way as has already been suggested for the diffuse bands and that they are different effects of the same cause.

Because 6180 Å has the same profile in each group, the three profiles have been averaged to increase the signal to noise ratio, as was done for 4430 Å. This mean trace is the third in Fig. 1 and is labelled "sum". The standard error per point is  $\pm 0.003$  magnitude, the total number of plates used being 85. Because of the curvature mentioned earlier, a smooth curve has been inserted through regions expected to be free from absorption. As can be seen, the curvature is slight and errors in it will not greatly affect the profile of 6180 Å.

The fourth trace is the sum trace rectified to the inserted curve. The smooth profile inserted in the 6180 Å band has not been fitted to the tracing but is the profile of 4430 Å derived in ref. 1, scaled down for the different central depth, and extended in the wavelength direction by the ratio 6180:4430. The fit is surprisingly good. There is a real deviation from this profile centred on 6203 Å. This feature is interstellar in origin<sup>8</sup>. Its position, profile and amplitude relative to 6180 Å appear to be constant and so it can be thought of as part of the composite 6180 Å profile, in which case the 6180 Å profile would not resemble that of the 4430 Å band. Wilson<sup>3</sup>, however, has pointed out that no other diffuse line is associated with a band, even if much more intense than 6203 Å. Probably they should be considered to be separate. With this in mind, the inserted profile has been subtracted from the composite around 6203 Å, giving the short trace below, labelled "Derived Profile 6203 Å Interstellar". Too much significance should not be attached to this profile. It may be noted that the profile has the same half-width as the adjacent strong interstellar line at 6284 Å.

The inserted profile derived from 4430 Å seems a good fit for 6180 Å. Its centre is, however, at 6175 Å, the wavelength of 6180 Å having been attributed on much less satisfactory profiles. The equivalent width of the whole feature 6180 + 6203 is 1.30 Angstrom units, of which 6180 is responsible for 1.03 Å. The equivalent width of the 4430 Å band derived in ref. 1 is 2.18 Å. It would seem that the relative strength of 4430 Å to 6180 Å is in the ratio 2:1, within the errors involved here. The width of 6180 Å at half the central depression is 29 Å, as compared with 21 Å for 4430 Å. It was noted in the earlier communication that 4430 Å profiles in the three regions of galactic longitude fortuitously cancelled out in strength as well as width. We see here that the same cancellation has taken place, that is to say, that the aforementioned ratio of strengths of 4430 to 6180 of 2:1 appears to be constant in these longitude regions at least, and may indicate that the two bands arise from the same process, and are to be expected always to have the same ratio. It may also be that the diffuse lines 6284 Å and 6203 Å are also closely connected, because they also cancel out. It has already been noted that the cancellation of 4430 Å profiles is frequently to be found with equal reddening and equal colour excess. It is not possible to say here much about the strength of the intense diffuse line 6284 Å, because it is so heavily involved in the  $\alpha$ -band absorption of atmospheric oxygen, and is in any event not resolved here from the other weak interstellar diffuse line at 6270 Å. The total combined strength of 6284 Å and 6270 Å is, however, close to 1.4 equivalent Angstroms after allowance has been made for atmospheric absorption, from corrections derived in the original sources<sup>4,7,9</sup>. The ratio of the strengths of 6284 Å and 6203 Å is then about 5:1.

In the earlier communication<sup>1</sup> on 4430 Å, a double Gaussian profile was assumed and fitted. The same profile has been shown to fit 6180 Å well, with the further assumption that  $\Delta\lambda/\lambda$  is equal for any two equivalent points on the two profiles, where  $\Delta\lambda$  is the wavelength difference of a point from the centre of the profile  $\lambda_0$ .

The validity of these two assumptions could lead to a decision between mechanisms.

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## PLANETARY SCIENCE

### Rare Earth Content of Green Fluorite— a New Source of Europium

A CONNEXION has already been noted between the rare earth content of fluorites, their colour and their fluorescence when viewed under ultra-violet light<sup>1</sup>. Europium has been indicated as probably responsible for the blue component of the observed fluorescence, and ytterbium for the green component.

From a series of approximate analyses of specimens from the North Pennine Orefield, it was reported that those fluorite samples exhibiting the greatest fluorescence were both rich in europium and poor in cerium. This combination favours the known solvent extraction procedures for europium. In view of this, and of the recent increased importance of europium, it has been considered desirable to re-examine the material from this orefield as a possible domestic source of europium compounds. Dr K. C. Dunham kindly provided some of the original sample material on which the earlier observations were based, and the content of europium has been re-determined.

The original determinations were made by a spectrographic method, but this was not considered to be sufficiently precise, and a neutron activation procedure was devised. This procedure involved irradiation in BEPO at a neutron flux of  $10^{12}$  n/cm<sup>2</sup>sec, precipitation of total rare earths as oxalates, a separation of europium by amalgam-exchange according to the method described by Malan and Münzel<sup>2</sup>, counting of europium-152 by  $\gamma$ -spectrometry, and finally a determination of the chemical yield by cathode ray polarography.

The results obtained are given in Table 1, where they are compared with the original results. It is clear from this work that samples of fluorite from this orefield do contain appreciable amounts of europium, although not all the specimens examined were as rich as had earlier been supposed. The specimen richest in europium was the only one to give a really intense fluorescence—the green material from Stotfield Burn Mine.

Table 1. A COMPARISON OF APPROXIMATE SPECTROGRAPHIC RESULTS WITH THOSE OBTAINED BY NEUTRON ACTIVATION ANALYSIS

Sample No.	Locality	Fluorescence under ultra-violet light	Europium (p.p.m.) Spectro- Neutron-graphic activation
63	Stotfield Burn Mine	Intense, bluish white	90 132
64	Stotfield Burn Mine	Moderate, purple	20 41
65	Stotfield Burn Mine	Weak, purple	15 7
1419	Boltsburn East Mine	Weak, bluish	50 24
1501	Rispey Mill, tailings	Weak, bluish	70 23
1502	Sharnberry, high level dumps	Weak, bluish	100 42
1503	California Mine dumps (non-uniform material)	Some grains strong, bluish; others moderate, purple	110 43
1505	Rotherhope Fell Mine	Moderate, purple	30 15

Table 2. APPROXIMATE SPECTROGRAPHIC ANALYSIS OF FLUORITE SAMPLES FROM REDBURN MINE, CO. DURHAM

Sample No.: Colour: Fluorescence:	67/1925 Green Intense, bluish	67/1926 Purple Strong, purple	67/1927 Colourless Weak, purple
Analysis (p.p.m.)			
Lanthanum	200	< 5	5
Cerium	< 5	5	5
Praseodymium	20	< 20	< 20
Neodymium	200	< 20	< 20
Samarium	20	< 20	< 20
Europium	80	5	8
Gadolinium	10	< 1	2
Terbium	< 20	< 20	< 20
Dysprosium	50	10	10
Holmium	10	< 10	< 10
Erbium	20	10	10
Thulium	< 10	< 10	< 10
Ytterbium	7	5	5
Lutetium	< 10	< 10	< 10
Yttrium	~ 500	~ 500	~ 500

At present the only mine in the area producing green fluorite in any quantity is Redburn Mine, working the Red Vein, north of Rookhope Village, which also produces purple and colourless varieties. An approximate spectrographic determination of individual rare earths in these three varieties (Table 2) has shown that, in this mine too, it is the green variety that is rich in europium. This variety also exhibits an intense bluish fluorescence similar to that observed from the green variety from Stotfield Burn Mine, which also works the Red Vein, but south of Rookhope Village.

The extent of variation of the europium content of green fluorite is not known, but analyses obtained so far have indicated that a general level of 100 p.p.m. can be expected in the material from the Red Vein. Any process designed exclusively to produce europium from this material is likely to prove economically unattractive. A process, designed to recover a rare-earth concentrate as a by-product from plants utilizing this fluorite for the production of fluorine chemicals, would give a material rich in europium and poor in cerium that would be a convenient starting material for the extraction of europium.

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### Graptolites of the *Monograptus hercynicus* Type recorded from Malaya

I RECORDED Lower Silurian graptolites from Malaya 8 years ago<sup>1</sup>. Since their discovery during the geological mapping of the Langkawi Islands large areas of north-west and central Malaya have been systematically mapped on a 1 in. : 1 mile scale by the Geological Survey of West Malaysia. As a result of this work more than 100 further graptolite localities have been found in Kedah, Perak and Pahang States, and among the graptolites found have been those of *Monograptus hercynicus* type, which has previously been described only from Central Europe, the Yukon Territory and Australia. I am now examining collections of these fossils.

The majority of the graptolites are within the range Llandoveryan to Wenlockian, but it is very interesting that material from at least seven localities contains recognizable monograptids of the *M. hercynicus* type. Although these latter fossils are usually poorly preserved there is enough detail to permit comparison with the following described species: *Monograptus hemiodon* Jaeger, *Monograptus praehercynicus* Jaeger, *Monograptus uniformis* Pribyl and *Monograptus yukonensis* Jackson and Lenz. Two new species of the group are also present.

The descriptive palaeontology, stratigraphical significance and distribution of *M. hercynicus* type monograptids, which are among the youngest graptolites to occur, have only recently been made known by the work of Jaeger<sup>2,3</sup>. Although Perner<sup>4</sup> in 1899 described two species from beds in Bohemia which he considered might be of Devonian age, it remained for Jaeger to show conclusively that these graptolites were confined to beds of post-Ludlow age. The researches of Jaeger in Central Europe and of Jackson and Lenz<sup>5</sup> in the Yukon Territory of Canada, together with other instances from North America and Central Asia quoted by Berry<sup>6</sup>, indicate that shelly faunas which are either intimately or closely associated with these graptolites are of early Devonian aspect and more precisely they allow correlation of the host strata with the Gedinian and part of the Siegenian Stages.

In Malaya the suggested Lower Devonian chronology is corroborated by the frequent association of *M. hercynicus* type monograptids with daeryoconarid tentaculites. Several forms of these pelagic organisms can be distinguished. Bouček (personal communication) has identified the ribbed species *Tentaculites matlockianus* Chapman and the smooth shell *Styliolina fissurella* (Hall). *T. matlockianus* is said to be very close, if not identical, to *Novakia acuarina* Richter of middle Lower Devonian age and the styliolinid is indicative of a Lower to Middle Devonian age.

The intention of this preliminary communication is to put on record a further global occurrence of monograptids of the *M. hercynicus* group. It is also gratifying to note that these and the abundance of the earlier Silurian graptolites have assisted greatly in determining the lower and middle Palaeozoic age of strata covering more than 2,000 square miles of Malaya which were previously classed as Late Palaeozoic and Early Mesozoic.

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## PHYSICS

### Atmospheric Electric Field as a Possible Cause of Radio Pulses from Extensive Air Showers

VARIOUS mechanisms have been proposed for the origin of the radio pulses which are found<sup>1-6</sup> to be associated with large cosmic-ray extensive air showers. While these proposed mechanisms differ in detail, they all depend essentially either on enhanced Cherenkov radiation from an electron excess in the shower<sup>7</sup> or on charge separation effects in the Earth's magnetic field<sup>8</sup>; these effects are believed to contribute about equally to the observed pulses<sup>2</sup>. It appears that charge separation by the electric field of the lower atmosphere may also play a significant part under certain atmospheric conditions.

The existence of a vertical electric potential gradient in the atmosphere has long been known; the surface of the Earth carries a negative charge and the upper layers of the air a positive one. At altitudes of up to a few kilometres the average fine-weather field is  $\sim 100$  V m<sup>-1</sup> and above this height the gradient falls steadily to a value  $\sim 1$  V m<sup>-1</sup> at an altitude of  $\sim 20$  km. The precise values vary with locality, time, season and weather. Under disturbed weather conditions the vertical gradient may be much higher. Gradients of  $\sim 500$  V m<sup>-1</sup> occur



near the ground in drizzle, while under thunderclouds the gradient may reach  $\sim 50,000 \text{ V m}^{-1}$  (see, for example, Schonland<sup>9</sup>). Reversals of the field direction with varying altitude also occur and the field may have considerable horizontal components<sup>10</sup>.

The particles of an extensive air shower will suffer a systematic transverse separation according to their charge under the influence of the component of the potential gradient which is perpendicular to the shower arrival direction. This results in the formation of a dipole and a transverse current which are exactly analogous to those caused by the magnetic separation effect discussed by Kahn and Lerche<sup>6</sup>. The transverse acceleration of a positive or negative electron moving at relativistic speed is  $eE_T/\gamma m_0$ , where  $e$  is the electron charge,  $\gamma m_0$  is the electron mass,  $m_0$  is the electron rest mass and  $E_T$  is the component of the field perpendicular to the shower motion. The resultant separation between a pair produced at one point is

$$\Delta_{TE} = \frac{eE_T}{\gamma m_0} \tau^2$$

where  $\tau$ , the particle lifetime, is  $\sim$  one radiation length/ $c$ , that is,  $\sim 10^{-6}$  sec near sea level;  $c$  is the velocity of light. This compares with a separation due to the Earth's magnetic field

$$\Delta_{TM} = \frac{eH_T c}{\gamma m_0} \tau^2$$

where  $H_T$  is the component of the Earth's magnetic field perpendicular to the shower motion. For the transverse separations due to the electric and magnetic fields to be comparable we find  $E_T \sim H_T c$  which with  $H_T \sim 0.2$  oersteds gives  $E_T \sim 6 \times 10^9 \text{ e.m.u./cm}$  or  $6 \times 10^3 \text{ V m}^{-1}$ . This is much higher than the normal fair weather field of the atmosphere but is frequently attained in thundery conditions when such fields may extend over many ( $\sim 10^3$ ) cubic kilometres of space. It appears then that under some atmospheric conditions the yield of radio emission from extensive air showers could be considerably enhanced by the transverse separation of charge caused by the atmospheric electric field.

We now consider the charge separation produced along the direction of shower motion by the component of the potential gradient,  $E_p$ , parallel to this direction; there is no magnetic analogy to this case. This separation

$$\Delta_{PE} = \frac{eE_p}{\gamma^3 m_0} \tau^2$$

Taking  $\gamma = 40$  (20 MeV electrons at the Cherenkov threshold for air),  $e/m_0 = 1.8 \times 10^{11} \text{ coulombs kg}^{-1}$  and  $E_p \sim 100 \text{ V m}^{-1}$  we find  $\Delta_{PE} \sim 3 \times 10^{-4} \text{ m}$ . Disregarding charge excess effects, the shower can now be approximated as an array of dipoles of this length, oriented with their axes parallel to the motion of the shower, the dipoles being distributed within the shower disk  $\sim 100 \text{ m}$  across and  $\sim 2 \text{ m}$  thick.

For wavelengths where  $\lambda \gg \Delta_{PE}$  we may write, following Jelley<sup>11</sup>, that the ratio of the yield of energy per unit bandwidth per unit time of dipole Cherenkov radiation,  $W_{PE}$ , to normal Cherenkov radiation,  $W_0$ , is given by

$$\frac{W_{PE}}{W_0} \sim \frac{p^2 \omega^2}{e^2 v^2} \sim \frac{\Delta_{PE}^2 \omega^2}{c^2} \sim \frac{4\pi^2 \Delta_{PE}^2}{\lambda^2}$$

where  $v$  is the particle velocity ( $\sim c$ ),  $\omega$  is the angular frequency of the radiation of wavelength  $\lambda$ , and  $p$  is the dipole moment.

Considering wavelengths where  $\lambda > 3 \text{ m}$ , all the dipoles will be radiating in phase for a suitably placed observer, because they are confined within a disk  $\sim 2 \text{ m}$  thick. The ratio of the dipole Cherenkov yield to the normal Cherenkov yield for the whole shower containing  $N$  particles is

therefore  $\frac{W_{PE}}{W_0} \sim \frac{N^2}{4} : N$ , that is, an enhancement of

$\sim \pi^2 \Delta_{PE}^2 N / \lambda^2$  occurs at metric wavelengths as a result of the longitudinal electric dipole effect. This compares with an enhancement  $\sim \epsilon^2 N$  for the charge excess mechanism, where  $\epsilon$  is the fractional charge excess. Putting  $\Delta_{PE} \sim 3 \times 10^{-4} \text{ m}$ , we find that the enhancement arising from the dipole mechanism for  $\lambda = 3 \text{ m}$  is  $\sim 10^{-7} N$  compared with  $\sim 10^{-1} N$  for the charge excess mechanism ( $\epsilon \sim 0.3$  assumed for 20 MeV electrons). Even with fields as high as  $10^4 \text{ V m}^{-1}$  ( $\Delta_{PE} \sim 3 \times 10^{-2} \text{ m}$ ) the dipole enhancement only rises to  $10^{-3} N$ .

We therefore conclude that, of the two types of charge separation by the electric potential gradient of the atmosphere, only the transverse separation is capable of producing yields of radio emission from extensive air showers which are comparable with those produced by the geomagnetic charge separation and charge excess mechanism. Both the direction and the amount of the electrically produced charge separation will depend on the local atmospheric electric field along the shower track and thus the magnitude and polarization of the radio emission produced by the mechanism will vary widely with local climatic and other factors. This may reduce the usefulness of the observed intensity of radio emission as a measure of the size of the extensive air showers.

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### Small Angle Filtered Electron Diffraction from Growing Films

WE have developed a method for studying the structure of thin films during the earliest stages of growth. The method is based on small angle electron diffraction<sup>1-3</sup> and is more sensitive than *in situ* electron microscopy.

At early stages of film growth the resolving limit for *in situ* microscopy is set by lack of contrast<sup>4</sup>. When silver is deposited, to an average thickness of about 10 Å on carbon in good vacuum conditions, the resolving limit is 20 Å (ref. 5). At this stage the films consist of globular well developed islands. The elastically scattered small angle ( $s \sim 0.01 \text{ Å}^{-1}$ ) intensity profile is proportional to

$$I(s) = N \bar{n}^2 f^2(s) \exp \left( -\frac{4}{3} \pi^2 s^2 \bar{R}_g^2 \right) \left[ 1 + \frac{\sigma_n^2}{\bar{n}^2} - \frac{P J_1(2\pi s r_0)}{2\pi s r_0} \right] \quad (1)$$

where  $s = 2 \sin \frac{1}{2} \beta / \lambda$ ,  $N$  is the number of islands irradiated,  $\bar{n}$  is the mean number of atoms per island,  $\bar{R}_g$  is the mean radius of gyration of an island and  $\sigma_n^2$  is the variance of  $n$ .

If the number of island centroids lying between  $r$  and  $r + dr$  from an arbitrary origin is  $2\pi r \rho(r) dr$ , then  $\rho(r)$  is found to be nearly a step function  $\rho(r) = 0$ ,  $r < r_0$ , and  $\rho(r) = \rho_0$ ,  $r \geq r_0$ ; the dimensionless parameter  $P = 2\pi r_0^2 \rho_0$ .

Equation (1) applies only to the scattering from the growing film; scattering from the substrate adds a profile of its own, measured before deposition is begun. Conditions are of course kinematic.

Recordings made during the growth of a silver film on to carbon, inside a scanning electron diffraction system, are shown in Fig. 1 with points calculated using equation (1). One difficulty is that the intensity profiles can be approximated by a cubic equation, whereas equation (1) contains five unknown parameters  $N\bar{n}^2$ ,  $\bar{R}_g$ ,  $\sigma_n$ ,  $\rho_0$  and  $r_0$ . It is found by trial in computer curve-fitting that the uncertainties in  $(\sigma_n/\bar{n})^2$  and  $P$  do not greatly affect the values determined for  $\bar{R}_g$  and  $r_0$ . The earliest stage of film growth for which useful results have so far been obtained is when the film's average thickness was 0.75 Å when  $\bar{R}_g = 8.3 \pm 1$  Å and  $r_0 = 33 \pm 8$  Å. The accuracy of the method has been checked by comparing the values obtained for  $\bar{R}_g$  and  $r_0$  for much thicker films, about 10 Å average, with values obtained from electron micrographs.

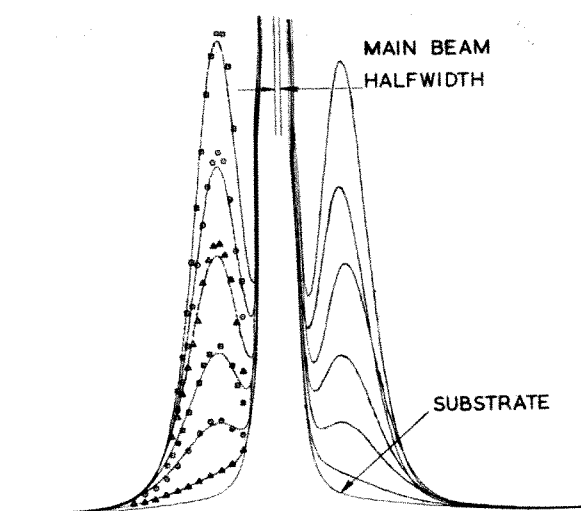


Fig. 1. Growth of silver on to carbon. The evaporation proceeds for 15 sec and is then stopped by a shutter while the intensity profile is recorded, and so on. Successive increments of average film thickness are 0.75 Å. 30 keV electrons, filtered 0.2 eV. The plotted points were obtained by curve fitting to equation 1.

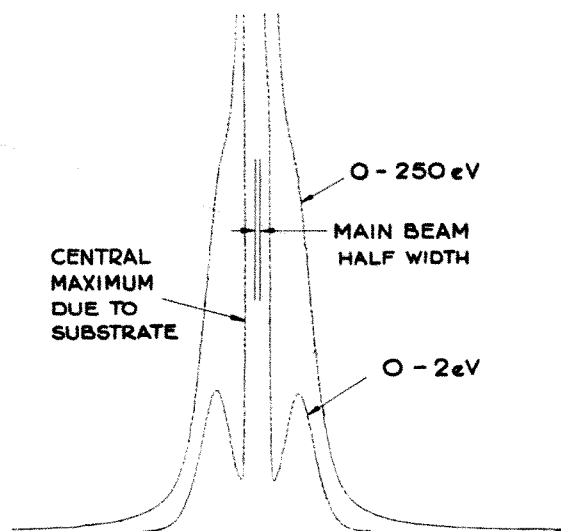


Fig. 2. Filtered and unfiltered diffraction profiles of 5 Å silver film on carbon substrate (200 Å). Filtered profile consists largely of elastically scattered electrons, unfiltered profile contains up to 250 eV loss electrons.

Previous work<sup>3</sup> has not emphasized the importance of using well filtered electrons or has ignored it<sup>1,2</sup>. A profile consisting largely of elastically scattered electrons compared with a virtually unfiltered profile containing electrons which have lost up to 250 eV energy is given in Fig. 2. Clearly if quantitative deductions are to be made well filtered data must be used. An earlier attempt<sup>3</sup> at the derivation of equation (1) omitted the exponential factor; as a consequence the theoretical profiles bore scant resemblance to the observed ones; moreover a different distribution function was used<sup>3</sup> which required well resolved micrographs for determining it and the assumption of spherical islands.

The advantage of this method of studying film growth is that statistical data may be obtained for films so thin that nothing quantitative can be obtained by *in situ* electron microscopy. The high sensitivity is explained by equation (1), which shows that in the small angle region  $I(s)$  is proportional to  $\bar{n}^2$ , while in electron microscopy the contrast is related to  $\bar{n}$  and in normal electron diffraction the intensity is proportional to  $\bar{n}$ .

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### Quasi-linear Dispersion Spherical Fabry-Perot Interferograms for Giant Pulse Laser Spectroscopy

SPHERICAL interferometer fringes have previously been considered unsuitable for the production of a spectrum extending over several orders of the spectral range, chiefly because when the spherical mirrors are separated by exactly their radius of curvature<sup>1,2</sup>, the order of interference of the fringe pattern decreases as the fourth power of the radius. (With a plane Fabry-Perot instrument, the order of interference varies with the square of the radius.) In practice, however, spherical interferometers have been used successfully to measure the time resolved spectra of ruby laser relaxation oscillations<sup>3</sup> and, more recently, for the direct spectral detection of off-axis modes in giant pulse ruby lasers<sup>4</sup> and an intensity dependent frequency shift<sup>5</sup>. In circumstances like these the spherical instrument has the advantage of the high intensity of the illumination of the detector and the fact that the plates are always well adjusted.

The fringes visible in the spherical interferometer are the spherical aberration diffraction pattern of the mirrors, sharpened by multiple reflexions. It is for this reason that they can only be seen when the instrument is illuminated by a monochromatic axial point source or by the equivalent laser wavefront. It is well known that the spherical aberration diffraction patterns of optical elements are radically altered<sup>6-8</sup> by the addition of a defocusing term.

The structure of the interference fringe pattern for a particular mirror separation is most easily calculated by considering the optical path difference between successively reflected rays as a function of the ray zonal radius. Fig. 1 represents an incoming plane wavefront, represented by the rays *AB*, *CD*, *EF*, passing through an interferometer consisting of two high reflectivity spherical mirrors of equal radii of curvature (*R*) and separated by the distance *EF*. *AB* and *CD* are parallel to and equidistant from the axial ray *EF* passing through



the centres of curvature of the symmetrically arranged mirrors.  $ABCDAB$  represents, to a first approximation, the path of a reflected ray and it is easily shown<sup>1,2</sup> that the total optical path difference between this reflected ray and the directly transmitted ray  $AB$  has the value

$$4(R + \rho) - (r^4/R^3) - (4\rho r^2/R^2)$$

where  $AC = BD = 2r$  and  $EF = R + \rho$ , and where  $\rho$  can be a positive or negative distance. The condition for constructive interference is then given by

$$4(R + \rho) - (r^4/R^3) - (4\rho r^2/R^2) = m\lambda \quad (1)$$

so that the relative positions of the fringes in the diffraction pattern are determined by the values of  $\rho$  and the zonal radius  $r$ .

If a "fringe number"  $p$  is defined by the relation

$$p\lambda = (r^4/R^3) + (4\rho r^2/R^2) \quad (2)$$

then  $p$  is zero for the confocal case  $\rho = 0$  when  $r = 0$ , corresponding to the axial ray  $EF$ . For the non-confocal arrangement, the fringe number is also zero, to fifth order terms in  $(r/R)$  and first order terms in  $(\rho r^2/R^2)$ , when

$$r^2 + 4\rho R = 0 \quad (3)$$

Differentiation and rearrangement of (1) give  $d^2\lambda/dr^2 = 0$  and  $d\lambda/dr$  constant for

$$r^2 + (2/3)\rho R = 0 \quad (4)$$

Choosing a suitable negative value for  $\rho$  to satisfy relation (4), a linear wavenumber dispersion with zonal radius, and hence with fringe radius, is obtained in the region of  $r = (-2\rho R/3)^{1/2}$ .

Adjusting relations (1), (3) and (4) to take into account the diverging lens effect of the plano-concave spherical mirrors (10 cm radius of curvature) used, and putting  $\rho = 150\mu$ , then  $r = 2.7$  mm—a convenient input zonal radius for a typical 1 cm diameter giant pulse laser beam. The linear dispersion position in the fringe field then corresponds to an output zonal radius  $r_0 = 4$  mm for this 10 cm interferometer, well within the 2.5 cm diameter mirror apertures. (The fringe number  $p$  is zero at  $r_0 = 7$  mm.) The resulting fringe pattern is illustrated in Fig. 2, showing an interferogram of three axial modes of a helium-neon gas laser ( $\lambda 6328$ ), the neighbouring axial modes of which are separated by 175 Mc/s (cavity length 85 cm). The second and third sets of three fringes, counting outwards from the centre of the pattern, are located in the linear dispersion region. Calculation<sup>3</sup> shows that the dispersion should be linear there to within 2 per cent over a frequency spread of 500 Mc/s, and this is verified by measurement of the interferogram. (Whatever the magnitude of the lens effect of the mirrors, the fractional change in the dispersion ( $d\lambda/dr$ ), for a fractional change  $kr$  in the zonal radius  $r$ , is given by

$$\Delta(d\lambda/dr)/(d\lambda/dr) = k^2(k+3)/2.)$$

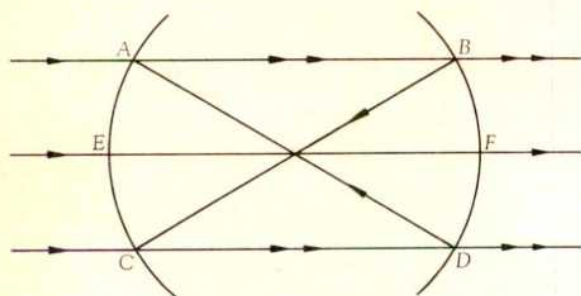


Fig. 1. Ray paths through spherical interferometer.

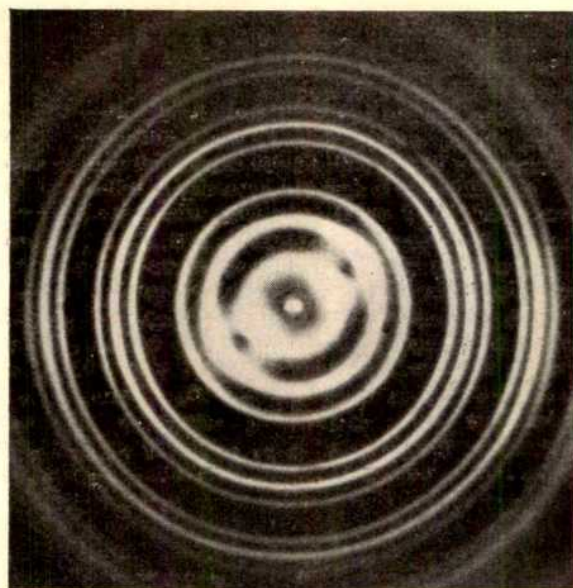


Fig. 2. Three axial modes of 85 cm gas laser ( $\lambda 6328$ ).

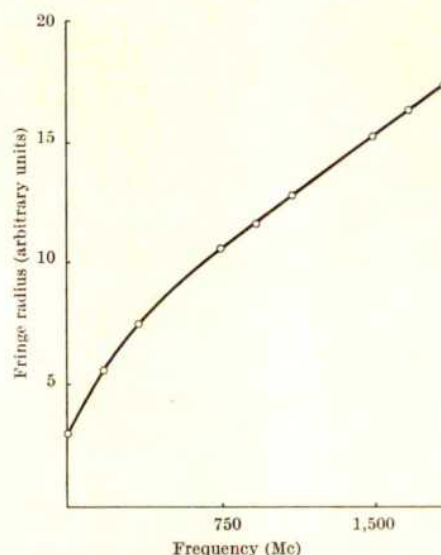


Fig. 3. Dispersion calibration curve of 10 cm interferometer for  $\rho = -150\mu$ , derived from Fig. 2.

As can be seen from the dispersion calibration curve (Fig. 3) obtained from microdensitometer traces of the etalon interferogram, linearity is, in fact, largely preserved over a spectral spread considerably greater than the free spectral range of the interferometer (750 Mc/s). For image tube and multi-channel photoelectric detection of laser spectra, particularly if ultra-fast time resolution of giant pulses is required, matching of the interferogram to the spatial resolution of the detector<sup>10</sup> is straightforward and, more important, quantitative interpretation of small frequency shifts is possible.

Several spherical Fabry-Perot interferometers of various separations are now being evaluated in this laboratory for ruby and neodymium giant pulse laser diagnosis<sup>9</sup>. The simple optical path difference calculation described here has been found adequate in determining the value of the optimum separation so as to place the linear dispersion region in the most useful part of the interferogram, usually at a zonal radius corresponding to half the exit aperture radius. (Various laser beam diameters can be

dealt with for this value of  $\rho$  by using the appropriate degree of telescopic magnification or demagnification of the beam aperture.) To determine the instrumental profile and finesse more precisely a computer wavefront calculation is being carried out, and the effects of coherent and incoherent illumination are also being studied.

The interferogram of Fig. 2 was photographed by Mr M. E. Engwell and the calibration curve was derived by Mr A. Durrant. This work is supported by the Science Research Council.

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## Relativistic Equations of State

WE offer a new approach to an old<sup>1</sup> question: "Given a material medium with its equation of state, what conditions must be satisfied by that equation of state if the existence of such matter is to be consistent with the principles of special relativity?" Our object is to determine one such condition. Our approach has the advantage that it is not necessary to consider the detailed structure of the matter, but it has the disadvantage that it is not applicable in its present form to the most interesting case—that of nuclear matter at high densities.

The two traditional approaches to this question may be summarized as follows:

(1) It has been claimed<sup>2</sup> that the assumption of causality\* implies that  $dp/d\rho \leq 1$ , where  $p$  is the pressure and  $\rho$  is the mass density of a fluid. (We have set  $c=1$ .) This inequality may be shown to hold rigorously for a perfect fluid characterized only by the two parameters  $p$  and  $\rho$ , with conserved stress-energy  $T_{\alpha\beta} = (p + \rho)\xi_\alpha\xi_\beta + p g_{\alpha\beta}$ . (Here,  $\xi_\alpha$  is the unit 4-velocity of a fluid element at each point.) Real matter, however, is more complicated. One must also consider temperature, composition and possibly other variables—the equation of state may, for example, depend on the past history of the matter. When these detailed properties of the matter are taken into account, no simple conclusion such as  $dp/d\rho \leq 1$  can be drawn.

(2) It has been claimed<sup>3</sup> that the "reasonable physical assumption" that the behaviour of high-energy particles is similar to that of photons implies that  $p \leq (1/3)\rho$  for all matter. This inequality is in fact satisfied for the case of a relativistic gas of non-interacting particles<sup>4</sup>. There is, however, no compelling reason why the equation of state for a gas of non-interacting particles should approximate that for matter (see, for example, the discussion of Zeldovich<sup>5</sup>).

Our approach may be illustrated by a simple example based on a spring characterized by the following three parameters: unstressed length =  $L$ ; mass per unit length =  $\rho$ ; spring constant  $\times$  length =  $p$ .

We assume energy conservation and causality. Accelerate the spring to a velocity  $v < 1$  so that, during the acceleration process, the length of the spring in its own rest frame is kept constant. We wish to entrap the spring

in a cylindrical container of length  $b$ , closed at the rear and with a sliding door at the front. At the moment the leading end of the spring strikes the rear of the container, a light signal is sent along the spring. If the

length  $b$  is chosen to be  $L \frac{\sqrt{1-v^2}}{1+v}$ , then this signal reaches

the trailing end of the spring just as this end enters the container. The sliding door is then shut. Thus the assumption of causality, which entails that the information that the leading end of the spring has struck the container does not reach the trailing end of the spring faster than light, determines the size of the container necessary to entrap the spring. After the spring has settled down and cooled off, the container is opened and the spring is allowed to expand. The energy released in this expansion is carefully collected. The requirement that the energy necessary to accelerate the spring should be greater than or equal to that released by the expansion is

$$\rho \left( \frac{1}{\sqrt{1-v^2}} - 1 \right) \geq \frac{1}{2} p \left( 1 - \frac{\sqrt{1-v^2}}{1+v} \right)^2$$

This inequality will hold for all  $v < 1$  if, and only if,

$$\rho \geq p$$

One would hope to apply a similar argument to nuclear matter at high densities. There is, however, a serious difficulty: it is necessary to introduce a second container to hold the matter under high pressure during the acceleration process. In order to obtain the inequality  $p \leq \rho$ , it is now necessary to find a *gedanken* procedure to recover the energy involved in accelerating this second container.

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## States in Neon-21 near 3 MeV

PROPERTIES of low lying levels of neon-21 have been the subject of extensive studies<sup>1-8</sup> in recent years and the strong coupling collective model with K band mixing has been used for their interpretation<sup>2,4,6,9,10</sup>. Two states, which are predominantly members of  $K=1/2$  and  $K=3/2$  rotational bands and have  $J^\pi=1/2^+$  and  $9/2^+$  respectively, are predicted<sup>9,10</sup> near to 3 MeV excitation in neon-21. In addition, Freeman<sup>10</sup> has predicted a  $J^\pi=5/2^+$  state near 3 MeV and a  $3/2^+$  state near 3.5 MeV, while Davidson<sup>9</sup> shows three states of  $3/2^+$ ,  $1/2^+$  and  $5/2^+$  between 3.5 and 4 MeV. In this calculation an additional  $K=1/2$  band, based on Nilsson orbit 6, was included.

Recent high-resolution  $\gamma$ -ray studies<sup>7,8</sup>, using the reactions  $^{18}\text{O}(\alpha, n\gamma)^{21}\text{Ne}$  and  $^{20}\text{Ne}(d, p\gamma)^{21}\text{Ne}$ , have established a doublet in neon-21 at 2.791 and 2.797 MeV. The latter state has been shown<sup>8,11</sup> to be  $J^\pi=1/2^+$  as the former is not populated in the reaction  $^{20}\text{Ne}(d, p\gamma)^{21}\text{Ne}$ .  $J^\pi=1/2^+$  was also suggested for the 2.791 MeV state by Pelte *et al.*<sup>4</sup> from an application of the  $(2J+1)$  rule to the averaged  $^{23}\text{Na}(d, \alpha)^{21}\text{Ne}$  cross-sections. He subsequently explained<sup>12</sup> the reduced M1 transition probabilities from the 2.791 state by accepting this assignment which was based on a hole in Nilsson orbit 6. Bent *et al.*<sup>6</sup> were unable to explain this hindrance on the assumption of  $5/2^+$ .

\* This implies that if initial data for any system are given on a space-like surface  $S$ , the state of the system at any point  $P$  of space-time is uniquely determined by the data on the intersection of  $S$  with the interior of the backward light cone of  $P$ .



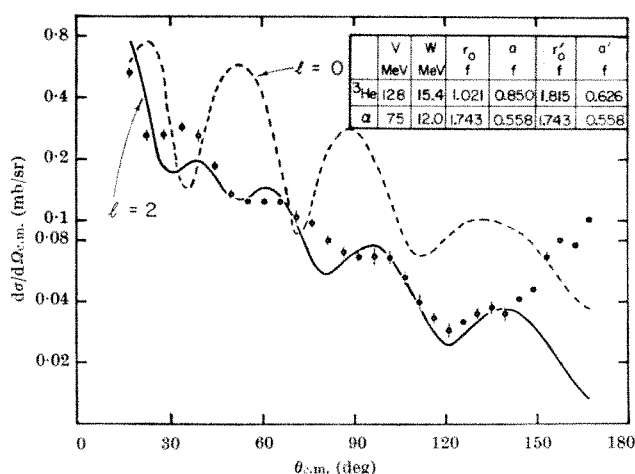


Fig. 1. Distorted wave  $l=0$  and  $l=2$  angular distribution fits to the 2.791/2.797 and 2.87 MeV "triplet" in the reaction  $^{22}\text{Ne}(^3\text{He}, \alpha)^{21}\text{Ne}$  at  $E(^3\text{He}) = 9.744$  MeV (lab.). The optical model parameters used are shown.

Thus it seemed likely that both members of the 2.80 MeV doublet were  $J^\pi = 1/2^+$  states. A more recent study<sup>13</sup> of  $\gamma$ - $\gamma$  and  $n$ - $\gamma$  angular correlations from the reaction  $^{18}\text{O}(\alpha, n\gamma)^{21}\text{Ne}$ , however, gives very strong evidence for an assignment of  $J = 3/2$  to the 2.791 MeV state. This experiment also confirms that  $J = 9/2$  for the state at 2.87 MeV. Thus the other possible spin allowed previously<sup>4-6</sup>, namely  $J = 5/2$ , can now be eliminated.

In the present experiment, a study of the reaction  $^{22}\text{Ne}(^3\text{He}, \alpha)^{21}\text{Ne}$  was undertaken to test the validity of the Nilsson model description of neon-21. The angular distribution shown in Fig. 1 corresponds to the unresolved group of the three states discussed here. It was analysed by the DWBA using the code DRC<sup>14</sup>. Fig. 1 shows the DWBA calculations for both  $l=0$  and  $l=2$  angular momentum transfers. The optical model potentials and cut-off radius are identical to those used by us in the analysis of the angular distributions for the ground state and first excited state of neon-21. The calculations clearly indicate an  $l=2$  transition, the contribution from an  $l=0$  transition being, apparently, negligible. This  $l=2$  transition indicates either a  $J^\pi = 3/2^+$  or  $5/2^+$  state. Thus the present experiment is consistent with the latest  $3/2$  assignment to the 2.791 MeV state<sup>13</sup> and, further, indicates that it is a positive parity state.

A similar measurement has been carried out at 15 MeV (C. M. Fou, personal communication) and preliminary analysis also indicates  $l=2$  for the transition.

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## CHEMISTRY

### Initiation of the Hydrogen Chlorine Reaction by Red Laser Light

DURING a recent discourse at the Royal Institution<sup>1</sup>, I wished to illustrate the effect of  $Q$  switching on the peak intensity of light emitted by a pulsed ruby laser and also to demonstrate the occurrence of intensity dependent two quantum processes. This was achieved in a simple and striking manner by using the best known of all photochemical demonstrations, the hydrogen chlorine explosion.

When a pulse from a ruby laser, of energy 30J, was projected across the theatre and focused onto a vessel containing hydrogen and chlorine at a total pressure of 1 atm., no reaction was observable, nor was any reaction expected because chlorine is transparent to the red light of the ruby laser which emits at 6943 Å. When the experiment was repeated with no change in the arrangement except for the interposition of a cell containing cryptocyanine dye between the laser rod and the mirror, the characteristic explosion of hydrogen and chlorine occurred.

The cryptocyanine  $Q$  switch reduces the total energy output of the laser pulse by a factor of ten and the effect must therefore be attributed to the increased intensity of the 20 nsec pulse which is emitted under  $Q$  switched conditions. The process of two quantum absorption which is proportional to the square of the intensity will therefore be greatly increased when the  $Q$  switch is inserted.

The two quantum process is equivalent to absorption of a quantum of wavelength 3476 Å, which is near to the first absorption maximum of molecular chlorine and is known to lead to dissociation. Although similar two quantum processes have been observed in systems where an intermediate energy level is available<sup>2</sup>, the chlorine system seems to be a clear case of a gaseous photochemical reaction induced by true frequency doubling.

I thank Mr M. Topp for invaluable help with the experiments.

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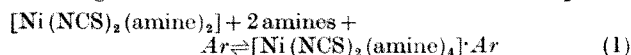
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### Purification of the Optical Antipodes of $\alpha$ -Arylalkylamines by the Formation of Clathrates

THE classical methods for resolving amines such as  $\alpha$ -phenylethylamine<sup>1,2</sup>,  $\alpha$ -(*p*-tolyl) ethylamine<sup>3,4</sup>, or  $\alpha$ -phenylpropylamine<sup>4</sup> into their optical antipodes involve salt formation with a naturally occurring acid such as *d*-tartaric or *d*-camphoric acid. By these methods, one of the antipodes can usually be isolated in a pure state by successive crystallizations of the less soluble diastereoisomeric salt. The antipode remaining in the mother liquor, however, is always contaminated by the other and another resolving agent is necessary for further purification. Theilacker<sup>1</sup> has purified *d*- $\alpha$ -phenylethylamine by sulphate formation; we did not, however, obtain the same result with other  $\alpha$ -arylalkylamines. In most cases, the isolation of *d*-amines by these methods requires the use of expensive reagents such as *L*-malic acid.

We have now found that the optical isomers of most  $\alpha$ -arylalkylamines can be efficiently purified through clathrate formation by their co-ordination complex  $[\text{Ni}(\text{NCS})_2(\alpha\text{-arylalkylamine})_4]$ . It is known that complexes of this type are used to form clathrates with a wide variety of aromatic compounds<sup>5-7</sup>. These clathrates can be formed simply by mixing an aqueous solution of nickel

thiocyanate with the stoichiometric amount of amine in the presence of an aromatic compound. The clathrate then separates as a crystalline precipitate. Another method is to react the corresponding diamine complex with two additional molecules of amine according to the following reaction, where *Ar* is the aromatic compound



We have observed that when this reaction is performed with an excess of amine in which one of the antipodes predominates, the amine co-ordinated into the clathrate is preferentially racemic. This phenomenon may be illustrated as follows, *d* and *l* representing the dextro and laevo isomer of the amine respectively



Thus by reacting the correct amount of complex with the amine to be resolved, the antipode in excess of the racemic composition will remain in the liquid medium from which it can be readily isolated. To obtain this antipode in a pure state, it is obviously necessary that the whole of the other should be co-ordinated into the clathrate. The operating variables such as temperature, contact time, nature of the aromatic compound, etc., should therefore be adjusted in order that the reaction may go to completion. For example, the aromatic compound which will be selected should form the most stable clathrate. For the same reason it is advisable to use an amount of diamine complex somewhat in excess of the stoichiometric quantity with respect to the racemic amine to be removed.

In a typical experiment, 3.75 mmoles of the complex  $[\text{Ni}(\text{NCS})_2(\text{dl-}\alpha\text{-phenylethylamine})_2]$  was added to 30 ml. of *t*-butylbenzene containing 12.66 mmoles of  $\alpha$ -phenylethylamine enriched to 75 per cent in the *d*-isomer ( $[\alpha]_D = +20.15^\circ$ ). After stirring for 10 min at  $0^\circ \text{C}$ , the resulting clathrate was filtered off. The filtrate was diluted with 40 ml. of *n*-heptane in order to precipitate any dissolved complex which was removed by a second filtration. The unreacted amine was extracted from the filtrate with 3 ml. of 20 per cent sulphuric acid and then liberated by neutralization with 12 ml. of 20 per cent sodium hydroxide. Its specific rotation was found to be  $+38.10^\circ$  which corresponds to an optical purity of 97.3 per cent based on the value  $+40.3^\circ$  as stated in the literature for optically pure  $\alpha$ -phenylethylamine<sup>1</sup>.

It is noteworthy that the selective co-ordination of racemic amine as demonstrated by the preceding example can only be observed when clathration takes place. Thus by substituting *n*-heptane for *t*-butylbenzene in a similar experiment, the unreacted amine was only 64 per cent in the *d*-isomer.

Table 1. PURIFICATION OF *d*- $\alpha$ -ARYLALKYLAMINES BY THE FORMATION OF CLATHRATES

Amine	Clathrated compound	<i>d</i> -Antipode purity (per cent)	
		Initial	Final
$\alpha$ -Phenylpropylamine	Methylnaphthalene*	67.8	92.7†
$\alpha$ -( <i>m</i> -Chlorophenyl)ethylamine	<i>p</i> -Xylene	79.6	99.2‡
$\alpha$ -Phenylisohexylamine	<i>m</i> -Xylene	71.2	94.0§

\* Percentage of isomers:  $\alpha$ -, 57;  $\beta$ -, 43.

† Based on  $[\alpha]_D = 20.20^\circ$ , highest specific rotation found in the literature for the optically pure *d*-amine<sup>1</sup>.

‡ Based on  $[\alpha]_D = 26.35^\circ$ , highest specific rotation obtained for the *l*-amine by successive crystallizations of its *d*-tartrate.

§ Based on  $[\alpha]_D = 10.50^\circ$ , highest specific rotation obtained for the *l*-amine by successive crystallizations of its *d*-camphorate.

Table 1 shows some results obtained with other  $\alpha$ -arylalkylamines. In each case, the sample treated was the residue of the *l*-antipode isolation from racemic amine by modifications of classical methods using *d*-tartaric or *d*-camphoric acid as resolving agent. These results show that, by the present method, high optical purity can be reached in only one step even from rather impure samples. Obviously the same procedure may be applied to the purification of both antipodes. For instance, the impure *l*-antipode first crystallized as the salt of *d*-tartaric or *d*-camphoric acid may be purified in the same way. Thus

by combining the present method with a crude resolution effected by any conventional means, both antipodes can be isolated in pure form without resorting to numerous crystallization steps.

Clathrates resulting from this procedure can be thermally decomposed by reversing the reaction shown in equation (1), either by heating *in vacuo* or by stripping with a hot fluid at a temperature depending on the nature of the co-ordinated amine<sup>5</sup>. The diamine complex is thus regenerated and the liberated racemic amine may be further treated for optical resolution.

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## A New Series of Isoprenoid Isoalkanes in Crude Oils and Cretaceous Bituminous Shales

In recent studies on the origin of petroleum much emphasis has been laid on the structural relationship between compounds occurring in petroleum and their possible natural precursors. A series of compounds, now well known as the isoprenoid isoalkanes, has received much attention, partly because of their sometimes strikingly high concentrations, partly because of their possible relationship to phytol. The members of the 2,6,10-trimethyl- and 2,6,10,14-tetramethylalkane series with 14 (ref. 1), 15 (ref. 1), 16 (ref. 2), 18 (ref. 2), 19 (ref. 3), 20 (ref. 4), and 21 (ref. 2) carbon atoms have been reported. The member of the 2,6,10-trimethylalkane series with seventeen carbon atoms proved either to be absent or to occur at best in only very small amounts<sup>5</sup>.

Johns *et al.*<sup>6</sup> have reported the occurrence of series of isoalkanes (2-methylalkanes), ante-isoalkanes (3-methylalkanes) and *n*-alkylcyclohexanes in shales and oils of very different ages ( $2.7 \times 10^9 \text{ yr} - 3 \times 10^6 \text{ yr}$ ). They discuss the possible relationship of these compounds to presumed natural precursors.

During a study of the gasoline/kerosene-range hydrocarbons from marine bituminous shales of Cretaceous age from Italy, our attention was attracted by a number of conspicuous peaks in the capillary gas chromatograms. (The hydrocarbons were liberated by stripping the rocks with carbon dioxide at  $150^\circ \text{C}$  for 1 h, and collected above a 40 per cent potassium hydroxide solution.) Regularities in the gas chromatograms suggested two homologous series (see Fig. 1). Two-stage gas-liquid chromatography in series with a mass spectrometer<sup>7</sup> revealed that some of these peaks could be attributed to the series of isoprenoid 2,6,10-trimethylalkanes and others to a series of 2,6-dimethylalkanes. The member of the last series containing nine carbon atoms had already been reported in 1958 (ref. 8) and the member with ten carbon atoms has been isolated from petroleum by Mair *et al.*<sup>9</sup>. We identified the members with 9, 10, 11, 12, 13 and 14 carbon atoms.

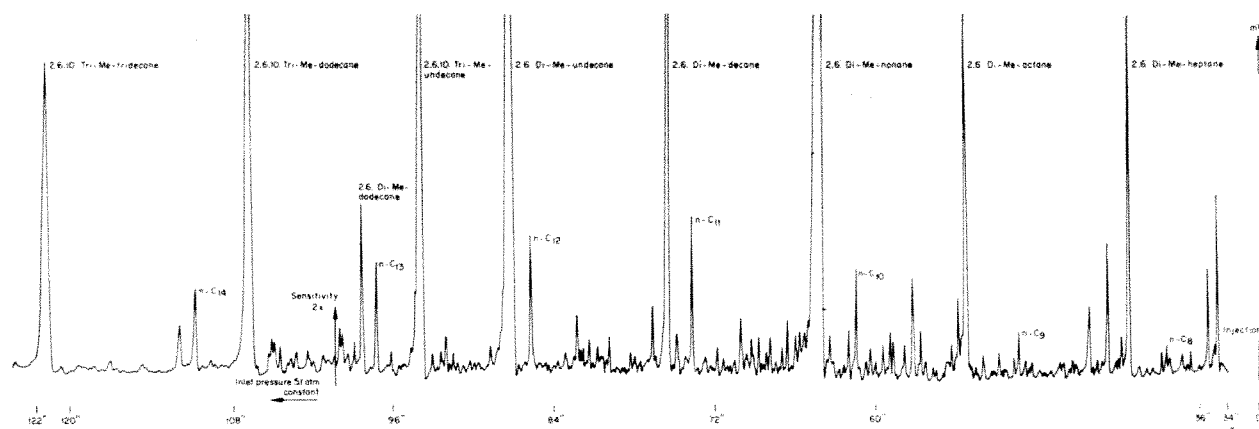


Fig. 1. Gas chromatogram of the volatiles liberated from a Cretaceous rock sample from Italy. 200 m copper capillary column coated with squalane. Starting temperature 30° C, rising by 37.5° C/h up to 140° C, then constant. Inlet pressure 1.5 atm, linearly increasing by about 1 atm./h up to 5.1 atm., then constant. Chart speed 24 in./h.

Our identification is based on data from gas-liquid chromatograms (see Figs. 1 and 2) and mass spectra (see Fig. 3).

The gas chromatograms reveal constant differences in the retention time for the *n*-alkanes as well as for the peaks under investigation. This indicates that these peaks may belong to a homologous series. The mass spectra show: (1) that the elemental compositions are  $C_nH_{2n+2}$ , where  $n=9-14$ ; (2) that the identity of the  $C_9$  compound is 2,6-dimethylheptane, as found by comparing the spectrum with American Petroleum Institute spectra; (3) relatively high intensities of fragments with eight carbon atoms and of fragments with ( $n-6$ ) carbon atoms, especially of  $C_{n-8}H_{2n-12}$ ; (4) relatively low intensities of fragments with more than eight carbon atoms.

These data strongly indicate a homologous series and that the structure of all compounds is  $C_6-C(C)-C_{n-8}$  containing more branches than the one already indicated.

Furthermore, we conclude from the fragmentation pattern that the  $C_6$  and  $C_{n-8}$  chains cannot contain quaternary carbon atoms and that the third or higher numbered carbon atoms in the chains cannot be tertiary carbons. Gas chromatographic retention data show that the unknown peaks cannot be ascribed to monomethyl-substituted alkanes. We can preclude 2,6, ( $n-1$ )-trimethyl-substituted alkanes containing 11, 12, 13 and 14 carbon atoms because of the relatively low intensities of the ( $n-1$ ) fragments. This is confirmed by the fact that the retention time of 2,6,10-trimethylundecane is shorter than that of the unknown  $C_{14}$ -compound.

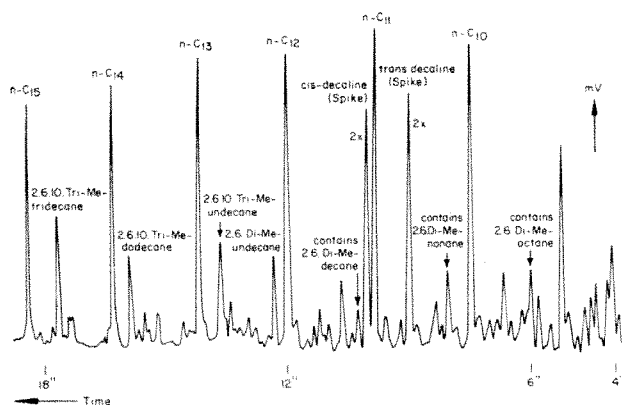


Fig. 2. Part of a gas chromatogram of saturates from paraffinic Nigerian crude oil. 100 ft. stainless steel capillary column coated with SE 30. Starting temperature 90° C, rising by 2° C/min up to 250° C. Chart speed 20 in./h.

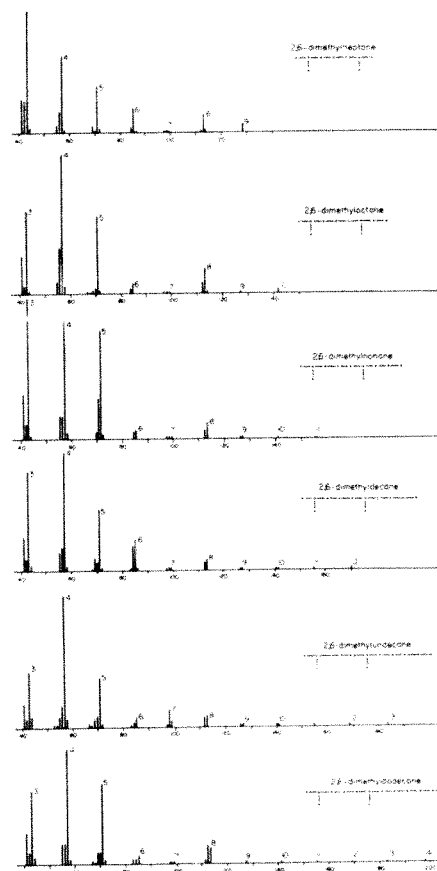


Fig. 3. Mass spectra of 2,6-dimethylalkanes.

From the definite identification of the  $C_9$ -compound as 2,6-dimethylheptane and from the impossibility of a trimethyl substitution already mentioned, we concluded that the homologous series must be the series of 2,6-dimethylalkanes.

The "isoprenoids" in our Cretaceous rock samples are remarkable from a quantitative point of view (see Fig. 1). First, the isoalkanes of the 2,6-dimethyl- and 2,6,10-trimethyl series form together the bulk of the fraction boiling up to about 285° C; the *n*-alkanes up to  $n-C_{15}$  are only present in strikingly small amounts. Second, 2,6-dimethyldecane and 2,6-dimethyldodecane are present in

significantly smaller concentrations than two other members of the same series, namely 2,6-dimethylnonane and 2,6-dimethylundecane.

Having thus been put on the track of the 2,6-dimethylalkane series, we also looked for these compounds in crude oil. In fact, we were able to identify them as a number of rather pronounced peaks in the gas chromatogram of the saturates of a paraffinic Nigerian crude oil (see Fig. 2). The presence of these compounds is not limited to this particular crude oil because these peaks are also quite common in the chromatograms of other paraffinic crude oils.

Because the structure of the compounds under investigation is very specific and the amounts in which they occur are often relatively high, they must be hydrocarbons that were either present as such in the source material or have been derived from their precursors by processes that left the original carbon skeleton, or at least part of it, unchanged. For the isoprenoids pristane and phytane, phytol has been suggested as a possible precursor<sup>3</sup>. For the lower members of the series, a genetic relationship to phytol is possible, although they might also be derived from compounds of lower molecular weight such as farnesol or farnesene<sup>6,10</sup>.

For the formation of the C<sub>17</sub> 2,6,10-trimethylalkane and the C<sub>12</sub> and C<sub>14</sub> 2,6-dimethylalkanes, not only a cleavage of a C-C bond of the chain is necessary, but also the splitting off of a methyl group<sup>5</sup>. This reaction is unlikely, however. The relatively low concentrations often found for these compounds might be seen to be in accordance with the lower probability of their formation from phytol.

On the other hand, other precursors for these and possibly other isoprenoid isoalkanes should also be looked for. Squalene shows a carbon skeleton from which many isoprenoid isoalkanes can be thought to have been formed. Another source might be found in the carotenoids. Both types of compound have been suggested earlier as precursors for petroleum hydrocarbons<sup>2,5,6,9,10</sup>. Van Hoeven *et al.*<sup>11</sup> have isolated two isoalkanes from an Australian oil, which they think to be 5,9- and 4,9-dimethyltetradecanes. They suggest that these isoalkanes may have originated from squalene. For example, from the carotenoids  $\beta$ -carotene has been suggested by Mair as the source of the compound 1,1,3-trimethylcyclohexane<sup>10</sup>. In this respect it is interesting to note the recent mention<sup>12</sup> of carotane in a Green River oil shale extract.

It seems quite reasonable to assume also that either squalene or the carotenoids or both have to be considered as the precursors of the 2,6-dimethylalkane series, described in this communication.

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## Synthetic Analogues of Polynucleotides: Interaction of a Polymeric Adenine Derivative with DNA

ANALOGUES of polynucleotides in which the naturally occurring bases are partly or completely replaced by analogues have been synthesized *in vivo* and by enzyme methods<sup>1,2</sup>. It appeared to us to be of interest, however, to synthesize polymers soluble in water which could be considered as analogues of polynucleotides in the possession of side chains of purines and/or pyrimidines but in which the backbone would not be composed of sugar residues linked by phosphodiester linkages. Previous work in this laboratory has already shown that a polymer of this type can be formed by copolymerizing 5'-O-acrylyl-uridine with acrylamide and that this polymer interacts with DNA in solution<sup>3</sup>; the polymer was obtained by polymerization of a suitable monomer containing a pyrimidine. We now report the synthesis of a similar type of polymer by the linking of a purine derivative and a pre-formed polymer, polyacrylic acid hydrazide<sup>4</sup>. The polymer was treated with the dialdehyde obtained by the periodate oxidation of adenosine, and gave a polymer containing adenine, a fraction of which was found to hybridize with denatured DNA in solution.

The polymer was prepared as follows. Polyethyl acrylate was prepared by polymerizing ethyl acrylate (3 ml.) in dry dioxan (30 ml.) at 60° C for 48 h with azobisisobutyronitrile (50 mg) as initiator. The resulting polymer was isolated and treated for 10 h in an oil bath at 140° C with 90 per cent hydrazine hydrate. The reaction solution was diluted with water, exhaustively dialysed against distilled water and freeze-dried to give polyacrylic acid hydrazide (2 g). (Found (after drying at 110° C *in vacuo*), C, 41.40; H, 6.90; N, 28.7 per cent. Calc. for C<sub>3</sub>H<sub>6</sub>N<sub>2</sub>O, C, 41.90; H, 7.02; N, 32.06 per cent.)

Sodium metaperiodate (126 mg) was added to a solution of adenosine (140 mg) in water (140 ml.). After 1 h at room temperature the solution was added to a solution of polyacrylic acid hydrazide (420 mg) in water (280 ml.) and left at room temperature for 24 h. The solution was dialysed against three changes of water. The resulting cloudy solution was centrifuged and the sediment freeze-dried to give an insoluble product (96 mg). A portion of the solution of the soluble polymer was freeze-dried to give a product which contained 13.4 per cent of adenine (determined on material dried at 110° C *in vacuo* by acid hydrolysis and separation of the adenine by paper chromatography). (Found, N, 32.0 per cent. Calc., N, 31.5 per cent.) The freeze-dried sample did not redissolve completely in water, so the bulk of the polymer (equivalent to 480 mg dry weight) was stored in solution. The proportion of insoluble to soluble polymer formed increased when the reaction was carried out in pH 4.6 and pH 7 buffers and when more adenosine dialdehyde was used.

The structure of this polymer has not been determined, but it would appear from the reaction of isonicotinic acid hydrazide with periodate oxidized RNA that the dialdehyde would react with only one hydrazide residue<sup>5</sup>. If this is so, analysis indicates that the polymer contains approximately one adenine residue for each 8 acrylic acid hydrazide residues. The molecular weight of the polymer was estimated by gel filtration on 'Sephadex'. About 55 per cent was excluded from both 'Sephadex G-200' and 'G-100'. The shape of the elution pattern indicated that both a high molecular weight fraction (> 2 × 10<sup>5</sup>) and a low molecular weight fraction (≤ 10<sup>5</sup>) were present. One explanation of this might be that cross linking had occurred because of the reaction of some dialdehyde residues with hydrazine residues on different polymer chains. A similar distribution of molecular weight was, however, observed with a polymer formed by the reaction of vanillin with polyacrylic acid hydrazide, so it is more probable that the effect is due to the molecular weight distribution of the polyacrylic acid hydrazide.



The interaction of the polymer with denatured DNA was carried out using the DNA-agar gel technique introduced by Bolton and McCarthy<sup>6</sup>, modified as previously described<sup>3</sup>. The results (Fig. 1) showed that 9 per cent of the polymer was retained by the DNA-agar at high salt concentration and was eluted at low salt concentration. When it was hydrolysed with alkali, this fraction gave adenine (corresponding to 75 per cent of the ultra-violet absorption of the fraction); DNA was not hydrolysed in these conditions. This showed that the fraction contained less than 15 per cent of DNA. Control experiments showed that none of the polymer was retained by agar alone. The fraction of the polymer eluted at low salt concentration was tested for its ability to hybridize

with denatured DNA in solution. The results (Fig. 2) showed that there was a maximum hypochromic effect of about 16 per cent after the mixtures had been kept at room temperature for 24 h; after standing for 4 h the hypochromic effect was about 7 per cent.

These results therefore extend those already obtained with the copolymer of 5'-O-acetyluridine and acrylamide. They show that certain polymers which contain non-nucleotide backbones and purine or pyrimidine side chains are able to interact with denatured DNA and might therefore have important effects in biological systems.

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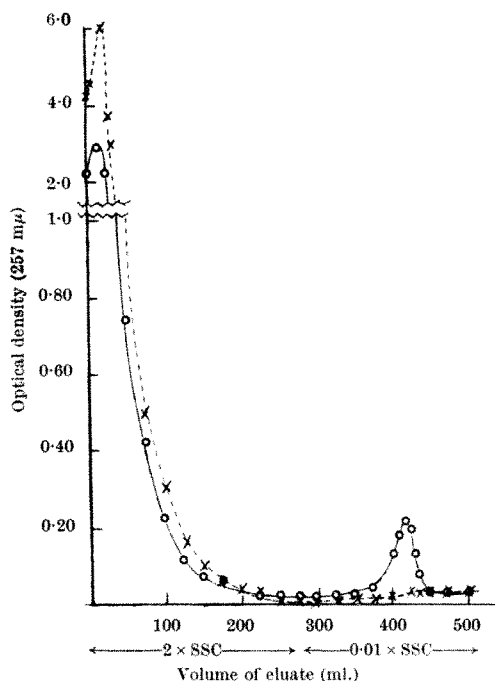


Fig. 1. Fractionation on DNA agar of the polymer soluble in water, formed by the reaction of polyacrylic acid hydrazide with adenosine dialdehyde. For details of the procedure see Cassidy and Jones<sup>3</sup>. O—O, Polymer on DNA-agar gel column; x—x, polymer on agar gel column.

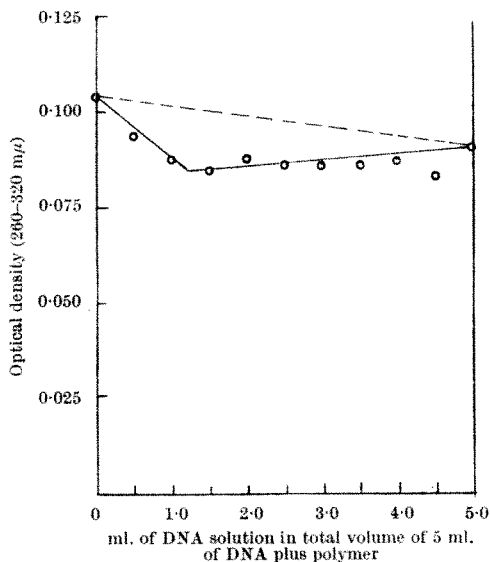


Fig. 2. Optical density of mixtures of solutions of denatured calf thymus DNA with solutions of the fraction of the adenine-containing polymer eluted from the DNA agar column with 0.01 x standard saline citrate (SSC). The solutions were prepared in 2 x SSC and the mixture kept at room temperature for 24 h. Optical densities were measured in 1 cm cells. O—O, Denatured DNA plus polymer; ----, optical densities of the mixtures if there were no hypochromic effects.

## Temperature Effects in Membrane Phenomena

THERMAL anomalies occurring over relatively narrow ranges have been described for many properties of water and aqueous solutions. In some cases only one such thermal anomaly has been claimed to exist between the freezing and the boiling point of water<sup>1-3</sup>. Drost-Hansen, however, has proposed<sup>9-13</sup> the existence of at least four anomalies in this temperature range, namely, near 15, 30, 45 and 60° C. Unfortunately, detailed data on the effects of temperature on membrane phenomena are notably lacking in the literature; however, anomalous temperature effects in some membrane properties have been reported. We propose that these anomalies reflect structural changes of the water in or adjacent to the membranes.

An interesting study of temperature effects was made on an exceedingly simple membrane by Rosano, Doby and Schulman<sup>14</sup> (see also Sears<sup>15</sup>). The rate of transfer of salt and water across a non-aqueous, liquid phase was studied at a number of temperatures. The liquid was *n*-butanol, separating two aqueous phases of different electrolyte concentrations. Fig. 1 shows the rate of transport of sodium chloride across the butanol membrane. There is an abrupt change in the rate between 30° and 39° C. The results obtained for the rate of transfer of potassium chloride (Fig. 2) very closely resemble the data for the

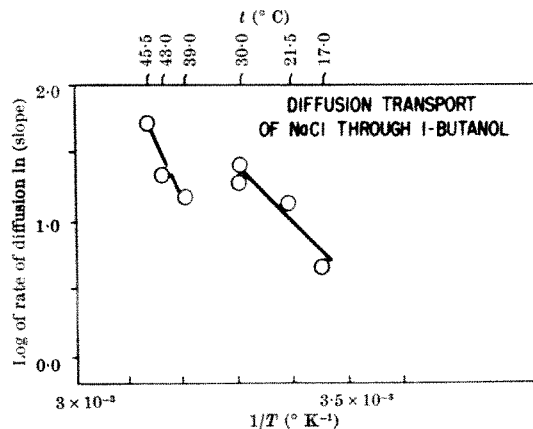


Fig. 1. Log rate of diffusion (equals log rate of change of potential) of sodium chloride across a 'butanol membrane' as a function of reciprocal, absolute temperature. Data points: Rosano, Doby and Schulman<sup>14</sup>. Curve redrawn by the present authors.

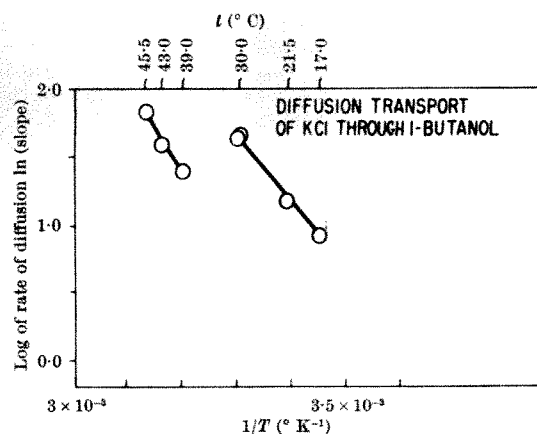


Fig. 2. Log rate of diffusion (equals log rate of change of potential) of potassium chloride across a "butanol membrane" as a function of reciprocal, absolute temperature. Data points: Rosano, Duby and Schulman<sup>14</sup>. Curve redrawn by the present authors.

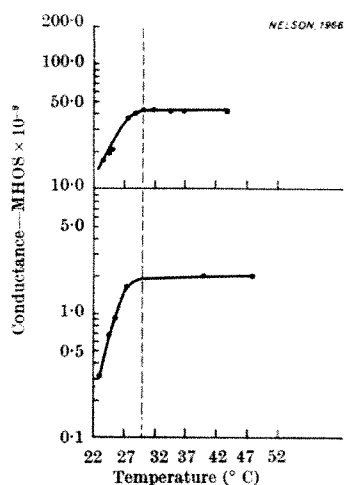


Fig. 3. Conductance of barium stearate multilayer membranes. Data by Nelson<sup>16</sup>.

transfer of sodium chloride, again revealing an abrupt change between 30° and 39° C.

A number of model membrane systems have been described<sup>16</sup> which relate to ionic transport in biologic systems, measured at closely spaced temperature intervals. A relatively wide range of membranes was used. This study disclosed the occurrence of several thermal anomalies. As an example, Fig. 3 shows the conductance of barium stearate multilayer membranes, suggesting a highly anomalous behaviour near 28°–32° C. It was speculated that this anomalous behaviour was the manifestation of a phase transition.

Thompson<sup>17</sup> studied the properties of a bimolecular phospholipid membrane and measured the resistance across this membrane at closely spaced temperature intervals. His results are shown in Fig. 4. One notices the large maximum near 23° C and a marked transition in the vicinity of 29°–30° C. We propose that the increase in resistance between 29° and 30° C is a reflexion of a change in water structure in or adjacent to the bimolecular membrane. Thompson independently suggested there may be a continuous aqueous phase across the membrane and that the anomalies may be caused by higher order phase transitions.

Finally, as an example of studies of temperature effects on a natural membrane, we mention two studies on the plasma membrane of the green alga, *Valonia*. Fig. 5 shows the results obtained by Blinks<sup>18</sup> on *Valonia macrophyssa*;

the ordinate is the potential difference across the membrane, the abscissa is the temperature. It is apparent that the values for the potential from 15° to 30° C are relatively constant, independent of temperature, while the E.M.F. increases both below 15° C and above 30° C. One of us carried out a similar study of a number of specimens of *V. utricularis* (collected in Tahiti by Professor Blinks). Again, we found potential across the cell to be relatively constant between 15° and 30° C (Fig. 6). An abrupt increase in potential was found above 30° C with an abrupt decrease below 15° C (the latter trend being opposite to the trend noted by Blinks using *V. macrophyssa*).

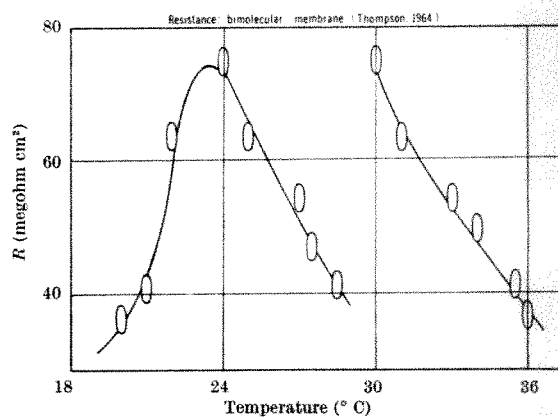


Fig. 4. Temperature dependence of the resistance of a phospholipid, bimolecular membrane. Data by Thompson<sup>17</sup>.

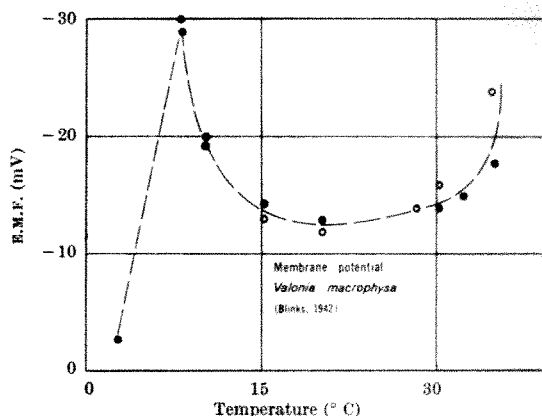


Fig. 5. Potential across plasma membrane, *Valonia macrophyssa*. ○, Increasing temperature; ●, decreasing temperature. Data by Blinks<sup>18</sup>.

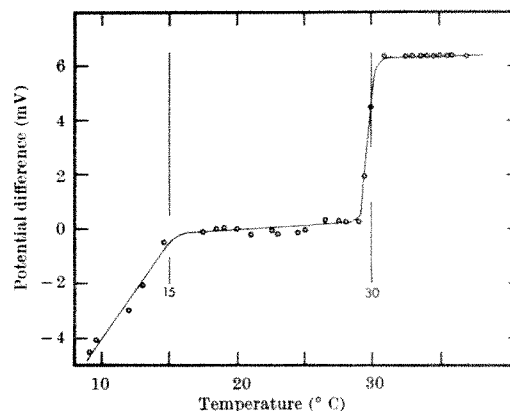


Fig. 6. Potential across the membrane of the alga *Valonia utricularis* as a function of temperature. Data by Thorhaug<sup>19</sup>.



While higher order phase transitions may be expected in some systems (such as some lipid systems), we propose that the general occurrence of anomalies in membrane properties near certain discrete temperatures is likely to reflect changes in the underlying water structures. That thermal anomalies have been observed with an *n*-butyl alcohol liquid membrane, cellulosic type membranes, bimolecular phospholipid membranes, and the plasma membrane of a living cell, suggest that these anomalies are caused by some common factor in these systems rather than by the specific effects of the matrix material. Because water is common to all the membrane systems enumerated above, and because the anomalies often occur at or near the temperatures for which the anomalies occur in the properties of bulk water and aqueous solutions, it is reasonable to suggest that the anomalies observed in such membrane systems are caused by structural changes in the water associated with the membranes. For further discussion of some of these aspects, see the articles by the senior author, in particular, regarding the observations made by Trapeznikov on palmitic acid monolayers and by Steinert and Haase on the properties of cellulose membranes.

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## MOLECULAR STRUCTURE

### Super-helical Model for Nucleohistone

DNA is combined, as nucleohistone, with histone proteins in the chromosomes and nuclei of all cells except bacteria. It has been suggested that histones are involved in regulating gene function<sup>1,2</sup>. The molecular structure of nucleohistone is basic to the structure of chromosomes. X-ray diffraction<sup>3</sup>, and physical chemistry<sup>4,5</sup> studies, suggest a coiled molecular structure for nucleohistone which is compatible with results from electron microscopy<sup>6-8</sup>. This communication discusses a coiled-coil structure and new experimental evidence that is compatible with it.

X-ray diffraction patterns from fibres of nucleohistone at high relative humidity (about 92-98 per cent) consist of three groups of reflexions (Fig. 1a). These are

(a) reflexions from the DNA double helix within the nucleohistone complex; these are poorly oriented, indicating that the DNA molecules are not well aligned; (b) an equatorial reflexion at 60 Å ascribed to lipid impurity<sup>3,9</sup>; (c) a series of low-angle diffraction rings, or slightly meridionally oriented arcs, corresponding to spacings of approximately 110, 55, 35, 27, 22 Å; the spacing and intensity of the rings vary with the relative humidity of the specimen. The rings arise from structural features of nucleohistone which have dimensions greater than those of histone or DNA molecules alone, that is, the complex has a tertiary structure. Consideration of these spacings<sup>10</sup>, together with the semi-meridional orientation, suggests that the structure is a coiled-coil (super-helix). The coiling accounts for the DNA diffraction not being well aligned.

Fourier transform calculations for helical models indicate that the super-helix has pitch 120 Å and diameter 100 Å<sup>11</sup>. The X-ray data at various humidities, including those of Luzzati and Nicolaieff<sup>12,13</sup>, considered in detail give general support to the model, but some features of the data are not satisfactorily accounted for, and the model must therefore be regarded only as a working hypothesis. It is encouraging, however, that Davies<sup>7</sup> has, by electron microscopy, found in chick erythrocytes hollow rods of about 150 Å outside diameter. These could be coiled nucleohistone threads. Allowing 30 Å diameter for the threads, the super-helix diameter measured from the axial centre of the threads would be 120 Å, which is in satisfactory agreement with the value of 100 Å for this dimension suggested by the X-ray data.

We have obtained further experimental evidence supporting the proposed super-helical structure by systematic study of the changes in the X-ray diffraction pattern brought about by stretching and relaxing fibre specimens of nucleohistone from calf thymus. A typical set of diffraction patterns is shown in Fig. 1; five separate experiments gave the same result. Fig. 1a was obtained from a fibre of diameter 120 μ at 92 per cent relative humidity. The reflexions characteristic of DNA are poorly oriented and the characteristic low-angle rings at 36, 26, 22 Å are present. The second pattern (Fig. 1b) was obtained after the specimen had been re-wetted with distilled water to form a stiff gel, and then redrawn until its diameter was 70 μ. This pattern shows improved orientation of the DNA reflexions but the low angle rings are not visible. Fig. 1c is the pattern obtained after the same fibre had been re-wetted with distilled water, and then allowed to relax without tension. It shows all the features of the original pattern.

The disappearance on stretching of the low-angle rings and the appearance of a well-oriented DNA pattern seem

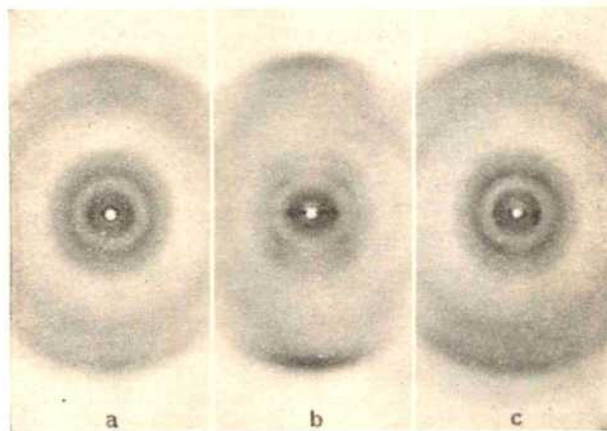


Fig. 1. Three X-ray diffraction patterns from the same fibre specimen of calf thymus nucleohistone, all at 92 per cent relative humidity. (a) From the fibre as originally drawn, with a diameter of 120 μ; (b) after re-wetting and further drawing, with a diameter of 70 μ; and (c) after further wetting and relaxing of tension, with a diameter of 100 μ.



to provide incontrovertible evidence that in native nucleohistone the molecules of nucleohistone are in some regularly folded configuration with the DNA molecules inclined to the fibre axis. Stretching destroys regular folding and aligns the DNA molecules along the fibre axis. No increase in the spacing of the low angle rings is observed on stretching; instead the rings are replaced by diffuse scatter: this shows that the folded structure does not stretch uniformly but breaks up and becomes irregular on stretching. The regular folding re-forms when the specimen is wetted and relaxed. The super-helical model is an obvious choice for the folded structure. The stretching experiments are entirely consistent with a super-helical model but do not eliminate other types of fold in which the DNA molecules are inclined to the length of the nucleohistone molecule.

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### Attempt to locate the $\alpha$ -Helical Segments of Ribonuclease

IN recent communications concerning the tertiary structure of ribonuclease two very different space models of this molecule were presented<sup>1,2</sup>. I wish to point out that, in circumstances such as these, a choice can be made between alternative structures or details of structures by a method based on a statistical matching of a given sequence of amino-acids with the sequences of proteins of known tertiary structure<sup>3</sup>. This method has been refined (my unpublished work) so that for every amino-acid position along the chain which is analysed the probability of that amino-acid belonging to an  $\alpha$ -helical segment can be calculated.

This method seems to give results which agree with X-ray data in the case of the hen egg-lysozyme<sup>4,5</sup>, but still more controls are required before the general validity of the method can be tested.

The  $\alpha$ -helical segments of ribonuclease, according to Kartha *et al.*<sup>1</sup>, should be located in the regions 5-12, 28-35 and 51-58 of the chain, but by statistical analysis I have obtained high probability figures for the residues 1-9, 14-23 and 47-59 (see Fig. 1).

Because of the difficulties and uncertainties involved in the determination of the tertiary structure of proteins

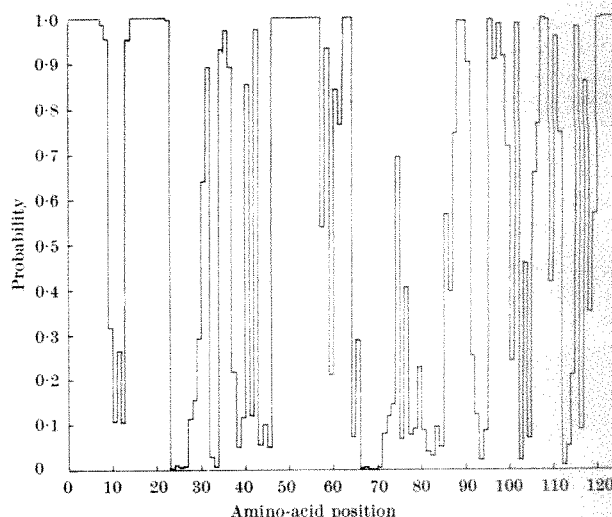


Fig. 1. Diagram showing the helical probability of the amino-acids of the ribonuclease. The abscissa shows the position on the chain from the N- to the C-terminal, and the ordinate shows the probability of the residue being in  $\alpha$ -helical conformation. Note the strong signals in the regions 1-9, 14-23 and 47-59. The last stretch overlaps completely with the third helical segment indicated by Kartha<sup>1</sup>, and the first one overlaps only partially. There is no agreement for the second stretch.

I hope that this technique will be of some use in this kind of problem.

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### Cross-linking of Gelatine by Dehydration

THE contribution of water to the stability of macro-molecular structures of biological origin is often difficult to assess. Besides its intrinsic theoretical interest<sup>1,2</sup>, this problem is of great practical importance in studies concerned with the preparation of single crystals of proteins<sup>3</sup> and nucleic acids from an aqueous mother liquor as well as in studies of such macromolecules in non-aqueous solvents<sup>4</sup>. For these reasons we believe that the detailed study of physicochemical changes which accompany the excessive dehydration of proteins and nucleic acids might be of considerable value. In this communication, we wish to report evidence that gelatine becomes covalently cross-linked when the water content falls below about 0.2 g/100 g protein.

Ossein gelatine having a weight-average molecular weight of about 300,000 was used in this work. The preparative procedure leading to this grade has been reported<sup>5</sup> and a number of dilute solution properties of this gelatine have been determined<sup>5,6</sup>. The glass transition temperature as well as the viscoelastic properties of films prepared from this grade of gelatine have also been reported<sup>5,7</sup>. We intend to show, however, that the observed behaviour of excessively dehydrated gelatine is apparently independent of source or preparative treatment.

The moisture content of gelatine samples was routinely determined using a modification of the procedure recommended by Eastoe and Courts<sup>8</sup>. The determination started with atmospheric oven drying at  $105 \pm 1^\circ \text{C}$  for 24 h and was followed by evacuation under a pressure of



$10^{-3}$  mm mercury at  $105^\circ \pm 1^\circ$  C over an additional 24 h. After such treatment, the weight reached a level which remained constant within  $\pm 0.05$  per cent even during control tests where the treatment under vacuum was extended over an additional 48 h. An independent determination of trace moisture content in gelatine by titration with the Fischer reagent could not be made because of the insolubility of gelatine samples which had been excessively dehydrated.

The solubility of gelatine films (about 1 g) of known moisture content was determined by immersing them in 50 c.c. of deionized water which was maintained at  $40^\circ \pm 0.2^\circ$  C. After immersion for 2 h, the portion of the film remaining undissolved had become a swollen, but coherent and distinct, mass. This mass was removed from the aqueous solvent by gentle suction and was exhaustively dehydrated by treatment in an atmospheric oven at  $105^\circ \pm 1^\circ$  C for 24 h followed by evacuation under  $10^{-3}$  mm mercury at the same temperature for an additional 24 h. The proportion of gelatine which had failed to dissolve was then computed and expressed as a percentage by weight of insoluble gelatine. It was observed that prolongation of the immersion time of films over several days did not significantly affect the proportion of gelatine which remained undissolved after the routine 2 h immersion test.

The relation between the moisture content of gelatine and its solubility in water was found using gelatine films 0.1 cm thick, which were prepared by casting. This simple procedure involved dissolution of gelatine powder of known moisture content in deionized water in aluminium dishes kept at  $65^\circ \pm 0.5^\circ$  C in an atmospheric oven. The initial protein concentration thereby attained was about 5 per cent by weight and the rate of evaporation of water was routinely monitored by frequent weighing. The treatment in atmospheric conditions was extended over several days after which the solubility of the resulting films was determined. We observed that films which were treated as outlined here were completely soluble even when the atmospheric treatment at  $65^\circ$  C was extended over 37 days (compare entries 1-4 in Table 1). Another set of control samples was treated in the atmospheric oven at  $65^\circ \pm 0.5^\circ$  C for 6 days; films were then transferred to a vacuum oven and were treated under  $10^{-3}$  mm mercury over such time intervals and at such temperatures as were found necessary to bring about distinct impairment of their solubility. It was observed that increasingly higher temperatures and longer residence times under vacuum made gelatine films progressively less soluble (compare entries 5-10 in Table 1).

It seems certain that the insolubility of gelatine following sufficiently prolonged evacuation is a consequence of the formation of a three-dimensional network resulting from interchain cross-linking. Such cross-linking cannot be the result of an initial oxidative reaction, because prolonged exposure to the atmosphere does not itself diminish the solubility of gelatine (Table 1). Neither, apparently, is this cross-linking reaction necessarily caused by pyrolytic decomposition which gelatine might undergo at temperatures exceeding  $65^\circ$  C; we have, in

fact, found that gelatine films can be made partially insoluble if evacuated under  $10^{-3}$  mm mercury at temperatures as low as  $25^\circ$  C over a period of 40-50 days. We believe instead that the cross-linking of gelatine is a direct consequence of the removal of water below a critical trace level which we estimate to be 0.1-0.3 g water/100 g gelatine.

The proposed mechanism of insolubilization of gelatine in terms of a critical level of trace moisture below which cross-linking occurs serves to explain several observations which have been previously made in a number of laboratories. In these reports<sup>9,10</sup>, the occasional insolubility of gelatine samples, derived from a wide spectrum of mammalian sources, which were subjected to a variety of heat treatments and drying procedures was attributed to a number of causes, but the effect of trace moisture was apparently not directly investigated.

The chemical nature of the cross-links can perhaps be inferred directly from the work of Bello and Riese-Bello<sup>10</sup> on the solubility of certain chemically modified gelatines. These workers observed that gelatine which had been modified either by esterification of the carboxyl groups or by acetylation of the amino groups remained soluble in water even after being heated at  $108^\circ$  C for three days; by contrast, gelatine which was not so modified became insoluble under identical treatment. These and other results guided these authors<sup>10</sup> to the conclusion that gelatine becomes insoluble exclusively by formation of interchain amide links; the role of trace moisture was, however, not considered explicitly<sup>10</sup>.

Our proposal that the cross-linking of gelatine is a specific consequence of excessive dehydration is consistent with the detailed, independent observations referred to briefly here<sup>9,10</sup>. Drastic removal of the aqueous product of an amide condensation reaction can cause the equilibrium concentrations to shift irreversibly towards the right (formation of interchain peptide links). W. Kauzmann has suggested to us that mass action considerations alone can therefore account for the cross-linking of gelatine by dehydration.

Several proteins are known to contain carboxyl, amino and hydroxyl groups which could potentially condense either by esterification or by amide formation. It should be noted here that such reactions occur relatively slowly at moderate temperatures in the absence of a catalyst. Despite these considerations, we suggest that future investigations of proteins in the highly anhydrous state may very well reveal the occurrence of such irreversible transformations.

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Table 1. SOLUBILITY OF AQUEOUS GELATINE FILMS

Sample No.	Treatment*		Time (days)	Percentage by weight water†	Percentage by weight insoluble gelatine†
	P (mm Hg)	T ( $^\circ$ C)			
1	760	65	1	8.1	0
2	760	65	4	3.3	0
3	760	65	6	1.9	0
4	760	65	37	1.7	0
5	$10^{-3}$	65	3	1.4	0
6	$10^{-3}$	65	6	0.9	0
7	$10^{-3}$	70	4	0.4	0
8	$10^{-3}$	70	15	0.2	$10 \pm 1$
9	$10^{-3}$	80	7	$< 0.1$	$60 \pm 5$
10	$10^{-3}$	105	4	$< 0.1$	100

\* Samples 5-10 were treated for 6 days in an atmospheric oven at  $65^\circ$  C before being subjected to evacuation.

† Determined after the indicated treatment.

## Chlorophyll - Water Interactions in the Solid State

A MARKED, reversible effect of water on the absorption spectrum of crystalline chlorophyll *a* in 'Nujol' mull has recently been observed<sup>1</sup>. Thin films of such mulls, dried by prolonged evacuation or standing over phosphorus pentoxide, absorb at 672 nm, but this absorption shifts to about 743 nm when the film is exposed to water, or if the chlorophyll contains water of crystallization. The infrared spectra of the wet and dry films are correspondingly different<sup>1</sup>. The 743 form shows only a single carbonyl absorption at 1,647  $\text{cm}^{-1}$ , similar to the band assigned to an "associated" carbonyl at the C(9) position in dissolved chlorophyll<sup>2</sup>, while the 672 species exhibits an additional infra-red peak at 1,698  $\text{cm}^{-1}$ , which has been assigned to a "free" carbonyl. The intensity of this band is about two-thirds that of the 1,647  $\text{cm}^{-1}$  peak. This suggests<sup>2</sup> that the 672 form contains dimers or other stoichiometrically associated forms. The differences in spectrum (both red and Soret bands) between the 672 species and the chlorophyll *a* dimer definitely identified in solution<sup>3</sup> may be attributable to effects of the medium or to exciton interactions in the solid phase. It is remarkable, however, that dimers in non-polar solvents are disaggregated by addition of small concentrations of Lewis bases (ethanol, pyridine, water)<sup>2,4</sup>, whereas in these chlorophyll crystal mulls, moisture increases the extent of dye interaction, as indicated by the bathochromic shift of the optical spectrum and, presumably, by the disappearance of the "free" carbonyl band. The water effect in solid chlorophyll has therefore been studied further, particularly to determine whether the 672-743 transition is sharp or whether intermediate forms can be obtained. Such intermediate forms are reported here.

Chlorophyll *a*, prepared from fresh spinach<sup>5</sup>, was brought into crystalline form by evaporation from solution in an ether layer on water. The material consisted of brilliant, purple microcrystals, and gave very sharp X-ray patterns agreeing with those of Donnay<sup>6</sup>. 'Nujol' mulls, spread evenly in thin films on 'Pyrex' plates, were equilibrated in absorption cells at various vapour pressures of water, controlled by connexion to water reservoirs which were maintained at temperatures lower than the films. The cell-reservoir assembly could be evacuated on the vacuum line and the cells closed off and removed for determination of the spectrum.

The position of the main red absorption band is found to depend on the water vapour pressure, the temperature of the film and equilibration time. The conditions in the previous work, in which the film was placed over water in a closed desiccator, were such that liquid water condensed on the film surface, thereby completely shifting the absorption from 672 to 743 nm. With more careful control of

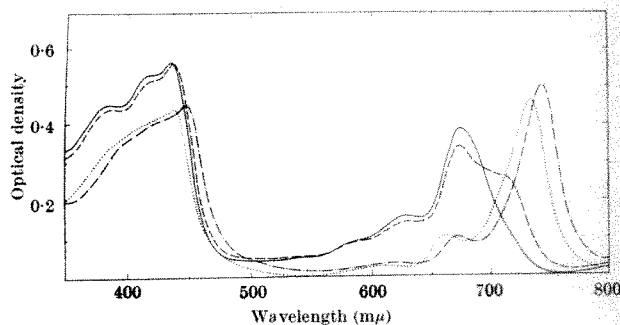


Fig. 2. Visible spectra of films of chlorophyll *a* suspended in paraffin oil: —, film dried by evacuation for 2 h; ----, film exposed to vapour pressure of 15.5 mm for 4 h, at 27° C; ····, film exposed to vapour pressure of 15.5 mm for total of 26 h, at 27° C; — · —, film exposed to vapour pressure of 15.5 mm for total of 50 h, at 27° C.

hydration of the dry film, new absorption peaks have been observed at 716 and 733 nm (Figs. 1 and 2). In other experiments in which diffusion was much slower, peaks at 722 and 736 nm have been found. While the complicated vapour pressure, temperature and time dependence of the spectral shift have not yet been fully established, it is evident that a continuous shift in the visible spectrum between the extreme limits of 672 (dry) and 743 (wet) may be obtained. The spectrum can always be reversed back to 672 nm, from any stage of hydration, by drying on the vacuum line, or over phosphorus pentoxide.

The spectrum of the water-saturated 743 form of chlorophyll *a* is practically identical with the limiting spectrum of ethyl chlorophyllide *a* microcrystals of increasing size, prepared by dilution of acetone solutions with water<sup>7</sup>. The correlation between the magnitude of the spectral shift and the size of the chlorophyllide microcrystals<sup>8</sup> suggests that, in the chlorophyll-'Nujol' mulls, hydration controls the spectral shift (Figs. 1 and 2) in the same way, by controlling the size of the aggregate in which effective intermolecular dye interactions can occur, or by influencing the degree of coupling between the dye molecules. Either effect could result from a "tightening" of the crystal structure by added water. The mechanism may resemble the large red-shift in chlorophyll *a* "monolayers", produced by the presence of calcium ions<sup>9</sup>. The involvement of water in the crystallization of chlorophyll has also been noted earlier<sup>5</sup>. The manner in which hydration leads to disappearance of the "free" carbonyl absorption is still not clear.

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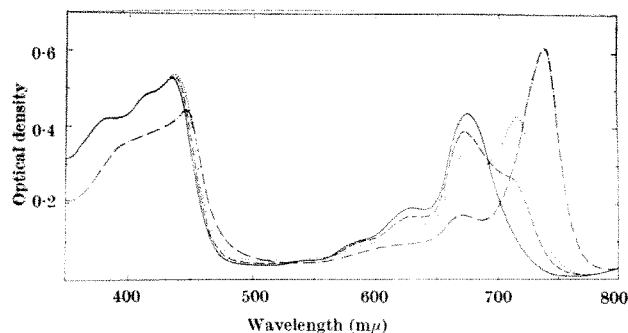


Fig. 1. Visible spectra of films of chlorophyll *a* suspended in paraffin oil: —, film in the presence of excess water; ----, film evacuated with high vacuum for 2 h; ····, film exposed to vapour pressure of 8-9 mm for 2 h, at about 20° C; — · —, film exposed to vapour pressure of 8-9 mm for total of 43 h, at about 20° C.

## BIOLOGY

### Ultrasound in Adult Rodents

PRODUCTION of ultrasound by baby rodents has been reported by Zippelius and Schleidt<sup>1</sup> in *Apodemus f. flavicollis* Melchior, *Mus musculus domesticus* Ratty, and *Microtus a. arvalis* Pallas, and by Noirot<sup>2,3</sup> in baby mice, *Clethrionomys glareolus* Schr., *Mesocricetus auratus* Waterhouse and albino rats. I have now confirmed this phenomenon for the young of *Clethrionomys*, *Mesocricetus* and laboratory rats and mice, and extended it to the young of *Acomys cahirinus* (Desmarest), *Meriones shawi* Rozet, *M. unguiculatus* Milne-Edwards, a species of *Gerbillus*, *Apodemus sylvaticus* (L.), *Mus minutoides* and a species of *Thamnomys*.

In earlier reports ultrasound emission in the species studied ceased on the day on which the eyes opened. I have found, however, that in *Mesocricetus*, *Acomys*, *M. shawi* and laboratory rats ultrasound can be elicited, by handling, after the eyes of the young have opened. In laboratory rats, emission of ultrasound has been followed well into adult life. A continuation of juvenile behaviour due to daily handling can be eliminated, because out of ninety-one adult rats taken at random from normal departmental stock, forty-eight (approx. 53 per cent) produced ultrasound.

Ultrasound was monitored visually on an oscilloscope using a capacitance microphone and wide-band amplifier, and, at the same time, audibly by a "bat detector" as described by Pye and Flinn<sup>4</sup>. Samples of the pulses were recorded directly on magnetic tape. Two distinct types of pulse were produced by these adult rats: "short pulses", of length 30–60 msec at a frequency of about 50 kc/s, were produced by thirty-eight animals when handled; "long pulses" lasting up to 700 msec at about 22 kc/s were produced by twelve animals without handling. Two animals gave both types of pulse.

To elicit short pulses from older rats, they were rolled on their backs and restrained in positions resembling the full submissive posture of rats as described by Grant and MacKintosh<sup>5</sup>. This suggested that ultrasound might be associated with aggressive or submissive responses. To test this, one male rat, previously isolated for 10 days, was introduced into the cage of another, and the actions and postures of the animals, together with the types of pulse produced, were noted for 1 h. Forty different introductions were studied in this way; attacking and fighting occurred in many cases.

Short pulses were heard in every case except one. Long pulses were heard in twenty-six cases and could always be correlated with the long exhalations often shown by the submissive rat. Such abnormal respiration is probably that referred to by Seward<sup>6</sup>. Although the long pulses were initially associated with a submissive or crouch posture<sup>6,7</sup> they were sometimes heard later when the same animal was feeding or cleaning. In only three introductions were long pulses followed by any sort of conflict, actual fighting or boxing. It was difficult to see which animal was producing the short pulses, although sometimes they were synchronous with the head shaking of the aggressive rat as it left the submissive one after typical aggressive postures. Short pulses were often heard during all phases of aggressive behaviour, including fighting, except when the animals were in full aggressive-submissive posture or when one animal was grooming the other. These short pulses were also detected while activities such as feeding, cleaning and sniffing the cage (often occurring as obvious displacement activities) were performed.

In similar conditions of introduction, long and short pulses were detected from male rats (*Rattus norvegicus*) which had been trapped in the wild. No attacking or fighting was seen and in three out of seven introductions both animals remained "frozen" and silent for the whole hour. Short pulses, heard in two other cases, were associ-

ated with one animal approaching and sniffing the other. Long pulses, heard in four cases, were always associated initially with a submissive or crouch posture. One rat produced long pulses for 30 min after the other had been removed.

Many short pulses were detected when male rats were introduced into the cages of lactating or pregnant females, which are noted for increased aggressiveness. In one case these pulses could definitely be associated with the lactating female, for they continued after both her litter and the male had been removed, and were repeatedly obtained by disturbances up to 25 min later.

Short pulses at 50 kc/s were also detected when wild and laboratory males attempted to mount laboratory females or other males. Similar signals were found during intraspecific fighting between adults in *M. shawi*, a species of *Gerbillus* and a species of *Thamnomys* and from a disturbed lactating female *M. unguiculatus* while she thumped one hind foot on the ground. Pulses at 70 kc/s have been heard from mice of Noirot's impure strain, during attempted mounting and sniffing of either sex by males.

These observations suggest that ultrasound plays an important part in the social life of these adult rodents. So far the short pulses seem to be aggressive and the long pulses submissive. Certain aspects of this work including analyses of the ultrasounds and correlation with particular behaviour patterns will be reported in more detail later.

I thank Professor D. R. Arthur and Dr J. D. Pye for their help, and Mr J. Greaves, Ministry of Agriculture, Tolworth, for supplying the wild rats. This work was carried out with the aid of a Layton Science Research Studentship at King's College and a Science Research Council studentship.

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### Circadian Periodicity of Blood Amino-acids in Adult Men

THE term circadian was introduced by Halberg<sup>1</sup> to describe a recurring sequence of events with a cycle of approximately 24 h, and the present knowledge of human circadian rhythms has been reviewed recently by Mills<sup>2</sup>. The circadian periodicity of a large number of constituents of blood, plasma, and urine has been described. Although a diurnal periodicity of whole blood amino-acids in man, in which concentrations at 2000 h were consistently greater than those at 0800 h on the same day, has been noted in this laboratory<sup>3</sup>, there were insufficient sampling times to establish a circadian pattern. A circadian periodicity of serum amino-acids in growing chickens<sup>4</sup> and whole blood tryptophan in mice<sup>5</sup> has been reported. We report here a circadian periodicity of whole blood and serum amino-acids in healthy adults.

Six healthy male volunteers, aged 20–23 yr, were placed in a hospital ward and subjected to uniform conditions of diet and activity. The subjects were informed of the nature and details of the investigations before they volunteered. Each subject was determined to be in excellent health by a check of his medical history, by a physical examination and by baseline laboratory determinations. Venous blood samples were collected at intervals of 4 h beginning at 0800 h on the day of admission and continued for 5 consecutive days. Amino-acids in 0.006 ml. of whole

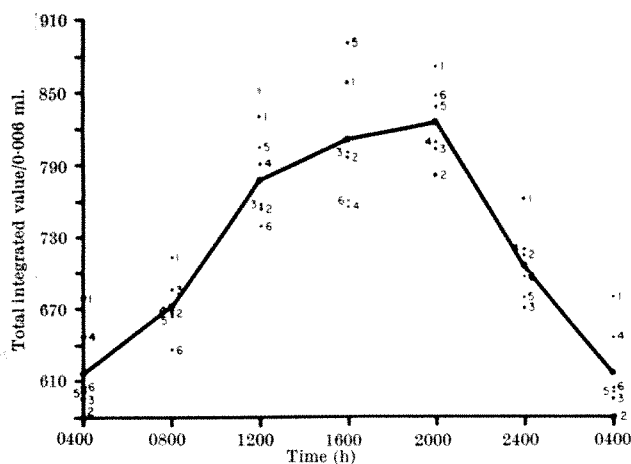


Fig. 1. The total integrated value of amino-acids/0.006 ml. of whole blood averaged for men and days, plotted against hours of the day. Each point on the line represents the mean of thirty determinations. The mean value for each man at each point in time is indicated by the subject's number.

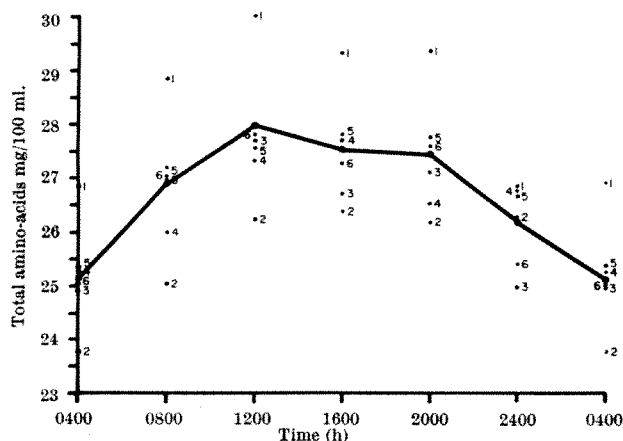


Fig. 2. Total serum amino-acids in mg per cent of a glycine nitrogen standard plotted against hours of the day. Each point on the line represents the mean of thirty determinations. The mean value for each man at each point in time is indicated by the subject's number.

blood were determined by the method of Efron *et al.*<sup>6</sup> and quantitated by densitometry. The sum of the integrated values for each whole blood amino-acid detectable as single spot (cystine, glutamine,  $\alpha$ -amino butyric, alanine and tyrosine) as well as those obtained for amino-acid groups (leucine and isoleucine; valine, methionine and tryptophan; glutamic acid and threonine; glycine, aspartic and serine; and lysine, arginine and histidine) was called the total integrated value. The error of the method as calculated from three standard deviations of the mean total integrated value of twenty replicate determinations of the same blood sample on the same day was 2.1 per cent. The error of the method calculated similarly from replicate determinations of the same blood samples on 20 different days was 5.7 per cent. Amino-acids which react with ninhydrin were determined on serum obtained at the same times of sampling according to the method of Moore and Stein<sup>7</sup>, with modification for the autoanalyser and the total value expressed as mg/100 ml. as compared with a glycine standard. The mathematical analysis of all data was conducted by Drs G. L. Jessup, L. W. Gaudette and W. D. Foster of the Biomathematics Division of Fort Detrick, using a 'Univac' solid state II computer.

Fig. 1 illustrates results obtained when the total integrated value in 0.006 ml. of whole blood is averaged for subjects and days with each point representing thirty determinations. The mean value for each man at each point in time is indicated by the subject's number. Fig. 2 presents a similar plot of results obtained from

autoanalyser analysis of serum. The periodicity observed by each method was characterized by the occurrence of maximal concentrations between 1200 and 2000 h and by minimum concentrations at 0400 h. The difference between results obtained at 0400 and either 1200, 1600 or 2000 h was statistically significant at less than 0.01. Similarly, the difference between concentrations at 1200, 1600 and 2000 h was not statistically significant with either analytical method. Figs. 3 and 4 illustrate results obtained when the mean concentrations of amino-acids of six subjects are plotted by the hour of the day for the 5 days of the experiment. The patterns seen on each day were similar.

Results plotted in Figs. 1 and 2 clearly show that the concentration of amino-acids in any single individual can be quite different from that obtained in any other individual although environment and diet are similar. Despite the differences in absolute concentrations, the periodicity patterns are alike. Results plotted in Figs. 3 and 4 demonstrate that the concentrations of amino-acids on any given day may be different from those obtained on any other day, but the rhythmicity observed is the same from day to day, that is, smallest and greatest concentrations occur at the same time each day. In addition, the individual amino-acids or groups of amino-acids listed here demonstrate the same periodicity as that illustrated for their sum.

Obvious differences exist between the large day-time values and the small night-time readings, but the reasons for these differences cannot be ascertained from these data or the experimental design employed. The hypothesis that a beta function or a Fourier series would represent

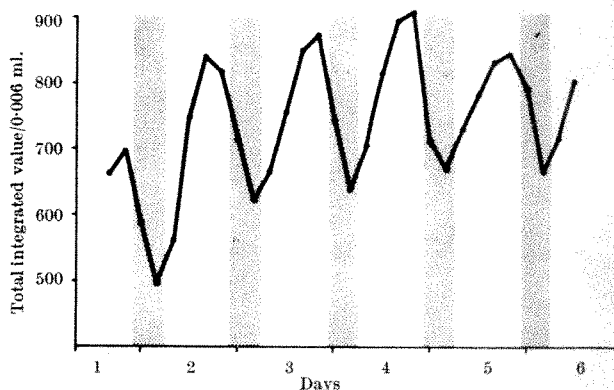


Fig. 3. Total integrated value of amino-acids/0.006 ml. of whole blood plotted against days of the observation period. Each point represents the mean value obtained for six subjects. Shaded areas indicate periods of sleep (2200-0600 h each day).

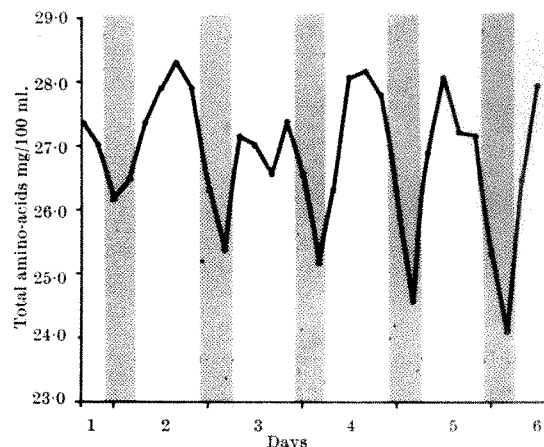


Fig. 4. Total serum amino-acids reported in mg per cent of a glycine nitrogen standard plotted against days of the observation period. Each point represents the mean value obtained for six subjects. Shaded areas indicate periods of sleep (2200-0600 h each day).



the data was not fully supported because of the tendency for values to form a plateau between 1200 and 2000 h.

There is a large range of reported values for plasma amino-acids<sup>8-14</sup>. We can find no information about sampling time in the literature detailing normal concentrations of amino-acids. Possibly the wide range of values reported is caused in part by differences in sampling time.

The influence of diet on concentrations of amino-acids in whole blood has been a source of considerable speculation. Rouser *et al.*<sup>15</sup> cite examples which demonstrate that dietary intake, provided it is in the form of food rather than a single amino-acid load, does not appreciably affect whole blood concentrations of amino-acids. Squibb<sup>16</sup> demonstrated that even in conditions of extreme protein deficiency in chicks, the free amino-acid pool remained at reasonably large levels and that large amounts of dietary protein did not result in an increase in this pool. In contrast, Frame<sup>17</sup> and others<sup>18</sup> reported that most amino-acids increase in concentration after a heavy protein meal, but there is no indication of the time of feeding and none includes control non-fed subjects who were sampled simultaneously. Frame<sup>17</sup> noted that the concentration of some amino-acids was higher 8 h after the test meal than in the fasting state. Although a large protein load may temporarily increase blood amino-acid concentrations, such a persistent 8 h increase may reflect an overall increase related to circadian periodicity rather than food intake.

The influence of exogenous rhythm synchronizers, for example, sleep and wakefulness, light and darkness, exercise, and the quality, quantity and time of food ingestion, must be considered. We are now trying to elucidate the contribution of each of these factors to the observed rhythmicity. In addition, the precise role of numerous individual hormones and other regulatory mechanisms in maintaining normal amino-acid periodicity remains undefined.

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### Morphological and Physiological Distinction between Two Populations of the Peach-Potato Aphid

INDIVIDUAL *Myzus persicae* (Sulz.) which had survived repeated spraying with organophosphorus insecticides were collected from plants grown in glasshouses in 1963

and 1964 and were allowed to multiply on *Brassica pekinensis* Rupr. Several populations, derived from aphids collected in different glasshouses and from different species of host plant, were reared on *B. pekinensis* in separate insect proof cages for more than 2 yr. Experiments showed that these populations were much more resistant to organophosphates than another designated NC (normal colony), which had not been exposed to these insecticides<sup>1</sup>. During 1965 and 1966 experiments were carried out to investigate whether aphids from one of these populations resistant to insecticides (*RR3*) differed from NC aphids in morphology as well as in physiology.

Microscopic examination of individual adult apterae from the two populations showed that the longest ventral hairs on the hind femur of *RR3* aphids were usually shorter than those of NC aphids (Figs. 1 and 2). The difference between the mean hair lengths of the two populations was  $3.8\mu \pm 0.36$  ( $P < 0.001$ ), indicating that the length of femoral hairs was a good criterion for distinguishing between them. To test the reliability of this distinction, a random sample of adult, apterous *RR3* or NC aphids was placed in a series of tubes which were identified only by code numbers. Every sample was identified correctly by comparing the mean lengths of the femoral hairs of aphids in the different tubes.

There was some correlation between the length of the femur and the length of the femoral hairs in the apterae of the NC aphids, but in the alatae of both samples and the apterae of *RR3* there was little increase in hair length with femur length. Small apterae (antennal segment III 300–375 $\mu$  long) of *RR3* aphids usually had the hind femur 1.3–1.4 times as long as antennal III, while similar sized NC apterae usually had the ratio 1.4–1.5. Large apterae (antennal III 425–525 $\mu$  long) of both populations had the hind femora 1.35–1.55 times as long as antennal III.

The experiments demonstrated that apterae of the *RR3* and NC clones could be distinguished by comparing their resistance to insecticides and also by comparing the length of femoral hairs. It would be necessary to examine the femoral hairs of other populations resistant to insecticides to establish whether there is a genetic linkage between resistance to organophosphates and short femoral hairs. Linkages between genes, which control specific

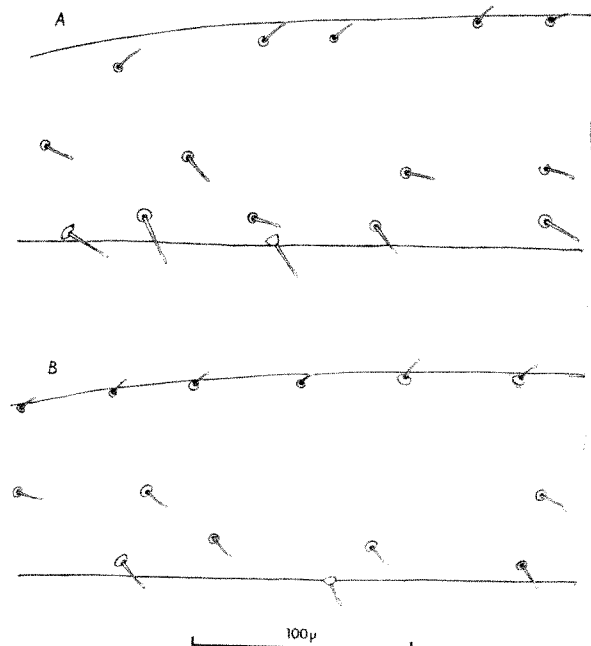


Fig. 1. Anterior surface of middle third of hind femora of *Myzus persicae*; A, population NC; B, population *RR3*.

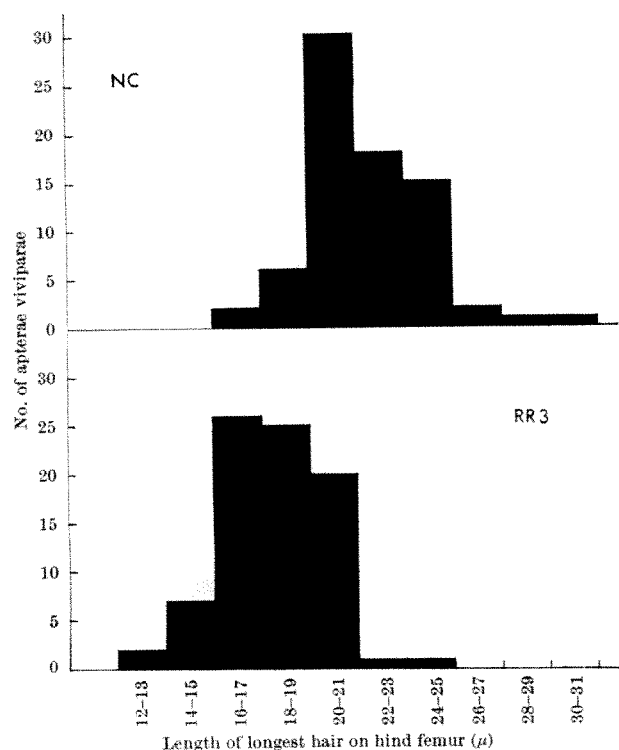


Fig. 2. Distribution of femoral hair lengths from NC and RR3 populations.

morphological and physiological characters, would be particularly useful in identifying individual aphid populations. *M. persicae* consists of a vast complex of clones which differ morphologically and physiologically<sup>2</sup>, and the taxonomy of populations such as RR3 presents special difficulties.

Systematic relationships are usually based on studies of a wide range of inter-related morphological characters; tests of interfertility are rarely used because of practical difficulties. Morphological characters can always be checked on preserved specimens and a system of nomenclature, based on type specimens, can conveniently be used. Aphid populations, however, which have genes of economic importance in common, are often considered to be of the same strain or race, although they may differ from one another in many morphological and physiological characters. Resistance-breaking populations of *Amphorophora rubi* (Kalt.) on raspberry are examples of such strains<sup>3</sup>. Resistance to insecticides, or the ability to overcome the resistance of host-plant varieties, is unlikely to be permanent in aphids, particularly in those species with wide host ranges. There is therefore little point in applying a formal system of nomenclature to populations such as RR3, although it is convenient to apply the terms "strain", "race" or "biotype" to them. It must, however, be emphasized that these terms do not imply that strains are homogeneous except for certain specific characters.

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## Energy Flow in *Euphausia pacifica*

To understand relationships within food webs in the sea, with an eye to predicting and perhaps ultimately improving the efficiency of the production of commercially important species, we must understand the energetics of the lower elements of the food web. Energy flow more accurately represents the significance of a species in a community than either the numbers or the biomass of the species<sup>1</sup>. Euphausiids (class Crustacea) are pelagic filter-feeders of world-wide distribution which serve as forage for a wide variety of marine animals, including whales<sup>2</sup> and salmon<sup>3</sup>. *Euphausia pacifica* Hansen, the principal species in the North Pacific, often represents the largest biomass of the macroplankton in the subarctic and transitional water masses<sup>4</sup>. The species is a vertical migrant, inhabiting depths of roughly 100–500 m during the day and ascending to surface waters at night<sup>4</sup>. It is exposed to water temperatures of about 5°–15° C off Oregon (depending upon season and depth of migration); 10° C is the approximate average<sup>5</sup>. Day length varies off Oregon from about 8 h in winter to 16 h in summer, so that the time spent in waters of different temperature varies seasonally<sup>6</sup>.

Enough is now understood about the metabolic requirements of *E. pacifica* in different environmental conditions to make possible a reasonably accurate assessment of annual energy flow in this species off Oregon, and some prediction of energy flow throughout its range in the North Pacific. Energy flow was calculated from the equation assimilation = respiration + growth, where assimilation is approximately equivalent to energy flow<sup>7</sup>. The calculations were first expressed as g cal / "average" animal / day on a monthly basis. To estimate the "average" animal weight each month, the percentage contributions of small, medium-sized, and large animals were computed on a monthly basis (Table 1). The weight-frequency distribution of animals in the area of collection was roughly bimodal from spring to early summer. One mode ranged from 0.3–1.8 mg dry weight (mean, 0.9 mg), and the second mode from about 6.1–15.5 mg (mean, 8.2 mg). The dry weight range of intermediate sized euphausiids was 1.9–6.0 mg, with a mean of 4.3 mg. The adjusted monthly mean weight of the population was estimated by multiplying the mean weight of each size group by its percentage occurrence in the population for the specified month, then summing the three products and dividing by 100.

Table 1. PERCENTAGE OF SMALL, MEDIUM-SIZED, AND LARGE *E. pacifica* IN THE POPULATION EACH MONTH

Month	Per cent of total population		
	Small	Medium	Large
January	96%	4%	0%
February	86	14	0
March	76	11	13
April	70	9	21
May	65	8	27
June	61	7	32
July	95	3	2
August	89	8	3
September	87	10	3
October	84	13	3
November	98	2	0
December	97	3	0

Small animals ranged from 0.3–1.8 mg dry weight (mean 0.9), medium-sized animals from 1.9–6.0 mg (mean 4.3), and large animals from 6.1–15.5 mg (mean 8.2).

Measurements of respiratory rates were made in a Gilson differential respirometer<sup>8</sup> at 5°, 10°, and 15° C, after the animals had been collected at sea with either 0.5 or 1 m nets (mesh size 0.239 mm). A mean respiratory rate for each temperature was calculated, in  $\mu$ l. of oxygen/mg of dry weight/h. The approximate daily rate of respiration of the population in any one month was obtained by first computing  $\mu$ l. of oxygen/mg consumed during that portion of the day spent in surface waters, then computing  $\mu$ l. of oxygen/mg consumed during the remainder of the day at maximum depth, and then summing the two estimates to yield  $\mu$ l. of oxygen/mg/day. Daily respiration

was converted to g cal/"average" animal/day by first multiplying by an oxy-caloric coefficient of 0.005 g cal/μl. of oxygen (ref. 9), and then by the mean weight of the population.

Growth rates were estimated from weight-frequency data on natural populations collected by trawling off the Oregon coast. There was no significant difference ( $P=0.05$ ) among the mean seasonal rates, or between growth rates of small (young) and large (adult) euphausiids. The overall mean rate of growth was used as the best estimate of euphausiid growth (0.0104 mg of dry weight/day). This overall mean rate agreed well with rates determined for euphausiids maintained at 10° C in the laboratory<sup>10</sup>. Conversions of daily growth rate to growth estimates in g cal/"average" animal/day for each month was accomplished as follows. Weight-specific calorific values of the three size groups of *E. pacifica* were determined in a Parr adiabatic bomb calorimeter (Table 2). On a dry weight basis the values varied by size group, a phenomenon noted also in *Daphnia pulex*<sup>9</sup>. Each increment in the growth attained of a large animal represented therefore a greater caloric addition than the same increment of growth in a small animal. Clearly, growth in the population, when expressed in calories, was a function of size structure of the population. To obtain g cal/"average" animal/day it was necessary to multiply the growth rate of each size group (in g cal) by the respective percentage contribution of each group to the total population, sum the products, and divide by 100.

Table 2. CALORIFIC VALUE OF SMALL, MEDIUM-SIZED, AND LARGE *E. pacifica*, IN G CAL/MG DRY WEIGHT

Determination	Small	Medium	Large
1	4.452	4.694	5.411
2	4.080	4.622	4.893
3	4.206	4.408	5.339
4	4.517	4.165	5.505
5	4.353	4.535	4.962
6	3.998	4.854	5.173
7	4.321	4.623	5.036
8		4.244	4.984
9		4.495	
10		4.780	
11		4.371	
Mean	4.275	4.526	5.163
Standard deviation	0.190	0.216	0.230
Coefficient of variation	4.44%	4.77%	4.45%

Weight ranges of size groups are as given in Table 1.

Respiration and growth, in g cal/"average" animal/day, were summed to estimate energy flow (Fig. 1). The increase in energy/"average" animal from January to early June was largely the result of an increase in weight of the "average" animal during this time. Spawning occurs mainly in June<sup>11</sup>, so the mean weight/animal, and thus

energy flow/"average" animal, decreased significantly after this month because of the appearance of many young animals in the population. A smaller decrease occurred after October, but this was because larger animals dropped out of the population in the collecting area rather than because young animals entered the area.

Because of the patchy distribution of euphausiids, the variation in total numbers/unit volume of water was great; thus energy flow/unit volume was not computed. If, however, energy/"average" animal for each month is divided by the mean weight for each month, g cal/mg/day can be plotted (Fig. 1). This curve can be considered indicative of the effects on respiration of changing environmental conditions (particularly temperature and photoperiod). A comparison of the two curves in Fig. 1 shows that size structure of the population has more influence on annual energy flow than temperature and photoperiod. The annual ranges of temperature and photoperiod off Oregon are reasonably representative for the whole oceanic region inhabited by *E. pacifica*, particularly when vertical migration is taken into account. The applicability of the energy flow estimates off Oregon to the rest of the North Pacific, however, depends almost entirely on whether changes in size structure of the population off Oregon are representative. This, of course, is not known, but Ponomareva's<sup>11</sup> account of the life cycle of euphausiids in the eastern North Pacific indicates that it may be so.

Integration of the area under the curve for g cal/"average" animal yielded an annual energy flow of approximately 100 g cal/"average" animal. The weight-specific value was slightly less than 60 g cal/mg dry weight/yr. Lasker<sup>10</sup> found that the assimilation of ingested carbon by *E. pacifica* was on average 84 per cent in animals maintained in the laboratory. If this figure generally holds for animals in the natural environment, the "average" animal ingests roughly 15 mg of carbon/yr, using the conversion of 8 g cal/mg of carbon and assuming complete biological oxidation of all assimilated carbon. On a weight-specific basis, ingestion is about 9 mg of carbon/mg dry weight/yr.

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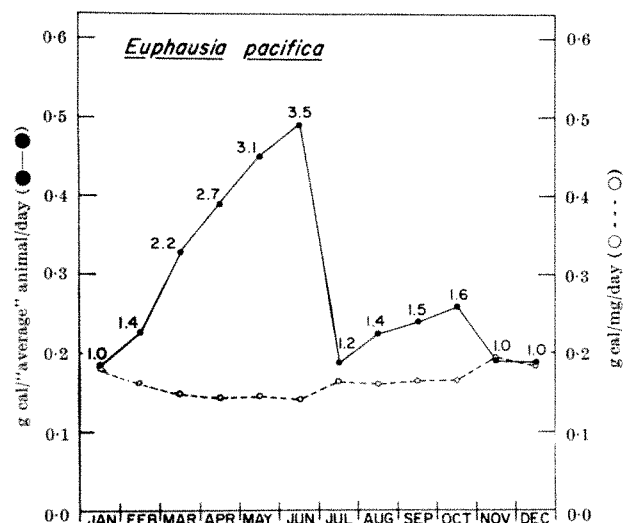


Fig. 1. Energy flow in *E. pacifica*. The values beside the data points refer to the adjusted mean dry weight/animal (mg) in the respective months.

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## Newt Orientation by Sun-compass

SALAMANDERS migrate long distances to breeding sites<sup>1</sup>, and apparently possess an accurate guidance mechanism<sup>2,3</sup>. The homing of blinded *Taricha* after being displaced downstream suggests that they rely on olfactory cues<sup>4</sup>. The ability of the normal animal to use vision in orientation, however, has received little attention. Three species of frogs and toads use a sun-compass<sup>5-7</sup>, and the toad, *Bufo woodhousei fowleri*, can learn a direction of escape relative to a light cue<sup>8</sup>.

We have trained newts and tested them in a circular arena (4 ft. diameter with 1.33 ft. high wall) with eight covered goal boxes attached at equal distances around the outside. The boxes opened into the arena through doors (4 in. by 4 in.) located at 45° intervals around the circular wall. The arena was surrounded by a wall of white curtains (10 ft. diameter). A 175 W sun lamp, situated at an angle of 51° above the base of the arena, provided the only asymmetry within the test area as viewed from the centre of the arena. Newts were trained under the sun lamp to move in a particular direction from the centre of the arena to an open goal box. During training, the angle between the open goal box and the light was the same, and a moist sponge (4 in. long, 3 in. wide, 1 in. thick) and pieces of earthworm were placed in the box as a reward.

The first group (A) of twelve newts was trained to find a goal box located 90° to the right of the sun lamp (directions expressed from the centre of the arena). These animals were given ten conditioning trials (1 h trial/day) with the same box (No. 0) open each day. After several training sessions, newts moved rapidly to the box and spent the remainder of the hour inside. They were tested with all the boxes open, the arena in the training position, but without a sponge or earthworms. In two tests, these newts preferred box No. 0 which was 90° to the right of the light (Fig. 1a). Scores were recorded when an animal entered a box. All data were analysed for randomness by the Rayleigh test ( $P = 0.05$ ) and the mean angles of scores shown in the figures were computed vectorially<sup>9</sup>.

Immediately after the previous test the part played by odour was investigated with a second group (B) of twelve newts. These newts were untrained and had never been in the arena. When released with the arena in the same position, eight newts moved to Box No. 0, where eleven newts had scored in the preceding tests (Fig. 1b). These untrained animals did not appear to follow scent trails, and explored the arena before entering a goal box.

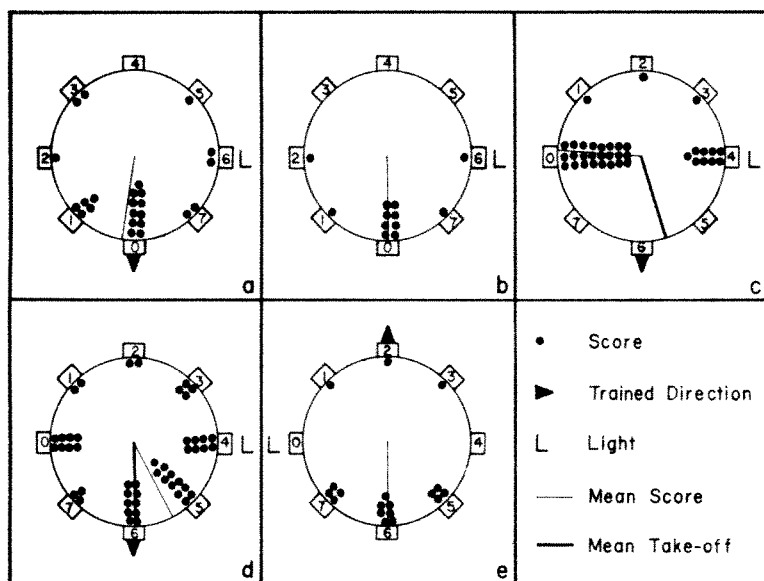


Fig. 1. Directional responses of newts trained to an escape direction relative to a sun lamp. (a) The arena in the training position; (b) untrained animals released immediately after the previous test; (c) familiar boxes (No. 0 and No. 4) rotated 90°; (d) familiar odours in each box; and (e) light moved 180° from the training position. The null hypothesis of random distribution was rejected for all tests.

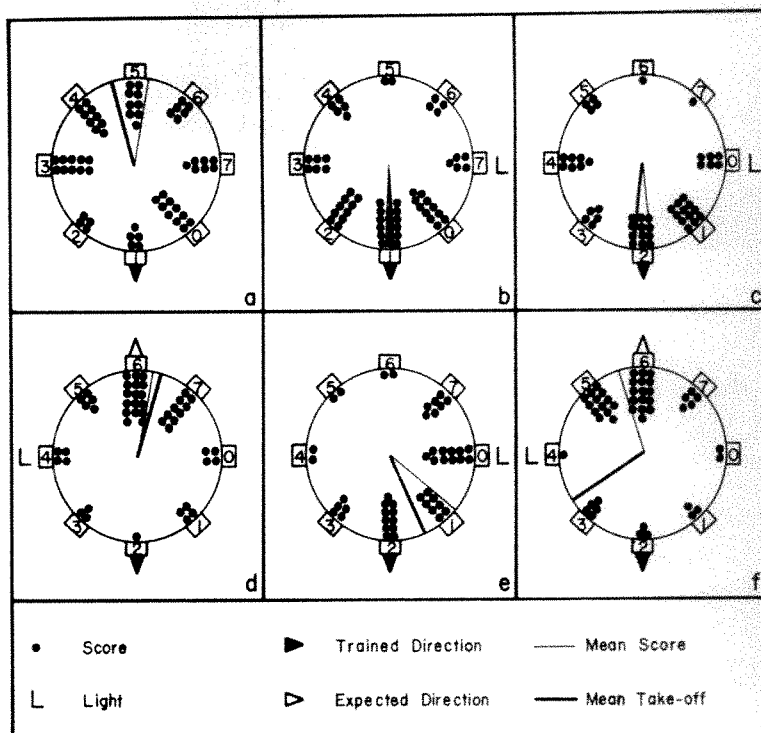


Fig. 2. Responses of newts to visual cues with odours minimized. (a) Test of trained animals with indirect illumination in place of a light cue; (b) light cue in training position. Tests with the light cue (c) in the training position and (d) moved 180°. Response of newts trained under a sun lamp and tested under (e) an infra-red lamp; (f) a red flood lamp moved 180°. The null hypothesis of randomness is accepted for scores in Fig. 2a and take-off directions in Figs. 2a and 2f; all others are rejected.

Group B was trained to enter a box 90° to the right of the light and was used in a test comparing visual and olfactory cues. Training was similar to that used earlier, except that boxes No. 0 and No. 4 were open on alternate days. After the arena was rotated to bring box No. 6 in the trained direction, these newts were tested three times and both the directions of take-off and the boxes they entered were recorded. The animal's position halfway to the arena wall constituted the direction of take-off. In these tests thirty-three newts scored in boxes No. 0 and No. 4, but the take-off directions were toward box No. 6 (Fig. 1c). If odours were detected from the centre of the arena, the take-off direction should have been toward the box entered.

The arena was cleaned thoroughly and repainted. Four sponges were placed in the aquarium holding group A for 48 h and four sponges were similarly placed with group B. These sponges should have provided "home" scents. Sponges from aquarium A were alternated in the goal boxes with those from aquarium B and newts (A and B) were tested twice. The scores and directions of take-off were to the right of the light and usually in the trained direction (Fig. 1d). The sponges did not appear to influence the choice of boxes.

To test the use of potential cues other than the light (for example, sound, kinaesthetic information, odours), nine newts, group C, were trained to locate a box 90° left of the light source. These animals were tested twice with the light moved 180°. Box No. 2 was in the trained direction (No. 0 and No. 4 used for conditioning) and sponges were placed in the goal boxes as described previously. Of the eighteen scores, fifteen were within 45° of the trained direction relative to the rotated light (Fig. 1e).



Groups *A* and *B* were conditioned twice to Box No. 4 and were tested on the third day with box No. 1 rotated to the trained direction (90° right of the light). The arena and boxes were painted daily, and before each test a new sponge, moistened in tap water, was placed in each box. During tests, the sun lamp was placed underneath the arena, where it illuminated the curtained area evenly but was not visible to the test animals. The scores and directions of take-off were scattered in these tests (Fig. 2a). When the sun lamp was placed in the original training position, these newts again favoured the direction which they had been trained to take (Fig. 2b).

Other tests have verified that *T. granulosa* uses light cues. Group *D* was trained to locate a box 90° to the right of the sun lamp and was tested four times with the light in the training position. The entire arena was painted before all group *D* tests and fresh sponges placed in each box. Most newts scored to the right of the light (Fig. 2c). After the light was moved 180°, seventeen of forty-eight newts moved in the trained direction relative to the new cue position (Fig. 2d). Take-off directions corresponded to the new light position.

The sun lamp was moved to the original training position and group *D* was conditioned six times. An infra-red lamp (250 W) was substituted for the sun lamp. When these newts were re-tested, twenty-four of forty-eight scores were within 45° of the trained direction (Fig. 2e). The infra-red lamp emitted less light but more heat. Take-off directions were more scattered than under the sun lamp. Next, a red flood lamp (150 W) was used and the light source moved 180°. In these tests, thirty-four of forty-eight scores were within 45° of the trained direction, but take-off directions were scattered (Fig. 2f). These newts were slow to score and seemed less responsive to the low light and heat intensities of this bulb.

*T. granulosa* uses information from light cues in its orientation and has a very well developed olfactory sense. Our findings and those of Twitty and his associates<sup>10</sup> suggest that further investigation could show the relative value of these two orientational mechanisms in the natural environment.

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### Behavioural Responses to Contact with DDT in *Anopheles atroparvus*

We have obtained two colonies of *Anopheles atroparvus* by selecting for escaping (colony *A*) and not escaping (colony *B*) from a tube lined with paper impregnated with DDT dissolved in 'Risella' oil<sup>1</sup>, and this selection has now reached the *F*<sub>32</sub> generation. Percentages of escapes, which differed widely in both males and females, have not changed significantly since the tenth generation. Neither DDT nor its solvent has been found to be essential for the escape reaction, but each greatly enhances the response.

In this communication we analyse some aspects of the behaviour underlying the escape flights. We started with a series of tests in which the length of the exposure tube and the diameter of the escape hole could be altered (Fig. 1). The results indicated that both the length of the tube and the diameter of the hole influence the percentage of the mosquitoes escaping but that there is no interaction between the effects of the two variables. We concluded therefore that the length of the tube and the diameter of the hole affect different components of escape behaviour. In each case more mosquitoes of colony *A* escaped than of colony *B*.

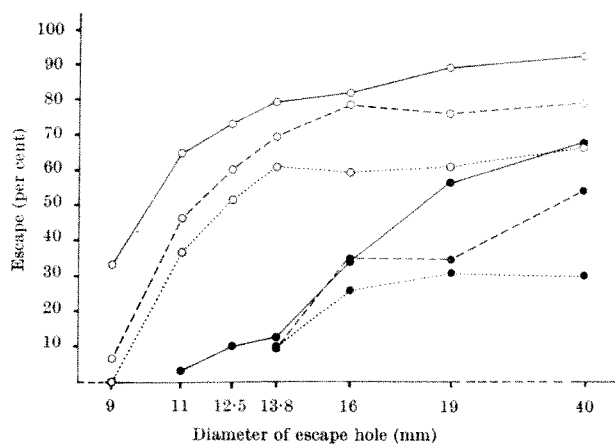


Fig. 1. Percentage of escapes of single 1 day old empty females of *Anopheles atroparvus* of colony *A* (○ ○ ○) and *B* (● ● ●) from tubes lined with filter-paper impregnated with 4 per cent DDT, put into different lengths and 40 mm in diameter, in relation to the diameter of the escape hole. Tests were run in complete darkness. Duration of tests was 1 min (preceded by 3 min of adaptation). Numbers used/point were for colony *A*, twenty-nine to forty-four; for colony *B*, thirty to fifty-eight. Tube lengths, ———, 12 cm; ---, 36 cm; ····, 60 cm.

The first component of escape behaviour which we investigated was flight within the tube. To record this a narrow slit along the length of the tube was left unlined, and with this part uppermost and a source of illumination placed below the tube we followed by hand, with a pencil on a paper moving at 22.5 cm/min, the movements of a single mosquito. In this way we obtained a 'kinetogram' for each mosquito tested. The two selected strains showed quite different flight patterns, two typical specimens of which are shown in Fig. 2. These patterns have been analysed for (a) activity expressed by the percentage of mosquitoes taking off at least once; (b) activity expressed by the number of take-offs/active mosquito; (c) activity expressed by the total time flown/active mosquito (a mosquito was considered to be flying as long as it was on the wing, part of the flying time being consumed by hovering and part by actual locomotion); (d) speed of flight; (e) number of turns/sec of 'productive flying-time', this being the time consumed by locomotion over a distance of at least 5 cm of tube length; and (f) number of turns/m covered. The results are shown in Table 1 and Figs. 2 and 3. They show that the mosquitoes of colony *A*

Table 1. DIFFERENT ASPECTS OF ACTIVITY OF SINGLE 1 DAY OLD EMPTY FEMALES OF *Anopheles atroparvus*, EXPOSED TO 4 PER CENT DDT

	Colony A	Colony B
Percentage of active mosquitoes	100.0 (25)	88.0 (25)
No. of take-offs/active mosquito	14.1 (25)	9.8 (22)
Time flown/active mosquito (sec)	107.8 (25)	63.7 (22)
Speed of flight (cm/sec)*	15.5 (25)	12.2 (20)
No. of turns/sec of "productive flying time"*	0.29 (25)	0.38 (22)
No. of turns/m flown*	1.87 (25)	3.13 (20)

The mosquitoes were exposed to the DDT in a tube 46 cm long. Activity was analysed by means of kinetograms. Experimental conditions were as described in the text. Duration of the tests was 3 min (preceded by 3 min of adaptation). Numbers of mosquitoes are in parentheses.

\* Determined during 3 min adaptation and 3 min test.

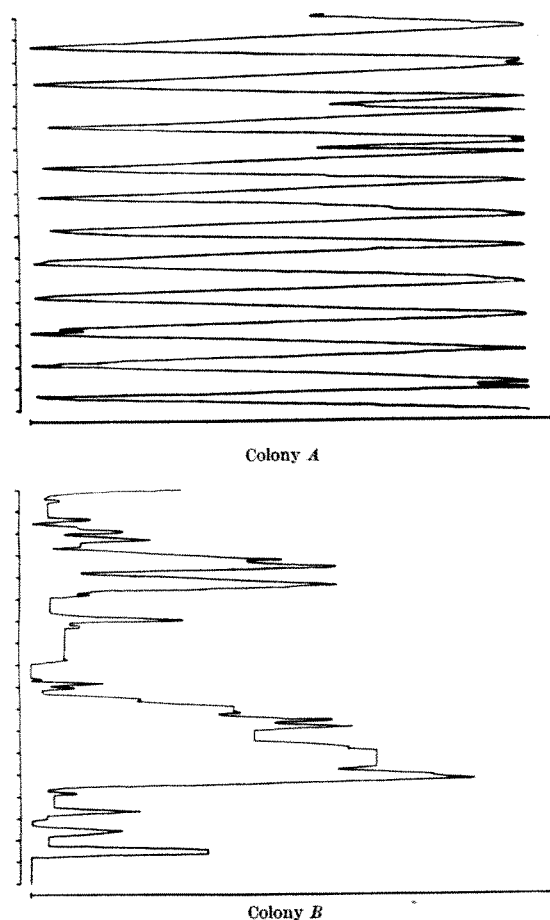


Fig. 2. Kinetograms of single females of *Anopheles atroparvus* of colony A and B, recorded in the conditions described in the text. Time is marked on the vertical scale with 5 sec intervals.

have a greater average speed of flight than those of colony B; there is even a sharply defined discriminating speed of flight, for only four mosquitoes of colony B flew faster than 14 cm/sec and only four mosquitoes of colony A were slower. The mosquitoes of colony A fly more directly from one end of the tube to the other. Those of colony B turn more frequently in each metre covered. Although there is apparently a negative correlation between the number of turns/m and the speed of flight, the former was excluded as a possible cause of the different speeds of flight of A and B mosquitoes. The average speed of flight in 100 long, straight flights recorded for B mosquitoes was found to be 12.8 cm/sec. This was exactly the same as the average speed of flight of B mosquitoes in flights with frequent turns. The activity, judged by the criteria (a)–(c), is greater in mosquitoes of colony A than in those of colony B.

The second component of escape behaviour which we investigated was flight in relation to the escape hole. In order to measure the capacity of a single mosquito for escaping, it should be given the opportunity of escaping repeatedly. This can be achieved when the mosquito, after escaping from the exposure chamber, finds exactly

Table 2. DIFFERENT ASPECTS OF ACTIVITY AND PERCENTAGES OF ESCAPING BY SINGLE 1 DAY OLD EMPTY FEMALES OF *Anopheles atroparvus*

	Colony A	Colony B
Percentage of active mosquitoes	98.0 (100)	94.0 (50)
No. of take-offs/active mosquito	11.2 (98)	8.2 (47)
Time flown/active mosquito (sec)	77.2 (98)	42.3 (47)
Percentage of active mosquitoes escaping	91.8 (98)	2.1 (47)

Activity was recorded in WHO irritability test funnel completed with its reflected image (bases covered with filter-paper impregnated with 4 per cent DDT in 'Risella' oil; diameter of escape hole, 11 mm).

Duration of the tests was 3 min (preceded by 3 min of adaptation). Numbers of mosquitoes are in parentheses.

the same conditions as before escaping. These conditions were met simply by using two World Health Organization irritability test funnels (slightly modified) joined together. The pair of funnels, top-holes combined so as to make one escape hole common to both sides, and their bases covered with DDT-impregnated paper, were illuminated from below. With this apparatus we made simultaneous observations of the number of escapes, the activity expressed by the number of take-offs and the activity expressed by the total time flown. The results are shown in Table 2 and Fig. 4.

This quantitative method of estimating escape reactions of single mosquitoes in short tests confirms that A and B mosquitoes differ in their capacity for escaping. The

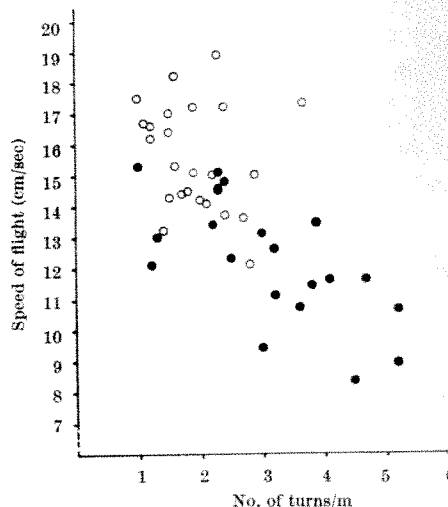


Fig. 3. The relation between the speed of flight and the number of turns/m flown by a single 1 day old female of *Anopheles atroparvus* watched for 6 min. Experimental conditions were as described in the text. ○, Colony A; ●, colony B.

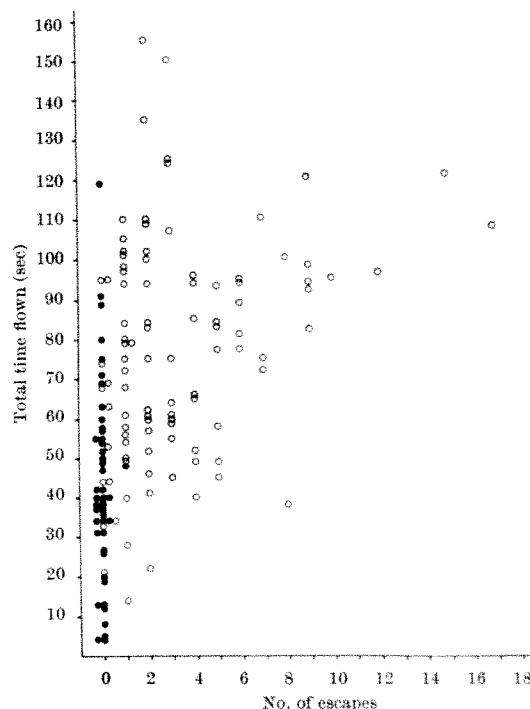


Fig. 4. The relation between activity (total time flown) and number of escapes of single 1 day old empty females of *Anopheles atroparvus*, exposed to 4 per cent DDT in two combined WHO irritability-test funnels. Experimental conditions were as described in the text. Duration of the tests was 3 min (preceded by 3 min of adaptation). ○, Colony A; ●, colony B.



activity is greater in mosquitoes of colony *A* than in those of colony *B*, although there are individual *B* mosquitoes which are very active. Although the number of escapes is related to the minimum activity, escaping is not a direct result of activity. Thus irritation by DDT accounts for increased activity, but the ability to escape is a separate component of behaviour.

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### Morphological Changes in a Parasitic Nematode due to Acquired Resistance of the Host

THE vulval flap of trichostrongylid nematodes is of interest to taxonomists and its presence or absence and its form are regarded as characteristic of different species. Recent work with experimental infections of *Ostertagia ostertagi* in calves has shown that the vulval flap, which in this species is normally large and well developed, may be greatly reduced or entirely absent in worms from resistant hosts. This is illustrated in Fig. 1.

The burden of adult worms of calves exposed to constant infection with this parasite is regulated by a loss of worms and their replacement. The rate of loss depends on the number of worms present, with the result that the worm burden is maintained at the level at which the appropriate loss balances the acquisition of new worms. Thus there is a rapid turnover of worms and consequently the worms present at any moment have developed recently.

In an experiment in which three groups of calves were infected daily at different rates, it was found that the percentage incidence of female worms, in which the vulval flap was greatly reduced or absent, increased with the passage of time. The increase in incidence appeared to be linear and its rate positively related to the infection rate (see Fig. 2). A closer study of the results suggests that the percentage of flapless females is proportional to the host's experience of the presence of adult worms as measured by the product of the mean number of adult worms and the time for which they have been present. In further

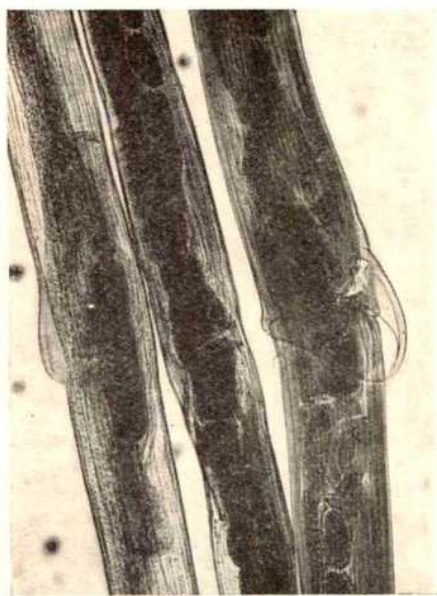


Fig. 1. Vulval region of three mature female *Ostertagia ostertagi*. The worm on the right has a normal vulval flap, that on the left a greatly reduced flap. The worm in the middle has no vulval flap.

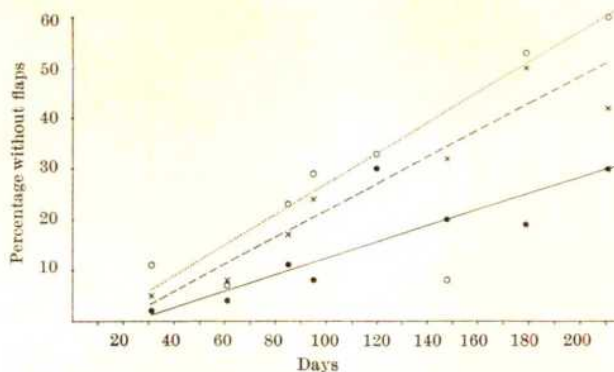


Fig. 2. The incidence of female *O. ostertagi* without vulval flaps in populations of worms from three groups of calves which received infective larvae daily at the rate of 500/day (●—●), 1,000/day (×---×) and 1,500/day (○...○) respectively.

experiments it was shown that the administration of cortisone to calves receiving infective larvae daily prevented an increase in the incidence of flapless females, even though a turnover of worms was taking place.

Other experiments showed that in calves infected on one occasion only, the percentage of flapless females in the resulting population was low and was not affected by the size of the infection. On the other hand, when a first infection established by the administration of a single dose of larvae was entirely removed by anthelmintic treatment and replaced by means of a similar single dose of larvae from the same culture, the incidence of flapless females was 7.3 per cent in the first infection and 26.9 per cent in the second. It was shown further that, as a population of worms of uniform age decreases in number, the percentage of flapless females remains unchanged.

These experiments were carried out with a strain of *O. ostertagi* grown from ova from females especially selected as having a well developed vulval flap. When larvae from this culture were administered to calves free from worms a small proportion of the resulting adult worms were without flaps and this incidence has not changed in successive passages through susceptible calves. It has been shown<sup>1</sup> that the worms present in calves exposed to constant infection with *O. ostertagi* become smaller with the passage of time. Within any population of worms from such calves the flapless worms are not merely the small worms. If anything, the flapless worms are longer, but this difference is sufficiently small to be explained by the fact that the different members of the population completed their development over a period of about a month.

The increasing incidence of flapless females in calves exposed to repeated infections cannot be attributed to the accumulation of members of a distinct variety of subspecies which is lost from the host more slowly than the more common form. Were this so, the size of flapless worms from such calves would be expected to decrease less rapidly than that of worms with vulval flaps. Furthermore, the percentage incidence of flapless females in populations, in which all the worms are of the same age, would be expected to increase as the number of worms decreases.

All the evidence suggests that conditions for the development of the vulval flap are less favourable in hosts that have had previous experience of infection and that the extent of this change in conditions may be proportional to the host's experience of the presence of adult worms. That immune mechanisms are involved in the deterioration of conditions is indicated by the effect of treating the host with cortisone. Thus the phenomenon of flaplessness resembles other phenomena of host resistance to infection with *O. ostertagi*. These also appear to be due to a deterioration in the environment which in turn is the consequence of immune reactions, and the extent

of the deterioration seems to be directly proportional to that of the cause by which it is elicited. In spite of this basic similarity, flaplessness and the various other manifestations of resistance can be shown to be distinct in their causation as well as in their effects.

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### Inhibition of Respiration of *Chlorella vulgaris* by Simultaneous Application of Cupric and Fluoride Ions

IN experiments of short duration, the respiration of many micro-organisms is diminished only a little, if at all, by cyanide or by fluoride at concentrations which inhibit the main respiratory system of higher animals. I have used a strain of *Chlorella vulgaris* in which the respiration is insensitive to fluoride, cyanide and (in aerobic conditions) copper ions, but when suitable amounts of copper sulphate and sodium fluoride are added simultaneously to a cell suspension, oxygen uptake almost ceases. No similar effect has been seen when other metals are substituted for copper, or when most other anions are substituted for fluoride. Cyanide and iodide are exceptions, but both of these interact with cupric copper. The addition of citrate, to avoid possible precipitation of sparingly soluble basic copper fluoride, does not make copper sulphate or sodium fluoride more toxic when these are added to cells singly, nor does it reduce the inhibition of respiration which occurs when they are applied simultaneously (Fig. 1).

In mixtures containing less copper sulphate or sodium fluoride, the respiratory inhibition is less and apparently can disappear at appropriate concentrations. Nevertheless, interaction is still taking place, for cell suspensions treated with even smaller concentrations absorb considerably more oxygen than do untreated suspensions. The figures in Table 1 represent the respiration of standard cell suspensions expressed as percentages of the respiration of untreated controls, the data being based on the oxygen uptake for the 60 min period after addition of the poisons. The experiments were carried out in aerobic conditions

Table 1. RESPIRATION, AS A PERCENTAGE OF THAT OF UNTREATED CONTROLS, AFTER ADDING MIXTURES OF COPPER SULPHATE AND SODIUM FLUORIDE TO A SUSPENSION OF CELLS OF *Chlorella vulgaris*

Copper sulphate (moles/l.)	Sodium fluoride (moles/l.)		
	0	$8 \times 10^{-3}$	$4 \times 10^{-2}$
$6.6 \times 10^{-3}$	110	226	43
$1.3 \times 10^{-2}$	111	256	82
$4.0 \times 10^{-3}$	112	237	175
$2.5 \times 10^{-3}$	101	140	160
$3.1 \times 10^{-4}$	100	—	144
0	100	121	146

in a Warburg respirometer provided with a light-excluding canopy and at a temperature of 30° C.

Insensitivity of respiration to fluoride has often been attributed to the presence of a respiratory pathway which is not dependent on enolase. *Escherichia coli*<sup>1</sup>, *Pseudomonas aeruginosa*<sup>2</sup>, *Bacillus subtilis*<sup>3</sup> and *Chlorella pyrenoidosa*<sup>4</sup> are among organisms, strains of which have been demonstrated to be fluoride-insensitive, though the techniques used have sometimes measured gas exchange and sometimes substrate utilization. The alternative pathway has been characterized tentatively as the pentose phosphate system<sup>5,6</sup>.

Perhaps the most striking respiratory peculiarity of *Chlorella* when it is treated with a respiratory poison is its ability to switch from a main to an alternative pathway capable of assuming the full respiratory load; in contrast, tissues of higher organisms in similar circumstances respond by undergoing a reduction in respiration to a "cyanide-resistant" or "fluoride-resistant" level. If fluoride blocks a main pathway dependent on enolase, the foregoing observations suggest that (a) an alternative respiratory pathway is disturbed or inhibited by concentrations of copper ions exceeding approximately  $2 \times 10^{-4}$  molar, and (b) both respiratory pathways are blocked when the two poisons are applied together. I am investigating the possibility that the pentose phosphate system is the alternative copper-sensitive system.

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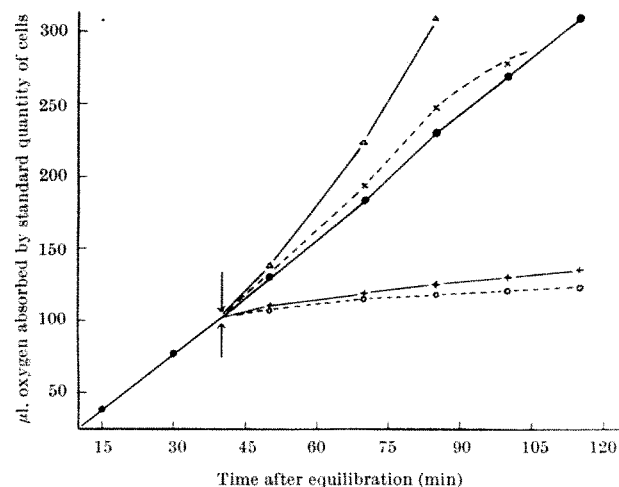


Fig. 1. Respiration of *Chlorella vulgaris* after applying sodium fluoride and copper sulphate singly or together. Side-arm contents were added at time indicated by the arrow. ●—●, Untreated cells (with or without citrate); △—△, sodium fluoride alone,  $4 \times 10^{-2}$  molar after addition to cells; ×—×, copper sulphate alone,  $8 \times 10^{-3}$  molar after addition; +—+, mixture of sodium fluoride and copper sulphate at the concentrations above; ○—○, same mixture, but  $3 \times 10^{-3}$  molar citrate also present.

## BIOCHEMISTRY

### Evidence for binding of Cytoplasmic Creatine Kinase to Structural Elements in Heart Muscle

THERE have been suggestions<sup>1,2</sup> that cytoplasmic creatine kinase is not always free in solution in the cell, but that it is in part bound to structural elements, for example myofibrils. Some evidence for this is provided *inter alia* by localization studies<sup>3-5</sup>. I have obtained direct evidence for the binding of some cytoplasmic creatine kinase by isolation and crystallization of the enzyme from the myofibrillar fraction of a heart muscle homogenate. The enzyme was differentiated from the mitochondrial creatine kinase, which forms about 50 per cent of the total creatine kinase activity in heart muscle<sup>6</sup>.

Beef heart (800 g) was homogenized in 4 l. of 0.25 mmolar sucrose, containing 20 mmolar triethanolamine buffer, pH 7.2, and 2 mmolar EDTA, according to the recommendations of Green and Ziegler<sup>7</sup>. The myofibrillar fraction was centrifuged down at 1,000g for 15 min and the supernatant, containing the mitochondria as well as the sap, was removed. The precipitate was suspended in



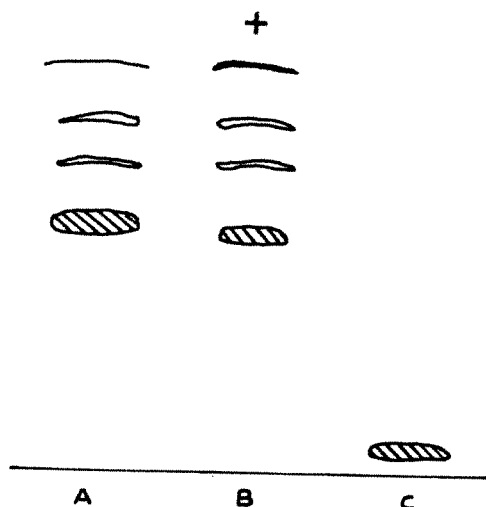


Fig. 1. Electrophoresis of creatine kinase preparations on acrylamide gel, pH 9, 2.5 h, 10 V/cm (cathode direction is indicated by +). A, Commercial creatine kinase; B, creatine kinase from myofibrillar fraction of ox heart; C, creatine kinase from ox heart mitochondria. The areas showing enzyme activity are shaded.

1 l. of sucrose medium, and rehomogenized in a Waring blender for 30 sec. The suspension was centrifuged for 20 min at 6,000*g* and the supernatant decanted off. This procedure was repeated five times. On each occasion the supernatant was tested for creatine kinase activity by the method of Bücher *et al.*<sup>8</sup>. After the fifth and sixth washes, the activity in the supernatant was negligible ( $< 0.1$   $\mu$ /ml. solution). (1  $\mu$  = 1  $\mu$ mole ATP disappearing/min, in the conditions of Bücher *et al.*<sup>8</sup>.) The gelatinous precipitate was then extracted by suspending in 1 l. of ice-cold 0.02 molar phosphate, pH 7.2, and standing overnight at 2° C. The precipitate was centrifuged off, and the creatine kinase activity of the supernatant was found to be about 2  $\mu$ /ml. and 5  $\mu$ /mg protein.

The enzyme was concentrated by precipitation with 2 mmolar ammonium sulphate at pH 7.8, and purified by fractional precipitation with acetone at pH 9. The fraction which precipitated between 35 and 45 per cent acetone was allowed to crystallize at pH 8.4 in a solution of very low ionic strength, as described by Jacobs, Klingenberg and Ottaway (in preparation). The activity of the crystals was about 18  $\mu$ /mg, and the yield was 8.6 mg.

Electrophoresis in acrylamide gel at pH 9 (0.04 molar glycine buffer, 0.01 molar sodium chloride, 0.01 molar creatine) showed that the preparation was not completely pure. The impurities were stained with naphthalene black and had electrophoretic mobilities very similar to those of impurities found in a commercial preparation of crystalline muscle creatine kinase (obtained from Boehringer and Söhne, Tutzing). All the enzyme activity detected on the gel by the method of Burger *et al.*<sup>9</sup> was found in a spot with slightly less mobility than that of the commercial sample. No trace of activity was found near the origin, where mitochondrial creatine kinase migrates in these conditions (Fig. 1).

A significant amount of creatine kinase, with electrophoretic properties similar to those of muscle (cytoplasmic) creatine kinase, is therefore strongly bound to myofibrillar structures of heart muscle, from which it can be released by treatment with hypotonic solutions. If the values given by Jacobs<sup>6</sup> for the partitioning of creatine kinase between mitochondria and cytoplasm in rat heart muscle are assumed to apply also to ox heart, it can be calculated that 100 g of heart tissue containing 13 per cent protein will contain about 1,800  $\mu$  of creatine kinase of which about 50 per cent will be extra-mitochondrial.

The original 800 g of heart tissue would therefore be expected to contain about 7,300  $\mu$  of extra-mitochondrial

enzyme. The original phosphate extract of the myofibrils contained about 2,000  $\mu$ , which amounts to 28 per cent of the estimated "cytoplasmic" enzyme in the tissue. The activity in the crystals was about 2 per cent of the "cytoplasmic" enzyme activity.

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### Action of Pemoline on RNA Metabolism in the Brain

MAGNESIUM-PEMOLINE produces an increase of RNA in the brain tissue of animals<sup>1</sup>. It has been confirmed<sup>2,3</sup> that this product is a psycho-stimulant which acts on the memory. It has also been suggested<sup>4</sup> that RNA acts as a "molecule of memory" and that RNase interferes with this action in planarians<sup>5</sup> and in mammals<sup>6</sup>.

The sequence of atoms from the carbonyl group to the imino group in the pemoline molecule is planar and sterically similar to the corresponding zone of the cytosine and guanine molecules (Fig. 1A). Both these bases are components of nucleic acids, assumed in the Watson-Crick model to be bonded by three hydrogen bonds. This was confirmed in 1965 by Pauling and Corey<sup>7</sup>.

Fig. 1B shows the hydrogen bonds of the chelate structure of magnesium-pemoline (ref. 8 and others). This partial similarity in structure suggested to us the possibility that pemoline might be fixed to the active site of RNase in a manner similar to that described by Findlay *et al.*<sup>9</sup> for the RNA cytidine during enzymatic hydrolysis of RNA.

Our experiments were carried out either with crystalline pancreatic RNase or with homogenized and centrifuged rat and guinea-pig brain tissue. Yeast RNA was used as a

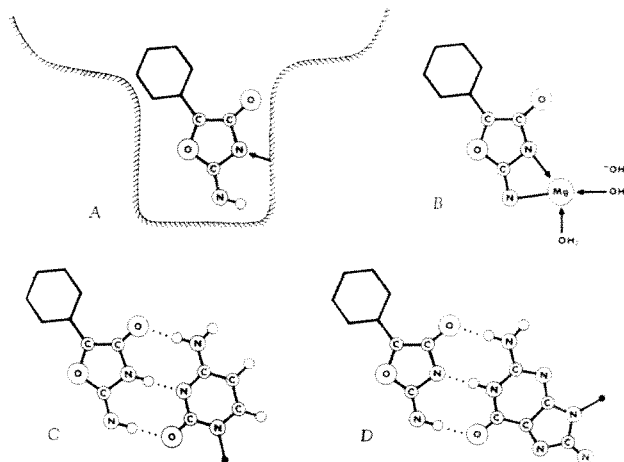


Fig. 1. A, Structure of pemoline (2-imino-5-phenyl-4-oxozolidinone); B, chelate structure of magnesium-pemoline (after Lange *et al.*<sup>8</sup>); C, possible interaction between pemoline and cytosine; D, pemoline and guanine. The shadowed line around the pemoline molecule in A represents the specific site of RNase, as suggested by Findlay *et al.*<sup>9</sup> to explain the mechanism of RNase activity.

Table 1

Assay	pH	None	With pemoline	Inhibition (per cent)	With magnesium-pemoline	Inhibition (per cent)
(A) Action on crystalline pancreatic RNase	—	5.0	0.036	0.023	36	—
(B) Action on brain RNase	Rat	5.4	0.125	0.119	5	0.061
	Rat	7.4	0.094	0.075	20	0.045
	Guinea-pig	5.4	0.084	0.076	10	0.067
	in vitro	7.4	0.200	0.126	37	0.036
(C) Action on brain RNase	Rat	5.4	0.192	—	—	0.090
	Rat	7.4	0.040	—	—	0.025
	Guinea-pig	5.4	0.075	—	—	0.066
	in vitro	7.4	0.090	—	—	0.070

(A) Determination by spectrophotometric method<sup>12</sup>. Results given in E/min at 300 $\mu$ . Final concentration of mixed solution: pemoline  $7.5 \times 10^{-4}$  moles; pancreatic RNase, 3.75 mcg/ml. Yeast RNA (purified, free from RNase) 0.1 per cent. Pemoline was prepared according to Traube and Ascher<sup>13</sup>; RNase was made by us from beef pancreas. The purity constants are described by Ledoux<sup>16</sup>.

(B) Recently excised brain tissue was homogenized (1:10) either in barbiturate (pH 7.4) or acetate (pH 5.4) buffer and then centrifuged at 525 g for 3 min. The assay for RNase activity was performed according to McDonald<sup>17</sup>. 5 ml. supernatant and 6 ml.  $3 \times 10^{-3}$  molar pemoline or, in some cases, magnesium-pemoline (in the blank run, 6 ml. buffer instead) were mixed and pre-incubated for 30 min at 37° C. Afterwards, 1 ml. of 0.5 per cent yeast RNA was added. After standing for another 30 min periodic samples were precipitated with McFadyen's reagent, and the extinctions of the filtrates read at 260 $\mu$ .

(C) Groups of six animals were injected intraperitoneally with 100 mg/kg of magnesium-pemoline suspended in saline (controls were run with saline only) and killed after 2 h. RNase activity on brain tissue was assayed by the method of Kunitz (*loc. cit.*)<sup>14</sup> and by Datta *et al.*<sup>15</sup>, as explained above (B).

substrate. Table 1A shows the results obtained with pancreatic RNase. It may be seen that pemoline produced a significant inhibition. The slight solubility of pemoline and of the corresponding magnesium chelate did not allow us to use higher concentrations of the drug. On the other hand, we did not think DMSO suitable for use as a solvent<sup>1</sup> because it is known to induce changes in cell wall permeability<sup>10</sup>, and also to liberate RNase from the lysosomes<sup>11</sup>. The inhibitory action on RNase of magnesium alone has long been known<sup>12,13</sup> and, although not shown in the table, magnesium-pemoline was also a powerful inhibitor.

The *in vitro* inhibition of alkaline and acid RNase of rat and guinea-pig brain tissue by pemoline and magnesium-pemoline is shown in Table 1B. The decrease in brain RNase activity after two hours of treatment with magnesium-pemoline *in vivo* can be seen in Table 1C.

The ultra-violet spectra of pemoline ( $4 \times 10^{-5}$  moles/l.), RNA (0.004 per cent), and a mixture of the two, at pH = 5.0, have been studied. No shifting of the maximum at 260 $\mu$  was observed; the A260 of the mixture, however, was significantly lower (10.5 per cent) than the sum of the individual components.

In order to investigate whether the observed difference could be attributed to a partial hydrolysis of RNA, another experiment was carried out in which RNA was incubated at pH 8.0 in the absence and presence of pemoline. The results shown in Table 2 suggest an interaction between pemoline and RNA; however, no significant alteration in the rate of hydrolysis of RNA was observed. Furthermore, consideration of the steric characteristics of the pemoline molecule suggests that pemoline may be fixed to the cytosine or guanine residues in the RNA chain. This possibility is illustrated in Fig. 1C and D.

Glasky and Simon<sup>1</sup> reported that pemoline increases RNA polymerase activity. This fact is not inconsistent with our finding that RNase is inhibited by pemoline and magnesium-pemoline, because it is well known that different enzymes react in different ways; there are several

Table 2

Samples at pH 8	Time (in min) at 37° C			
	0	30	60	240
Pemoline ( $4 \times 10^{-5}$ moles/l.)	0.211	0.220	0.220	0.220
RNA (0.004 per cent)	0.314	0.332	0.353	0.360
Pemoline + RNA ( $4 \times 10^{-5}$ moles/l. and 0.004 per cent)	0.333	0.358	0.368	0.379

At known intervals, portions were removed and added to a similar volume of McFadyen reagent. The solutions were centrifuged and the extinctions of the supernatants read at 260 $\mu$ .

chemical and physical agents which bring about opposing reactions in RNA-polymerase and RNA-depolymerase or RNase (for example, magnesium ions<sup>12,13,18</sup>, freezing and thawing<sup>12,19</sup>, radiation<sup>20,21</sup>, actinomycin<sup>22,23</sup>, etc.).

The biochemical and pharmacological properties of pemoline seem to be due to an increase in the concentration of brain RNA. This increase in RNA may be the result of enhanced biosynthesis, or diminished degradation, or both. Our results suggest that decreased degradation may be due to inhibition of RNase or stabilization of the RNA configuration.

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## Phosphogluconate Oxidative Pathway in Glial Metabolism

INVESTIGATION of the distribution of oxidative enzyme activity in the optic nerve of the cat<sup>1,2</sup> has revealed that oligodendrocytes, which are restricted to the post-laminar part of the optic nerve<sup>3,4</sup>, are characterized by reactivity for the enzymes of the citric acid cycle, succinic and malic dehydrogenases. Astrocytes, which are found throughout the optic nerve<sup>3,4</sup>, show much less reactivity for these enzymes. Succinic and malic dehydrogenases are also strongly active in the fibre bundles of the optic nerve within and anterior to the lamina cribrosa: within the lamina the bundles are composed of finely myelinated and unmyelinated nerve fibres, but in the prelaminar part of the optic nerve the fibres are almost all unmyelinated. In contrast, the post-laminar fibre bundles show negligible activity of these enzymes and are composed of myelinated nerve fibres. Nicotinamide-adenine dinucleotide (NAD) and NADP tetrazolium reductases have a mixed pattern of activity: they show up in oligodendrocytes, in astrocytes, and in the laminar and prelaminar nerve fibre bundles.

In the first 2 weeks after birth the optic nerve of the kitten is extremely cellular and its glial cells, revealed by histochemical staining, are of irregularly stellate form.

These undifferentiated glial cells are best referred to as glioblasts<sup>5</sup>. They exhibit strong activity of the reductases and of non-NAD linked  $\alpha$ -glycerophosphate dehydrogenase, but reactivity for succinic and malic dehydrogenases is slight or absent. Reductase activity first appears in cells identified as oligodendrocytes during the third week; in the fourth week they show strong succinic dehydrogenase activity. At corresponding times the same enzymes become demonstrably active in the nerve fibre bundles of the prelaminar part of the optic nerve and within the lamina cribrosa. Later, malic dehydrogenase shows an identical distribution<sup>6</sup>. These findings are consistent with the working hypothesis that oligodendrocytes are involved in the energetic support of myelinated, post-laminar nerve fibres<sup>2</sup>.

Present findings concern the distribution pattern of enzymes of the phosphogluconate oxidative pathway. The activity of glucose-6-phosphate dehydrogenase and of 6-phosphogluconate dehydrogenase has been investigated in the optic nerves of adult cats and of kittens grouped according to age: 6-7 days, 10-12 days, 15-16 days, 23 days, 28-29 days and 38 days. Longitudinal cryostat sections, 15 $\mu$  thick, were cut from the orbital parts of the optic nerves; they were incubated at 37°C for between 20 and 120 min. Incubation media were as follows. The stock solution consisted of 0.05 molar glycyl glycine buffer, 0.01 molar potassium cyanide and 0.01 molar magnesium chloride. To this was added 0.25 mg/ml. of nitro blue tetrazolium, 0.25 g/ml. of polyvinylpyrrolidone, 1.0 mg/ml. of NADP and 2.5 mg/ml. of substrate. The final pH was adjusted to 7.1-7.2. Control incubations were carried out in the absence of co-enzyme.

At each of the developmental stages which we investigated the distribution of glucose-6-phosphate dehydrogenase activity was identical with that of 6-phosphogluconate dehydrogenase. In the adult cat there was evidence of strong enzyme activity in astrocytes throughout the optic nerve and in oligodendrocytes of the post-laminar region. There was no distinguishing enzyme activity in the nerve fibre bundles within the lamina cribrosa and in the prelaminar part of the optic nerve. In the kitten optic nerves of the three earlier stages the hexosemonophosphate dehydrogenase response was confined to the glioblasts: at three later stages it was found in both astrocytes and oligodendrocytes. No differences of reactivity distinguished the post-laminar nerve fibre bundles from those at the head of the optic nerve.

The distribution of the hexosemonophosphate dehydrogenases in the optic nerve of both kitten and cat thus differs significantly from the distribution of enzymes of the citric acid cycle and of the tetrazolium reductases. In contrast with enzymes of the citric acid cycle those of the phosphogluconate oxidative pathway are present in the glioblasts that initially populate the optic nerve; at later stages of development astrocytes and oligodendrocytes seem to be equally reactive, and there is no correlation between the reactivity of oligodendrocytes in the post-laminar part of the nerve and that of the laminar and prelaminar nerve fibre bundles. In this respect the distribution of hexosemonophosphate dehydrogenase differs also from that of tetrazolium reductases. These differences throw further light on the relationship between myelinated axons and oligodendrocytes and the metabolically linked cytophysiological unit which they form. The reciprocal distribution of enzymes of the citric acid cycle suggests a linkage concerned with processes involving rapid production of energy. The fact that the enzymes of the phosphogluconate oxidative pathway are not similarly distributed is not unexpected, for the pathway may be concerned not so much with the production of energy as with synthetic activity in nucleotide metabolism and in the metabolism of fatty acids and steroids<sup>7</sup>.

Evidence that the astrocytes may be the cells which support the myelin sheath within the central nervous system has been presented elsewhere<sup>1,4</sup> and is consistent

with our findings. The presence of glioblasts within the optic nerve of the kitten during the first 2 weeks is associated with centrifugal advance of myelination demonstrable by staining with 'Luxol' fast blue; their hexosemonophosphate dehydrogenase activity is therefore likely to be associated with a need for intense synthesis of lipid at this time. The presence of hexosemonophosphate dehydrogenases in the astrocytes of the optic nerve of the later three stages in the development of the kitten and the adult cat may be significant in view of the fact that glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase are demonstrable in the cells in myelinating cultures which support the sheath<sup>8</sup>.

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### Inhibition of Lysozyme by N-Acyl-D-glucosamine Derivatives

LYSOZYME (N-acetyl muramyl hydrolase, E.C.3.2.1.17) is one of several proteins of which the three-dimensional structure is known<sup>1</sup>. The detailed mechanisms of the enzyme function and specificity are not completely established, but it is known to hydrolyse the  $\beta$ (1 $\rightarrow$ 4) glycosidic linkages, between N-acetyl-D-muramic acid and N-acetyl-D-glucosamine in the glycoaminopeptide component of the cell walls of *Micrococcus lysodeikticus*<sup>2</sup> and those of chitin<sup>3</sup>. The action of lysozyme is inhibited by N-acetyl-D-glucosamine<sup>4,5</sup> and the crystallographic structure of a lysozyme-N-acetyl-D-glucosamine complex has been described<sup>6</sup>. The activity and structure of lysozyme have been surveyed recently in a discussion organized by the Royal Society<sup>7</sup>.

Our present work has been concerned with the inhibitory power of some N-acyl-D-glucosamine derivatives. We assumed that they would bind at or near the site responsible for enzyme hydrolysis. In this work lysozyme (crystallized three times) from hen's egg white was used and the progress of hydrolysis was followed by the reduction in turbidity at 450 m $\mu$  of cell suspensions of *Micrococcus lysodeikticus* at pH 6.2 (ref. 8).

By preparing the  $\alpha$  and  $\beta$  anomers of N-acetyl-D-glucosamine the inhibitory power of each was investigated separately and it was found that the  $\beta$  anomer was more inhibitory than the  $\alpha$  anomer. The results with a mutarotated solution indicated that the inhibitory power of the two anomers was additive (Table 1). The methyl N-acetyl-D-glucosaminides resembled the N-acetyl-D-glucosamines in their inhibitory power, that is, the  $\beta$  anomer was the stronger inhibitor (Table 1). The ethyl N-acetyl-D-glucosaminides showed a weak but definite inhibition. The N-formyl and N-propionyl-D-glucosamines

Table 1. CONCENTRATION OF INHIBITOR ( $10^{-3}$  MOLAR) GIVING 30 PER CENT INHIBITION AT pH 6.2 AND pH 9.2

Inhibitor	pH 6.2	pH 9.2
N-Acetyl- $\alpha$ -D-glucosamine	15.0	—†
N-Acetyl- $\beta$ -D-glucosamine	4.6	—†
N-Acetyl-D-glucosamine*	12.0	6.6
Methyl N-acetyl- $\alpha$ -D-glucosaminide	42.0	26.0
Methyl N-acetyl- $\beta$ -D-glucosaminide	3.5	0.5
N,N'-Diethyl chitobiose*	0.2	—†

\* Mutarotated solution.

† Not determined.

were tested and found to have no inhibitory power up to a concentration of 0.1 molar. N-Methyl-N-acetyl-D-glucosamine also inhibited, although the inhibitory power was weaker than that of the homologue N-acetyl-D-glucosamine.

There was some correlation between the inhibitory power and the effect on the difference spectrum of lysozyme in the region 250–300 m $\mu$ , which can be associated with a red-shift in the spectrum of tryptophanyl residues in the protein<sup>9</sup>. Thus chitobiose gave the biggest spectral change, followed successively by methyl N-acetyl- $\beta$ -D-glucosaminide and a mutarotated solution of N-acetyl-D-glucosamine corresponding to their relative inhibitory power.

These results can be interpreted on the assumption that the derivatives tested bind at position C in the cleft, so designated by Blake *et al.*<sup>1</sup>. N-Formyl-D-glucosamine is of particular interest, for it showed no inhibition or characteristic difference spectrum with lysozyme. This clearly suggests that the methyl moiety of the acetamido group is involved in hydrophobic interactions with residues 108(try) and 98(ileu) of the protein.

Johnson<sup>10</sup> observed an additional optimum for lysozyme activity at pH 9.2 and we have carried out some experiments at this pH. The results showed that the inhibitory power of the derivatives tested at this pH was enhanced (Table 1). We suggest that at pH 9.2 the  $\epsilon$ -ammonium group of lysine 97 acts as a proton donor to form the glycoside carbonium ion and that the negative charge on the carboxylate group of aspartic acid 101 stabilizes the intermediate carbonium ion in accordance with present views on the mechanism of cleavage<sup>11</sup>.

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### Morphine cancels Effect of Magnesium on Hormone-sensitive Uptake of Glucose by Muscle

It has been shown that the acute *in vitro* effects of adrenal hormones on the rate of uptake of glucose by diaphragms of chronically morphinized rats differ markedly from the normal effects<sup>1-3</sup>, and the evidence suggests that the changes induced by morphine which affect hormone sensitivity involve a modification of membrane properties<sup>4</sup>. The possibility that such changes depend, at least in part, on an ability of morphine specifically to interfere with the formation and function of metal ion complexes within membranes encouraged us to investigate the effects of

varying magnesium concentrations in the incubating medium used in our experiments.

The effect of adrenaline on the uptake of glucose by muscle is known to be influenced by magnesium concentration<sup>5</sup> and it has been suggested that hormones act as sequestering agents for ions such as those of magnesium<sup>6</sup>. The activity of thyroxine<sup>7</sup>, hydrocortisone<sup>8</sup> and insulin<sup>9</sup> is known to be affected by magnesium concentration. The formation of chelates of magnesium with adenosine triphosphate (ATP) and catecholamines has recently been discussed in relation to membrane function<sup>10,11</sup>. Magnesium-dependent enzymes, including an ATPase sensitive to sodium and potassium ions<sup>12</sup> and a phosphatidic acid phosphatase<sup>13,14</sup>, have been detected in membranes where they are believed to function in a variety of membrane transport mechanisms.

In preliminary experiments with excised rat diaphragm in Krebs phosphate-saline, modified by Herman and Ramey<sup>5</sup>, we found that omission of magnesium from the medium has no effect, but increasing the content of magnesium markedly increases the rate of uptake of glucose, and a maximum effect was attained with double the normal concentration of magnesium. With diaphragm from chronically morphinized rats, on the other hand, the rate of glucose uptake is not affected by changing the concentration of magnesium, even if it is a fourfold increase. The results are summarized in Table 1. As already established, the mean basic rate of glucose uptake by diaphragms of chronically morphinized rats is not significantly different from that of normal rat diaphragm<sup>4</sup> and it can be stimulated by hormones<sup>2,3</sup>.

Table 1. *In vitro* EFFECTS OF MAGNESIUM CONCENTRATION ON UPTAKE OF GLUCOSE BY DIAPHRAGM OF NORMAL AND CHRONICALLY MORPHINIZED RATS

State and (No.) of rats	Ratio of [Mg] B/A	Uptake of glucose (mg/100 g wet tissue/h) (mean $\pm$ SE)		
		A (Mg, 1.28 mM)	B (Mg, varied)	Difference (B-A)
Normal (5)	0	162 $\pm$ 12	164 $\pm$ 7	+2 $\pm$ 13
CM (8)	0	229 $\pm$ 14	230 $\pm$ 12	+1 $\pm$ 12
Normal (5)	0.5	180 $\pm$ 3	175 $\pm$ 9	-5 $\pm$ 10
Normal (5)	1.5	180 $\pm$ 13	241 $\pm$ 15	+61 $\pm$ 9 ( $P=0.002$ )
Normal (5)	2	190 $\pm$ 4	302 $\pm$ 5	+112 $\pm$ 12 ( $P<0.001$ )
CM (7)	2	216 $\pm$ 8	210 $\pm$ 18	-6 $\pm$ 19
Normal (5)	3	194 $\pm$ 2	294 $\pm$ 7	+100 $\pm$ 5 ( $P<0.001$ )
CM (7)	3	216 $\pm$ 11	225 $\pm$ 11	+9 $\pm$ 14
Normal (5)	4	190 $\pm$ 6	296 $\pm$ 9	+106 $\pm$ 9 ( $P<0.001$ )
CM (6)	4	206 $\pm$ 8	215 $\pm$ 10	+9 $\pm$ 14

Hemi-diaphragms were incubated at pH 7.4 and 37° C for 1 h in 2.0 ml. of oxygenated Krebs-Ringer phosphate containing glucose (0.15 per cent). In each experiment, half the excised diaphragm served as a control (A) for the other half (B), in which the magnesium content of the medium varied from 0 to  $5.1 \times 10^{-3}$  molar. CM, Chronically morphinized rats, which had received daily injections of morphine (30 mg/kg body weight) for 5–6 weeks.

In further experiments, details of which will be published elsewhere, to compare the acute effects of morphine, adrenaline and hydrocortisone at different concentrations of magnesium, we find that a significant effect of either the drug or a hormone on the rate of uptake of glucose by normal diaphragm is obtained only with a normal or near normal concentration of magnesium in the medium. With chronically morphinized diaphragm, on the other hand, the effect of either the drug or a hormone appears to be quite independent of extracellular concentration of magnesium.

These findings encourage the view that morphine induces changes in membrane transport systems that are sensitive to adrenal hormones, and that by virtue of its structural analogies to both catecholamines and steroids it competes or otherwise interferes with the formation and function of metal complexes which are involved in the hormone-sensitive transport systems. We are investigating the mutual effects of morphine and adrenal hormones as sequestering agents in model systems, and comparing magnesium distributions in tissues of normal animals with those in chronically morphinized animals.

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### Increase in Apparent Peroxide ("Pro-blue") in Pregnant Mice

EARLIER we showed that an oral contraceptive, acting by inhibition of ovulation, decreases the total apparent peroxide in mice<sup>1</sup>. As a corollary to this observation it seemed worth while enquiring whether pregnancy, which also inhibits ovulation, similarly decreases peroxide.

Albino hairless mice (*hr hr*) were weighed daily, with precautions against alarm, for 3 weeks. After the first week experimentals were mated, the male being left with the female throughout the second week. Controls were unmated. Pregnancy was apparent from the increase in weight during the third week and was confirmed *post mortem*. Twelve control and twelve pregnant mice, initially 13 weeks old, were used for this experiment. Control and pregnant mice were used alternately. In three of the experiments, vaginal lavages were taken from each mouse immediately after it was killed with nitrogen.

The term "apparent organic peroxide", or "peroxide" for short, refers to "pro-blue"<sup>1</sup>, a substance or substances extractable from biological material by *n*-butanol and capable of oxidizing leucobright cresyl blue in the absence of air. The peroxide estimations of whole mice and the precautions against alarm were as previously described<sup>2</sup>. For the peroxide estimations of mouse organs, a modified form of the anoxic box<sup>2</sup> was used.

Because the results of the experiment with intact mice showed a significant increase in total peroxide, separate organs were examined to determine its location. As shown in Table 1, there were significant increases of peroxide in the uterus, the ovarian fat plus ovaries and the mammary tissue. The increase in weight of the uterus plus fetuses in the pregnant mice was about 45 per cent of the total increase in weight. The lowered average weight of the

ovarian fat plus ovaries in the pregnant mice suggests a utilization of ovarian fat during pregnancy, the weight of the ovaries being negligible by comparison. The mammary tissue included the skin in the area of the nipples. As far as possible a similar area of skin was taken from each mouse. The pituitary body and adrenals were also examined. The two organs were taken from the same mice but their peroxide contents were estimated separately.

The total increase of peroxide in the organs so far tested, although statistically significant, accounts for only about 6 per cent of the whole increase. Further work is being done on other organs of pregnant mice.

It is not clear why inhibition of ovulation due to pregnancy increases organic peroxide although that due to an oral contraceptive decreases it. One obvious difference between pregnancy and oral contraception is that of hormone activity. Lipid peroxidation is believed to be associated with fatty acid metabolism<sup>3</sup>, and pituitary activity, which is increased in pregnancy, stimulates fatty acid release.

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## MICROBIOLOGY

### Effect of Mixtures of Atabrine and Antibacterial Agents on the Emergence of Resistant Strains of *Mycobacterium tuberculosis*

THERE have been numerous descriptions of the successful use of mixtures of antibiotics and atabrine in preventing the emergence of resistant strains of *Staphylococcus aureus* and *Escherichia coli*<sup>1-4</sup>. When a sensitive strain of these organisms was subcultured in an antibiotic, however, the mixture of antibiotic and atabrine was no longer effective in eliminating resistance.

The emergence of strains resistant to chemotherapeutic agents used in the treatment of tuberculosis has narrowed the usefulness of such agents. It seemed worthwhile to investigate the effect of atabrine in suppressing the emergence of strains of *Mycobacterium tuberculosis* resistant to isonicotinic acid hydrazide (INH), dihydrostreptomycin sulphate (DHSM), and 4-aminosalicylic acid (PAS).

The H37Rv strain of *M. tuberculosis* was used. The strain, maintained on Dorset egg agar slants, was sensitive to INH, DHSM and PAS. Drug resistance was determined after the strain had been grown for 14 days at 37°C in Dubos oleic acid liquid medium. The tests were carried out by sub-inoculating 0.1 ml. of a 10<sup>-2</sup> dilution of a 7 day culture in 9 ml. portions of Dubos medium containing atabrine (10 µg/ml.) and serial two-fold dilutions of INH, DHSM and PAS. Atabrine alone inhibited this strain of *M. tuberculosis* at 25 µg/ml. From the tube containing the highest concentration of the drug which exhibited growth comparable with that of the control tubes, 0.1 ml. was sub-inoculated into 9 ml. of medium containing the same and higher concentrations of the drug.

The results of this experiment are presented in Table 1. Although an increase in tolerance to the mixture of anti-bacteria and atabrine does eventually occur with repeated passages, there is every indication that the extent of this resistance is small compared with that obtained

Table 1. µMOLES OF PEROXIDE IN MOUSE AND ORGANS

		Mean weight	Mean peroxide	Peroxide/g
Whole mouse	Control	26.52 g	7.29	0.27
	Pregnant	29.96 g	13.38	0.45
			<i>P</i> < 0.01	
Uterus	Control	0.16 g	0.10	0.63
	Pregnant	1.73 g	0.26	0.15
			<i>P</i> < 0.01	
Ovarian fat plus ovaries	Control	0.54 g	0.14	0.26
	Pregnant	0.32 g	0.23	0.72
			<i>P</i> = 0.02	
Mammary tissue plus adjacent skin	Control	1.88 g	0.10	0.05
	Pregnant	1.80 g	0.18	0.10
			<i>P</i> = 0.01	
Adrenals	Control	4.97 mg	0.03	6.04
	Pregnant	4.87 mg	0.08	16.36
			<i>P</i> = 0.01	
Pituitary	Control		0.02	
	Pregnant		0.03	
			<i>P</i> = 0.20	

Table 1. PREVENTION OF *Mycobacterium tuberculosis* H37Rv RESISTANCE TO INH, DHSM AND PAS BY TREATMENT WITH ATABRINE (10 µg/ml.)

Drug	Treatment	Minimal inhibitory concentration (µg/ml.)					
		No. of passages					
		1	2	3	4	5	6
INH	With atabrine treatment	0.005	0.01	0.01	0.05	0.05	1.0
	Without atabrine treatment	0.005	5.0	100	100	100	100
DHSM	With atabrine treatment	0.1	0.05	0.5	0.1	0.5	0.1
	Without atabrine treatment	0.1	0.1	5.0	5.0	5.0	5.0
PAS	With atabrine treatment	0.1	0.1	0.5	0.1	0.1	5.0
	Without atabrine treatment	0.1	0.5	0.1	0.5	10	>100

with the systems containing the antibacterial drugs alone. It can be seen that the susceptibility of *M. tuberculosis* H37Rv to INH in the absence of atabrine has decreased by a factor of two thousand in the course of six passages, whereas in the presence of a sublethal concentration of atabrine it has decreased only by a factor of two hundred. Resistance to DHSM and PAS also developed, but more slowly than to INH, and represented an increase in the tolerance of the organism to these drugs by a factor of between fifty and a thousand in the absence of atabrine compared with an increase by a factor of between 0 and 50 in its presence. A comparable elimination of drug resistance was obtained when *Mycobacterium bovis*, strain Vallée, was passed in systems containing the atabrine-drug combinations. The significance of these observations is being investigated *in vivo*.

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### Effect of Berberine Sulphate on *Entamoeba histolytica*

BERBERINE, an alkaloid derived from the plant *Berberis aristata* Linn., has been shown to be useful in the treatment of experimental cholera in the infant rabbit model<sup>1</sup>. It compares well with chloramphenicol in the chemotherapy of cholera and severe diarrhoea in humans<sup>2</sup>. Results of the investigations on *Entamoeba histolytica* reported here suggest that berberine and its salts, such as berberine sulphate, may have a place in the chemotherapy of amoebiasis.

The original stock culture of *E. histolytica* provided by Dr N. K. Dutta of Haffkine Institute, Bombay, was grown in Boeck and Drbohlav (B.D.) medium and subsequently maintained in this, in LMS<sup>3</sup> and in Pavlova's media. Testing of the drugs *in vitro* was carried out in monophasic fluid media by the standard test-tube method and/or by a capillary tube method described by Woolfe<sup>4</sup>. A culture of *E. histolytica* grown in LMS medium for 48 h, in the presence of mixed bacterial flora, was used. Aqueous solutions of two samples of berberine sulphate, one supplied by E. Merck, Darmstadt, and the other prepared in our organic chemistry laboratory, were investigated.

'Intestopan Forte' (Sandoz) containing broxyquinoline and brobenzoxaldine was used as a standard for comparison. The results, shown in Tables 1 and 2, indicate that berberine sulphate is amoebicidal at a concentration of 0.5–1 mg/ml. and that it acts rapidly. Separate and independent experiments have shown that the observed amoebicidal action is not the result of bacteriostatic or

bactericidal action affecting the associated microflora. The growth of the latter was affected only in concentrations greater than 5 mg/ml. In the same conditions of testing, 'Intestopan' exhibited an amoebostatic action in concentrations of 0.05–0.10 mg/ml.

The morphological changes in the trophozoites after the addition of berberine sulphate include encystation, degeneration and finally lysis. Although the rate at which these steps followed each other differed from sample to sample, at the end of 12 h of incubation at 37° C, very few intact trophozoites could be observed. Preliminary results indicate that berberine sulphate may also prove to be cysticidal.

Experiments on 3–4 week old golden hamsters (average weight 40 g) (Table 3) confirm the results obtained *in vitro*. A dose of 2 mg/kg, given intramuscularly, and of 3 mg/kg when fed orally, administered in a regimen of 3 doses at 4 h intervals (first dose before infection, second dose at the time of infection and the third dose about 4 h after infection), prevents the development of hepatic amoebiasis. Similar results were obtained in rats infected intraeally with trophozoites of *E. histolytica* in which the control groups of animals developed an intestinal form of amoebiasis, while the group treated with berberine sulphate did not.

Berberine and its derivatives are well tolerated by animals in large doses as evidenced in the case of rabbits which tolerate a dose of up to 100 mg/kg administered

Table 1. EFFECT OF BERBERINE SULPHATE (MERCK), BERBERINE SULPHATE (ALEMBIC) AND 'INTESTOPAN FORTE' (SANDOZ) ON TROPHOZOITES OF *E. histolytica*

Drug	Growth of trophozoites in concentration (mg/ml.)								Remarks
	10	5	4	2	1	0.5	0.1	0.05	0
Berberine sulphate (Merck)	—	—	—	—	—	±	±	±	+
Berberine sulphate (Alembic)	—	—	—	—	±	±	±	±	+
'Intestopan Forte'	—	—	—	±	±	±	±	±	+

—, None; ±, occasional (< one/field) trophozoite or encysted amoebae observed; +, good growth (> twenty/field). All the tests were carried out in LMS<sup>3</sup> medium which is further modified by the inclusion of egg albumen to a final concentration of 1 per cent in place of normal horse serum. The density of the suspension of trophozoites was adjusted to an average of ten/microscopic field at a magnification of 100. We found that standardization by this method gave reproducible results. Observations on growth were made at the end of 24 and 48 h of incubation at 37° C.

Table 2. TIME COURSE OF THE AMOEBICIDAL ACTION OF BERBERINE SULPHATE ON THE TROPHOZOITES OF *E. histolytica*

Drug	Time after the addition of drug (h)	Growth of trophozoites in concentration (mg/ml.)					Remarks
		5	2	1	0.5	0	
Berberine sulphate (Merck)	1	±	+	+	+	+	Decrease in the number of trophozoites observed in all tubes except the one without the drug
	2	±	±	+	+	+	
	4	±	±	+	+	+	
	6	—	±	±	+	+	
	24	—	—	—	±	+	
Berberine sulphate (Alembic)	1	+	+	+	+	+	In all the tubes with the drug there was an initial increase in number followed by a rapid decrease
	2	+	+	+	+	+	
	4	±	+	+	+	+	
	6	±	±	+	+	+	
	24	—	—	±	±	+	

Symbols and conditions of experiment are the same as in Table 1.

Table 3. EFFECT OF BERBERINE SULPHATE (MERCK) ON HEPATIC AMOEBIASIS IN GOLDEN HAMSTERS

Route of administration	Dose/kg body weight	No. of animals in each group	Deaths	Post-mortem observations 4 days after infection
Intramuscular	2 mg	4	0	Normal liver. No trophozoites
	Control	4	1	Liver abscess and trophozoites (five to ten/field) observed in all the survivors and the dead hamster
Oral	5 mg	4	0	Normal liver
	3 mg	4	0	No trophozoites
	Control	4	0	One out of three showed small liver abscess; rest normal
	Control	4	0	Three out of four showed liver abscess with trophozoites; remaining one with normal liver

Hamsters were infected intrahepatically under ether anaesthesia. The drugs were administered in three doses, the first one about 4 h before infection, the second one at the time of infection and the third one about 4 h after infection. The controls received a placebo which was either Locke or Ringer solution. All the survivors were killed after 4 days. Infected livers were examined both macroscopically and microscopically.



subcutaneously<sup>1</sup>. Because of its lack of toxicity at amebicidal concentrations, berberine sulphate may prove to be a promising drug in the chemotherapy of amebiasis and other dysenteries. Clinical trials to test these and other possibilities of prophylactic chemotherapy in areas of endemic amebiasis are now being carried out.

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### Agent of Marek's Disease in Tissue Culture

ATTENTION has recently been drawn to the differences between avian leukosis and Marek's disease (neural lymphomatosis) in the chicken<sup>1</sup>. Avian leukosis is caused by a closely related and well characterized group of RNA viruses, but the nature of the causative agent of Marek's disease is not known<sup>2,3</sup>. The *in vivo* assay of blood and tumour material from chicks infected with both the *HPRS B14* classical strain and the *HPRS 16* strain of acute Marek's disease indicates that under the conditions of the experiments the infective agent was strictly associated with the cells and could not be separated from them<sup>3,4</sup>.

Work at these laboratories has been directed towards the development of a tissue culture system for the detection and assay of the infective agent of Marek's disease. Because of the apparent cell-bound nature of this agent, viable cells from affected chickens were either cultured as monolayers or were used for inoculating cultures of cells from uninfected birds. The experiments have been carried out using the *HPRS 16* acute strain of Marek's disease<sup>5,6</sup>. The most striking effects were noticed in cultures of chicken kidney cells prepared as previously described<sup>7</sup>, except that monolayers were grown in 60 mm plastic Petri dishes which were seeded with  $1.5 \times 10^6$  freshly trypsinized cells. Cultures were incubated at  $38.5^\circ\text{C}$  in a humidified atmosphere of 5 per cent carbon dioxide in air. The same effects were noticed both in uninoculated monolayers prepared from the kidneys of diseased chickens and in normal monolayers after the inoculation of cells from affected birds. A virus-like cytopathic effect (CPE) was observed under these conditions 7–10 days after the inoculation of infected material into the cultures. The changes are regarded as a slowly progressing CPE rather than a transformation. Foci of rounded, highly refractile cells appear in the cell sheet. Each focus extends outwards during a further 7–10 days incubation, in which time the cells either retract or detach from the centre giving rise to a microscopic plaque (Fig. 1).

The incidence of CPE in monolayers treated with various cell inocula and in monolayers prepared from the kidney cells of affected birds is shown in Table 1. Of sixteen inocula derived from chickens infected with Marek's disease, thirteen produced CPE in normal cultures of

chicken kidney. Of eighteen different batches of normal chicken kidney cultures, two showed the spontaneous occurrence of CPE. No more than three microplaques were found on each plate in these cases, and after killing the remainder of the group from which these two birds were taken, histological lesions of Marek's disease were found in the nerves of one of six birds examined.

Affected monolayers fixed with methanol and stained with May-Grünwald-Giemsa show that the rounded cells may be mono-, bi- or multi-nucleate. The cytoplasm of such cells is intensely basophilic while the nuclei may contain pink staining Cowdry type A inclusions. Multi-nucleate cells which have not rounded up may be found associated with plaques. Varying numbers of nuclei within these giant cells show nuclear inclusions (Fig. 2). Cytochemical observations on the nuclear inclusions using methyl green-pyronin, Feulgen and fluorescent coriophosphine staining<sup>8</sup> indicate that they contain DNA. Treatment of monolayers with 0.01 per cent crystalline DNase in phosphate buffer (pH 7.2) containing  $10^{-3}$  magnesium chloride at  $37^\circ\text{C}$  for 30 min removes the nuclear inclusions from affected cells when subsequently stained with the coriophosphine fluorochrome.

The addition of 100  $\mu\text{g}/\text{ml}$ . of 5-iododeoxyuridine to the medium of monolayers at the time of infection prevented the development of microplaques showing that the cytopathic effect of the agent was sensitive to an inhibitor of DNA synthesis.

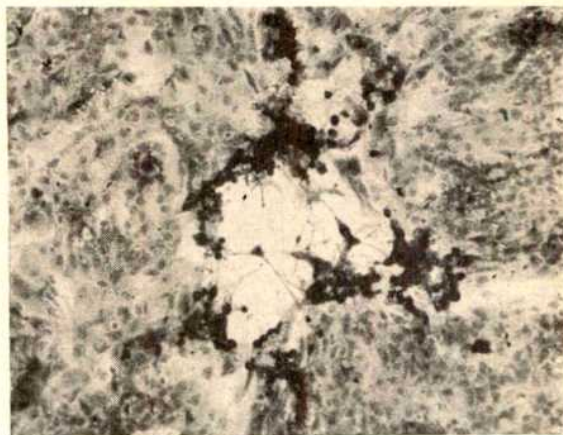


Fig. 1. Chicken kidney cell monolayer showing a microplaque 14 days after the inoculation of ovarian tumour cells. Note the rounded, intensely staining cells surrounding the plaque. (Stained with May-Grünwald-Giemsa,  $\times c. 125$ .)



Fig. 2. A giant cell found associated with a microplaque showing Cowdry type A inclusions within some of the nuclei. (Stained with May-Grünwald-Giemsa,  $\times c. 1,000$ .)

Table 1. INCIDENCE OF CPE IN CHICKEN KIDNEY TISSUE CULTURES INOCULATED WITH MAREK'S DISEASE TUMOUR CELLS

Test monolayer	Inoculum	CPE†
Normal chicken kidney	$2 \times 10^6$ ovarian tumour cells	4/5
" "	$2 \times 10^6$ testicular tumour cells	5/7
" "	Infiltrated liver cells	1/1
" "	MD infected blood	3/3
" "	Uninfected blood	0/1
" "	None	2/18*
Infected "	None	4/4

\* Marek's disease was diagnosed histologically in the group of birds from which the 2 positive cultures were prepared.

† The number of indicated inocula inducing CPE in tissue culture over the number tested. Each was inoculated into a different batch of tissue culture. Uninoculated control cultures from each batch were free from CPE.



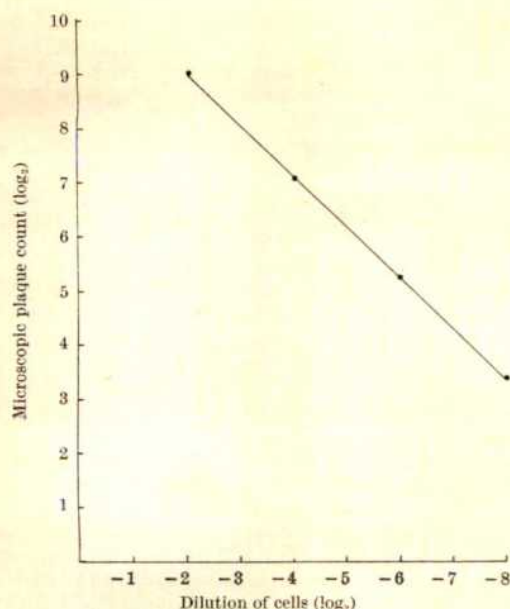


Fig. 3. Linear relationship between the number of versenized cells from an infected culture plated on normal kidney cells and the number of microplaques counted 6 days later.

Plaques which develop under fluid overlay can be counted microscopically after fixation and staining. An inoculum of  $10^6$  tumour cells was found to give rise to between ten and a hundred foci when counts were made 10–14 days later. Further incubation failed to reveal the development of significant numbers of secondary foci, which suggests that spread through the cell sheet occurs by cell to cell contact. The effect was passaged onto fresh monolayers by using cells from cultures showing a cytopathic effect, but not by using the supernatant free of cells. Cells were removed from Petri dishes by treatment with 0.1 per cent trypsin in versene (EDTA), counted, and inoculated onto fresh cultures in a series of four-fold dilutions. On passage small foci of refractile cells were observed 3–4 days after the inoculation of cells. Microplaques were counted 6 days after inoculation. The relationship between the number of cells plated and the number of microplaques obtained is shown in Fig. 3. The slope obtained by regressing these points is  $-0.95$  and indicates a linear relationship.

The infectivity of cultures showing a CPE and of control cultures was tested by the intra-abdominal inoculation of day old chicks from the Houghton Poultry Research Station line of Rhode Island Reds which are susceptible to Marek's disease<sup>4</sup>. Chicks were killed 3 weeks later, and results assessed by histological examination of nerves and gonads<sup>4</sup>. No infectivity was found in supernatant from affected cultures when cells had been removed by centrifugation, but the inoculation of day old chicks with between  $2 \times 10^4$  and  $2 \times 10^5$  cells from affected cultures produced specific lesions of Marek's disease. The result of one experiment is given in Table 2. In addition to microscopic lesions gross tumours of the gonad were found in some instances at 3 weeks, while the controls remained negative. These findings add support to the view that the agent producing the CPE is the cause of Marek's disease. The effects observed in tissue culture may result, however,

Table 2. INCIDENCE OF HISTOLOGICAL LESIONS OF MAREK'S DISEASE IN CHICKS INOCULATED WITH TISSUE CULTURE CELLS SHOWING CPE

Inoculum*	No. of days in tissue culture	CPE inoculated chicks	Proportion of †
Testicular tumour cells	16	++	6/9
Infiltrated liver	15	++	5/9
Infected CK tissue culture cells	35	+	3/9
None	16	—	0/9

\* Original inoculum causing CPE in chicken kidney monolayers.

† The number of chicks showing histological lesions of Marek's disease 3 weeks after inoculation with tissue culture cells over the number inoculated.

from a latent infective agent commonly found as a passenger in tissue from cases of Marek's disease. Biggs and Payne<sup>3,4</sup> have shown that the infectivity for chicks of blood from cases of Marek's disease is destroyed by ultrasonic disintegration and homogenization, that is, treatments which disrupt the cells. In the present investigation it was found that ultrasonic disintegration or freezing and

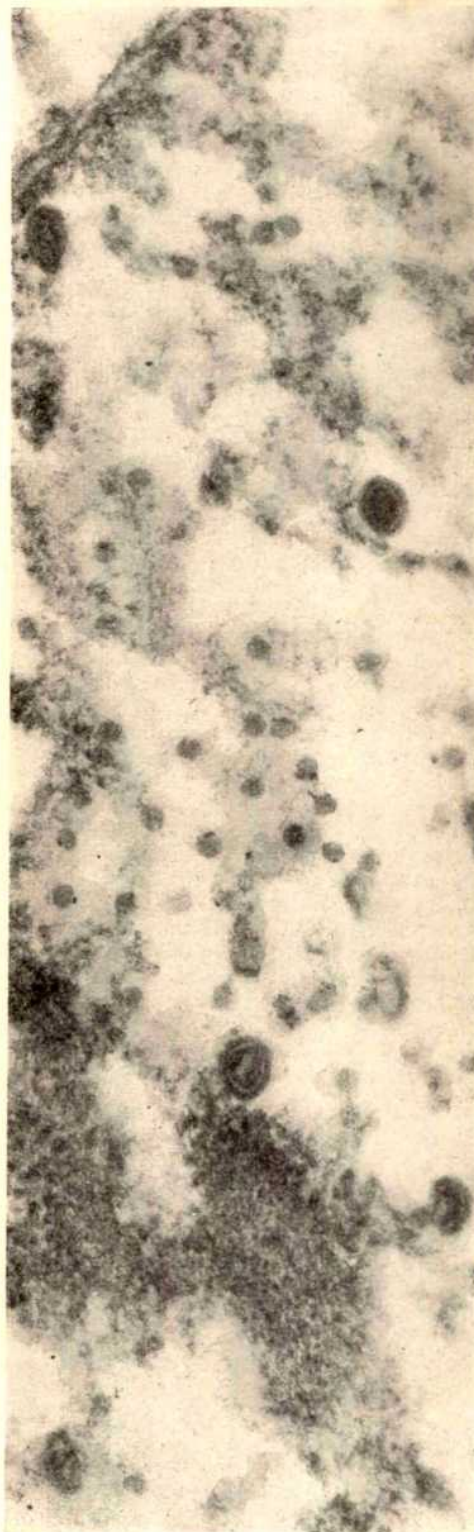


Fig. 4. Section of a cell from an infected tissue culture, showing intranuclear particles resembling herpes virus, and smaller granules measuring approximately  $30 \text{ m}\mu$ . The nuclear membrane is in the upper left corner. ( $\times 80,000$ .)



thawing of the tumour cells before inoculation onto tissue culture monolayers prevented the appearance of CPE.

Infected tissue cultures were examined by thin sectioning techniques of electron microscopy and by negative staining. Cell cultures were fixed in 1.5 per cent glutaraldehyde in phosphate buffer, and then fixed in Palade's osmium tetroxide. Embedding was done in methacrylate with 0.5 per cent uranyl nitrate added as a stain. Negative staining was performed by subjecting the infected tissue culture cells to freezing and thawing, suspending the cells in about 1 ml. of distilled water, and centrifuging the resulting suspension at about 3,000 r.p.m. with a desk centrifuge. The sediment was taken up in a small amount of 2 per cent sodium silicotungstate, and immediately applied to a grid.

Sections of cell cultures showed particles in the nuclei measuring about 100 m $\mu$ , and possessing an internal nucleoid and an external membrane (Fig. 4). These particles were consistent with the appearance of the herpes group of viruses. Smaller particles were also present, however, and those in the nuclei measured about 30 m $\mu$  in diameter and were often hexagonal in shape. These may correspond to the granules present in nuclei of cells infected with herpes virus, as described by Morgan *et al.*<sup>9</sup>. Particles have not yet been observed in the cytoplasm or outside the cells.

The negatively stained preparations showed two types of particle, apparently "hollow" (Fig. 5) and "full" (Fig. 6), and both measured about 100 m $\mu$  in diameter. Although capsomeres could be seen in the particles, their symmetry could not be established from the electron micrographs. The 30 m $\mu$  granules were not found in the negatively stained preparations. The conclusion from the electron microscopy was that a virus of the herpes type was present in the infected tissue cultures.

The present findings show that a cytopathogenic agent for chicken kidney tissue culture can be regularly isolated from the tissues of birds experimentally infected with Marek's disease. The effects in tissue culture are only obtained when intact viable cells are inoculated and, so far, no cell free infective agent has been recovered from these cultures. The CPE appears to spread in monolayers by cell to cell contact rather than by the release of free infectious particles into the supernatant. These findings closely resemble those described for the group B herpes viruses<sup>10</sup>, notably the varicella-zoster virus<sup>11,12</sup>. Electron micrographs of infected cells in this study support the thesis that the CPE is caused by a virus of this group. Herpes virus-like particles have been described in the Burkitt lymphoma of man<sup>13</sup> and in the cells of the Lucké renal carcinoma of the leopard frog, *Rana pipiens*<sup>14,15</sup>, which suggests that some herpes viruses are potentially oncogenic. The isolation of these viruses and the experimental demonstration of their oncogenicity, however, have yet to be achieved. The other

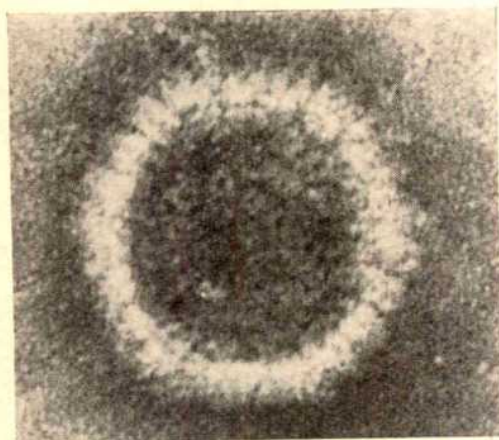


Fig. 5. Negatively stained preparation showing a "hollow" particle. ( $\times 400,000$ .)

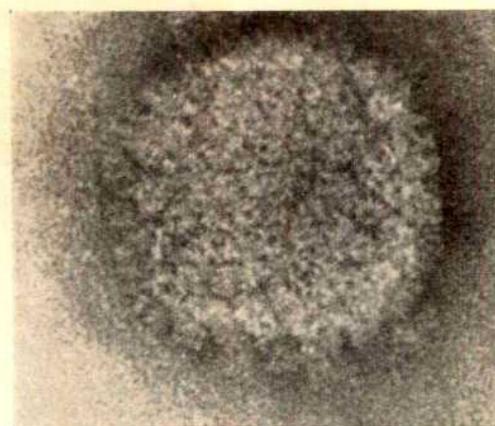


Fig. 6. Negatively stained preparation showing a "full" particle. ( $\times 400,000$ .)

viruses which have been isolated from frog tumours have not been shown to reproduce the disease and may only be passenger viruses<sup>16,17</sup>. In this study, cultures of cells showing a herpes virus-like CPE have been shown to be oncogenic when inoculated into chicks, reproducing Marek's disease. While these cultures clearly contain the agent of Marek's disease, the observed CPE could result from a passenger virus commonly found in the tissue of chickens. It is felt, however, that a comparison of the results presented here with those found for the agent of Marek's disease *in vivo*<sup>3,4</sup> suggests that the causal agent is a herpes virus which is not readily separated from cells in an infective form.

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### Effect of Suspending Medium on Heat Resistance of Spores of *Clostridium perfringens*

THE degree of heat resistance of spores of the various strains of *Clostridium perfringens* is a matter of both theoretical and practical importance, particularly because the organism is a potential food poisoning species. Studies carried out in various laboratories, by, among others, Hall *et al.*<sup>1</sup>, have indicated that spores of selected strains, when produced in Ellner's medium<sup>2</sup>, are less heat resistant than those produced in SEC broth. Hall *et al.* stated, "It seems probable that the atypical spores noted so frequently in this medium did not possess the heat resistant



characteristic of those produced in foods by the parent strain".

This communication reports further observations of this phenomenon. A strain of *C. perfringens*, NCTC 8238, which was originally isolated from salted beef and is one of the heat-resistant Hobbs strains, was used throughout. Spores were defined as those cells which survived heating at 75° C for 20 min. Spores were produced in Ellner's medium and in SEC broth.

The heat resistance of a part of the spores was initially determined at 100° C in the respective sporulating media, in 16 x 150 mm screw-cap tubes. The tubes, containing 3 ml. of suspension, were immersed in propylene glycol maintained at 100° C to within a few mm of the top of the cap. Before heating, the tubes containing the spore preparations were kept in ice water.

The remaining organisms were separated from their suspending media by centrifugation, washed, resuspended in physiological saline and the heat resistance of a portion was again ascertained. The remaining organisms were recovered from the saline solution; those originally obtained in SEC broth were suspended in the spent Ellner's medium and those from the Ellner's medium were suspended in the spent SEC broth and the heat resistance of each was determined once more.

In a second series of tests, spores produced in Ellner's medium were used to inoculate Noyes's veal broth<sup>3</sup> and were allowed to germinate and multiply. A sample of this culture was used to inoculate SEC broth. Spores forming in the SEC broth were tested for their heat resistance in this broth.

Eight replications of these experiments were carried out. Table 1 summarizes the results from the first series of experiments. The spores produced and suspended in Ellner's medium are much less heat resistant than those in SEC broth; however, when the same two preparations of spores are suspended in physiological saline, their heat resistances are very similar. Utilizing the two spent sporulating media as suspending media for spores had a very drastic effect on the heat resistance of the spores. Those produced in Ellner's medium, but suspended in SEC broth, were as heat resistant as those spores initially produced in SEC broth, whereas those spores originating in SEC broth were quite heat sensitive in Ellner's medium.

Table 1. HEAT RESISTANCE OF SPORES OF *C. perfringens*, STRAIN NCTC 8238, AT 100° C IN VARIOUS SUSPENDING MEDIA

Medium For sporulation	For suspension during heating	Number of positive samples in eight determinations				
		Min of heating at 100° C ‡				
		10	30	60	90	120
Ellner*	Ellner	8	0	0	0	0
SEC†	SEC	8	8	8	8	8
Ellner	Saline (0.85% NaCl)	8	8	2	1	1
SEC	Saline (0.85% NaCl)	8	8	6	0	0
Ellner	Spent SEC	8	8	8	8	8
SEC	Spent Ellner	6	3	0	0	0

\* 2.8 x 10<sup>8</sup> spores/ml.

† 7.0 x 10<sup>8</sup> spores/ml.

‡ Includes come-up time.

In the second series of experiments when, by passing the spores formed in Ellner's medium through a full cycle of germination in Noyes's veal broth and re-sporulation in SEC broth for a total of eight replications, the numbers of positive samples were 8, 8, 7, 7 and 5 for 10, 30, 60, 90 and 120 min of heating at 100° C, respectively. In this case, the suspending medium during heating was SEC broth. A comparison between the results obtained and those presented in Table 1 for spores produced and suspended in SEC broth again suggests the environmental effect on the measured heat resistance of spores.

There are various possible explanations for the results obtained. For example, the effect of the spent SEC and spent Ellner's medium may be only a reflexion of the varying pH of the media; or it is possible that unequal amounts of nutrients which could support germination remained in the two spent media. On the other hand, the heat sensitivity of the spores when they were suspended

in physiological saline during thermal stress suggests that the behaviour of spores of a given strain of *C. perfringens* is predicated by the effect of suspending medium, rather than by basic changes in heat resistant characteristics of the organism.

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### Genetic Association of Determinants controlling Resistance to Mercuric Chloride, Production of Penicillinase and Synthesis of Methionine in *Staphylococcus aureus*

It is generally accepted that the genetic determinant governing penicillin resistance in *Staphylococcus aureus* is located on an extra-chromosomal fragment<sup>1,2</sup>. This genetic determinant may exist in two alternative states, either as an extrachromosomal element or integrated into the chromosome<sup>3,4</sup>. Strains of staphylococci which are resistant to penicillin are often found to be resistant to mercuric chloride<sup>5</sup>. In addition, there is a genetic relationship between penicillin resistance and the chromosomal loci which govern the ability to synthesize methionine<sup>6</sup>.

This article describes evidence for the genetic relationship between the complex loci determining synthesis of methionine and the determinants governing resistance to mercuric chloride and penicillin. Data are also presented to show that both of these may exist either integrated into the chromosome or on an extrachromosomal fragment.

The parent strains of *Staphylococcus aureus* were resistant to penicillin (Pase<sup>+</sup>) and mercuric chloride (Hg-r) and were able to synthesize methionine (Met<sup>+</sup>). The strain designated as U9W was lysed by bacteriophages 80 and 81 of the International Typing Series. Strain U71 was lysed by phages 47 and 53. Strains sensitive to penicillin and mercuric chloride were designated Pase-Hg-s. The methionine dependent mutants which we used were isolated from strain U71 and described by Humbert and Baldwin<sup>7</sup>. Group C mutants (MetC-5 and MetC-9) grew when supplied with either methionine, homocysteine or cystathionine.

The Pase-Hg-s mutants were isolated after treatment with acridine orange or at an increased temperature of 44° C. After treatment, master plates were prepared and were replicated onto media containing 1 u of penicillin/ml. Colonies which did not grow on the medium containing penicillin were replicated onto brain heart infusion agar (Difco) containing a 1 : 20,000 concentration of mercuric chloride, and were phage typed to ensure derivation from the parent strain.

Methods used for propagation of bacteriophages and for transductional experiments were the same as those used by Pattee and Baldwin<sup>8</sup>. The transduction mixture was inoculated onto the surface of media containing the following selective agents: 0.15 u/ml. of penicillin G potassium; a 1 : 40,000 concentration of mercuric chloride; synthetic media<sup>9</sup> devoid of methionine. Sodium citrate (0.5 per cent) was also added to the transducing media to prevent further phage adsorption.

When strain U9W was treated with acridine orange or exposed to a temperature of 44° C, seventy penicillin and

mercuric chloride sensitive mutants were obtained. In contrast, only a single *Pase*<sup>+</sup>-*Hg*-s mutant was isolated after treatment of strain *U71*. Loss of a function after treatment with acridine dyes<sup>2,10</sup> and increased temperature<sup>11,12</sup> has been accepted as indirect evidence to distinguish between nuclear and extranuclear genetic structures. The results of this investigation have shown the mechanisms which control resistance to penicillin and mercuric chloride exhibited strain differences as demonstrated by the effect of acridine orange and increased temperature on their loss. Neither mutagenic agent appeared to have any demonstrable effect on strain *U71* in contrast to strain *U9W*. The ready loss of both determinants in strain *U9W* after treatment suggested autonomous replication. Failure of strain *U71* to lose the markers suggested that the markers are integrated or closely associated with the bacterial genome.

Although all mutants tested were found to be competent recipients of the *Pase*<sup>+</sup> and *Hg*-r determinants, no resistant recombinants were recovered when various penicillin and mercuric chloride sensitive mutants were used as donors in transduction. These data further support the suggestion that these mutants are sensitive due to loss of the entire genetic fragment.

Employing sensitive mutants of either strain, 100 per cent co-transduction of both the *Pase*<sup>+</sup> and *Hg*-r determinants was obtained when the initial transductants were selected for penicillin resistance. If, however, transductants resistant to mercuric chloride were selected, 100 per cent co-transduction of the penicillinase determinant did not occur (Table 1). The reasons for this variation are unclear although plasmid dissociation proposed by Novick and Richmond<sup>13</sup> is a possible explanation.

In some transduction crosses, all recombinants were initially selected for their ability to synthesize methionine. When *MetC*-9 was used as the recipient, 21 per cent of the transductants recovered were co-recipients of the methionine marker and either the *Pase*<sup>+</sup> or *Hg*-r determinant. These results were obtained after replication of the original *Met*<sup>+</sup> transductants onto media containing either penicillin or mercuric chloride. As shown in Table 2, about 1 per cent of recombinants simultaneously received all three markers. When *MetC*-5 was employed as the recipient, however, all three markers were simultaneously transduced at a frequency of 13 per cent. These results indicated that the determinant for mercury resistance was closer to the *MetC*-5 region.

Table 1. CO-TRANSDUCTION OF THE PENICILLINASE AND MERCURIC CHLORIDE DETERMINANTS TO PENICILLIN AND MERCURIC CHLORIDE SENSITIVE MUTANTS OF STRAINS *U9W* AND *U71*

Donor	Recipient	Frequency of transduction*		
		<i>Pase</i> <sup>+</sup>	<i>Hg</i> -r	<i>Pase</i> <sup>+</sup> and <i>Hg</i> -r
80/ <i>U9W</i>	<i>U9W</i> <i>Pase</i> <sup>+</sup> - <i>Hg</i> -s 1	10,020		10,020
80/ <i>U9W</i>	<i>U9W</i> <i>Pase</i> <sup>+</sup> - <i>Hg</i> -s 13	12,640		12,640
80/ <i>U9W</i>	<i>U9W</i> <i>Pase</i> <sup>+</sup> - <i>Hg</i> -s 44		1,260	1,240
80/ <i>U9W</i>	<i>U9W</i> <i>Pase</i> <sup>+</sup> - <i>Hg</i> -s 46		1,400	1,220
80/ <i>U9W</i>	<i>U9W</i> <i>Pase</i> <sup>+</sup> - <i>Hg</i> -s 48		3,340	2,980
53/ <i>U71</i>	<i>U71</i> <i>Pase</i> <sup>+</sup> - <i>Hg</i> -s 1	6,540		6,540
53/ <i>U71</i>	<i>U71</i> <i>Pase</i> <sup>+</sup> - <i>Hg</i> -s 1		6,920	6,140

\* Expressed as number of transductants recovered/10<sup>10</sup> phage particles in the transduction mixture.

Table 2. CO-TRANSDUCTION OF PENICILLINASE AND MERCURIC CHLORIDE DETERMINANTS OF METHIONINE SYNTHESIZING TRANSDUCTANTS BY PHAGE 53/*U71*

Recipient	<i>Met</i> <sup>+</sup>	Frequency of transduction*		
		<i>Met</i> <sup>+</sup> and <i>Pase</i> <sup>+</sup>	<i>Met</i> <sup>+</sup> and <i>Hg</i> -r	<i>Met</i> <sup>+</sup> , <i>Pase</i> <sup>+</sup> and <i>Hg</i> -r
<i>U71</i> <i>MetC</i> -9 <i>Pase</i> <sup>+</sup> - <i>Hg</i> -s	18,790*	2,570 (14%)†	1,320 (7%)	130 (1%)
<i>U71</i> <i>MetC</i> -5 <i>Pase</i> <sup>+</sup> - <i>Hg</i> -s	400	70 (17%)	140 (35%)	50 (13%)

\* Expressed as number of transductants recovered/10<sup>10</sup> phage particles in the transduction mixture.

† Per cent of *Met*<sup>+</sup> recombinants.

Table 3. THREE FACTOR RECIPROCAL CROSSES AMONG MUTANTS OF STRAIN *U71*

Donor	Recipient	Frequency of transduction*	
		<i>Met</i> <sup>+</sup>	<i>Met</i> <sup>+</sup> and <i>Hg</i> -r
<i>MetC</i> -5 <i>Hg</i> -r	<i>MetC</i> -9 <i>Hg</i> -s	4,940	360 (7%)†
<i>MetC</i> -9 <i>Hg</i> -r	<i>MetC</i> -5 <i>Hg</i> -s	260	120 (46%)

\* Expressed as number of transductants recovered/10<sup>10</sup> phage particles in the transduction mixture.

† Per cent of *Met*<sup>+</sup> recombinants.

<i>Met</i> C	<i>Met</i> B	<i>Met</i> A
9	5	7
		6 8 4

*Pase* *Hg*

Fig. 1. Diagrammatic representation of the relation of the penicillinase and mercuric chloride determinants to the chromosomal loci determining synthesis of methionine in *Staphylococcus aureus*.

The association between the mercuric chloride determinant and the *MetC*-5 region was determined by three factor reciprocal crosses. When strain *U71* *MetC*-5 *Hg*-r was used as the donor and *MetC*-9 *Hg*-s was used as the recipient, 7 per cent of the transductants recovered were *Met*<sup>+</sup>*Hg*-r (Table 3). These results were obtained after replication of the original *Met*<sup>+</sup> transductants onto media containing mercuric chloride. In contrast, 46 per cent of the *Met*<sup>+</sup> recombinants were *Hg*-r when *MetC*-9 *Hg*-r was used as the donor and *MetC*-5 *Hg*-s as the recipient.

The results of these crosses indicated that the locus governing mercuric chloride resistance was to the left of the *MetC*-5 region and thus located between *MetC*-5 and *MetC*-9. The location of the *Pase*<sup>+</sup> locus in this strain had previously been shown to be to the left of *MetC*-9<sup>6</sup>. A diagrammatic representation of the relationship between these two resistance determinants and the chromosomal loci determining synthesis of methionine is shown in Fig. 1.

The results of this investigation confirm the work of Novick and Richmond<sup>13</sup> in showing that the determinant governing resistance to mercuric chloride and penicillin may be located on an extrachromosomal fragment in strain *U9W*. In strain *U71*, however, this fragment is closely associated or integrated with the host chromosome near the locus which controls the formation of cystathionine. Such data indicate that in addition to the *Pase*<sup>+</sup> locus, the determinant governing mercuric chloride resistance may be episomal in nature.

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### Serological Differences between Apparently Typical Pepper and Tomato Isolates of *Xanthomonas vesicatoria*

This communication describes the use of serological techniques to distinguish apparently typical isolates of *Xanthomonas vesicatoria* (Doidge) Dows, obtained from pepper (*Capsicum frutescens* L.) and tomato (*Lycopersicon esculentum* Mill.). Isolates from pepper and tomato are culturally similar, although only tomato isolates hydrolyze starch<sup>1</sup>. A tomato isolate has been found to utilize mannose whereas two pepper isolates do not<sup>2</sup>. Pepper isolates have been found to be more virulent on pepper than on tomato, and tomato isolates were more virulent



on tomato than on pepper<sup>3,4</sup>; such isolates have been distinguished by means of phages. In another report, however, tomato and pepper isolates were shown to be culturally and pathologically similar and could not be distinguished with phages<sup>5</sup>.

We decided to determine whether serological techniques could be used to identify such isolates. Lyophilized cultures of a pepper isolate (Culture No. 11633) and a tomato isolate (Culture No. 11551) of *Xanthomonas vesicatoria* were obtained from the American Type Culture Collection, Rockville, Maryland, so that the isolates studied would be generally available. On potato-dextrose agar, the pepper isolate was bright yellow and the tomato isolate dull yellow or cream; our previous observations suggest that these colours are typical of pepper and tomato isolates. Also the pepper isolate was more virulent on pepper than on tomato, and the tomato isolate was more virulent on tomato than on pepper. Thus the isolates obtained seemed to be representative of pepper and tomato isolates.

Bacterial suspensions were prepared and frozen until used in preparing and evaluating antisera. Antisera were prepared in rabbits, with two rabbits for each isolate. All antisera had titres of between 1:2,000 and 1:4,000, and were frozen without preservative until tested in immuno-agar diffusion plates at room temperature (20°–35° C). In all comparisons, undiluted antiserum was added to the central well and bacterial antigens from disintegrated cells were added to peripheral wells. Sources of antigens tested included original frozen bacterial suspensions, isolates grown for 2 months on potato-dextrose agar, and isolates maintained for several weeks in their respective initial hosts.

For each isolate, antisera from each of the two rabbits reacted identically, and antigens from bacteria maintained by freezing, by growth on potato-dextrose agar, and by infections of host plants gave similar reactions. Illustrations of results are shown in Fig. 1. Pepper isolate antisera versus homologous antigens produced two prominent bands in 1 day, pepper isolate antisera versus tomato isolate antigens produced no bands in 1 day and two faint bands in 2 days. Tomato isolate

antisera versus pepper isolate antigens produced one band in 1 day, tomato isolate antisera versus homologous antigens produced two bands in 1 day. No additional bands were detected in the above tests during several days of incubation.

Results were consistent and show that these isolates can be distinguished serologically. Reactions between homologous antisera and antigens were the only ones which produced two bands in 1 day. Cultural growth and infection of initial hosts did not alter the results, which suggests that differences are relatively stable. Similarities between antisera from rabbits inoculated with a particular isolate suggest that the differences noted are not a result of variations in animals or techniques.

The reactions of the isolates were not reciprocal and perhaps were largely the result of differences in concentrations of antigens. Bands were broad and very likely included confluent factors. Further studies planned include the absorption and electrophoretic separation of factors.

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## CYTOLOGY

### The Nucleolus and Trisomy

EVANS<sup>1</sup> has argued that in the human oocyte attachment of acrocentric chromosomes to a persistent nucleolus during the first metaphase of meiosis may be an important cause of non-disjunction involving these chromosomes. The critical stages of meiosis in oocytes are difficult to examine, but Evans's hypothesis may receive indirect support from examples of the mechanism in mitosis. We have recently observed what appears to be such an example.

Fig. 1 is a photograph of a human lymphocyte in mitotic metaphase in which a G group chromosome is

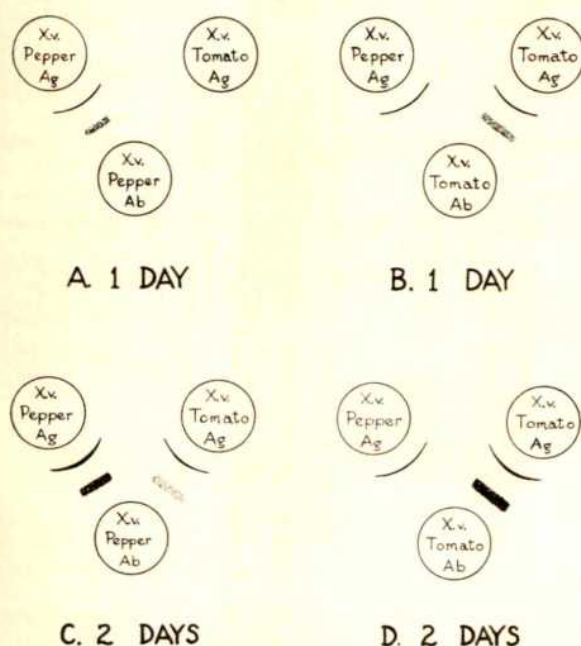


Fig. 1. Band formation after immunodiffusion reactions of *X. vesicatoria* pepper isolate (A, C) and tomato isolate (B, D) antisera with pepper isolate and tomato isolate antigens. Only one band formed between the tomato isolate antisera and pepper isolate antigens, and the production of bands was delayed and faint between the pepper isolate antisera and tomato isolate antigens. Reactions between homologous antisera and antigens always produced two bands in 1 day.

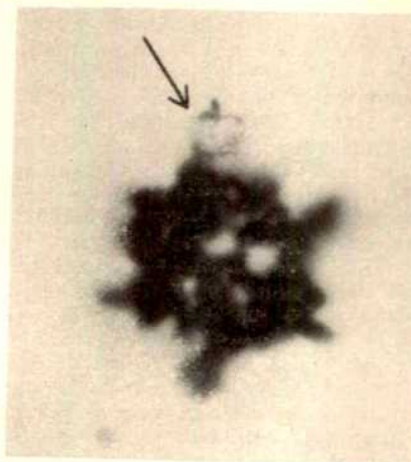


Fig. 1. Metaphase of a human lymphocyte showing a G group chromosome attached to a nucleolus.



detached from the other chromosomes and related by its short arms to a persistent nucleolus.

The preparation was made from a lymphocyte culture stimulated with phytohaemagglutinin and incubated for 72 h. No treatments with colchicine or hypotonic solutions were used. The cells were fixed in a solution consisting of 3 parts methanol and 1 part acetic acid and the chromosomes were spread by an air-drying technique.

The lymphocytes were obtained from a married woman aged 26 who has one son aged 3 and has aborted five times at 8-9 weeks. Cultures treated with colchicine and hypotonic solutions yielded cells of normal karyotype apart from one unusual A.1 chromosome in all the cells examined. This chromosome had an exceptionally obvious secondary constriction which was further from the centromere than usual. The patient denied a history of jaundice or of an illness suggestive of non-icteric hepatitis. Her father gives a history of jaundice and has had several surgical operations on his bile ducts. Her mother, who has had three children but no miscarriages, also had the unusual A.1 chromosome.

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### Cellular Distribution of Serum $\alpha$ -Foetoprotein in Organs of the Foetal Rat

THE mammalian foetus synthesizes a serum  $\alpha$ -globulin, called  $\alpha$ -foetoprotein, which is not found in the adult of the species<sup>1-7</sup>. Although interspecies structural differences among the  $\alpha$ -foetoproteins have been noted<sup>4-7</sup>, the protein of each species appears to be a homologue of foetuin, or bovine  $\alpha$ -foetoprotein<sup>5,7</sup>. Synthesis of serum  $\alpha$ -foetoprotein has been shown to occur in the liver in both the rat foetus<sup>8,9</sup> and the human embryo<sup>9</sup>, and in the yolk sac of the rat<sup>9</sup>. The cells responsible for this synthesis are not known, however, and it has been suggested that the haematopoietic tissue of the liver may be the site of synthesis<sup>8</sup>. In the present investigation, the cellular distribution of  $\alpha$ -foetoprotein in organs of the rat foetus was investigated by means of the fluorescent antibody method.

Albino rat foetuses of a Wistar strain were removed from their membranes after gestation for 15-19 days, frozen in tubes immersed in solid carbon dioxide and 95 per cent ethyl alcohol, and sectioned while frozen at 4-8 $\mu$ , either sagittally or in cross-section; the placenta and membranes were similarly frozen and sectioned. The sections were placed on glass slides, thawed and dried, placed in acetone for 10 min and again dried; they were then stained either with rabbit antiserum specific for rat serum  $\alpha$ -foetoprotein (anti-R $\alpha$ ) labelled with fluorescein, or with the same labelled antiserum from which reactive antibodies against  $\alpha$ -foetoprotein had been removed by adsorption with foetal rat serum. The rabbit antiserum had been prepared as described elsewhere<sup>7</sup>; it was labelled with fluorescein isothiocyanate<sup>10</sup> and made specific for rat  $\alpha$ -foetoprotein by adsorption with maternal rat serum<sup>7</sup>. The stained sections were washed in 0.1 molar sodium chloride, mounted in glycerol buffered at pH 8, and examined under the dark field microscope using ultra-violet light from a mercury arc lamp filtered through a Corning '5840' filter as the excitation beam. After selected fields had been photographed, the sections were stained with haematoxylin and eosin and the fields compared with their photographs.

None of the tissues of the rat foetus displayed specific fluorescence after staining with labelled anti-R $\alpha$  except those of the liver and yolk sac (Figs. 1A and C). In the

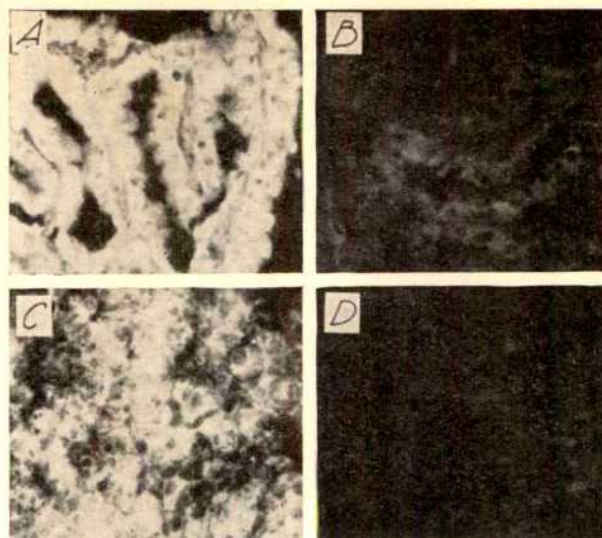


Fig. 1. A, Section of yolk sac treated with anti-R $\alpha$ , labelled with fluorescein, displaying specific fluorescence in cytoplasm of yolk sac cells. B, Section of yolk sac treated with anti-R $\alpha$ , labelled with fluorescein, adsorbed with foetal rat serum to bind antibodies against  $\alpha$ -foetoprotein; relatively little or no specific fluorescence is apparent. C, Section of foetal rat liver treated with anti-R $\alpha$ , labelled with fluorescein; most of the specific fluorescence is associated with the cytoplasm of parenchymal cells. D, Section of foetal rat liver treated with anti-R $\alpha$ , labelled with fluorescein, adsorbed with foetal rat serum. (All  $\times 150$ .)

liver, the fluorescence was found to be primarily in the cytoplasm of the parenchymal cells (Fig. 1C). Although slight fluorescence of the cytoplasm of some of the haematopoietic cells appeared to be present, the larger portion of the haematopoietic tissue did not show specific fluorescence. In the yolk sac, the fluorescence was associated with the cytoplasm of the yolk sac cells (Fig. 1A). Specific fluorescence was not found in the brain, heart, lungs, thymus, muscle, gastrointestinal tract, pancreas, spleen, kidneys, bone marrow, or placenta. Little or no fluorescence was observed in either the yolk sac (Fig. 1B) or the liver (Fig. 1D), when the fluorescent antiserum was adsorbed with foetal rat serum to bind the antibodies against  $\alpha$ -foetoprotein before staining.

The presence of serum  $\alpha$ -foetoprotein only in the yolk sac and in the liver of the rat foetus as determined in these experiments agrees with the finding that foetal rat liver and yolk sac will synthesize radioactive serum  $\alpha$ -foetoprotein when cultured in the presence of leucine labelled with carbon-14, whereas the other foetal rat tissues will not<sup>9</sup>. The results also suggest that hepatic synthesis of serum  $\alpha$ -foetoprotein probably takes place, primarily at least, in the parenchymal cells.

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## Effects of *N*-Methyl-*N*-nitrosourethane on Cells in Tissue Culture

A COMMUNICATION on the morphological conversion of cells *in vitro* by *N*-nitrosomethylurea by Sanders and Burford<sup>1</sup> prompts me to report similar experiments performed about 5 years ago in which the related and very effective carcinogen, *N*-methyl-*N*-nitrosourethane (MNU)<sup>2</sup> was used. Its action *in vitro* was tested on various mammalian cells grown in tissue culture. These included the Syrian hamster fibroblast *BHK* 21/13 *C* cell line<sup>3</sup> made available by Professor M. Stoker, and also fresh cultures of trypsinized baby (or foetal) rat heart fibroblasts. The cultures were maintained in Roux bottles, in Eagle's medium (containing double concentration of amino-acids and vitamins) and Hanks tryptose phosphate broth with 10 per cent calf serum. The cultures were trypsinized and sub-cultured once a week or more often. Explants, 24 h or 48 h old, were used for treatment with MNU of concentrations  $1 \times 10^{-2}$ – $1 \times 10^{-5}$  molar. For this purpose, the medium was removed from the cultures and replaced by MNU dissolved in ethanol (1:10 v/v) and diluted to the desired concentration with phosphate buffer. After 30 or 60 min at 37° C, the MNU solution was decanted, the cultures were washed once with phosphate buffer, and left in fresh medium at 37° C.

Concentrations of MNU of less than  $2 \times 10^{-4}$  molar allowed the cells to survive and to grow. These were collected by trypsinization, centrifuged and suspensions containing  $7$ – $10 \times 10^6$  cells/ml. were injected subcutaneously into homologous animals (0.1–0.2 ml./animal). Control animals were injected with cells grown and collected in a similar way which, however, were not treated with MNU, only with phosphate buffer.

Hamsters injected subcutaneously with *BHK* 21/13 *C* cells treated with MNU developed tumours at the site of injection, which became apparent in a few days, and formed large lumps, about 2 cm in diameter, 1 month after the injection. Hamsters injected with *BHK* 21/13 *C* cells which were not exposed to MNU developed similar tumours, however, though these grew at a somewhat slower rate. In both cases the tumours proved microscopically to be anaplastic sarcomata, which could be transplanted into other hamsters.

In contrast, no tumours were seen in rats which were injected with suspensions of rat fibroblasts, and maintained in tissue culture through only two or three sub-cultures, regardless of whether or not these were treated with MNU. The injected rats were observed for 7 months, during which time no tumours developed and at autopsy no residual surviving tissue was seen at the site of injection.

A single treatment with MNU can have profound effects on cells, which, however, manifest themselves first as inhibition of cell division, and probably correspond to the known "latent period" of carcinogenesis *in vivo*. The inhibitory action of MNU on mitotic division has been observed to last up to several weeks in the case of *Amoeba proteus*<sup>4</sup>; the affected amoebae retained their ability to grow, became greatly enlarged, and when they started dividing, they did so in a most irregular and unpredictable way.

Variations in the size of cells and of their nuclei could be clearly seen in cells of *BHK* 21/13 *C* cultures, when they assumed a rounded up form after trypsinization and were spread on microscopic slides, fixed in neutral formal and stained with, for example, the Feulgen stain. In *BHK* 21/13 *C* cultures treated with MNU the number of enlarged cells were more numerous. The cultures of *BHK* 21/13 *C* cells must have undergone malignant transformation in the course of prolonged subculturing *in vitro*. In some laboratories *BHK* 21/13 *C* cultures are said to contain a virus<sup>5</sup>.

The possibility has to be considered that the malignant transformation of cells in tissue culture occurs in stages<sup>3</sup>, and that MNU accelerates the process. It would be

desirable to establish at which stage this takes place. In the experiments described here, rat fibroblasts that had been subcultured two or three times when they were treated with MNU, and collected a few days later, failed to induce tumours when injected subcutaneously into young rats.

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## Time Sequence of DNA Replication in Heteropycnotic X

RESULTS obtained with different techniques suggest that heteropycnotic X, that is, the late-replicating X, in human female cells is not completely inactivated<sup>1-5</sup>. If the chromatin which is not late-replicating can be genetically active, then the observation of bands with little or no labelling by tritiated thymidine in the terminal phase of the *S* period may also indicate the presence of non-inactivated X linked genes on heteropycnotic X. By means of autoradiographic techniques<sup>6</sup>, non-late-replicating regions have been shown on this chromosome, but the validity of this observation is questioned by the finding, in the same experiment, of X chromosomes intensely labelled over their whole length<sup>7</sup>.

Such contradictory findings may simply result from the fact that precursors were available for different times in different cells in the same culture. The greatest difficulty involved in the study of patterns of DNA synthesis in chromosomes results from the necessity to either synchronize or time human cells in culture. Nevertheless, in cultures of asynchronous cells and with tritiated thymidine present in the medium at a constant concentration, it is now possible to arrive at a reasonable approximation of how much of *S* period each cell has spent in contact with the precursor. This is done either by evaluating relative incorporation in a late replicating chromosome<sup>8</sup>, or by using the cumulative curve method<sup>9</sup>. We have attempted to analyse in detail the way in which the synthesis of chromosome X is completed during the final 0.5 h of the *S* period.

Lymphocytes from a healthy woman were stimulated with phytohaemagglutinin by Moorhead's technique<sup>10</sup>. Tritiated thymidine was added to the culture at a final concentration of 1  $\mu$ Ci/ml. 3 h and 45 min before fixation; colcemid at a final concentration of 1  $\mu$ g/ml. was given 1 h and 45 min before fixation. The chromosomes and autoradiographic slides were prepared by the usual methods.

Random examination of 264 mitotic figures showed significant (greater than the background) labelling in sixty-six of these and absence of labelling in 198. The duration of the *G*<sub>2</sub> period was estimated as 3 h and 19 min and the *S* period as 26 min. The labelled cells were arranged in cumulative distribution, by Gilbert's method<sup>9</sup>, to establish the time of contact with precursor in each cell. The cells were then sub-divided into groups of thirteen, equivalent to 5 min of the *S* period, on the basis of a time scale plotted along the abscissa of the curve. All the grains present on the hot chromosome X (the hot-test of group C) of each of these groups were transferred

onto a diagram according to their real position (Fig. 1A). The exact site and intensity of DNA synthesis in each period was obtained by subtracting from each of these diagrams the grains present in the diagram of the period closest to the end of *S* (Fig. 1B).

These data show that the patterns of labelling observed at the end of the *S* period on hot *X* chromosomes are related to the degree of labelling in the cell to which the chromosomes belong and that each pattern pertains to a particular fraction of the terminal period of DNA synthesis. *X* chromosomes of cells which have been in contact with the precursor for about 26 min are almost completely labelled (Fig. 2A). Three non-late-replicating bands appear progressively on *X* chromosomes of the cells of successive periods and these are clearest in the group of cells which have been in *S* for not more than 15 min (Fig. 2B). Of the three bands which emerge from this analysis the widest is at the centromere, and the other two are at the distal extremity of the short arms and between the middle and distal third of the long arms. In the *X* chromosomes of cells that have been in *S* for 5 min or less, the three bands are no longer detectable and labelling is only present at the proximal part of the long arms (Fig. 2C). A more detailed view of how the sequence appears in the various zones of the *X* chromosome is shown in Fig. 1B; in each of the diagrams the number of grains expresses the relative amount of DNA synthesized in the corresponding sub-periods.

Previous investigations<sup>6,7</sup> showed that some *X* chromosomes with zones which are not late labelling are present at the end of the *S* period. Our results indicate that the finding of "cold regions" is an obligatory step in the

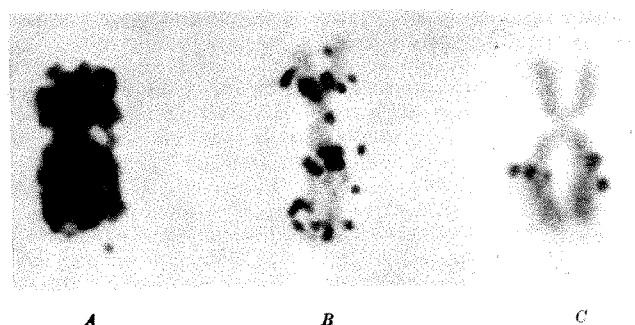


Fig. 2. Examples of different labelling patterns of *X* chromosomes belonging to cells which have been in contact with tritiated thymidine (A) for the last 25 min, (B) for the last 15 min, and (C) for the last 5 min of the *S* period.

sequence of the DNA duplication of heteropycnotic *X* and that the synthetic process is completed in those regions at least 25 min before the end of *S*. Thus the presence in the same culture of cells showing different labelling patterns on this chromosome is the consequence of the asynchrony among the cells.

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## GENETICS

### Immunochemical Studies on Bisalbuminaemia

BISALBUMINAEMIA is a rare inherited condition, with no clinical features, in which the plasma contains two albumins of different electrophoretic mobilities, usually in equal concentrations: the total concentration of albumin is normal. In all the families so far described, one of the albumins has migrated at the same rate as normal albumin; in some families the second albumin has migrated more slowly and in others more rapidly. Some of the reported variations in the characteristics of the two albumins have been discussed by Tárnoky<sup>1</sup>. The recent finding of homozygotes for the fast variant in two American Indian families has supported the hypothesis that the determinants for the albumins are alleles at a single locus<sup>2,3</sup>.

Earle *et al.*<sup>4</sup> made the first attempt to find an immunological difference between the separated albumins of bisalbuminaemia and normal albumin, using an antiserum to normal serum albumin in immunodiffusion studies. The three albumins showed reactions of identity with each other. Robbins *et al.*<sup>5</sup> suspected an immunological difference when they found that the ends of the albumin precipitin arc were split when immunoelectrophoresis was carried out using large dilutions of whole serum from patients with bisalbuminaemia. No splitting was observed with similar dilutions of normal serum. Split

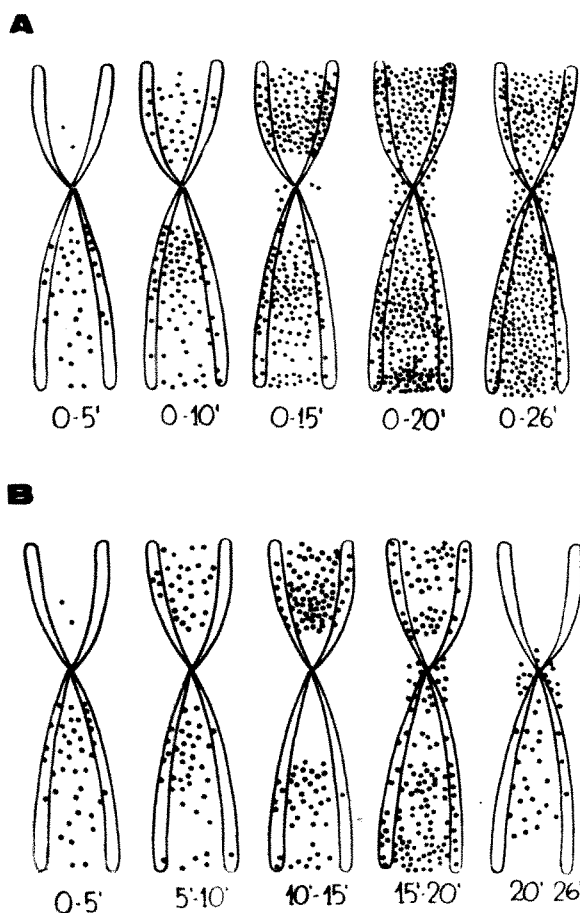


Fig. 1. Sequence in which labelling bands appear in the five successive sub-periods of the final 26 min of the *S* period. (A) Grains present on the *X* chromosomes of cells labelled between 0 and 5 min, 0 and 10 min, or longer, before the end of *S*. (B) In each sketch the grains actually formed in the corresponding sub-periods.

precipitin arcs can be observed in pure antigen-antibody systems when the antibody is in great excess<sup>6</sup>, however, and this finding alone is not proof of an immunological difference. The reactions of identity observed by Earle *et al.*<sup>4</sup> between the two albumins from patients with bisalbuminaemia and between these albumins and normal albumin, using an antiserum to normal serum albumin, show that the abnormal albumins have lost none of the antigenic determinants of normal albumin.

In a new family with bisalbuminaemia of the slow type, we have tested—we believe for the first time—the possibility that the variant albumin has gained an antigenic determinant as the result of the supposed amino-acid substitution which has altered its electrophoretic mobility<sup>7</sup>. We have shown, by the use of antisera to sera from normals and from patients with bisalbuminaemia, that both the albumins of the affected members of this family are immunologically identical with normal albumin.

The slow albumin of the affected members of the family we tested (Fig. 1) had an electrophoretic mobility relative to the other albumin of 0.90, both on paper and on cellulose acetate membrane in 0.07 molar barbitone buffer, pH 8.6. Potent antisera to sera from patients with bisalbuminaemia were raised in rabbits by the repeated intramuscular injection of sera emulsified in Freund's complete adjuvant. Pure samples of the two albumins were prepared in the following way. A little bromphenol blue was added to a serum, and electrophoresis was carried out in a horizontal starch block using 0.07 molar barbitone buffer, pH 8.6. The albumins appeared to bind bromphenol blue equally, and separated clearly. The bands were eluted from the starch and the albumin-dye solutions in buffer were separately applied to columns of 'Sephadex G-25' equilibrated with water. The albumins were eluted with water, appearing in the void volume of the columns free from dye and from buffer. Each albumin was shown by cellulose acetate electrophoresis to be electrophoretically homogeneous.

Immuno-electrophoresis of whole sera from patients with bisalbuminaemia showed in each case an albumin precipitin arc in the shape of a Cupid's bow, with no evidence of spur formation (Fig. 2). Both antisera were diffused in Ouchterlony plates against the two separated albumins and against pooled normal human serum albumin, each at a variety of dilutions. Reactions of identity were obtained between all the albumins, with no evidence of spur formation.

We conclude that the slow albumin and the normally migrating albumin from the affected members of this new family with bisalbuminaemia have lost none of the



Fig. 2. Immuno-electrophoresis of a 1:5 dilution of serum from a patient with bisalbuminaemia against its own antiserum, showing the precipitin arc of the albumins.

antigenic determinants of normal serum albumin, and have gained no new determinants.

We thank Dr Mary E. Cawley and Dr C. M. Dunn of Saltburn, Yorkshire, who provided details of the family and collected the blood samples.

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### Fundamental Theorem of Natural Selection

Li<sup>1</sup> has pointed out, by means of a neat algebraic formulation, that Fisher's fundamental theorem of natural selection does not apply exactly in a discrete-generation random-mating situation unless there is no dominance in fitness, and that the mean fitness cannot decrease from generation to generation for a single diallelic locus.

Li writes, "Fisher obtained his result on the basis of a continuous time model with logarithmic fitness", but it should be added that Fisher<sup>2-4</sup> never suggested that his theorem should be applied to discrete-generation models; indeed, he most carefully stipulated, in the sentence following his original formulation<sup>2</sup>, that "the rigour of the demonstration requires that the terms employed should be used strictly as defined". In particular, time was to be taken as continuous, and fitnesses were to be measured by Malthusian parameters of population increase. It is thus a misrepresentation to refer to any discrete-generation formulation as "Fisher's fundamental theorem".

These remarks should not be taken to imply that the interpretation of the fundamental theorem, correctly formulated, is simple. A further condition was later introduced by Fisher<sup>3</sup> and discussed by Kempthorne<sup>5</sup>. Kimura<sup>6</sup> has given a very general formulation, which Mode<sup>7</sup> has extended to the stochastic case. Several points remain to be cleared up and I shall examine them elsewhere<sup>8</sup>.

With regard to Li's second point, that in the discrete-generation model the mean fitness for a diallelic locus cannot decrease, it should be noted that this has already been proved by Moran<sup>9</sup>, and for any number of alleles by several investigators<sup>10-13</sup>. The fact that the general theorem preceded the special theorem for two alleles is due to the latter having been assumed for many years to be an obvious consequence of Wright's formulation<sup>14</sup>. In fact, as Moran<sup>9</sup> mentioned, monotonic convergence of the

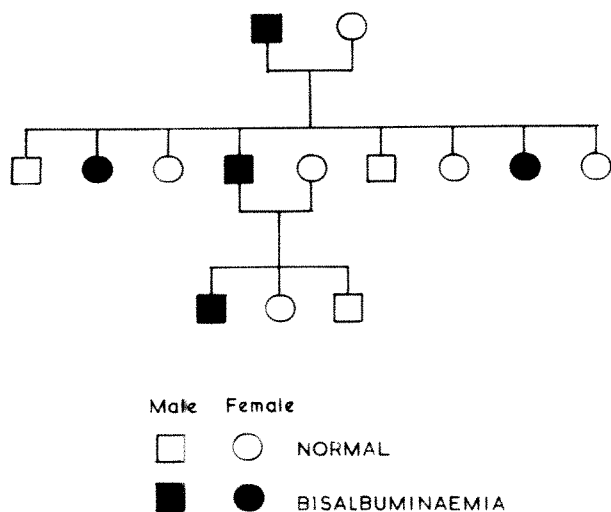


Fig. 1. Family tree.



gene frequency must be proved, and not assumed. Cannings has pointed out to me that in the case of heterozygote advantage monotonic convergence is implicit in Fisher's original treatment<sup>2</sup>. This property of the mean fitness should not, at the present state of our knowledge, be taken as a manifestation of any supposed fundamental theorem in discrete time.

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## HAEMATOLOGY

### Use of Human Lymphocytes in Studies of Drug Action

LYMPHOCYTES from venous blood synthesize immunoglobulins<sup>1</sup> and a wide range of other proteins<sup>2</sup>. Lymphocyte suspensions are a useful preparation for studying the effects of drugs on protein synthesis *in vitro*. Their value has been enhanced by using a medium free of plasma, so that substances in the plasma which affect protein synthesis<sup>3</sup> can be avoided. Also interactions of drugs with plasma proteins are avoided; alternatively it is possible to detect modifications of the effects of drugs by substances present in the plasma.

Lymphocyte suspensions were prepared by a modification of the method of Rabinowitz<sup>4</sup>, using cotton instead of glass wool. The cells were washed twice with Hanks balanced salt solution before suspension in culture medium (Eagle's medium<sup>5</sup> made up without leucine, and supplemented with "non-essential" amino acids, as described by Ambrose<sup>6</sup>). Triplicate cultures were set up with  $1-4 \times 10^6$  lymphocytes and  $0.25 \mu\text{C}$ .  $^{14}\text{C}$ -leucine (specific activity  $150 \text{ mc./mmole}$ ) in  $1 \text{ ml.}$ , for  $24 \text{ h.}$  To measure protein synthesis plasma was added, precipitated and washed with trichloroacetic acid, and its radioactivity was determined.

The plateau effect frequently seen with increasing concentrations of drug is illustrated in Fig. 1. Many drugs did not suppress the protein synthesis by lymphocytes *in vitro*. Cyclophosphamide, a drug which has been shown to inhibit immune responses *in vivo*<sup>7</sup> but is inactive *in vitro*<sup>8</sup>, was ineffective at concentrations up to  $160 \mu\text{g/ml.}$  No significant inhibition was observed in the presence of chloramphenicol at concentrations up to  $640 \mu\text{g/ml.}$  Salicylate ( $500 \mu\text{g/ml.}$ ) inhibited protein synthesis five times out of six, whereas *para*-hydroxybenzoate had no effect (Fig. 1). Two purine analogues, 6-mercaptopurine and azathioprine, inhibited significantly (25–75 per cent). Azathioprine ( $60 \mu\text{g/ml.}$ ) inhibited approximately twice as much as 6-mercaptopurine. Lymphocyte protein synthesis was completely abolished by puromycin, and actinomycin D immediately inhibited all but a small fraction of synthesis, which ceased after 6 h.

The inhibition by hydrocortisone may be related to its immunosuppressive effects. Hydrocortisone was inhibitory even at a very low concentration, an effect which seems to be at variance with the finding of Ambrose<sup>9</sup> that it is essential for antibody synthesis by fragments of rabbit lymph nodes. Chloramphenicol inhibits antibody synthesis in Ambrose's system<sup>9</sup>. These discrepancies have been resolved to some extent by subsequent studies of Ambrose which show that hydrocortisone and chloramphenicol exert their greatest effect during the inductive phase of the secondary immune response<sup>10</sup>. These substances had little effect on the established response. It is therefore possible that these drugs are effective on cells other than lymphocytes in Ambrose's system.

Synthesis of protein is stimulated 2.5–4 times by phytohaemagglutinin (PHA) in this system. The degree of inhibition of protein synthesis by all of the drugs used in these experiments was of the same order in the presence of PHA as in cultures without PHA.

The inhibition of protein synthesis by drugs in a medium free of plasma may be caused simply by the death of cells under these conditions. It was shown, however, that lymphocytes survived as well in the presence of concentrations of salicylate, hydrocortisone and chlorambucil which depressed protein synthesis significantly, as in the absence of these drugs. By contrast indomethacin killed most of the cells within 24 h (Fig. 2).

The inhibitory effect of drugs on protein synthesis may be modified by the presence of plasma. Synthesis in cultures free of plasma which contained drug was com-

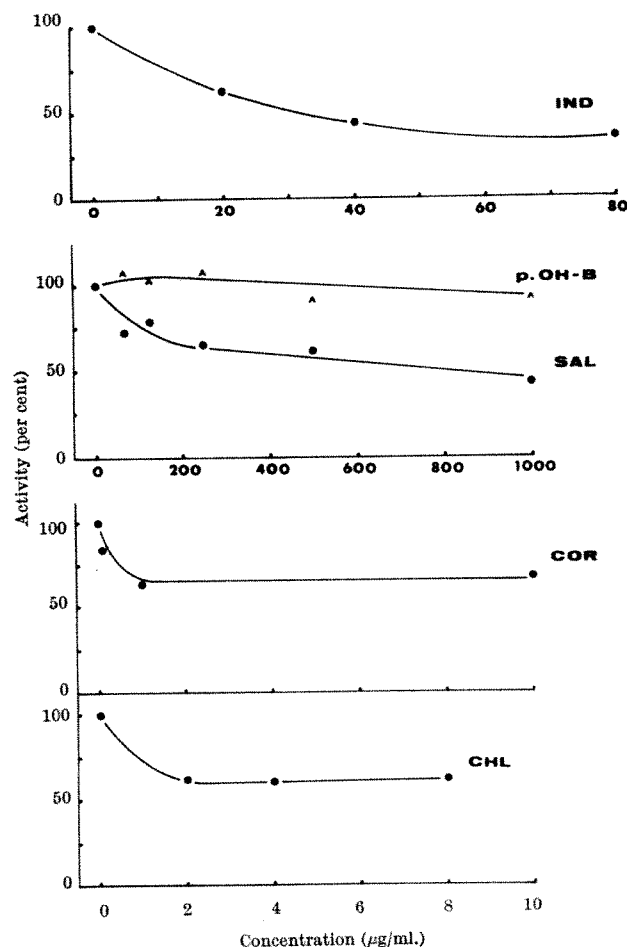


Fig. 1. Inhibition in the presence of increasing concentrations of indomethacin (IND), salicylate (SAL), hydrocortisone succinate (COR) and chlorambucil (CHL). The isomer of salicylate, *p*-hydroxybenzoate (p.OH-B), caused no significant depression of protein synthesis. Triplicate cultures set up in each experiment contained no drug. Protein synthesis in these cultures is represented as 100 per cent activity.

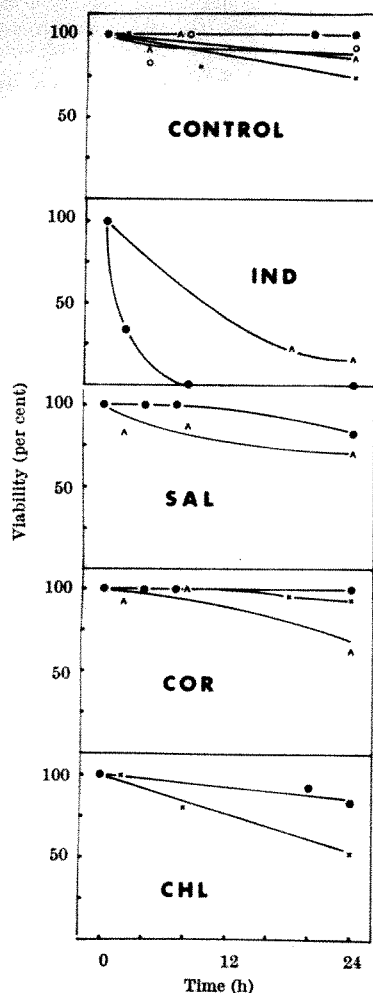


Fig. 2. Curves showing the survival of lymphocytes in cultures with a medium free of plasma. Survival was measured by counting the cells at intervals in a Coulter counter and determining viability by observing their ability to exclude trypan blue. The survival in the presence of salicylate, hydrocortisone and chlorambucil was not significantly reduced below that seen in control cultures. In contrast indomethacin was highly cytotoxic. Concentrations of drugs used were: indomethacin (IND) 40  $\mu\text{g}/\text{ml}$ ., salicylate (SAL) 500  $\mu\text{g}/\text{ml}$ ., hydrocortisone succinate (COR) 1  $\mu\text{g}/\text{ml}$ ., chlorambucil (CHL) 4  $\mu\text{g}/\text{ml}$ .

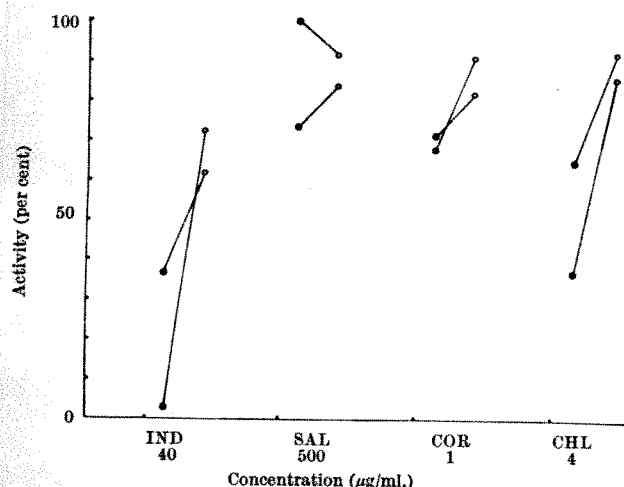


Fig. 3. Showing the inhibition of protein synthesis by lymphocytes by drug in medium free of plasma (●), and in medium containing 25 per cent autologous plasma (○). Lines join paired observations on lymphocytes cultured simultaneously from the same donor.

pared with synthesis in cultures in medium containing drug and 25 per cent autologous plasma (Fig. 3). The reduced potency of indomethacin, hydrocortisone and chlorambucil is probably caused by binding of drugs by plasma, which reduces their effective concentration in the medium. Oxyphenbutazone is known to be bound to plasma proteins<sup>10</sup>. When concentrations of this drug were increased to levels of 560  $\mu\text{g}/\text{ml}$ , the suppression of protein synthesis was similar to that caused by a concentration of 56  $\mu\text{g}/\text{ml}$  in medium free of plasma.

The mode of action of anti-inflammatory drugs is little understood. Auto-immune mechanisms, mediated by lymphocytes, are held to play a part in some of these diseases, such as rheumatoid arthritis. These data focus attention on the effects of anti-inflammatory drugs on cells of the lymphoid system. Of course, the effect *in vivo* may not be of the same order as that seen *in vitro*, but it is interesting that 6-mercapto-purine and azathioprine, which inhibit the homograft reaction<sup>7</sup>, are effective inhibitors of lymphocytes *in vitro*.

Screening for capacity to inhibit protein synthesis by lymphocytes may be a useful procedure in selecting drugs for both anti-inflammatory and immunosuppressive activity.

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## IMMUNOLOGY

### Modification of the Skin Allograft Response across the *H-2* Locus by Pre-treatment with Transplantation Antigens

WE have investigated whether or not pre-treatment with a sub-cellular fraction containing transplantation antigens can increase survival of skin allografts across the *H-2* histocompatibility locus in inbred adult mice. Graft survival was assessed by histological scoring at a standard time interval, rather than by relying on cross evaluation of graft rejection. Congenic resistant strains of mice were used so as to minimize non-*H-2* antigenic disparity between donor and recipient. *C57BL10/SnJ* mice (*H-2<sup>b</sup>*) were used as donors of antigen and skin, and *B10D2/SnJ* mice (*H-2<sup>d</sup>*) as recipients. All mice were obtained from the Jackson Laboratory, Bar Harbor, Maine, and were males between 10 and 30 weeks of age.

Antigenic extracts were prepared from lymphoid tissue by a modification of the magnesium-sucrose method of Mann *et al.* (unpublished results). Donors were killed by cervical luxation, and spleen, thymus, mesenteric, cervical and axillary lymph nodes were rapidly removed and immersed in cold Ringer's lactate solution. The tissues were washed in cold magnesium-sucrose solution (0.25

molar sucrose, 0.03 molar potassium chloride, 0.0005 molar magnesium chloride and 0.01 molar *tris-tris*-chloride, pH 8.0 at 5° C), cut into fragments, pressed through a 40 mesh stainless steel screen, and suspended in magnesium-sucrose solution in volume of 3–6 ml./donor. The suspension was dispersed in a glass hand homogenizer with 0.002 in. clearance and centrifuged at 1,500*g* max. for 15 min. The supernatant was centrifuged at 5,000*g* max. for 10 min, and the supernatant from this step was centrifuged at 105,000*g* max. for 1 h. The sediment of this last step, later used as the antigenic fraction, was resuspended in *tris* buffer (0.01 molar *tris-tris*-chloride, 0.0005 molar magnesium chloride, pH 8.0 at 5° C), freeze-dried, and stored at –20° C. The freeze-dried antigenic fraction, resuspended in 1 ml. of *tris* buffer, was injected intraperitoneally into prospective test graft recipients. A single injection of 1.6 mg given intraperitoneally 4 days before grafting was a moderately effective sensitizing dose (mean epithelial survival of 16 per cent at 6 days, *n*=11). Greater epithelial survival was seen after administration of either smaller or much larger doses.

Prospective recipients of graft were injected intraperitoneally with a large or small dose (8 mg or 8 µg) of the antigenic fraction on 6 days a week. After 3 weeks of pre-treatment, a test skin graft 1 × 1 cm was placed on the lateral chest wall. Antigenic injections were continued for 10 days. The grafts were then excised, and the raw graft bed was re-grafted with syngeneic skin to facilitate healing. Injections were continued one a week for 4 weeks after excision of the first-set grafts. The mice then received a second test graft on the contralateral chest wall. These grafts were also excised after 10 days. After excision, all skin grafts were fixed in 10 per cent formalin, processed, cut in six to nine planes and stained with haematoxylin and eosin. Grafts were coded, and epithelial survival was assessed histologically without knowledge of the origin of the graft. Criteria previously described for histological assessment of skin allografts were used<sup>1</sup>, but required modification because of the 10 day survival interval used in these studies. Grafts were assigned an epithelial survival percentage of 100, 90, 75, 50, 25, 10 or 0 based on microscopic examination.

Table 1. EFFECT OF HIGH AND LOW DOSE ANTIGENIC PRE-TREATMENT ON HISTOLOGICAL SURVIVAL OF *H-2* INCOMPATIBLE SKIN ALLOGRAFTS AT 10 DAYS (C57BL10/*SnJ* → B10D2/*SnJ*)

First-set skin grafts: recipients received intraperitoneal injections six times a week for 3 weeks before skin grafting.

Group	Injection	Epithelial survival percentage	Mean epithelial survival percentage
1a	8 mg of Antigen/ 1 ml. of <i>tris</i>	25, 25, 50, 50, 75	45
2a	8 µg of Antigen/ 1 ml. of <i>tris</i>	25, 50, 50, 75, 75, 90, 90	65
3a	1 ml. of <i>tris</i>	0, 0, 0, 25, 25, 25, 25	14

Second-set skin grafts: after excision of first-set grafts, intraperitoneal injections were continued once a week for 4 weeks and a second skin allograft applied.

Group	Injection	Epithelial survival percentage	Mean epithelial survival percentage
1b	8 mg of Antigen/ 1 ml. of <i>tris</i>	0, 50, 75, 90	54
2b	8 µg of Antigen/ 1 ml. of <i>tris</i>	0, 0, 10, 25, 25, 50, 75, 90	34
3b	1 ml. of <i>tris</i>	0, 0, 0, 0, 0, 0, 0	0

Three mice were lost from peritonitis (two in Group 1a and one in Group 1b). Two mice died from intraperitoneal bleeding after injection (one each in Groups 1a and 3b). Two mice were excluded because of technical failures in grafting (one each in groups 2a and 3a).

Results are shown in Table 1; mice which received either large or small doses of antigen showed an increase in first-set graft survival. This was particularly striking in the low dose group (*P* < 0.001 for Group 2a, as compared with control Group 3a). Improved survival of second skin grafts was also seen in all animals which received antigenic pre-treatment (*P* < 0.05 for Group 1b as compared with control Group 3b and *P* < 0.02 for Group 2b, as compared with 3b). The improved first-set

graft survival seen after injection of a small dose (2a) has since been confirmed in further experiments, but smaller doses of antigen (0.8 µg) have not resulted in increased allograft survival.

Previous attempts to induce tolerance across the *H-2* locus solely by antigenic extracts have generally been unsuccessful, although Martinez reported occasional striking prolongation of graft survival using massive doses of antigen<sup>2</sup>. Attention in the current experiments was focused on small doses of antigen, based in part on the results reported by Dresser<sup>3</sup> and Michison<sup>4</sup> using soluble non-transplant antigens (bovine serum albumin and bovine gamma globulin). The results reported here suggest that small doses of predominantly particulate transplantation antigens can diminish the host immune response to a test graft.

In summary, the survival of skin allografts has been increased in adult mice differing at the *H-2* locus by repeated intraperitoneal injections of either small or large doses of an allogeneic antigenic fraction. Graft survival was increased even after a strongly sensitizing stimulus in the form of a previous skin graft.

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### Monotypic Cold Agglutinins in Infection by *Mycoplasma pneumoniae*

ISOLATED cold agglutinins from patients with chronic idiopathic autoimmune haemolytic anaemia are macroglobulins, the majority of which have type-K and the minority type-L light chains<sup>1,2</sup>. Because they are homogeneous in individual patients with respect to their light chain types they resemble the "paraproteins".

Transient elevations of cold agglutinin titre occur in association with *Mycoplasma pneumoniae* infection<sup>3</sup>. Although overt haemolytic anaemia is uncommon in such situations<sup>4</sup> a transient autoimmune haemolytic anaemia can occasionally occur<sup>5</sup>. Transient post-infective cold agglutinins would be expected to be heterogeneous with respect to their light chain types. In twenty out of thirty isolated transient cold agglutinins so far reported in patients with *M. pneumoniae* infection or atypical pneumonia, both types of light chain were detected<sup>6-8</sup>. One type of light chain (either type-K or type-L) was detected in the remaining ten but their homogeneity was not definitely established<sup>6,8</sup>.

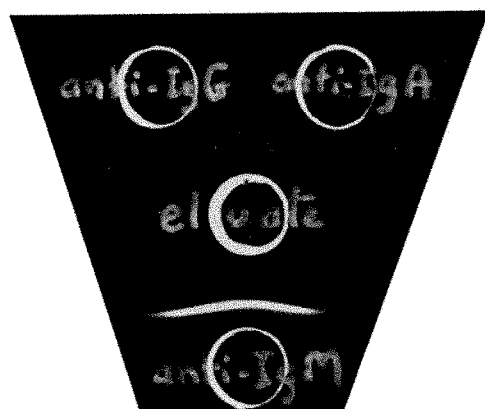
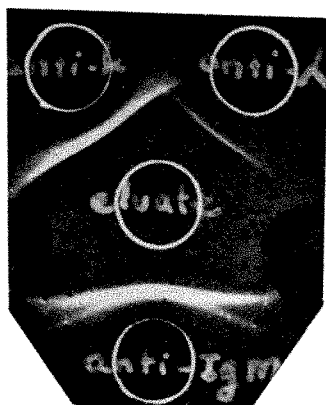
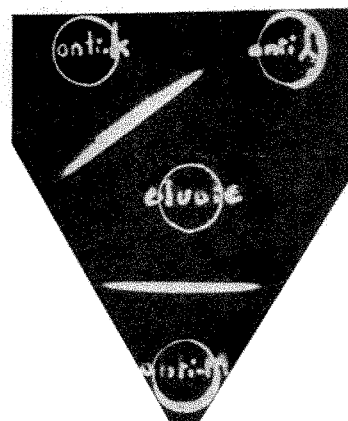
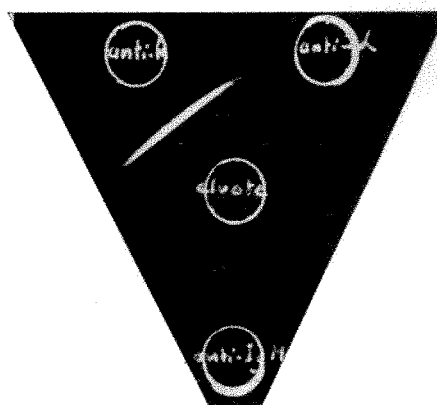
Ten cold agglutinins from patients with respiratory infections have been isolated by a thermal elution method<sup>9</sup> and their light and heavy chain types determined by the Ouchterlony technique in agar gel plates using anti type-K and anti type-L antisera provided by Dr J. Fahey and antisera to IgG, IgA and IgM obtained from the Netherlands Red Cross Laboratories. With one exception these were transiently elevated cold agglutinins, eight of which were associated with *M. pneumoniae* infection

Table 1. LIGHT CHAIN TYPES OF TEN ISOLATED IgM COLD AGGLUTININS FROM PATIENTS WITH RESPIRATORY INFECTIONS

Clinical picture	No. tested	Light chain types detected	
		K	K+L
<i>Mycoplasma pneumoniae</i> infection without anaemia	4	—	4
<i>Mycoplasma pneumoniae</i> infection with transient autoimmune haemolytic anaemia	4	3	1
Atypical pneumonia (cause not established) with transient autoimmune haemolytic anaemia	1	1	—
Chronic autoimmune haemolytic anaemia presenting after atypical pneumonia (cause not established)	1	1	—

(Table 1). The diagnosis of *M. pneumoniae* infection was based on a four-fold or greater increase in complement fixing antibody titre against this organism or a titre of 256 or greater during a respiratory infection. Tests for complement fixing antibodies against the antigens of *Rickettsia burneti* and the following virus antigens were negative: adenovirus, psittacosis, influenza A, B, and C, parainfluenza 3, respiratory syncytial, and sendai (parainfluenza 1). In four cases there was no anaemia; in five cases there was transient autoimmune haemolytic anaemia; in one case an episode of atypical pneumonia (aetiology not established) was followed by chronic unremitting autoimmune haemolytic anaemia.

All ten cold agglutinins were of anti-I specificity<sup>10</sup>; they were IgM antibodies forming precipitin lines with antiserum to IgM and not with antisera to IgG or IgA as in Fig. 1. The concentrations of IgM in the isolated cold agglutinin solutions ranged from 0.1 to 0.3 mg/ml. as determined by radial immunodiffusion (Hyland Immunoplates).

Fig. 1. Ouchterlony test with an isolated cold agglutinin (centre) from a patient with *Mycoplasma pneumoniae* infection and no anaemia, showing a precipitin line with antiserum to IgM but not with antisera to IgG or IgA.Fig. 2. Demonstration of type-K and type-L ( $\lambda$ ) light chains in an isolated IgM cold agglutinin (centre) from a patient with *Mycoplasma pneumoniae* infection and no anaemia.Fig. 3. Isolated cold agglutinin (centre) from a patient with *Mycoplasma pneumoniae* infection and transient autoimmune haemolytic anaemia, showing precipitin lines with antisera to IgM and type-K light chains but not with antiserum to type-L ( $\lambda$ ) light chains.Fig. 4. Isolated cold agglutinin (centre) from a patient with chronic autoimmune haemolytic anaemia presenting after atypical pneumonia (cause not established) showing precipitin lines with anti IgM and anti type-K antisera but not with anti type-L ( $\lambda$ ).

In the Ouchterlony tests the antigen wells were refilled three times with the more concentrated eluates and up to eight times with the more dilute ones. In this way approximately equal amounts of IgM were present in the various antigen wells. Both types of light chain were detected in all four of the transient cold agglutinins associated with *M. pneumoniae* infection without anaemia and in one of the four patients with this infection complicated by transient autoimmune haemolytic anaemia (Table 1, Fig. 2). There was no indication that the K and L determinants were present in the same molecules (as suggested by Costea *et al.*<sup>7</sup>) in so far as the precipitin lines with anti type-K and anti type-L antisera crossed one another without partial merging. In three of the four cases in whom the *M. pneumoniae* infection was complicated by transient autoimmune haemolytic anaemia and in the two cases with haemolytic anaemia (one transient and one chronic) in whom the aetiology of the pneumonia was not established, type-K light chains only were detected (Figs. 3 and 4).

Increase in cold agglutinin associated with *M. pneumoniae* infection is probably an indirect rather than a direct effect of the infection<sup>11</sup>. Whatever the mechanism of production of cold agglutinins with this infection, the finding of exclusively type-K light chains in some of them is interesting. It is not known whether such monotypic, post-infective cold agglutinins can, in some cases, persist; the observations in the last case (Table 1) indicate that such a possibility exists.



I thank Dr J. Fahey for the antisera to type-K and type-L light chains, Professor J. V. Dacie and Drs D. Garrow and H. P. Lambert for sera from patients with high titre cold agglutinins, Dr R. G. Sommerville for the mycoplasma and virus complement fixation tests, Mr C. Gilson and the staff of the Royal Free Hospital photographic department for their assistance.

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## PHYSIOLOGY

### Liebreich's Sign for Defective Colour Vision among Artists

LIEBREICH<sup>1</sup> pointed out that in a London exhibition of 1871 a painting showed roofs an oxen red on the sunny side but green where shadowed. He suggested that it indicated that the painter was a red-green colour vision defective. Angelucci<sup>2</sup> called this "Liebreich's sign" when he described the works of six painters known to be red-green defectives, whose pictures showed this characteristic. Trevor-Roper<sup>3</sup> and Kalmus<sup>4</sup> also mention Liebreich's sign.

I have examined a number of paintings by the red-green defective artists mentioned elsewhere<sup>5-8</sup>, and by six of the art students discussed later<sup>9</sup>, together with paintings by seven more art students. The resulting data are shown in Table 1, each type of defective being noted separately. Of the ninety-eight pictures which did not show Liebreich's sign twelve had no shadows because they were abstract designs. Liebreich's sign is not more often shown by deuterans than by protans because the observed difference is not statistically significant.

Table 1

Type	Artist	No.	No. of pictures	Liebreich's sign	
				+	-
DA	5	39	6	33	
EDA	5	25	4	21	
D	1	5	2	3	
Totals	11	69	12	57	
PA	2	19	0	11	
EPA	5	17	7	10	
P	1	12	1	19	
Totals	8	48	8	40	
Undiagnosed	1	1	0	1	
Grand totals	20	118	20	98	

Among six paintings reproduced in colour by Rabkin<sup>10</sup>, which are copies by red-green defectives of other pictures also shown, only Plate 4, by a deuteranomal, and Plate 6, by a protanomal, show Liebreich's sign. In Plate 10 a green shadow in the original has been more emphasized in the copy by a protanomal, while in Plate 12 green shadows of the original have become brown in the copy by a deuteranomal.

It appears that Liebreich's sign is sometimes present in the paintings of red-green colour vision defectives, but, at least in contemporary artists and students of art, it is too infrequent to be a reliable guide.

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### Effect of Laparotomy on Plasma Renin Activity in the Rabbit

WHILE trying to find an effective stimulus for the release of renin in the rabbit, we inserted a catheter into the left lumbo-adrenal artery and pushed the tip into the left renal artery close to the kidney. With no further manipulation the plasma renin activity increased within a few hours and subsequently fell to normal or less than normal values during the next few days. This unexpected observation encouraged us to investigate the changes in plasma renin brought about by opening the abdomen.

Eight rabbits were anaesthetized with 30 mg/kg of sodium pentobarbitone given intravenously. A 4-in. incision was made in the midline of the abdomen and the peritoneum was opened. The incision was covered with gauze swabs soaked in warm saline. After 20 min the abdomen was closed in two layers, and blood samples were taken by puncture of the central artery of the ear. Three samples were taken from each animal: after anaesthesia but before incision; 4 h after incision; and 48 h after the first sample. The volume of blood taken was 4.5 ml. with 30 U of heparin/ml. to prevent clotting. A normal diet with free access to water was allowed before and after operation. For comparison arterial blood samples were taken from four rabbits at time intervals which were the same as for the operated animals, but these rabbits were not anaesthetized nor were they operated on. A further four rabbits were anaesthetized and blood samples were taken at similar intervals.

Plasma was separated by centrifugation and assayed by a method described in more detail elsewhere<sup>1</sup>. Each sample of plasma was treated in the following way: 2 ml. of plasma was added to 1.0 ml. of soybean trypsin inhibitor (1 mg/ml.). To this mixture was added 1.0 ml. of  $4 \times 10^{-2}$  molar disodium ethylene diamine-tetraacetic acid (EDTA), and 0.1 ml. of  $4 \times 10^{-1}$  molar dimercaprol, which was in arachis oil. Soybean trypsin inhibitor and EDTA were in 0.1 molar sodium phosphate buffer, pH 6.0, containing chlorhexidine gluconate, 0.01 per cent w/v. The EDTA, dimercaprol and chlorhexidine inactivate plasma angiotensinases, and soybean trypsin inhibitor prevents the formation of plasma kinins. To this buffered plasma mixture was added sufficient renin substrate to give a final substrate concentration of 900-1,000 µg/ml. The final mixture was incubated for 6 h at 42° C. The supernatant collected after snap freezing or heating at 100° C was assayed to find the concentration of angiotensin.

Renin substrate was prepared from plasma of rabbits 48 h after bilateral nephrectomy. Ammonium sulphate was added to the plasma to 60 per cent saturation. The precipitate was separated by centrifugation and redissolved in a quarter the volume of distilled water. This

solution was dialysed at 4°C first against distilled water containing 0.01 per cent chlorhexidine gluconate for 24 h and then against  $3 \times 10^{-3}$  molar EDTA, also containing chlorhexidine, for a further 24 h. The clear, faint brown solution was used as renin substrate.

Plasma renin activity was estimated in the anaesthetized ganglion blocked rat<sup>2</sup> by direct comparison of the amount of angiotensin formed in the reaction mixture against the total substrate angiotensin available. We have defined for this reaction an arbitrary unit (our unpublished results)<sup>2</sup>. One unit is that net enzyme activity causing the release of 1 per cent of the angiotensin content of substrate in 1 h at 42°C when the initial concentration of substrate is within the first order range.

Table 1. EFFECT OF LAPAROTOMY ON PLASMA RENIN ACTIVITY

Rabbit No.	Control (U/ml.)	4 h (U/ml.)	48 h (U/ml.)
1	3.3	10.0	2.7
2	4.7	14.6	2.8
3	1.3	8.0	1.3
4	4.0	14.4	3.3
5	2.7	9.2	3.3
6	3.3	7.7	2.5
7	3.3	13.3	2.5
8	2.2	5.3	2.5

Mean plasma renin, controls = 3.1 U/ml. ( $\pm 0.42$  standard error)

Mean plasma renin at 4 h = 10.3 U/ml. ( $\pm 1.21$  standard error)

Mean plasma renin at 48 h = 2.6 U/ml. ( $\pm 0.22$  standard error)

By Student's paired *t* test the mean increase between the control group and the 4 h group is very significant ( $P < 0.01$ ). By the same test the decrease at 48 h was not significant.

Table 2. PLASMA RENIN ACTIVITY DURING SAMPLING AND ANAESTHESIA

Sample	Sampling only (4) mean (U/ml.)	Anaesthesia (4) mean (U/ml.)
Control	2.0 (4)	1.9 (4)
After two or three samples at 30-min intervals	2.3 (4)	1.8 (4)
4 h after control sample	2.4 (4)	1.2 (2)
2 days after control sample	1.8 (4)	1.6 (3)

Numbers in brackets indicate the number of rabbits in each group.

The effect of opening the abdomen is shown in Table 1. Plasma renin activity increased on average three-fold in 4 h. By 48 h plasma renin activity had returned to control levels and in five out of eight animals it was less than the control values.

Systolic blood pressure was measured using the Grant-Rothschild ear capsule in six rabbits which had been subjected to operation. In no case did the pressure fall to less than the pressure observed before anaesthesia, and never was it less than 80 mm of mercury. Sampling and anaesthesia produced no significant change in plasma renin activity, which confirmed previous observations<sup>3</sup> (Table 2). A feature of the control experiments was the remarkable constancy of plasma renin activity in a particular healthy rabbit although renin activity varied widely from rabbit to rabbit.

These results indicate that laparotomy causes a marked increase in plasma renin activity. Such change is in contrast to the results in rabbits subjected only to blood sampling and anaesthesia. The mechanisms involved in the changes in renin activity with operation are obscure. The increase in plasma renin activity seems to be too prolonged to be associated with the small systemic changes in blood pressure seen during operation. Opening the abdomen could act as a form of decompression stimulating a baroreceptor mechanism which in turn reduces renal blood flow. For example, a decrease in pressure in the superior mesenteric artery has been shown to produce reflex vasoconstriction in the cat<sup>4</sup> and dog<sup>5</sup>.

Increased plasma renin activity could stimulate the secretion of aldosterone leading to post-operative sodium retention<sup>6,7</sup>. Laparotomy in the anaesthetized dog has

produced a four-fold increase in secretion of aldosterone<sup>8</sup>. There are high concentrations of aldosterone in the plasma of the adrenal vein in man during operation<sup>9</sup>. Spironolactone has been shown to suppress almost completely post-operative sodium retention<sup>10,11</sup>.

Clearly future experiments on renin and aldosterone which require intra-abdominal manipulation should take into account the acute increase of plasma renin activity produced by laparotomy alone.

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## Fluid Dynamics of Excretion in *Anodonta*

URINE is produced in the bivalve mollusc *Anodonta* by the filtration of blood plasma into the pericardium and the pericardial fluid so formed is directed into the renal organs by the cilia of the reno-pericardial funnels<sup>1</sup>. The output of urine from the external urinary pore was until recently considered to be caused by filtration pressure and ciliary action. Trueman<sup>2</sup>, however, confirms the hypothesis of Ramsay<sup>3</sup> and Krijgsman and Divaris<sup>4</sup> that a negative pressure tends to occur in the pericardium at systole and suggests that the suction produced favours auricular filling. This suction would also oppose the flow of urine through the renal organs. Using transducers to record pressures in the pericardium and haemocoel of various bivalves, Trueman<sup>2,5</sup> has shown that there are considerable hydrostatic pressures corresponding to adduction of the shell and has established that there is a pressure gradient between the pericardium and the mantle cavity immediately after adduction. The possibility of these pressure gradients being involved in the mechanism of urine flow in *Anodonta* prompted this investigation.

Simultaneous pen recordings of pressures in the pericardial cavity, mantle cavity and renal organs, and of movements of the shell were obtained by the use of two Statham 'P23BB' pressure transducers (maximum sensitivity 0.4 cm water/cm pen deflection) and of an isotonic myograph. The cannulae, No. 16 hypodermic needles, were carefully inserted through small holes in the shell valves until satisfactory recordings were obtained, whereupon they were firmly fixed in position with wax. After recording, the locations of the cannulae were checked by dissection.

On adduction of the shell valves, simultaneous pressure peaks of equal amplitude were recorded from the pericardial cavity and mantle cavity (Fig. 1A). The amplitude of the pressure recorded from the renal bladder on adduction was, however, equivalent to about one-fifth of those of the pericardial and mantle cavities, though of similar duration to that of the pericardial cavity (Fig. 1B). Secondary peaks of longer duration and smaller amplitude followed in specimens where the foot was originally extended, coinciding with pedal retraction (Fig. 2).

To substantiate these results, the urinary pore of one side of *Anodonta* was displayed by the removal of a small square of shell and tissue. To aid observation, a little pericardial fluid was withdrawn and replaced by an equivalent quantity of starch in isotonic saline to which a few drops of iodine solution had been added. It was then possible, after allowing a sufficient period for the dye to distribute throughout the renal system, to observe the actual excretion of urine by the appearance of the blue fluid through the urinary pore. During periods of siphoning with the valves gaping widely, the urinary pore could be observed to be occluded by its sphincter muscle, occasionally opening briefly to allow a slight outflow of urine. Adduction of the shell valves resulted in a jet of urine being expelled through the urinary pore which ceased whenever pedal retraction took place.

The morphology of *Anodonta* (Fig. 3) implies that the increase in pericardial pressure at shell closure must effect a change in pericardial shape, either as the contraction of the heart and rectum or by the ventral displacement of the renal organs which lie immediately below the floor of the pericardium. Because natural adductions invariably occur during systole (Fig. 4), the ventricle is unlikely to be effective in buffering much of the force produced by further contraction but the rectum probably undergoes compression. Ventral displacement of the pericardial floor together with the lateral force imparted

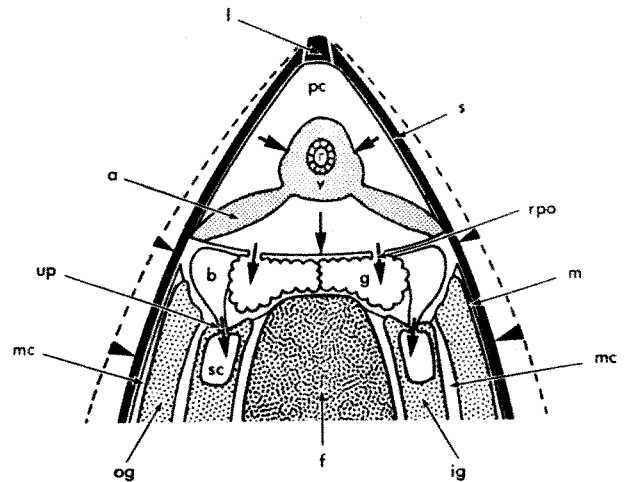


Fig. 3. Diagrammatic transverse section of *Anodonta* with valves adducted showing the probable effect of adduction on the pericardium. The dotted lines represent the original position of the shell valves before adduction and the large arrows indicate the effect of the pressure at adduction. *a*, Auricle; *b*, renal bladder; *f*, foot; *g*, glandular region of the renal organ; *ig*, inner gill; *l*, ligament; *m*, mantle; *mc*, mantle cavity; *og*, outer gill; *pc*, pericardial cavity; *r*, rectum; *rpo*, reno-pericardial opening; *s*, shell; *sc*, suprabranchial cavity; *up*, urinary pore; *v*, ventricle.

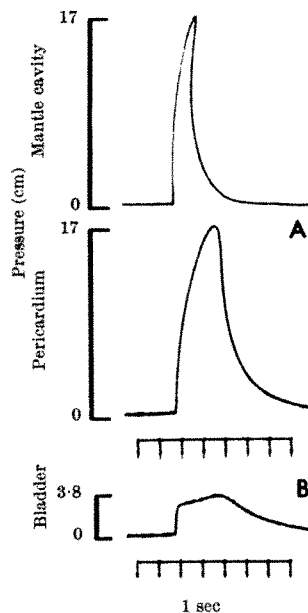


Fig. 1. Recordings demonstrating the relationship, at adduction, between pressures (in cm of water) in the mantle cavity and (A) the pericardium and (B) the renal bladder.

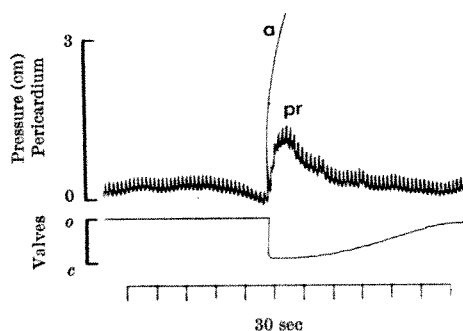


Fig. 2. Recording of the pericardial pressure, upper trace, showing peaks caused by adduction (*a*) and by pedal retraction (*pr*). Shell movements are also recorded, (*o*) indicating valves maximally open and (*c*) completely closed.

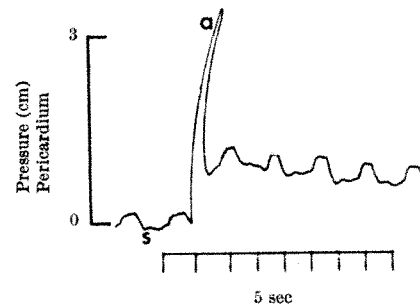


Fig. 4. Recording of pressure changes in the pericardium. The negative pressures (*s*) correspond to systole; the large peak (*a*) is brought about by adduction at systole.

on the bladder by the closing of the shell valves will apply an external force on the fluid within the renal organs which will produce a pressure gradient between the bladders and the suprabranchial cavities of the inner gill lamellae. This occurs because the positive pressure in the mantle cavity returns more rapidly to normal after adduction than does that in the pericardium and renal bladders because there is no ventral fusion of the mantle lobes in *Anodonta*, the mantle cavity being continuously open to the exterior (Fig. 1A and B). Opening of the urinary pores occurs at adduction and to equilibrate the pressure gradient urine is ejected under pressure into the suprabranchial cavities to be voided through the exhalant siphon. Pericardial fluid may also pass at adduction, under pressure, into the glandular parts of the renal organs through the reno-pericardial openings, but the resistance to flow in this region may be great because of the convoluted nature of the glandular epithelium.

Shell and pedal movements resulting in an increase in tone of the general body musculature are shown to play a significant part in the excretion of urine in the freshwater mussel *Anodonta*. Indeed, shell movements occur with sufficient frequency that they may well prove to account for most of the urine excreted. Further experiments are required to ascertain whether there is any direct relationship between shell or foot movements and osmotic regulation.

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### Ganglion Cells in the Frog Retina: Inhibitory Receptive Field and Long-latency Response

The ganglion cells of the frog retina can be sub-divided into different functional classes<sup>1</sup>. When investigated under light adapted conditions, class I cells (sustained edge detectors) respond to sharp edges moving through their receptive field, giving a stronger response when the object causing the edge is small than when it is large. A sustained response occurs when an edge is stopped within the field. In darkness, with a standing edge present in the receptive field, a response can be elicited by turning on the light or by a short flash of light.

Our investigation examines the properties of class I cells in dark adapted conditions with non-moving stimuli. Varying the intensity of light flash stimuli we found that the latency of the spike response becomes longer with increasing light intensities, the delays becoming abnormally long under certain conditions. A simple empirical equation adequately describes the relationship between light intensity and latency.

European water frogs, *Rana esculenta*, were immobilized with curare and given xylocain for local anaesthesia so that the optic tectum could be surgically exposed. Using metal filled microelectrodes, spikes were recorded from the terminal endings of class I axons in the outer layers of the exposed tectum (right hemisphere). The contralateral (left) eye faced the stimulus pattern (the ipsilateral eye was covered) and the frog was dark adapted for at least 30 min before measurements were taken. The experiments were carried out at room temperature, which ranged between 23° and 27° C. The stimulus pattern consisted of a stationary circular black disk against a matt white paper background with a contrast ratio of 1:10, centred in the receptive field 25 cm from the eye. According to Grüsser *et al.* (personal communication) an object at this distance will be in focus for curarized frogs. The diameter of the black disk subtended a visual angle of 5° from the eye. A stroboscope supplied single light flashes of high intensity with a half width of 50  $\mu$ sec. The peak intensity ( $I_0$ ) of the flash was 40,000 lux, as measured in a plane across the frog's eye, and neutral density filters were used to vary the intensity.

Readings were taken from thirteen cells in six different frogs, and a total of 149 measurements were made. After each light flash there followed a "rest period" of at least 60 sec before the next stimulus. As long as the rest period was greater than 30 sec the measured latency was

independent of the previous flash. The series of light flashes were given both in order of increasing and of decreasing intensities; the results did not differ. Fig. 1 *a-f* shows the results for one cell for six different intensities of flash. In this initial report only the latency between stimulation and the onset of firing will be considered; other parameters such as frequency and total activity have not yet been quantitatively investigated. In this case, at the lowest light intensity ( $I/I_0 = 0.0032$ ), the cell responded 1.2 sec after stimulation. As the intensity is increased the latency becomes progressively longer so that at the greatest intensity ( $I/I_0 = 1.0$ ) a delay of 13 sec is observed between the application of the light flash and the onset of major activity of the cell. Occasionally, one or two spikes can be seen during the first second after the stimulus.

When intensity and latency are plotted logarithmically, the relationship displayed in the graph in Fig. 1 is seen. The points represent each of the measurements made on the thirteen cells and the heavy unbroken line is a curve described by a power function of the form  $T = kI^\alpha$ , where  $k$  and  $\alpha$  are constants,  $T$  is latency and  $I$  is intensity. This line fits the experimental results within the intensity range investigated. It represents the least square fit applied to the logarithms of  $I$  and  $T$ , with resulting values for the constants of  $k = 10.1$  and  $\alpha = 0.36$ . The other lines represent those individual curves which have extreme values of  $k$  and  $\alpha$ . The two dashed lines show the consecutive measurements made on each of two different cells which had the largest (maximal and minimal) deviations from the average value of  $k$ . The two unbroken (thin) lines are correspondingly drawn through points from two cells with the largest deviations from the average slope.

The size of the stimulus disk affects the total number of spikes and the rate of spike discharge. We used the 5° disk because it produced more spikes at a given intensity than smaller or larger disks. Furthermore, we found a spatial organization of the receptive field. In order to evoke activity with a light flash it was necessary for a contrast to be present in the central excitatory part of the receptive field (ERF), whereas, when additional

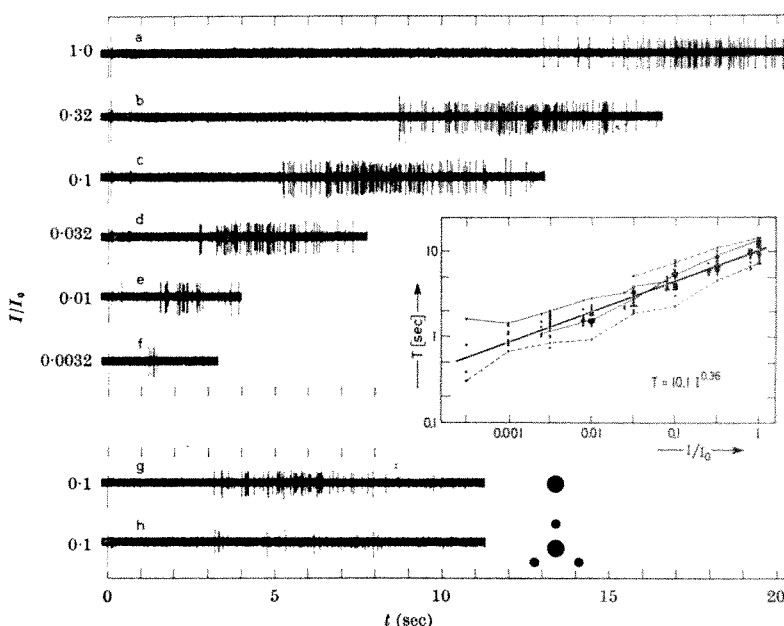


Fig. 1. Class I neurones. Records *a-f*: responses of one cell to short flashes of light of varying intensity, when a 5° black disk is placed against a white background and centred in the receptive field. First spike: stimulus artefact. The numbers to the left indicate the flash intensity in relative units. Records *g* and *h*: response of another cell to flashes of light of the relative intensity 0.1. Stimulus pattern as indicated to the right. Inset: the latency (in seconds) between stimulus and onset of spike response plotted versus the intensity of flash stimuli (in relative units). Data obtained from thirteen different cells. Lines drawn across the points are explained in the text.



contrasts were present in the area surrounding the ERF, the activity of the cell was reduced. This inhibition is thus seen only as a diminution of activity already evoked by a contrast in the ERF proper. An example of this effect is shown in records *g* and *h* of Fig. 1. Record *g* shows the response of the cell to a light flash ( $I/I_0 = 0.1$ ) with only a  $5^\circ$  black disk in the ERF. Record *h* was obtained under the same conditions, with the exception that three  $3^\circ$  disks were placed peripheral to the  $5^\circ$  central disk—as indicated by the small diagram beside the record. The distance between the centres of the central and peripheral disks was  $7^\circ$ . The general activity has been strongly inhibited. It was possible by placing still more disks in the surroundings to suppress the general activity completely. When a contrast was present only in the area surrounding the ERF, the cell did not fire.

Thus the ERF of class I ganglion cells is surrounded by a concentric inhibitory receptive field (IRF), similar to the spatial receptive field organization of class II ganglion cells<sup>2</sup>. The inhibition extends spatially at least over  $5^\circ$ , thus contradicting Schipperheyn's conclusion<sup>3</sup>. He concluded after using black and white stripe patterns with varying widths that the inhibitory interaction extends only over a distance of about four receptor cells ( $0.34^\circ$ ). The presence of the IRF may explain the observation of Maturana *et al.*<sup>1</sup> that large objects evoked less activity than small objects when moved through the receptive field—it may be that the large objects cause excitation in the IRF as well as the ERF and therefore produce a general reduction of activity.

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## Multi-ionic Action Potentials in Molluscan Giant Neurones

IN extension of the "sodium theory" of nerve impulse production<sup>1</sup>, a number of authors have described excitable membranes in which normal inward action currents are carried chiefly by calcium ions in crustacean muscles<sup>2-6</sup> and amphibian heart tissue<sup>7</sup>. It has been suggested that some gastropod giant neurones have "calcium spikes"<sup>8,9</sup>. Oomura *et al.*<sup>8</sup> showed that supra-oesophageal ganglion cells in *Onchidium* continue to produce spikes in media free from sodium or calcium, but become inexcitable if both sodium and calcium ions are removed from the perfusing solution. This behaviour is also seen in *Aplysia* giant neurones and, together with the quantitative effects of varying external ion concentrations, suggests that both sodium and calcium act as carriers of current during the normal action potential.

The experiments were performed on giant "A-cell" somata<sup>10</sup> in the visceral ganglion of *Aplysia californica*. The ganglion was isolated, mounted in a lucite chamber of volume 0.5 ml., and cut open to expose the giant cell to the perfusing solution. Intracellular recording and stimulating electrodes, and the extracellular micropipette used as a reference, were of 3–10 megohms resistance, filled with 2 molar potassium chloride. The normal saline solution contained 494 mmolar sodium chloride; 11 mmolar potassium chloride; 11 mmolar calcium chloride; 19 mmolar magnesium chloride; 30 mmolar magnesium sulphate; and 10 mmolar *tris* hydrochloric acid, pH 7.7.

Test solutions were made by replacing sodium or calcium chloride by an osmotically equivalent amount of sucrose. Before each measurement, the new solution was continuously perfused until 40 times the volume of the chamber had passed through. All experiments were done at 20–23°C.

The effects of varying external sodium while calcium was held constant at three different concentrations are shown in Fig. 1. Some variation of the resting potential was seen after each change of solution, but this was not consistently related to the concentration of sodium chloride. The spike amplitude, measured from the resting potential, varied almost logarithmically with  $[Na]_0$ ; the slope of this relationship was greatest at the least value of the external concentration of calcium,  $[Ca]_0$ , reaching 9.9 mV/ten-fold change in  $[Na]_0$  at  $[Ca]_0 = 11$  mmolar. The maximum rate of rise of the spike varied strongly with external sodium. Surprisingly, the critical depolarization for spike initiation increased abruptly with reduction of  $[Na]_0$ . This increase in threshold may reflect some change in the axonal spike-initiating membrane<sup>11</sup>; the resistance of the somatic membrane, by contrast, was not affected significantly by changing  $[Na]_0$  between 0 and 494 mmolar.

Alteration of the external concentration of calcium while holding  $[Na]_0$  constant at three different values produced the results shown in Fig. 2. Spike amplitude, measured from the resting potential, varied almost logarithmically with  $[Ca]_0$ . As with sodium replacement,

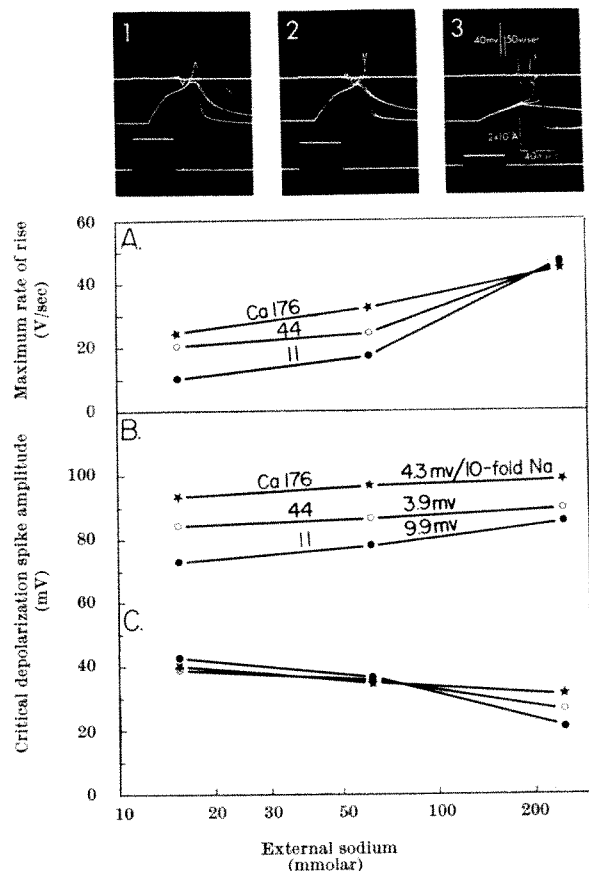


Fig. 1. Effects of partially replacing external sodium with sucrose when  $[Ca]_0$  was held constant. Inserts 1–3: one experiment at  $[Ca]_0 = 11$  mmolar.  $[Na]_0$  was 15.5 mmolar in 1, 62 mmolar in 2, and 248 mmolar in 3. Top trace shows zero potential and rate of rise of spike; middle trace is transmembrane potential, shifted 20 msec to right of top trace; lower trace shows stimulating current, synchronized with top trace. Four sweeps superimposed in each case, to display critical depolarization for spike initiation. Graphs: (A) maximum rate of rise; (B) spike amplitude measured from resting potential; and (C) critical depolarization, as functions of  $[Na]_0$  at three different constant values of  $[Ca]_0$ . Average slopes indicated for the two segments in each curve. Each point is the average of experimental results in three different cells.

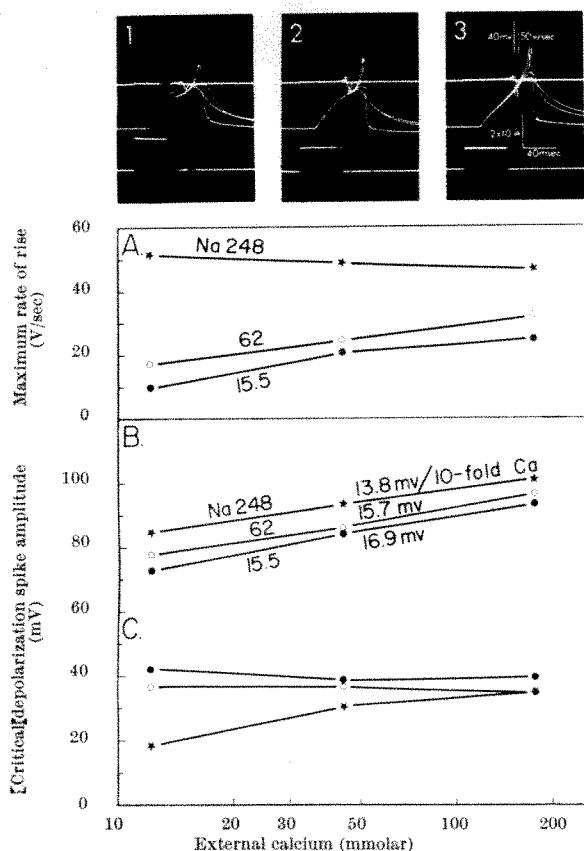


Fig. 2. Effects of increasing external calcium in exchange for sucrose when  $[Na]_0$  was held constant. Inserts 1-3: one experiment at  $[Na]_0 = 15.5$  mmolar.  $[Ca]_0$  was 11 mmolar in 1, 44 mmolar in 2, and 176 mmolar in 3. Top trace, zero and rate of rise; middle trace, transmembrane potential; lower trace, pulse current. Graphs: (A) maximum rate of rise; (B) spike amplitude; (C) critical depolarization, as functions of  $[Ca]_0$  at three different constant values of  $[Na]_0$ . Average slopes indicated; each point is the average of results in three different cells.

the slope of this relationship was greatest at the least concentration of the constant species. The slopes with changing  $[Ca]_0$ , however, were greater in every case than those obtained with changing  $[Na]_0$ , reaching 16.9 mV/tenfold change in  $[Ca]_0$  when  $[Na]_0$  was 15.5 mmolar. These calcium slopes are in the range observed by Gerasimov *et al.*<sup>9</sup> for *Helix* giant neurones. (Note that, as  $[Na]_0$  was increased to more nearly normal values, the spike amplitude showed approximately the same sensitivity to changes in  $[Ca]_0$  as was seen in Fig. 1 to changes in  $[Na]_0$  at the normal concentration of calcium.) The maximum rate of rise was far less sensitive to  $[Ca]_0$  than to  $[Na]_0$  at the more nearly normal concentrations. When plotted, however, for all values of  $[Na]_0$  and  $[Ca]_0$ , the maximum rate of rise was a monotonically increasing

function of the EMF-value (spike amplitude - critical depolarization). This suggested that the maximum rate of rise was determined principally by the size of the rapidly rising part of the spike, and not by the particular ion present in greatest concentration. The critical, or threshold, depolarization was elevated at low values of  $[Na]_0$ , and in that condition was practically independent of  $[Ca]_0$ . Somatic resistance, however, always increased with increasing  $[Ca]_0$ , as was seen by Gerasimov *et al.*<sup>9</sup>. Comparison of a high-sodium ion, low-calcium ion spike (Fig. 1, inset 3) with a low-sodium ion, high-calcium ion spike (Fig. 2, inset 3), indicates that calcium may substitute for sodium, restoring the action potential to normal or greater than normal amplitude, as has been found for neurones in *Onchidium*<sup>8</sup> and *Helix*<sup>9</sup>.

In experiments where sodium was completely replaced by a non-electrolyte, sucrose, it was always possible to obtain all or none action potentials for periods of up to 1 h. In five different cells, the spike height was reduced 12 per cent, 15 per cent, 21 per cent, 30 per cent and 37 per cent on replacing a normal sodium ion medium with a medium lacking sodium ions. The possibility that these spikes could have resulted from a diffusional barrier, preventing the complete removal of sodium ions, was examined by the method shown in Fig. 3. In these solutions free of sodium ions, rapid (1 min) changes from normal calcium to zero calcium caused the cell to become inexcitable, the spike returning with restoration of calcium. Apparently, the action potential in solutions free of sodium ions is largely due to the remaining calcium ions. The converse experiment of removing calcium ions from a normal medium containing sodium ions resulted in a slight reduction in spike amplitude, in agreement with Oomura *et al.*<sup>8</sup>.

To assess further the contribution of sodium to the spike, the puffer-fish poison, tetrodotoxin, was applied in the external solution. This drug blocks the production of spikes dependent on sodium at external concentrations of  $3 \times 10^{-10}$  molar in the frog single node of Ranvier<sup>12</sup>,  $1.5 \times 10^{-8}$  molar in the giant axon of the lobster<sup>13</sup> and  $3 \times 10^{-9}$  molar to  $1.5 \times 10^{-6}$  molar in the squid axon<sup>14</sup> (for further review see ref. 15). The mechanism of action of tetrodotoxin may be a specific occlusion of sodium channels<sup>16</sup>. In my experiments, the addition of tetrodotoxin to the normal saline solution at a concentration of  $3 \times 10^{-6}$  molar did not block the generation of all or none spikes. In four different cells, it caused reductions in spike amplitude of 2 per cent, 3 per cent and 22 per cent after application for about 5 min, and 18 per cent after 30 min. Recovery of spike amplitude was almost complete after removal of the drug. The reduction in spike height caused by  $3 \times 10^{-6}$  molar tetrodotoxin is similar to that obtained here with complete replacement of sodium, and this suggests that tetrodotoxin may act by blocking the sodium ion component of the spike only.

The dependency of spike amplitude on both  $[Na]_0$  and  $[Ca]_0$ , when varied individually, and the persistence of

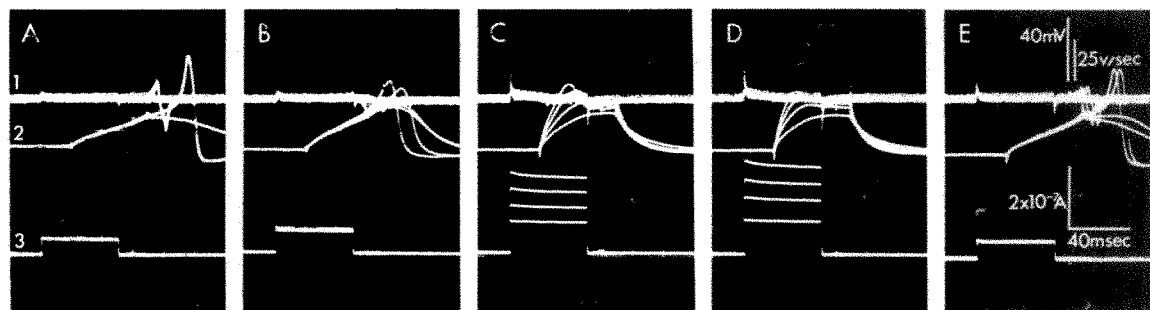


Fig. 3. Abolition of all or none spikes in sodium free medium by removal of calcium.  $[Ca]_0$  was 11 mmolar in (A), 2.75 in (B), 0.69 in (C), 11 in (D) and returned to 11 in (E). Trace 1, rate of rise and zero level; trace 2, transmembrane potential, shifted to right of trace 1; trace 3, stimulating current pulse. Four sweeps superimposed in each photograph.



the spike in the absence of either cation, are presumptive evidence for a multi-ionic mechanism of carrying action currents. In such a system, the rate of change of action potential size with concentration of calcium, in the presence of a constant amount of sodium, for instance, would be expected to be less than the 29 mV/tenfold change in  $[Ca]_0$  predicted for a calcium electrode. Published data concerning the effect of external calcium on muscle spike heights deviate widely from this simple relationship<sup>6,7</sup>. In each case, it has been possible to explain this discrepancy very well by treating the active membrane as a multi-ionic system.

I thank S. Hagiwara for the research facilities and for his help. This work was supported by a grant from the US Public Health Service.

*Note added in proof.* Another class of molluscan nerve cells has recently been described<sup>17</sup> in which spike amplitude depends on both external sodium and calcium concentrations.

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## PATHOLOGY

### Growth of Two Human Tumour Cell Lines in Mice treated with Antilymphocyte Serum

TISSUE culture lines of human tumours can be transplanted into animal hosts conditioned with immunological depressants such as cortisone or irradiation<sup>1</sup>. Several reports<sup>2,3</sup> describe the immunosuppressive action of antilymphocyte serum; mice treated with antilymphocyte serum have a reduced capacity to reject skin homografts. Antilymphocyte serum seems to be the most effective immunosup-

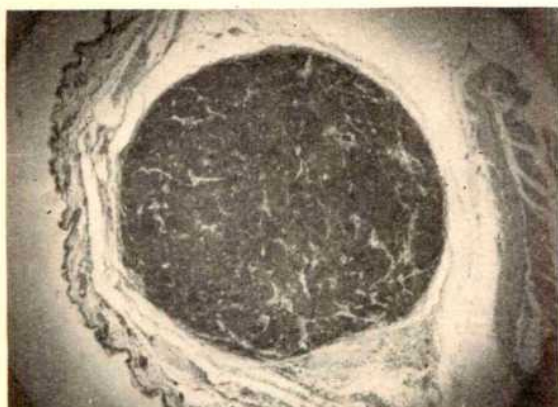


Fig. 1. HeLa cell tumour, day 15. (Haematoxylin and eosin,  $\times 60$ .)

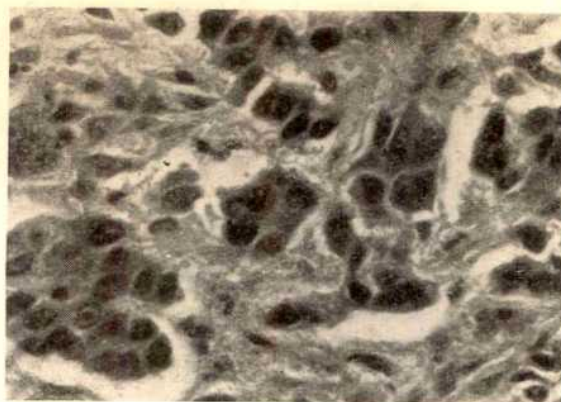


Fig. 2. Hep 2 cell tumour, day 15. (Haematoxylin and eosin,  $\times 945$ .)

pressant yet discovered, and because of its specificity and low toxicity it may provide an improved alternative to methods of host conditioning at present used in tumour transplantation. We have been able to grow two human tumour cell lines in mice treated with antilymphocyte serum.

Antiserum was produced as follows. Thymuses from between six and eight young female *Balb/C* mice were minced in cold normal saline and passed through sterile surgical gauze. The resulting suspension was injected intravenously into New Zealand white rabbits which were given a second injection 14 days later. Serum was obtained 7 days after this, heated to 56°C to destroy complement, and stored at -20°C.

Newly weaned *Balb/C* mice were subcutaneously injected in the ventral midline with a single cell suspension of  $0.5 \times 10^6$  HeLa\* or Hep 2† human tumour cells in 0.1 ml. Eagle's medium, and in the nape of the neck with 0.25 ml. antilymphocyte serum. They were then given a similar injection of antilymphocyte serum three times a week. Control groups were given tumour cells only.

**HeLa cells.** Tumours grew in eleven out of twelve animals given HeLa tumour cells and treated with antilymphocyte serum (Fig. 1). Mice were killed on day 15, by which time most tumours had grown to 5 mm in diameter, and one to 8 mm. Metastases were not found. Tumours did not grow in any of a group of ten control animals. In seven of these animals a small nodule formed subcutaneously, but this had regressed in five animals by day 3, and in all animals by day 10. There was no evidence of tumour growth in any of these animals when examined microscopically.

**Hep 2 cells.** Tumours grew in six out of ten mice given Hep 2 cells and treated with antilymphocyte serum (Fig. 2). In two animals the tumours showed signs of regression by day 13. The remaining four reached a diameter of 4–6 mm by day 15; there were no metastases. No tumour growth was observed in any of the ten animals in the control group.

Secondary transplantation from the growing tumours was attempted. The tumours were minced in normal saline, filtered through sterile gauze, and the resulting suspension subcutaneously injected into mice treated with antilymphocyte serum. No growth was obtained with transplanted Hep 2 tumours. Transplanted HeLa tumours reached a diameter of 5 mm but showed signs of regression by day 12. The mice were killed on day 15. When examined microscopically, the tumours showed a large central area of necrosis not present in the original tumour.

We hope that antilymphocyte serum may prove of value in growing primary explants of human tumour in laboratory animals. We have so far been able to grow one primary explant out of five implanted in mice con-

\* From a line cultured in the Department of Bacteriology in this hospital.  
† Burroughs Wellcome Laboratories.



ditioned with antilymphocyte serum. This was an adenocarcinoma of the stomach.

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## Unusual Susceptibility of Lingual Epithelium to Tumour Induction by 4-Nitroquinoline-1-oxide

4-NITROQUINOLINE-1-OXIDE (NQO), a simple bicyclic compound which was synthesized by Ochiai in 1945, is a potent carcinogen. Application of NQO to the skin of mice, rats or hamsters has been shown to induce local tumours<sup>1-5</sup> and also neoplasms of lung<sup>6-8</sup>, stomach<sup>9</sup>, uterus<sup>10</sup> and the lymphatic system<sup>11</sup>. The present report describes the unexpected induction of epithelial tumours of the tongue in mice following the application of NQO and croton oil to the dorsal skin; the doses of these two agents were insufficient to induce more than a few tumours of the treated skin or any neoplasms at other sites. Similar findings have not previously been encountered in this laboratory in numerous experiments in which known carcinogens have been applied to mouse skin.

Two groups of C57 × DBA2 mice were studied, each of thirty female animals which were housed in boxes of six. Mice in Group 1 received 0.2 ml. of 0.2 per cent NQO in acetone applied to the dorsal skin at weekly intervals for 16 weeks; the first dose was given when the animals were 20 weeks old. This was followed by weekly applications of croton oil, initially of 0.3 ml. of a 0.1 per cent solution in acetone. After 12 weeks, the concentration of croton oil was reduced to 0.05 per cent and treatment was continued for a further 53 weeks. Mice in Group 2 acted as controls and were painted with 0.2 ml. acetone alone for the first 16 weeks. Thereafter, they received croton oil for 65 weeks in the same way as the animals in Group 1. Mice in Group 1 died or were killed because of their poor condition, at intervals ranging from 291 to 489 days after the beginning of the experiment. During the same period, only three of the animals in Group 2 had to be killed and the remaining twenty-seven were killed at the end of the experiment, at 563 and 574 days. A full post-mortem examination was carried out on all mice.

The distribution of neoplasms in the two groups is shown in Table 1. The results in Group 1 are of considerable interest: only four skin tumours were produced but neoplasms of the tongue developed in six instances. The animals in which these lesions developed became strikingly cachectic and, in each case, the only abnormality found at post-mortem examination to account for their condition was the lesion in the tongue. Macroscopically, the tumours appeared as ulcerating outgrowths on the dorsum of the tongue. Five of the tumours were squamous

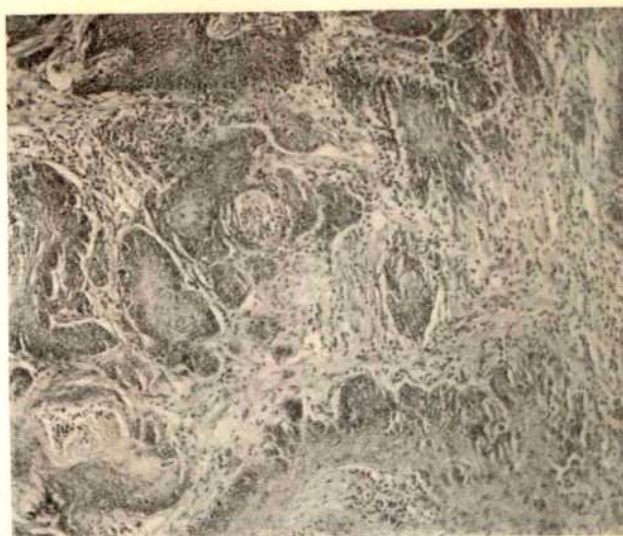


Fig. 1. Tongue from mouse in which NQO and croton oil were applied to the dorsal skin; a keratinizing squamous carcinoma which is widely infiltrating the substance of the tongue. Haematoxylin and eosin × c. 100.

carcinomata and two of these showed extensive invasion throughout the substance of the tongue (Fig. 1). Three tumours were regarded as carcinomata *in situ*; such lesions were confined to the epithelium and the basement membrane was intact but the component cells were grossly disorganized with loss of normal polarity, cellular atypia, and increased mitotic activity. One basal cell tumour was seen. In a seventh mouse, a tumour was diagnosed on gross examination of the tongue but sections at several levels showed only focal hyperplasia of the lingual epithelium with no evidence of malignancy. No metastases from any of these carcinomata were seen and no other lingual tumours, such as sarcomata, were encountered. Apart from one animal which developed an anaplastic lymphoma, no distant tumours were found. Various neoplasms were observed in the control mice but no lingual carcinomata were seen.

These results indicate that an unexpectedly high proportion of C57 × DBA2 female mice develop lingual carcinomata after application of NQO and croton oil to the dorsal skin; this undoubtedly reflects a direct effect on the lingual epithelium of NQO, acquired as a result of licking. Two points are of particular interest. First, the experimental production of tumours of the tongue is apparently difficult. Only two successful reports have been found and, in both of them, the carcinogen was applied directly to the tongue. In one account<sup>12</sup>, 3,4-benzopyrene induced sarcomata (but not carcinomata) in rats. In the other, direct application of NQO in mice was combined with chronic irritation from an implanted metal wire<sup>13</sup>; in the absence of this added mechanical trauma, local painting with NQO produced only one lingual carcinoma. Spontaneous tumours of the tongue in mice have not been observed in this department, nor have such tumours been recorded in the literature. The comparatively large number of lingual carcinomata in the present experiment is therefore most unusual. Second, the increased incidence of these neoplasms is even more impressive when compared with the unexpectedly low yield of tumours of the skin. Unfortunately, it is impossible to assess the degree of exposure of the tongue to NQO but it is improbable that high concentrations of this material remained there for any length of time. This may indicate that, in some circumstances, lingual epithelium is more sensitive to the carcinogenic effects of NQO than the skin. The basis for such a difference in susceptibility is obscure and several factors may be involved. The absence of other tumours commonly induced by NQO such as pul-

Table 1. INCIDENCE OF LINGUAL AND OTHER TUMOURS IN C57 × DBA2 MICE FOLLOWING APPLICATIONS OF NQO AND CROTON OIL TO DORSAL SKIN

Treatment	No. of mice	Skin tumours	Tongue tumours	Other tumours
NQO + croton oil	30	Papilloma Papilloma → carcinoma	2 2	Lymphoma 1
Croton oil only	30	None	Squamous carcinoma 2 Carcinoma <i>in situ</i> 3 Basal cell tumour 1 None	Hepatoma 3 Lymphoma 2 Pulmonary adenoma 1 Carcinoma of breast 1 Carcinoma of ovary 1



monary adenomata suggests that genetic factors may be important; the range of tumours induced by NQO is known to vary in different strains of mice<sup>5,14</sup> and the effects of NQO on *C57 × DBA2* mice do not appear to have been studied previously. Furthermore, local factors in the lingual epithelium itself may predispose to tumour induction. There is evidence, for example, that NQO is mainly active in its reduced form, as 4-hydroxyaminoquinoline-1-oxide<sup>15-17</sup>, and it is possible that conditions in the lingual epithelium favour a conversion of this kind. Conversely, it may be argued that epidermal elements in the dorsal skin of these mice are unusually resistant to the carcinogenic effects of NQO. Some support for this view is provided by recent work by Searle and Spencer<sup>5</sup>, who painted the dorsal skin of *C57Bl* mice with NQO; little epidermal damage was caused but there was a high incidence of local sarcomata. No sarcomata were, however, seen in the present study and it appears that differences in sensitivity to the carcinogenic properties of NQO may occur not only between epithelial and connective tissues but also between two closely similar epithelial surfaces—the squamous epithelium of the skin and the tongue.

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### Transplantable Mouse Neoplasm Control by Neutron Capture Therapy

AFTER several years of effort based on the application of the principles of neutron capture therapy, we have empirically evolved a procedure which now can be added to the few effective treatments for established invasive transplantable neoplasms of animals. Previously, we discussed the biological utilization of thermal neutrons to generate alpha particles and lithium atom fragments from boron-10. We noted the pathways of these fragments are of cellular dimensions<sup>1-5</sup>, and how, in principle, one might thereby attain sharply localized intense effects. Our present observations on 611 neoplasms may serve to point towards achievement of these goals. In this communication we report the thermal neutron flux which must be incident on the skin over solid neoplasms 8 mm–17 mm in diameter to result in permanent regression after giving sodium pentaborate intravenously in a dose equivalent to 35 µg/g body weight of boron-10. Only a single treatment was required by this procedure.

Elemental boron enriched to 96 per cent of boron-10 was converted to borax, and then to the more soluble sodium pentaborate. For injections, sufficient glucose was added to the sodium pentaborate solution to attain a molar ratio of glucose : borate of 2 : 1. Glucose has been found to

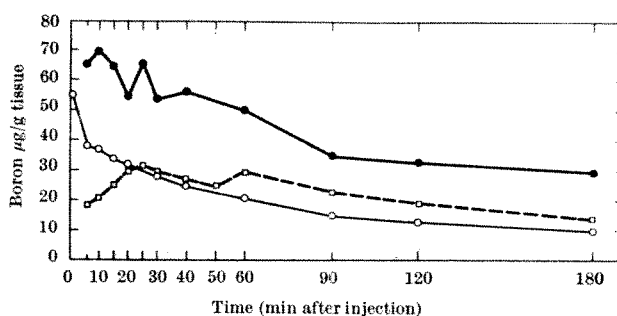


Fig. 1. Tissue boron concentration changes after prompt intravenous injection in the mouse. Drug, sodium pentaborate decahydrate; dose, 35 µg as boron-10/g body weight. Each point represents an average obtained from ten animals. The analyses of sarcoma were upon homogenates of the dissected neoplasm. □ - - □, Sarcoma implanted in muscle; ● - ●, normal muscle; ○ - ○, blood (mixed).

minimize borate toxicity. Minimum toxicity for sodium tetraborate is attained with a glucose : borate ratio of 1 : 2, but for closely related sodium pentaborate it is 2 : 1 (refs. 6 and 7). The solution for injection was also adjusted to a boron content of 35 µg/0.01 ml. to facilitate administration of the body dose of 35 µg/g from the observed weight. Each solution injected was analysed for boron by the method of Konikowski and Farr<sup>8</sup>. Because excretion of sodium pentaborate by mice has been previously reported<sup>9</sup> as part of the present study, tissue and neoplasm boron concentration were measured on comparable animals at intervals after injection up to 3 h (Fig. 1).

The medical reactor facilities used at Brookhaven National Laboratory have been described elsewhere<sup>10,11</sup>, as has also the field defining aperture unit to hold the mice<sup>12</sup>.

In studies of radiation spectra at the port used, activation of sulphur foils by fast neutrons was negligible. Gold foil activation consistently accounted for nearly all of the neutron flux. Exposure to gamma rays was less than 200 r. for 300 sec exposure. We have previously reported that equivalent neutron exposure without boron-10 has not caused complete regression of the neoplasm<sup>13</sup>, and boron administration by itself was ineffective. The lithium sheet used to line the animal holder in the exposure unit significantly attenuated the thermal neutron flux so that no whole body effects resulted. At each run, radiometric parameters were established by activation of gold foils affixed to the skin over the tumour, at various places on the animal, and about the exposure unit. Counts of the activated gold foils from the skin surface over the tumour indicate the incident thermal neutron flux range in these studies was from  $0.8 \times 10^{12}$  to  $3.2 \times 10^{12}/\text{cm}^2$ .

The neoplasm used was originally obtained in 1951, by intracranial implantation of methylcholanthrene crystals in mice. Subsequently, it has been maintained in young mice by intracranial passage. By use of young mice, not only was intracranial injection made easier, but the incomplete calcification of the skull permitted the enlarging tumour to expand modestly the cranial cavity which permitted a slight, but useful, increment of longevity. Under these conditions a satisfactory passage tumour can be obtained every 8 or 9 days. The details of the procedure used in passage have been reported elsewhere<sup>12</sup>.

When an intracranial passage neoplasm is implanted into the thigh muscle and takes, it grows in a solid mass up to 30 mm diameter (Fig. 2). First passage intramuscular implants about 10 mm in diameter were used to provide sufficient inoculum to develop the number of experimental neoplasms required for each series of reactor runs. The histological cell type, whether of muscle or brain implant, resembles an anaplastic sarcoma (Fig. 3). Maintained in the fashion noted<sup>12</sup> the cell type and invasive quality have remained quite uniform since 1951.

Healthy animals with tumours implanted in the thigh were weighed immediately before injection. The weights ranged from 18 to 24 g. The tail vein was used for injection.



tion. The warmer incorporated into the mouse holding device<sup>13</sup> encouraged dilatation. Unless delivery into the vein was complete and easy within a nominal three seconds, the animal was discarded.

After injection, the animal was positioned in a plastic centrifuge tube (1.377 in. diameter, 4 in. long), while a hole in the bottom allowed the leg bearing the neoplasm to extrude and project. The tube containing the animal was then placed in the exposure holding device; the leg bearing the neoplasm projected into the open central core area of the unit. Approximately 30 min later the neoplasm, together with the entire leg, was bathed in a thermal neutron cloud. The elapsed time between injection and exposure among each battery of 5 animals run at a time range from 30 min after completion of the injection of the first animal to 26 min for the fifth.

After exposure, ranging from 100 to 300 sec, depending on the flux planned, each animal was promptly removed from the holder, the gold foils were collected for counting and the mice returned to their cages. No immediate effects were noted. Subsequently, the animals were observed until their death, which for many was over 2.5 yr later.

Of the 611 animals bearing neoplasms, in 378 (62 per cent) the neoplasm regressed permanently (Table 1). In none of these animals was any mass detectable at the tumour site by one month after treatment. Most of these 378 animals were observed for their remaining life span, approximately 2.5 yr, but a few were killed earlier for histological study. The percentage of permanent regression increased with increasing neutron flux, while increasing the diameter of neoplasms resulted in decreasing per-

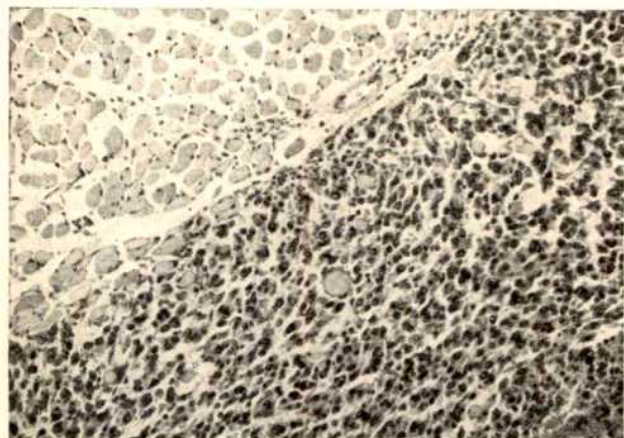


Fig. 2. Transplantable sarcoma showing compact arrangement of neoplasm ( $\times 165$ ).

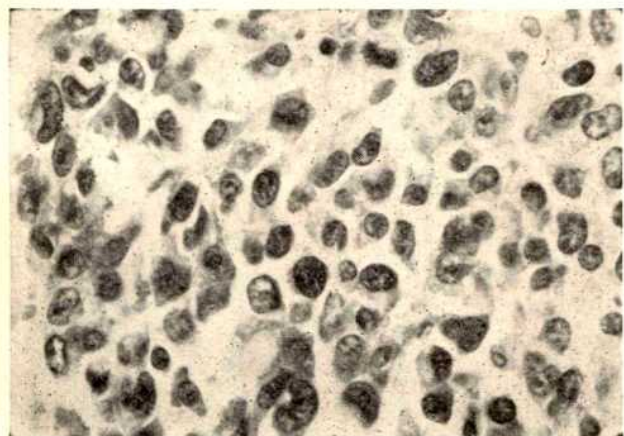


Fig. 3. Histology of anaplastic sarcoma of the mouse.

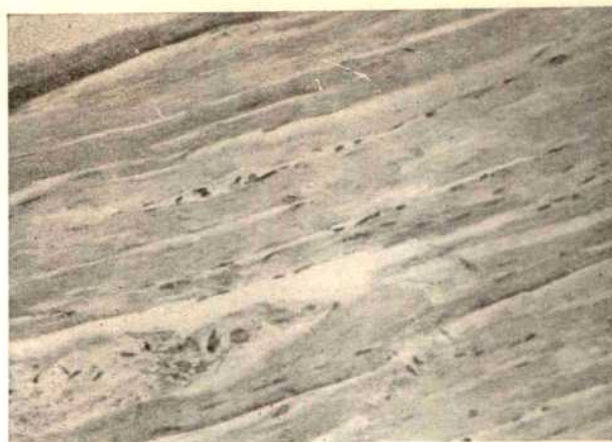


Fig. 4. Site of neoplasm implant in muscle following total regression of tumour. Animal killed 22 months after treatment ( $\times 125$ ).

centages of permanent regression. These data are completely compatible with observed thermal neutron attenuations in tissue where the half-distance is less than 1.5 c.

Of the 233 (38 per cent) of the animals dying from the neoplasm, in 205 (33 per cent) the tumour was observed to regress for a short, but variable period after treatment.

In only twenty-eight neoplasms (5 per cent) was there no evidence of any regression. In all of the animals with apparent regression, but recurrence, the recurrence was clearly evident by the end of the second month following treatment. Average longevity of an animal with a neoplasm growing at its usual rate was 6-7 weeks.

Leg sections of selected animals with complete regression studied histologically after killing, or after completion of the life span, revealed no evidence of residual neoplasm. The muscle, which had abutted and surrounded the site of the neoplasm, and its vascular structures did not show evidence of damage from either the neoplasm or the treatment (Fig. 4).

Studies are reported on 611 mice bearing a transplantable neoplasm as a thigh implant treated according to the principles of neutron capture therapy. Permanent and total regression for the animal's life span (up to 2.5 yr) was observed in 378 (62 per cent). In 205 animals (33 per cent) transient regression of the neoplasm was observed during the first month after treatment. In twenty-eight animals (5 per cent) there was no evidence of regression.

Data are given to show relation between thermal neutron flux and results upon neoplasms ranging in diameter from 8 mm to 17 mm in stepwise increments of 1 mm. For any given neutron flux, decreasing effects were seen as the neoplasm diameter increased. This is in agreement with expected attenuation of thermal neutron clouds in tissue. The boron-10 dose administered intravenously was 35  $\mu$ g of boron-10/g body weight. It was given 30 min before neutron exposure.

Table 1. RELATION OF TUMOUR DIAMETER AND THERMAL NEUTRON FLUXES TO PERMANENT NEOPLASM REGRESSION OF MUSCLE IMPLANTED TRANSPLANTABLE MOUSE SARCOMA

Drug, sodium pentobarbital ( $^{10}$ B) with glucose (molar ratio 2:1). Dose, 35  $\mu$ g  $^{10}$ B/g, body weight. Injection-irradiation interval, 26-30 min neutron exposure time, proportional to flux (100-300 sec)

Neutron flux cm <sup>2</sup> $\times 10$	Number of tumours permanently regressed/ Number of tumour bearing animals treated									
2.81-3.2							1/1	4/4	5/7	2/2
2.41-2.8							2/2	7/12	10/18	2/3
2.01-2.4	1/1	1/1	3/3	7/7	7/7	12/16	7/11	7/15	3/3	1/3
1.61-2.0	10/10	13/13	21/25	37/44	17/28	9/13	10/27	1/14	0/3	0/1
1.21-1.6	27/31	32/40	40/55	11/25	3/12	4/17	0/7	—	0/2	0/2
0.8-1.2	35/43	12/21	6/14	4/10	1/14	0/5	1/3	0/2	0/4	0/2
Totals	73/85	58/75	70/97	59/86	28/61	28/59	29/64	23/56	7/17	3/11
	8	9	10	11	12	13	14	15	16	17

(Tumour diameter on day of treatment—mm)

611 treated neoplasms; 378 (62 per cent) permanently regressed; 205 (33 per cent) temporarily regressed and 28 (5 per cent) no evident regression.



Histological study revealed no neoplastic cells at neoplasm sites after successful treatment. Under the conditions of the experiment, the normal muscle and vascular tissue at the site of the regressed neoplasm appeared unaffected by the treatment.

Longevity of successfully treated animals was not significantly altered from normal expectancy.

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### Absorption of Cholera Toxin into Blood from a Separated Jejunal Segment

ONE of the chief problems in cholera research is whether the toxic agents primarily responsible for the diarrhoea are confined to the intestinal tract, or are absorbed into the blood. Finkelstein<sup>1</sup> has suggested that there is only one important toxin, cholera toxin, which produces diarrhoea in infant rabbits, and that this does not leave the intestine, because he has found that cholera toxin injected intravenously does not cause cholera. The question is of practical importance because, if important toxins do not reach the blood, circulating antibodies will have no effect on them, and it has been suggested that prophylaxis should concentrate on an attempt to build up local antibodies in the intestine (sometimes called copro-antibodies), for example, by means of an oral vaccine.

Dutta and Habbu<sup>2</sup> showed that infant rabbits infected intra-intestinally with vibrios, suffered from a disease which very closely resembled human cholera. Furthermore, it was shown that sterile vibrio extracts

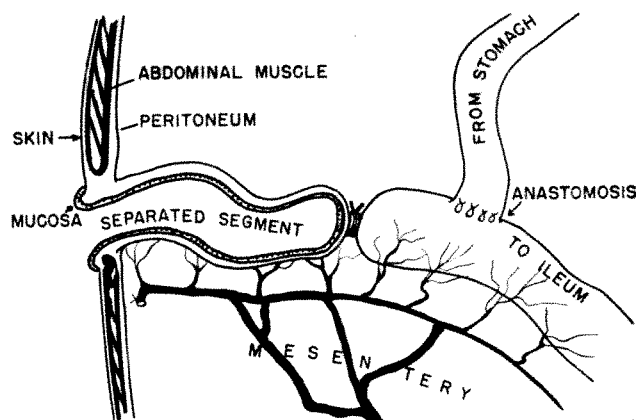


Fig. 1. Diagram of operation.

could produce cholera-like diarrhoea in infant rabbits<sup>3</sup>. The background to the present experiments was that one of us, who had had experience of making fistulae and intestinal loops of various kinds<sup>4</sup>, thought that if it were technically feasible to prepare similar intestinal fistulae in infant rabbits, a definite answer might be found to the question as to whether one of the important routes for the distribution of toxin was the blood. We proposed to separate off a segment of jejunum, with one end blind and the other opening to the exterior as a fistula ("Thiry loop") and to restore the continuity of the remainder of the gastrointestinal tract by anastomosis. Vibrios, or their lysate, could then be introduced into the separated segment, which had its blood supply intact, and if the animal later suffered from characteristic cholera-like signs, the toxins must have travelled by the only route available to them, the blood (Fig. 1).

In the event, a hundred and two operations were performed. The first twenty had to be rejected for various reasons (obstruction, leaking anastomosis, and so on), but ultimately thirty operations altogether were completely successful, in the sense that either the animals survived for a long period, or, if they died after infection, no other possible cause of death could be found at post-mortem. The animals were always returned to the mothers after the operations.

In the animals used as controls, 0.2 ml. of sterile medium was introduced into the segment opening on to the skin, either at operation or 24 or 48 h later. In three of them, loose stools were passed for a brief period, but all animals themselves looked perfectly healthy and lively throughout. Only one control died spontaneously, for no obvious

Table 1. SUMMARY OF RESULTS  
The ages and weights given were those obtaining at the time of operation

Time of installation after operation (h)	Experiment no.	Controls		Time of death after installation of sterile medium (h)	Experiment no.	Weight (g)	Age (days)	Infected		Diarrhoea	Rice water in caecum and/or large intestine
		Weight (g)	Age (days)					Time of death after installation of vibrios (h)	Approximate no. of vibrios		
0	46	142	12	96	56	94	12	11	10 <sup>4</sup>		+
	57	141	12	120	65	102	11	48	5 × 10 <sup>4</sup>	++	+
24	61†	126	13	> 330	67	186	10	> 140	8 × 10 <sup>4</sup>		
					22	135	12	24	6 × 10 <sup>3</sup>	++	+
					24	125	12	20	10 <sup>4</sup>	++	+
					31	115	11	16	2 × 10 <sup>4</sup>	+	+
					63	117	13	10	5 × 10 <sup>4</sup>	++	+
					64	107	11	31	5 × 10 <sup>4</sup>	++	+
48	69	198	12	70	48	149	12	13	1.5 × 10 <sup>6</sup>	+	+
	71	164	12	Killed at 24	70	165	12	24	10 <sup>5</sup>		+
	82†	124	10	> 200	72	117	12	8	10 <sup>5</sup>		+
	96	98	10	96	79	108	10	27	10 <sup>4</sup>	++	+
	101	122	10	> 96	88	131	10	10	10 <sup>4</sup>	+	+
	100‡	111	10	96	90	101	10	15	1.5 × 10 <sup>6</sup>		+
					102	107	10	15	1.5 × 10 <sup>7</sup>	++	+
					28	117	12	44 killed	3 × 10 <sup>4</sup>		
					80**	98	10	> 220	10 <sup>5</sup>	+	Normal
					83*	104	10	> 200	10 <sup>5</sup>		Not applicable
					84	103	10	148	10 <sup>5</sup>	+	
					92*	91	10	> 240	1.5 × 10 <sup>6</sup>		
					98*	121	10	> 96	1.5 × 10 <sup>7</sup>		

† Animals were subsequently given toxin.

‡ No installation of sterile medium.

reason (possibly because of dehydration through loss of secretions from the separated segment). The remainder, having lived for longer than the infected animals, were either killed for comparison or used for further investigations (Table 1). The control animals gained weight. At post-mortem the intestines of the killed animals were normal apart from a few adhesions.

In twenty-one animals, rabbit passaged *V. cholerae* Inaba 569B organisms were introduced into the separated segments at operation, or 24 or 48 h later. Fourteen of the infected animals were dead within 48 h of infection (Table 1). Ten of the fourteen had diarrhoea before death and in seven of them the diarrhoea was severe. At post-mortem, a distended caecum and colon, containing clear "rice water" fluid, and flakes of intestinal contents were found in all but one of the animals. Of the seven infected animals which lived more than 48 h, two had diarrhoea. No. 67 was reinfected, 77 h after operation, through the separated segment with  $2 \times 10^5$  vibrios, and later had diarrhoea, but recovered. No. 28 was killed 44 h after infection but was apparently normal.

In the control animal No. 82, 6 days after the operation, 50 mg sonicated toxin<sup>5</sup> of rabbit-passaged Inaba 569B was introduced into the separated segment, but the animal did not develop diarrhoea and lived another four days. In control No. 61, 16 days after the operation, 75 mg toxin was introduced into the separated segment. The animal developed copious diarrhoea, and was dead within 15 h. At post-mortem the distal caecum and the colon were distended with clear rice water, in which flakes of intestinal contents were floating.

Of the seven animals (Nos. 28, 67, 80, 83, 84, 92 and 98) which survived for more than 48 h after infection, two developed diarrhoea, and it was argued that all of them had probably suffered from cholera to some degree. If so, they should have become immunized and resistant to toxin.

In infected animal No. 80 (marked with two asterisks (\*\*)) in Table 1, 11 days after the operation, when it had gained weight to 113 g, 50 mg of toxin was introduced into the separated segment, and it did not develop diarrhoea.

When infected animals Nos. 83, 92 and 98 (marked with a single asterisk (\*) in Table 1), had been alive for 10, 6 and 5 days after operation respectively, three normal rabbits of the same ages were selected, and all six animals were anaesthetized, and 100 mg of sonicated toxin was injected intra-intestinally. After 8 h all the animals were alive. The three normal animals, but not the operated ones, had copious diarrhoea. All six were dead within 15 h. At post-mortem, the caecum and large intestines were enormously dilated with clear rice water fluid and flakes in the normal animals, but were unaffected in the operated animals.

Fluid was taken from the caecum of fourteen infected animals under strictly sterile conditions and cultured. In no case were *V. cholerae* grown, but there was growth of *Escherichia coli* or *Aerobacter aerogenes*, and some unidentified Gram-negative bacteria.

Organisms from the caeca of five animals were cultured on agar for 18 h. A loop-full (1 mm) from this growth was sub-cultured on 10 ml. broth for 3 h, and 1 ml. was then injected intra-intestinally into normal 10 day old rabbits. None showed diarrhoea or any other sign of toxicity. Additional evidence is thus provided that the deaths of the animals infected with *V. cholerae* in the separated segments were due to absorbed toxin, and not to any organisms in the main intestinal tract.

Fluid was also taken from the separated segments of eight animals and cultured. In every case, the fluid grew cultures of *V. cholerae*. We conclude therefore that the most probable explanation for these results is that a toxin of principal importance is absorbed into the blood of infant rabbits from *V. cholerae* in a separated segment of jejunum, and causes characteristic signs of cholera in the whole animal.

In three of the animals which survived infection, evidence was obtained which would be consistent with the view that their intestines had acquired protection against a large dose of sonicated toxin introduced intra-intestinally. The animals died, however, and evidently did not acquire adequate protection against those fractions of the total toxicity of *V. cholerae* which are lethal, but do not produce diarrhoea<sup>6</sup>.

We thank Dr H. I. Jhala for his invitation to E. M. V. W. to study this problem at the Haffkine Institute, and Dr N. K. Dutta for his advice and facilities provided. We also thank Miss Ratna Motwani for the gift of the sonicated toxin of *V. cholerae*.

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## PSYCHOLOGY

### Detection of Visual Stimuli located within Angles

THE upper member of two horizontal lines, identical in length, drawn within an inverted V-shaped bracket appears to be somewhat longer than the lower. This figure is one example of many two-dimensional spatial patterns known as "the geometrical illusions". These patterns undergo distortion in such a way as to assume an appearance markedly at variance with their physical form. This particular figure is usually described as the Ponzo illusion, the distortion characterizing it being seen clearly in Fig. 1. The geometrical illusions have provided the topics for a very large number of experimental enquiries. In these, attempts have been made to understand the spatial distortions seen in them, according to features of the structural organization and functional repertoire of the visual projection system. The purpose of the present communication is to initiate consideration of the problem of perceiving visual stimuli seen in the context of illusions, the Ponzo figure being used as a convenient example.

The facility with which visual stimuli can be seen depends on many factors. Most important of these is the size on the retina of the image resulting from a particular stimulus. Visual acuity is usually specified in terms of the threshold of angular resolution of the eye with respect to a given spatial pattern. Accordingly, it is customary to calculate acuity from knowledge of the size of the

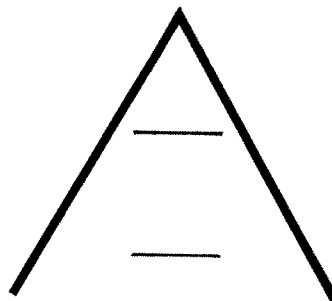


Fig. 1. The Ponzo illusion.



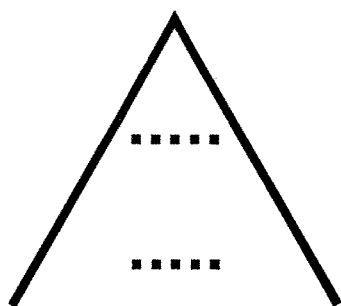


Fig. 2. The experimental materials.

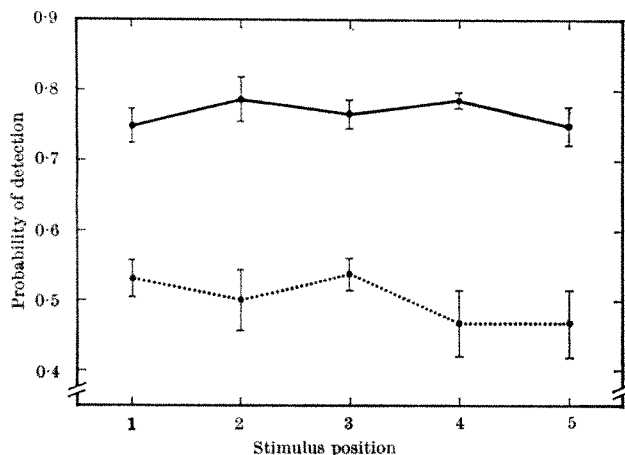


Fig. 3. The probabilities of detecting each of the ten stimuli.

stimulus resolved and the distance at which it is placed in relation to an observer. The two horizontal lines of the Ponzo figure, however, are of the same length. Thus they subtend similar angles at the eye and the retinal image resulting from each is of the same size. Nevertheless, the two lines appear to differ appreciably in length. Thus the problem under consideration is whether detection is determined by the real or apparent characteristics of this figure.

The materials used for preparing the experimental stimulus patterns are shown in Fig. 2. Eleven different stimuli were used. In ten of these one of the square blocks was placed within the 60° angular bracket, in the remaining pattern the block was omitted. Prepared as photographic slides, these figures were exposed in a tachistoscope for a period of 1/125 sec to subjects who were asked to indicate whether or not a block was present. In each experimental run forty angles containing blocks and forty angles without blocks were included, the random order in which they were presented being changed on each occasion.

The results of this experiment, completed on a total sample of thirty-three subjects, are shown in graphical form in Fig. 3. They refer to the upper and lower rows of stimuli illustrated in Fig. 2, numbered along the abscissa from left to right, from 1 to 5. The ordinate of the graph refers to the facility with which stimuli were seen in each position. The probability function for detection of the upper row of blocks is plotted in solid line and for the lower row in broken line. The standard error of estimate of each point defining both graph lines is indicated by a vertical solid bar.

It will be readily seen that these two graphs do not describe a common relationship. For each position the probability of detecting a stimulus in the upper row significantly exceeds that of a corresponding stimulus in the lower row. Taken as a whole, detection of stimuli in the upper row, in comparison with the lower, is facilitated by a factor of the order of 10 per cent. This approximates

to the extent of attenuation of the lower line in relation to the upper in this particular version of the Ponzo figure. Accordingly, it appears justifiable to conclude that the apparent features of illusory spatial patterns rather than their actual physical characteristics determine the probability with which near-threshold stimuli can be detected.

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## ANIMAL BEHAVIOUR

### Sexual Attractiveness and Receptivity in Rhesus Monkeys

THE catarrhine monkeys and the apes alone among animals have a clearly defined menstruation with a cycle lasting about 30 days. They do not show well-circumscribed periods of oestrus (heat), but will copulate throughout the cycle. This has led to the idea that the great development of neocortical mechanisms in the primate has resulted in an emancipation of its brain from the influence of gonadal steroids<sup>1</sup>. Earlier work in both the rhesus monkey<sup>2,3</sup> and the chimpanzee<sup>4-6</sup>, however, has indicated that the level of sexual interaction shows some variation with the phases of the menstrual cycle. Furthermore, studies of the rhesus monkey<sup>7,8</sup> have shown that well marked rhythms of mounting behaviour by males occur in relation to the menstrual cycles of their female partners. These rhythms are abolished by bilateral ovariectomy, and so endocrine mechanisms in primate sexual behaviour seem to have been underestimated<sup>9</sup>.

Copulation in the rhesus monkey consists of a series of sexual mounts by the male on the female, each with an intromission and thrusting, the series being terminated by a final, ejaculatory mount. The mounting sequence is not haphazard, but results from initiating movements by the male (for example, courtship postures, mounting attempts or claspings), and from initiating movements by the female (sexual presentations and various invitational gestures). Endocrine-dependent changes in the mounting activity of the males could therefore be mediated by changes in this initiating behaviour. This communication attempts to account, in behavioural terms, for the conspicuous decline in male mounting activity that occurs early in the luteal phase of the menstrual cycle.

Mature, intact male and female rhesus monkeys, weighing 8-14 kg, were studied in glass-fronted observation cages for 60 min periods by two observers, in carefully standardized conditions. Between tests animals were housed singly. Four females were used, each was tested with two males for a total of fifteen menstrual cycles involving 176 h of observation, and in eight cycles rhythmic changes in mounting occurred. A system, previously described, was used for scoring components of behaviour at 30 sec intervals giving their temporal sequence<sup>10</sup>. The present report is confined to the way in which the hormonal status of the female influences the outcome of mounting attempts by the male and of sexual invitations by the female.

Fig. 1 shows the changes in each test during four menstrual cycles: (a) in the number of mounts made by the male on the female; (b) in the number of sexual invitations made by the female to the male, and (c) in the proportion of these invitations that stimulate the male to mount. The latter is termed the female success ratio—successful mounting invitations (those followed by a mount) expressed as a percentage of total mounting invitations made by the female. Although rhythmic changes in the number of mounts in a test occurred in relation to the menstrual cycle, with a well marked decline early in the luteal phase, the number of female sexual

invitations remained at about the same level throughout the cycle, and did not decline when male mounting activity did so. The proportion of these invitations that were accepted by the male and resulted in a mount (the female success ratio), however, diminished markedly during the luteal phase when male mounting activity declined. In such cases, type *A*, the decrease in mounting activity was clearly related to the diminished effectiveness of the female sexual invitations, reflecting her diminished value as a sexual stimulus at this time.

This is not, however, the only mechanism responsible for the decline in male sexual activity observed early in the luteal phase. Fig. 2 shows the changes in each test during three further menstrual cycles: (a) in the number of mounts made by the male on the female; (b) in the number of mounting attempts made by the male, and (c) in the proportion of these attempts accepted by the female and followed by a mount. The latter is termed the male success ratio—successful mounting attempts (those followed by a mount) expressed as a percentage of total mounting attempts made by the male. Although the number of mounts declined in the luteal phase of these cycles also, this was not associated with any corresponding decline in the number of male mounting attempts. The

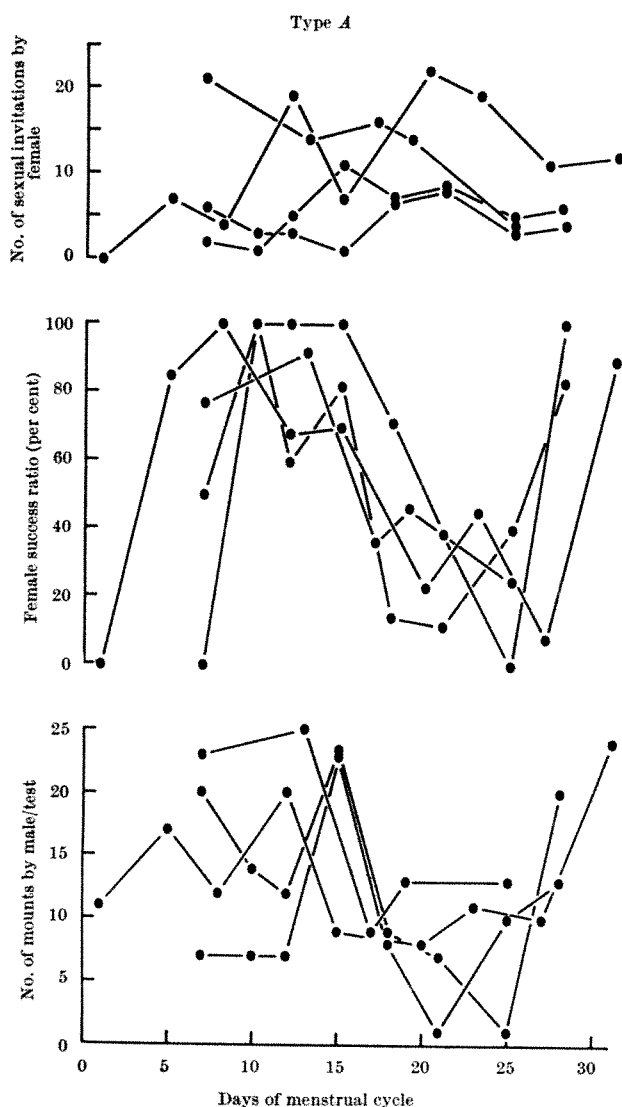


Fig. 1. Although sexual invitations in these females do not decline in the luteal phase of the menstrual cycle, the proportion accepted by the male (female success ratio) does so. This decline in female "attractiveness" correlates with the decline in male mounting (four of five cycles are illustrated).

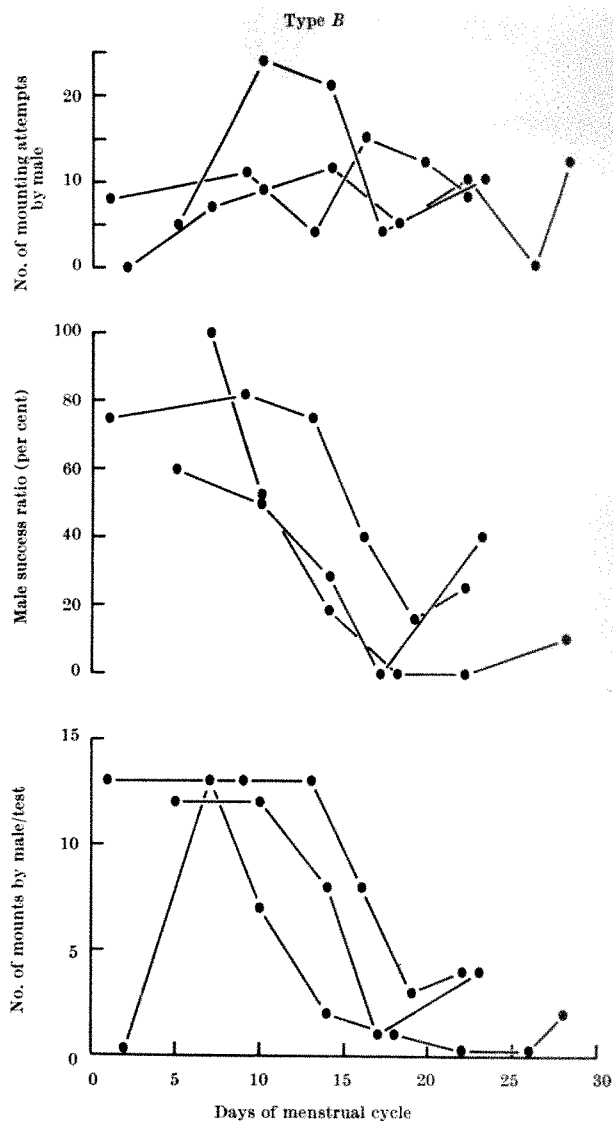


Fig. 2. Although mounting attempts by the male with these females do not decline in the luteal phase, the proportion resulting in a mount (male success ratio) does so, and the number of mounts declines. This is because of increased refusals by the female (three cycles).

proportion accepted by the female, the male success ratio, however, conspicuously declined when the number of mounts declined, and this was caused by a corresponding increase in the number of active refusals of male mounting attempts made by these females. In these cases, type *B*, the female had not lost her attractiveness, for the male continued to attempt to mount, and a female refusal mechanism was therefore brought into play during the luteal phase.

By analysing primate sexual behaviour in this way, it was possible to differentiate between the sexual "drive" of the female, on the one hand, expressed by the number of sexual invitations, and her value as a stimulus for the male, on the other, expressed by the female success ratio. Two distinct mechanisms thus seemed to underlie the decline in male mounting activity early in the luteal phase: the first depended on a decrease in the attractiveness of the female, indicated by the failure of her sexual invitations to stimulate mounting; the second depended on a decrease in the receptivity of the female, indicated by an increase in the number of female refusals. Further studies of ovariectomized females given oestrogen and progesterone have shown that these mechanisms can be brought into operation in different females by progester-

one; this may account for its inhibitory effect on ejaculation reported elsewhere<sup>11</sup>.

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## GENERAL

### An Early Reference to Genetic Coding

SCHRÖDINGER's little book *What is Life?*<sup>1</sup> is usually thought to contain the first reference to genetic coding<sup>2</sup>. It is therefore surprising to find that Johann Friedrich or "Fritz" Miescher (1844-1895), the discoverer of nucleoprotein and nucleic acid, expressed his belief in the existence of a chemical code within the hereditary material 52 yr before the publication of Schrödinger's discussion on the "hereditary code-script". Miescher's remarks are to be found in two letters which he wrote to his uncle, Professor Wilhelm His, the first in December 1892 and the second in October 1893. They are included in the letters which Miescher's friends collected together and published in 1897 (ref. 3). In the twenty-eight-page biography which Wilhelm His contributed to this volume they are mentioned very briefly in a footnote. We have also found one reference to Miescher's comments on the isomerism of the hereditary material by Jack Schultz in 1941 (ref. 4). The following is an extract from our translation of the first letter.

"To me the key to the problem of sexual reproduction is to be found in the field of stereo-chemistry. The 'gemmules' of Darwin's Pangenesis are no other than the numerous asymmetrical carbon atoms of organic substances. As a result of minute causes and external conditions these carbon atoms suffer positional changes and thus gradually produce structural defects. Sexuality is an arrangement for the correction of these unavoidable stereometric architectural defects in the structure of organized substances. Left-handed coils are corrected by right-handed coils, and the equilibrium restored. In the huge molecules of albumen compounds or in the yet more complicated molecules of haemoglobin, etc., the many asymmetric carbon atoms provide a colossal amount of stereo-isomerism. In them all the wealth and variety of hereditary transmissions can find expression just as all the words and concepts of all languages can find expression in twenty-four to thirty alphabetic letters. It is therefore quite superfluous to make the egg and sperm cell a storehouse of countless chemical substances each of which carries a particular hereditary quality (the Pangenesis of de Vries). My own research has convinced me that the protoplasm and the nucleus, far from consisting of countless chemical substances, contain quite a small number of chemical individuals [compounds] which are likely to be of a most complicated chemical structure."

There is no suggestion of a chemical code in the specific sense of the repetition of varied sequences of a few bases; this is hardly surprising because only two of the four common bases had been isolated from nuclei when this letter was written and there existed no information about their relative proportions until 1902 (ref. 5) (for adenine uracil and guanine) and 1909 (ref. 6) (for all four). Nor does Miescher identify his "chemical individuals" as nucleic acids or nucleoproteins. But the researches to which he refers showed that four-fifths of the sperm head is composed of nucleoprotein, so we may be fairly confident that he had this class of compounds in mind. His choice of proteins as examples of compounds with a high potential for stereoisomerism resulted probably from the fact that more was known about their composition than about that of nucleic acids.

Miescher was possibly the first biochemist to perceive the biological possibilities of Le Bel and van't Hoff's theory of the isomerism of the carbon tetrahedron (1874) and to speculate that stereometric alterations may be the cause of mutations. Remarkable, too, is the fact that this discoverer of nucleic acids was thinking in terms of right- and left-handed threads or coils. This conception of the hereditary material may have been pure speculation, but it could have been based on the knowledge that chromosomes at division show a coiled structure since this fact was clearly demonstrated by Baranetzky in 1880 (ref. 7). Admittedly this is at a very different level, but the analogy was there. Thus while other biologists were still talking about "arrangements" in vague terms, Miescher had arrived at a more sophisticated conception. He complained that men like Weismann "speculate and torment themselves with half-baked concepts which are either ill-defined or belong to an antiquated state of chemistry". The great questions relating to genetic continuity, he forecast, "will have to be fought out between the morphologists and biochemists of the twentieth century. Is it only substance or is it form as such which is inherited? By form I mean internal morphological structure, with well-defined external borders, and I do not care whether or not that structure is at present open to analysis". This quotation comes from a letter which he wrote 3 weeks before his death from consumption in 1895 at the age of fifty-one. It is tempting to speculate on what he would have thought of the erroneous and, to a man of his perceptiveness, naive tetranucleotide theory of nucleic acid structure had he lived to see its birth in 1909 (ref. 6). Well did his Uncle Wilhelm say: "The appreciation of Miescher and his studies will not decrease but increase with time, and the facts he discovered and the ideas he conceived are germs for which a fruitful future is approaching"<sup>8</sup>.

We wish to thank Professor Darlington for advice. This work forms part of a general history of nucleic acids which one of us (R. C. O.) is preparing with support from the Royal Society.

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- <sup>8</sup> Chargaff, E., and Davidson, J. N., *The Nucleic Acids*, **1** (Academic Press, Inc., New York, 1955. Originally stated in ref. 3 above).

## BOOK REVIEWS

## ENGLISH HIPPOCRATES

Dr Thomas Sydenham (1624-1689)

His Life and Original Writings. By Kenneth Dewhurst. Pp. viii + 191 + 8 plates. (London: The Wellcome Historical Medical Library, 1966.) 35s.

DR DEWHURST has continued his researches into medical biography. He made good use of the Lovelace Collection of John Locke's papers in the Bodleian Library for his admirable life of that physician and philosopher; and now has found them of value, together with other original material, for a study of Sydenham's life and writings. Sydenham called Locke from chemical research to study the natural history of disease at the bedside.

The first part of this book is devoted to the life of Sydenham, and the second part contains the original drafts of his writings which were penned in English. Medical works were expected to be published in Latin in the seventeenth century, and so several persons (identified by Dr Dewhurst) translated them. The translations were not always accurate, and we are now able to appreciate fully the teaching of Sydenham as he set it down in his mother tongue. He wrote some of his later works in Latin; for instance, his *Treatise on Gout and Dropsy*, translated by Dr John Drake into English in 1683, and his *Processus integri*, published posthumously in 1693. The concluding pages of the book contain correspondence including some letters from Sydenham to Locke.

This remarkable man of genius seems to have been born a physician, for he owed little to academic training in medicine, and, indeed, despised it: "Physic," he said, "is not learned by going to universities."

Thomas Sydenham was born at the manor of Wynford Eagle in Dorset. He came of a well-to-do Puritan family. His father, William Sydenham, Thomas himself and his three brothers served in the parliamentary army in the civil war. Two of the brothers were killed, while the eldest brother, William, a staunch adherent of Cromwell, became Governor of Weybridge and the Isle of Wight, and a member of parliament. Much new information is given on Sydenham's early life and war experience.

He was educated at Dorchester Grammar School. In 1643 he entered Magdalen Hall, Oxford, but soon left to become a trooper in the parliamentary cavalry, rising to the rank of captain. After the war he returned to Magdalen Hall in 1647, to migrate a few months later to Wadham College. Being on the winning side he did not undergo the frustrations that Locke afterwards experienced in obtaining the M.B.Oxon. The Earl of Pembroke, the Chancellor, conferred this degree on him in 1648; he was then made a fellow of All Souls' in place of an ejected royalist.

Sydenham resided for a few years at Oxford. He returned to the army for a brief period and was at the Battle of Worcester. Robert Boyle became his friend, but otherwise he seems to have held aloof from the experimental scientists, the precursors of the Royal Society in the University. He never mentions Harvey's discovery of the circulation of the blood. In 1655 he resigned his fellowship to marry Mary Gee. Soon afterwards he settled in practice in King Street, Westminster, removing to Pall Mall in 1664. He was a licentiate of the College of Physicians from 1663 (never a fellow) and

later M.D.Cantab. (1676). Dr Dewhurst considers that he never studied clinical medicine under Professor Barbeyrac at Montpellier as is often stated.

Sydenham's abiding fame as a physician rests on his study of fevers in which he well and truly laid the foundations of epidemiology. He accepted the Hippocratic theory of "humours", but ignored the erroneous beliefs and disputations of many seventeenth century physicians. He went back to Hippocrates, the source of clinical medicine, and investigated the natural history of disease. Studying prevailing fevers at the bedside of his private patients and in the wards of London hospitals, he found that diseases can be identified as clinical entities, that they pursued a regular course, and that their prognosis could be estimated from their characteristic symptoms. He was able to define a number of diseases, for example scarlet fever, measles, smallpox and gout; and described their "constitutions" as due to inexplicable changes within the Earth, climatic, atmospheric and seasonal variations. "The Epidemic Constitution" of any year determined its endemic or stationary fever, and this fever had an effect on any epidemics in that year. He also studied plague in the great plague of London.

Sydenham's treatment of disease was simple and enlightened; it comprised riding in suitable cases, living in the open air, little purgation and bleeding, rest and sleep. He also prescribed opium and other drugs, but mainly relied on the *vis medicatrix Naturae*. Curiously for so careful an observer he did not believe that smallpox was contagious. His "cooling treatment" of that disease under which his patients did well was criticized by the physicians of the day, who alleged that his therapy consisted in doing nothing. But his teaching and fame became world wide.

Thanks to Dr Dewhurst we now know much more about Thomas Sydenham, "the English Hippocrates".

ARTHUR MACNALLY

## WITTGENSTEIN MISCELLANY

## Zettel

By Ludwig Wittgenstein. Edited by G. E. M. Anscombe and G. H. von Wright. Translated by G. E. M. Anscombe. Pp. x + 248. (Oxford: Basil Blackwell, 1967.) 37s. 6d. net.

THE late provost of Queen's College, Oxford (Canon B. H. Streeter), once remarked on the value of having at hand an intellectual workshop, where ideas could be left lying about, and there was no need to tidy them up. When the right moment for assembly arrived, any number of stray thoughts could be brought to life, and thus become significant. Such a collection is sometimes of unique value, even if the author is dead. This is, in outline, the situation before us in the notes left in a box-file by Ludwig Wittgenstein (1889-1951), and now published by his literary executors. In fact, these 717 clippings (or *Zettel*) are fragments of typescripts on which, it seems, the philosopher intended to work at length when the mood occurred and the opportunity offered. Evidently, he considered some of them at least to be capable of extension and possibly of systematization. A portion of the material, however, is largely repetitious: Wittgenstein wrote a vast amount, too, in both these respects a little like Newton. The editors have tried to follow a plan, according to the subject matter. It might have rendered the reader's task a little less difficult if divisions could have been indicated in the text by a horizontal line, or some such device. The whole is simply a bundle of thoughts. Without comment and without criticism—some of them fairly loose and likely to fall out, others tightly bound and most intractable. Nobody could read these pages effectively who did not venerate Wittgenstein,



or understand how fiercely he sought for truth even when, as he himself would have admitted, he was near to writing nonsense.

Supposedly, he intended to elaborate these cogitations into further full-scale studies concerning the meaning of sentences, the theory of signs, the implications of symbolic logic, and especially the methodology of Frege. He shows great respect for Goethe's theory of colour; its metaphysical subjectivity utterly opposed to the mechanics of Newton, and all that followed. This in itself is a manifestation of *Sturm und Drang*; one recollects how Wittgenstein started as an engineer, and even in these jottings speaks almost affectionately of the steam-roller. Again, he likens passing thoughts to the progress of a film-show; an unconscious hark-back perhaps to his habit at Cambridge of making straight for the cinema after his exhausting twice-weekly "supervisions" at Trinity. On these occasions he was probably acting on sentence No. 460—"In certain cases, one cannot take too much care in handling philosophical mistakes, they contain so much truth". This is Wittgenstein at his best, and his most illuminating. Did he realize that in those words he was outlining the whole course of history in relation to the origin and treatment of heresy? In moments crucial for Church or State, it was not the fact that heresy was "wrong" that mattered so much as the nearly universal agreement that its very activity left the residual belief the stronger for its interventions. And thus, the infinite care lavished by Wittgenstein on philosophical mistakes.

This sentence is preceded by two observations regarding metaphysics; the first is that this subject cancels out the distinction between factual and conceptual investigations, whereas the second (pointing to the same basic effect) notes that, according to age-long metaphor, one cannot step into the same river twice. The Wisdom literature, of course, made the same point about birds and arrows in their flight through air. The importance of these entries lies in their refutation of the idea that Wittgenstein abhorred metaphysics. As a former disciple of the Vienna Circle this might have been so (a book is in preparation about this topic), but in his later work he seems to have extended almost a welcome to the blurring of the line between fact and concept. Centuries ago, St. Anselm came to grief just here, with his ontological argument for the existence of God.

Again and again Wittgenstein, aided perhaps by his passion for condensation, sees the difficulty exactly of making a clean sweep in the metaphysical sense, when at the same time he knows perfectly well that some system of mechanics (not necessarily classical) is, as it were, looking over his shoulder. Here writes the man who once upon a time delighted in the details of aeroplane construction, and when driven too hard by his semantics turned back again to screws and bolts. It would be asking too much to explain exactly why he did this: one guesses that brute fact had retained the same place in his mind as it had occupied in the days of the *Tractatus*. But this is only once more to accept the strong *a priori* element in his fundamental approach to the world, namely, that nobody is likely to understand his thoughts as he jots them down unless the "other mind" (in effect, the *alter ego* of Husserl's phenomenology) had indeed experienced them before.

In sentence No. 613 Wittgenstein discusses the starting and finishing point of a system, noting the exclusion of the intermediate states. This is clearly what usually happens in classical thermodynamics wherein *a* and *b* are states independent of the path. In this language-game sentence Wittgenstein absorbs most of the limitations to which traditional heat theory is prone, but this is not the case, methodologically, with Carathéodory's adiabatic surfaces between which exists total impassability.

The object of reading Wittgenstein in this way is to show how far-reaching his thought is. No physicist is likely to consult him on purely technical grounds, but

ever since the days of Leonardo da Vinci—at least—there has been a veneration for natural law, and incidentally for the use of the subjunctive. Maybe this is it, but if so, it accords ill with modern efforts to display structure rather than to rest content with something "independent of a mechanism".

When we consider, as we are told, that these meditations represent Wittgenstein's thoughts during 1929–48 (but mostly the last three years of that period), a natural question to ask is whence came such an avalanche of creative strife? Looking back at the Vienna of Wittgenstein's youth and formative years, the atmosphere must have been quite extraordinary—Johannes Brahms a friend of the family, Mach, Boltzmann, Schlick, Gödel, a galaxy of talent if ever there was one. Yet Wittgenstein was to know several suicides and one murder, even in his own relatively small circle. I suspect that the language-game which he played all his life arose in part as a counterweight at once challenging and grievous. And the lengthy references to the nature and influence of pain are long-range reflexions of so deep a tension.

The editors have succeeded in keeping some of the best until last. The thoughts on the propositional calculus and the antimonies begin at No. 681 and continue almost up to the end. Additionally, there is the warning remark to the effect that it is stupid to embark on the investigation of an infinite regress: there is no goal, and therefore no sense in setting out to reach it. In this context, however, one should perhaps bear in mind the possibility of an escape clause such as that in some "comparative" philosophy like the second "way" of St. Thomas Aquinas. Whether it is legitimate to see in this (as the late Sir Edmund Whittaker did) a "proof" of the existence of God, conceivably compatible with modern physics, is another matter. The point is that in cases wherein "greater than" or "less than" occurs, the end-point is not necessarily excluded from the start. A mathematical question (No. 697) is seen as a stimulus to imagination: in the next note a parallel is drawn with the act of translation from one language into another, and the great difficulty of doing so, if judged as a matter of replacement. Wittgenstein seizes on the essential element, namely, the absence of a systematic method of solution in both cases. History shows clearly enough that great advances are often made by inspired guesses, whereas "system" followed later, in much the same way that technical routine follows in the wake of original discovery in natural science.

The reader who can feel his way through this collection of ruminations will reap his reward and proffer his thanks to the editors. As a by-product he will be able to test his grasp of German idiom as he goes along, and noting, *en route*, the four-dimensional cube displayed on the jacket and illustrated in colour in the text.

Wittgenstein is full of surprises, even to his last remark (No. 717)—"You cannot hear God speak to someone else, you can hear him only if you are being addressed".

F. I. G. RAWLINS

## RADIATION PHYSICS IN MEDICINE

### Radiological Physics

By M. E. J. Young. Second edition. Pp. xii + 601. (London: H. K. Lewis and Co., Ltd., 1967.) 84s. net.

THE first edition of this book, which appeared in 1957, was welcomed as filling a need for a textbook covering those aspects of radiation physics which should be understood by medical practitioners specializing in radio-diagnosis or radiotherapy. This new edition attempts to bring the subject up to date, which is no mean task in a rapidly developing field. There is, naturally, much new material, but also some of the earlier work has been

rewritten or rearranged. The result is most successful and the author is to be congratulated on the considerable improvement achieved.

The chapters relating to radiotherapy, perhaps the most authoritative, now cover the more recent developments in high energy techniques. It is disappointing, however, that the chapter on radiodiagnostic procedures, while giving some space to recent developments in image intensifier and television techniques, has no mention of what is perhaps the most significant of recent advances, the application to this field of the principles of information theory. An elementary account of this, together with its implication of quantum limitations in the X-ray image, should surely have been included. In the chapter on the diagnostic use of artificial radioactive materials, the author has wisely elected to discuss a few established procedures rather than to catalogue the many possible applications. Some reference might well have been included, however, to clinical uses of whole body counting; this technique receives only a brief mention in relation to personnel monitoring.

There is a carefully revised chapter on chemical and biological effects of ionizing radiations. From the student's point of view, the chapter on radiological protection would have benefited from more specific detail, for example on the methods of calibrating and interpreting film badges; and it might have been stressed that the data on maximum permissible burdens relate to maintained levels. The selection of examination questions and the references following each chapter have been brought as up to date as publishing time permits.

The book has commendable clarity and the care which seems to have been devoted to its style of expression makes such a lapse as "increasing quality" of radiation all the more remarkable. There is a tendency to over-punctuation, particularly in the insistence on a period after every unit abbreviation. Among errors and misprints noticed is the rather curious substitution of ytterbium for yttrium on page 166 and on page 466 it is implied that the  $k$  factor is identical with the specific gamma ray constant, rather than a particular value of it. Presumably Fig. 11.43 refers to energy absorption in "average soft tissue elements" within bone, but the caption does not say so; in consequence a student might well be puzzled at the apparent discrepancy with Fig. 7.03. The text would have helped to avoid this confusion had it cross-referred to the earlier Section 11.14 on "dose absorbed in soft tissue elements in bone". Such oversights are considerably fewer than in the earlier edition, although the new book is more than half as long again.

This textbook can be warmly recommended to doctors preparing for their professional examinations in radiotherapy and radiodiagnosis and to new recruits in the field of medical physics.

G. W. REED

## IONIZATION AGAINST MAN

### Health Physics

Principles of Radiation Protection. By D. J. Rees. Pp. x + 242. (London: Butterworth and Co. (Publishers), Ltd., 1967.) 70s.

AN increasing number of persons is now employed in duties concerned with protection against ionizing radiations. They range from graduates, mainly in physics and chemistry, to technicians or, indeed, industrial workers engaged in the day to day control of radioactive contamination and other aspects of radiation protection where considerable amounts of radioactive materials are manipulated. In addition, other groups of persons, such as teachers, administrators and public health officials, now require a knowledge of the principles of protection against ionizing radiations. There is thus an increasing need for education and training in the principles and tech-

nology of health physics. It is also noteworthy that there is a conspicuous lack of appropriate textbooks in this field and the present volume is therefore very much to be welcomed.

This book is intended to provide a reasonable level of technical training for persons with no previous knowledge of the subject and with limited basic training in physics, chemistry, biology and mathematics. The book admirably achieves this objective and provides a clear and concise introduction to the subject. Starting with some basic physics such as the structure of matter, radioactivity and the interaction of ionizing radiations with matter, we are led on to a discussion of radiation dosimetry, the biological effects of radiation, standards of protection against radiation arising both internally and externally, the design of radioisotope laboratories and radiation protection measurements. There are many diagrams, some taken from specialized physics textbooks but others constructed especially by the author, and these contribute considerably to the value of the book in teaching and training.

Extensive reference is made to the recommendations of the International Commission on Radiological Protection, but it is unfortunate that the only reference to Publication 6 (1964) is in a postscript and no reference is made to Publication 9 (1966) which offers valuable flexibility in the method of compliance with the maximum permissible doses established by the Commission.

The chapter on biological effects of ionizing radiation provides an introduction to the structure of cells and cell division and devotes several pages to a discussion of the genetic effects of ionizing radiation. Somatic effects, however, are dealt with only briefly and attention is given mainly to the acute effects of whole body irradiation. The only reference to cancer is to induction of cancer of the skin after protracted exposures to high dose rates. There is no mention of the possible induction of cancer from significant amounts of radioactive nuclides lodged in the body, such as radium in the skeleton or radon decay products deposited in the lung, or of the induction of leukaemia from substantial whole body irradiation. The risks of the occurrence of these effects have been reviewed in a report of a Task Group to the International Commission on Radiological Protection, published in February 1966, and also in the reports of the United Nations Scientific Committee on the Effects of Atomic Radiation. Although the doses received, within the permissible limits for workers with ionizing radiations, are so low that the risk of these long-term effects is negligible, it is important that they should be recognized in any discussion of the biological effects of ionizing radiation.

The book contains a very extensive appendix on "Data useful for radiation protection problems". This is an admirable collection of data reproduced from a number of sources. This collection of information, however, is rather badly organized. There are examples of repetition of material; for example, beta particle ranges, beta absorption data, mass absorption coefficients, biological response and standards for radiochemical laboratories are each dealt with in more than one place, often with different figures, in the collected material, apparently as a result of quotation from a number of sources. There is no list of contents to this part of the book. Moreover, many of the data do not quote the original source of publication. (This is partly the fault of the International Atomic Energy Agency, from which some of the data are derived.) The individual graphs and data, however, are useful and must be considered as part of the stock in trade of any operational health physicist. Despite its shortcomings, this is a valuable volume, providing an introduction to the subject and some measure of basic training in health physics as well as being a useful volume of reference for the practising health physicist.

W. G. MARLEY

## STIMULUS IN SOIL SCIENCE

### Soil Chemistry and Fertility

Edited by G. V. Jacks. (Transactions of the Meeting of Commissions II and IV of the International Society of Soil Science, Aberdeen, September, 1966.) Pp. vii + 415. (Amsterdam: International Society of Soil Science, c/o Royal Tropical Institute, 1967.) n.p.

THIS book, which deserves only praise, contains a good sample of current work and thought for those interested in the chemistry and fertility of soils. The forty-three contributions, forty of which are in English, are in six main sections: soil organic matter; major nutrients—split into non-metals and metals; trace elements; nutrient diffusion and flow in soils; and the principles of experimentation in soil-crop studies. Each section is introduced with a review-type paper, but most of the other contributions are based on the results of recent research. An appendix contains a brief account of the soils of Scotland.

The dividend from basic research on topics of agricultural relevance is believed to be among the highest paid by any research activity, mainly because a measure of real understanding can go such a long way. Those who are biologically minded might like to ponder on just one point quoted from page 137. "Because the N : S ratio of soil organic matter is substantially less than that of plant protein, it appears likely that any crop that depends entirely on nitrogen from soil organic matter will obtain an adequate supply of sulphur from the decomposition of organic matter". Clearly, this statement should spark off a chain of thought; for example, "it does not necessarily mean that soils receiving fertilizer N should have fertilizer S because there are natural sources of S, other than organic matter . . . however, when large quantities of fertilizer N are added or when legumes are being grown, the S supply should be carefully evaluated to ensure a proper balance of N and S for plant protein production". Acute sulphur deficiency in crops is, in fact, not all that widespread, but how often is plant protein production quietly limited by supplies of sulphur?

The Aberdeen meeting fulfilled its function by providing a stimulus to research and by permitting specialists to broaden their interests; the book can do the same. Much credit is due to the secretary of the organizing committee, Dr. J. Tinsley, to the editor, Mr. G. V. Jacks, and, not least, to the contributors from many parts of the world who must have submitted manuscripts promptly; the Aberdeen University Press also did a first-class job. In all, a most worthwhile effort under the auspices of the International Society of Soil Science.

P. W. ARNOLD

## BOOK OF PALMS

### The Natural History of Palms

By E. J. H. Corner. (The World Naturalist.) Pp. 393 + 24 plates. (London: Weidenfeld and Nicolson, 1966.) 105s.

A FIRST-RATE account of the palms should be expected from a man of Professor Corner's reputation and long experience in the tropics. The palms are reputed to be second only to the grasses in economic importance and, as Corner rightly points out, they are a family which has been badly neglected. Most unhappily, this book falls short of expectations. In a sense, the author, commenting on Seemann's *Popular History of Palms* (1856), provides a pithy review of his own work when he writes: "It is good reading just so far as one does not go to the original sources".

*The Natural History of Palms* is not faithful to many of its written sources nor to many of the palms themselves. Errors of fact, lack of attention to detail, unqualified

and often contradicted generalizations, inconsistency and a florid style, perhaps designed for but misleading to the lay reader and repugnant to the professional, mar the book. It does, however, draw attention to these remarkable plants, dispels any concept that the coconut is representative of the palms in all their diversity, and focuses attention on the many questions yet to be answered before we understand the palms. Written with obvious enthusiasm, it is regrettable that it was not also written with greater care.

Fifteen chapters devoted to general topics, morphology, geography, evolution, generic notes and classification are followed by two appendices, a glossary, chapter references, bibliography and index. The typography is pleasing, the dust-jacket and halftone plates are handsome, but the line figures, particularly those of habit, inflorescences, and flowers, are sometimes "impressionistic" rather than faithful to detail.

It is possible to comment on only a few of the marginal notes in my copy of the book. *The Natural History of Palms* includes the startling statement on the first page that "A fan palm has been reported from the Triassic of Colorado . . ." It is not documented in the references for the first chapter, but I infer from reference 255 to the tenth chapter that Corner refers to *Sanmiguelia lewisii* which Brown very carefully described only as "palmlike" or "tentatively regarded as a primitive palm" and nowhere as a fan palm. The reconstruction and photographs of the actual impressions do not lend credence to the idea of a fan palm on the model of living palms.

In the same chapter appears this generalization: "The floral parts of the monocotyledon are arranged in threes, not fours, fives, or some higher number as in dicotyledons." Then are the Araceae, Cyclanthaceae, Pandanaceae, some palms, *Paris*, among others with floral parts not in threes, also not monocotyledons? The palm flower is said to have " . . . eventually three sepals, three petals, six stamens, and an ovary with three carpels or three cavities . . ." but " . . . this finality has been experimented with in many different lines of palm evolution from a greater number of stamens and carpels. . . ." The more numerous sepals and petals of *Phytelephas* suggest that the perianth might also have been included with stamens and carpels in the last.

Corner's peculiar style also appears in the first chapter where, considering the growth of the monocotyledonous leaf, he writes: "The bigger the leaf, as in palms, pandans and bananas, the more conspicuous the thrust [of the young leaf upwards by means of basal growth]; in palms it is pre-eminent and it is the key to understanding what is going on in their heads". On page 151 he writes: "The ovary of *Phytelephas* is syncarpous. It consists, that is, not of separate carpels but of an ovary-box or carpellary tube, on which the primordia of the separate carpels are raised on a style to form its five to ten branches or stigmata; into the box the ovules have been transferred. How this is done is not known; . . ." Surely the answer must be "It is not done!"

Professor Corner has missed a splendid opportunity to elucidate the nature of the palm inflorescence. Worse, he dwells at length on the significance of the bipinnate leaf of *Caryota*, yet has incorrectly figured and described the terminus of its main axis which is normally a pair of leaflets rather than a single leaflet as on the lateral axes.

"The flower is the meanest bud that can be made" introduces the chapter on the flower. Meanest does not seem an apt adjective for the female flower of *Lodoicea* stated on page 137 to be " . . . one of the most massive flowers of all. . . ." The diversity of floral morphology among the genera of palms is only partly brought out and there are some strange lapses as: "The exceptional subfamilies without multistaminate flowers are those of *Nipa* [sic], *Phoenix*, and the Coryphoid palms; yet the

Coryphoid *Thrinax* is said to have six to twelve stamens". *Thrinax*, when limited in concept, usually has flowers with six stamens, but the segregate genera *Coccothrinax* and *Zombia* usually have more than six stamens as an examination of descriptions or specimens will definitely show. The author does raise provocative questions about the androecium which are not yet definitively answered.

Three chapters on generic notes deal with selected genera but without any explanation of the basis for selection. Professor Corner disclaims in his preface any intent to provide a book which is systematic or taxonomic. It is regrettable, however, that he did not take some stand on the genera allied (or some of them, at least) to *Cocos* which in the unreliable Appendix B is listed as monotypic, for he gives the impression of a larger genus. This ambivalence is not comforting to the reader. The chapters do not contain useful comparative descriptions but instead comprise a series of miscellaneous jottings including paragraphs on how to climb palms. They, with the sketchy and not always correct characterizations of the family and sub-families in a chapter on classification, as well as careless keys in the fifteenth chapter and Appendix A, might well have been omitted.

Appendix A consists of keys to the "commoner" fan palms and pinnate-leaved palms. What a "commoner" palm might be is nowhere stated. The keys serve best as examples of what to avoid: for example, the couplet (7, page 352) "tropical" versus "temperate" to separate the American *Acoelorrhapha* from *Chamaerops* and *Trachycarpus* in the Old World. The last two are distinguished from each other in the succeeding couplet by "leaf-stalk strongly spiny" versus "slightly spiny". *Chamaerops*, however, is more readily distinguished by the orientation of the longer marginal teeth on the petiole in the vegetative state. *Chamaedorea elatior*, sometimes cultivated, keys to *Desmoncus* in the couplet "climbing" versus "not climbing" (6, page 354). *Euterpe precatoria*, one of the most abundant and widespread American palms, keys to the Old World genus *Rhopaloblaste* because of its pendulous pinnae. Perhaps the flowers (and fruits) which provide characteristics leading to accurate identification are not so mean after all.

Where Professor Corner has worked from first-hand observation, as in his description of the development and fall of leaves in *Actinorhynchus calapparia* (page 43), he has made a distinct contribution. I can only agree with him when he writes that "Palms must be studied in their immensity in nature. They cannot be deduced in a metropolis. . . . The hugeness of palms restores the lesson, so easily forgotten, that biological classification deals not with figment but with living things which were before man". Perhaps it is not only the hugeness of the plants but of the scope and complexity of detail to be considered that has defeated a bold and at least thought-provoking attempt to reduce the essential nature of palms to words.

HAROLD E. MOORE, JUN.

## FUNGI WITHOUT WALLS

### The Cellular Slime Molds

By John Tyler Bonner. Second edition, revised and augmented. Pp. x+205. (Princeton, N.J.: Princeton University Press; London: Oxford University Press, 1967.) 60s.

THE description of this second edition as a revised and augmented version of the first edition needs some amplification, particularly because my review of the first edition was somewhat severe (*Nature*, 184, 1976; 1959). In the eight years since the first edition a substantial amount of pertinent and detailed experimental investigation has been carried out on the Acrasiales. The number of refer-

ences cited in the first edition (up to 1959) was 124, and this included the older descriptive work from 1902 onwards as well as the newer experimental work starting with Raper about 1936. This second edition contains 341 references; a marked increase in a relatively short period, and one which reflects a considerable increase in interest in the group.

Dr Bonner gives his prime interest in writing the book as its usefulness for future experimental research. This edition should help considerably in that direction, for it reviews all the published work that is significant and relevant. Dr Bonner has probably had to compromise between comprehensiveness and reasonable size, but he includes the implications of the different approaches that have been made. These include cell biology, biochemistry, fine structure, physiology, developmental studies and taxonomy. In some connexions the discussion is tantalizingly short. For example, more could easily have been written about the control of orientation in the growth of the sorocarp away from the substratum, but within the space he has used for the book Dr Bonner has hit a good balance between the various aspects. Interest is stimulated, and references to the original work are given and can be followed up.

More than half of the book has been completely rewritten for this edition. The first two chapters, which present a perspective of the Mycetozoa one group with another, and describe the better known members of the Acrasiales, are little changed. There is some additional material where necessary, as in the inclusion of the Proto-steliales, and in the fuller description of the species of *Dictyostelium*. The remaining two chapters of the first edition have been expanded to three chapters, and follow the logic which the author has elsewhere established, by separately discussing the three phases of development, which are growth, morphogenetic movement, and differentiation. As he has stated before, these three phases are very clearly demarcated in the Acrasiales, and this is one of their advantages as material for the study of development, permitting individual and separate analyses of each phase.

The short chapter on growth includes some useful detail on laboratory methods, although I feel that it would have been helpful to have given more in a book with the stated aim of this one. A description of methods of isolation and some discussion of the natural history of the organisms (the outstanding omission of the book) would have been particularly appropriate. I wonder also whether some suggestions for class experiments with *Dictyostelium* might not have been worth while. The organisms are fascinating for this purpose and an introduction to them at an early stage might serve to recruit more contributors to future research.

The remaining two chapters comprise the best of the book. A good deal of the work that has been done with these organisms still does not allow of very firm conclusions, and may be interpreted in more than one way. The author seems to me to be very fair in his discussion and evaluation of the evidence. He is also generous in his consideration of the contributions of the different workers in the field. Where work is inconclusive he points this out, and points the way, often with reasonable suggestions, in which research might be directed. One of the attractive features of this book is the way in which research problems and points of uncertainty are given special attention. I found this part a great improvement on the corresponding part in the first edition. The quality of photographic reproduction is poor; much worse than in the first edition.

While the book is rather short, particularly in view of its price, I think the author, in maintaining the interest at a high level and in permitting the several aspects of research to be seen in proximity and in perspective, has produced a book which should fulfil his aim. More than ever, I find these creatures of absorbing interest.

DAVID PARK



## CLIMATES FOR RESEARCH

### Scientists in Organizations

Productive Climates for Research and Development. By Donald C. Pelz and Frank M. Andrews. Pp. xii+318. (New York and London: John Wiley and Sons, Inc., 1966.) 80s.

THIS is the work of two American social psychologists and it deals with the factors which are favourable to progress in original scientific research when the work is done both by individuals and teams. Observations and opinions of the work of more than 1,300 scientists in university, industrial and government laboratories in the United States were obtained. The numbers of scientists considered were large enough for a proper statistical analysis of the results. The authors claim that the book is based on more extensive objective and quantitative data than has ever before been examined in this area. The study is particularly useful in that the crucial laboratory factors can be modified to some extent by the heads of research departments, managers, and the scientists and technicians themselves. There can be no doubt that much of this investigation is valid for work in British scientific research institutions, but there are differences. The great difference in esteem between the Master's postgraduate degree and the PhD which is held in America does not obtain to the same extent in Britain. The deeper purse which finances many investigations in the United States and the generous staffing which is conducive to adequate teamwork with a well-spread set of complementary specialisms, common in America, are rarer and ideal in Britain. Reasonable financial rewards will operate as a stimulus both to the individual as a person and to the team as an organization which often requires highly sophisticated and expensive apparatus, which on occasion will try the resources of heavy engineering. Research, even in subjects other than physics, has moved far from Rutherford's "tobacco-tin stage".

The authors have portrayed their findings with many charts and tables, and have provided a complete description of the research on which the findings are based. This, in itself, is useful as a model and guide for other workers in sociological fields, who at times have used statistical formulae with little background knowledge of experimental techniques and the interpretation of results. It should be kept in mind that all the statistical analysis in the world may be helpless if the very rare Newton, Planck or Rutherford is caught in their net!

The chief areas which were explored by the authors of the book were freedom, communication, motivations, satisfactions, creativity, age, groups and co-ordination. The principal factors of the results of the researches show that effective scientists were self-directed by their own ideas and valued freedom. But at the same time they allowed several other people a voice in shaping their directions; they interacted vigorously with colleagues. Effective scientists did not limit their activities either to the world of "pure science" or of "applied science" but maintained an interest in both. Their work was diversified. Effective scientists were not fully in agreement with their organization in terms of their interests; what they personally enjoyed did not necessarily help them to advance in the structure. Effective scientists tended to be motivated by the same kinds of thing as their colleagues. At the same time, however, they differed from their colleagues in the styles and strategies with which they approached their work.

In effective older groups, the members interacted vigorously and preferred each other as collaborators; yet they held each other at an emotional distance and felt free to disagree on technical strategies. Thus, in numerous ways, the scientists and engineers who were studied did effective work in conditions which were not completely comfortable, but contained "creative tensions" among forces pulling in different directions.

Among the interesting by-products of the research, which appeared when it seemed important to remove the effects of certain extraneous factors, was the fact that PhDs in government research in America published 50 per cent more than university PhDs, and assistant scientists in government research published twice as much as those in industry. This appeared to stem from the obligations of government laboratories to let the public know where their money was going. Again, the relatively low publication rate among scientists in industrial laboratories could be attributed to "company security".

W. L. SUMNER

## OBITUARIES

### Professor H. N. Green

HARRY NORMAN GREEN, who died on May 16 at the age of 64, published many papers on nutrition, traumatic shock and the immunological aspects of cancer.

He graduated MB, ChB from the University of Sheffield in 1924 and the next year gained his BSc, with first class honours. His MSc and MD followed in 1926 and 1927. He then combined the posts of clinical assistant to Sir Edward Mellanby at the Royal Infirmary, Sheffield, with that of research assistant in the department of pharmacology. After two years as lecturer in pathology at Cambridge, he returned in 1935 to Sheffield as professor of pathology. In 1953 he became director of cancer research at the Universities of Leeds and Sheffield and professor of experimental pathology and cancer research at the University of Leeds.

Green was interested in that part of experimental pathology which has a bearing on clinical medicine. His earlier work, in collaboration with Sir Edward Mellanby, concerned the effects of vitamin A deficiency in the spread of infection. The advent of the sulphonamides as clinically effective antibacterial agents led him to study their mode of action. He demonstrated that bacteria produced one, or possibly more, factors capable of inhibiting the action of the drug. He made considerable progress towards the characterization of these factors.

The advent of the Second World War produced an immediate interest in traumatic shock; Green was approached by the Medical Research Council to investigate the problem. On the basis of conclusions drawn from the clinical examination of cases of industrial injury, he and his collaborators began to examine the problem experimentally. It was shown that adenosine triphosphate and related nucleotides accounted for the shock inducing properties of muscle extracts. A period as leader of British Shock Team 2, Royal Army Medical Corps, enabled him to demonstrate the release of nucleotides from the injured tissues of battle casualties and thus to confirm in man the experimental findings. The background to this work was described in a monograph with Dr H. B. Stoner, entitled *Biological Actions of the Adenine Nucleotides*.

Green's interest in cancer started with a series of investigations on the carcinogen, 2-acetylaminofluorene. These were followed by the study of those fractions of coal tar which were able to inhibit the growth of transplanted tumours in animals, work which was never published in full because of the fear of raising false hopes of an impending cure for cancer. A number of chemically pure tumour inhibiting but non-carcinogenic compounds were isolated and were also shown to be without effect on induced or "spontaneous" tumours in rodents. It was the consideration of this work which led, in 1954, to the immunological theory of cancer. The idea that immuno-

logical mechanisms might be responsible for the integrity and balance of the many different tissues of the body and that cancer might be the expression of a failure of these mechanisms was, at the time it was published, revolutionary and was one of the milestones leading to the resurgence of interest in immunology applied to cancer. The explosive growth of interest in cancer immunology led Green and his colleagues to prepare a text on an immunological approach to cancer which is due for publication within a few months.

Green's great strength was his ability to join together facts and concepts from apparently unrelated fields and thereby to add to the understanding of the subject in which he was interested. He took great pride in the activities of his son and daughter and of his wife, whose increasing reputation as a painter delighted him.

D. B. CLAYSON

### Friedrich Frans Koczy

Fritz Koczy, who died on April 18, was born in Vienna in 1914. He studied mathematics and physics at the University of Vienna during 1934-38; in 1936-38 he worked on his PhD thesis at the Institute for Radium Research under the direction of Dr Berta Karlik. At that time this institute was one of the leading centres for the study of natural radioactivity; its exciting scientific and intellectual atmosphere made a lasting impression on Koczy. His research was directed towards testing a hypothesis of Otto Hahn, that the coloration observed in certain samples of rocksalt was caused by the presence of RaD-F-G—these salts supposedly deposited from radioactive waters emanating from thermal springs. Koczy measured the helium contents of blue and white rocksalts, found no significant differences and disproved the hypothesis. In 1939, at the invitation of Hans Pettersson, the noted Swedish oceanographer, Koczy left Nazi occupied Austria to become a research fellow and to assist in establishing the Oceanographic Institute at Göteborg, Sweden. His chief interest was the application of radioactive methods to oceanographic problems. In particular, he was interested in the distribution of uranium and thorium and their decay products in the oceans, the sediments, and on the continents. On the basis of his measurements of lead isotopic ratios, Koczy calculated the age of the Earth to be 5.3 billion years. This value disagreed considerably with the favoured estimates of the time; however, it is in excellent agreement with the age of 4.5 billion years, generally accepted in the past few years. Koczy simultaneously engaged in a variety of scientific projects involving many disciplines; he cultured plankton on a large scale, investigated the factors influencing light propagation in sea water and the relationship of light levels and biological activity, and was one of the earliest users of underwater photography as a scientific tool.

Shortly after the end of the Second World War, when plans for the now-famous Swedish deep-sea expedition of 1947-48 were approved, Koczy played an important part in the preparations; he was especially active in the conversion of the *Albatross* into a research vessel and in the acquisition of scientific equipment, particularly difficult at that time. He participated in the entire fifteen month expedition which greatly profited from his scientific insight and experimental skill. The organization and analysis of the experimental material collected during the expedition occupied Koczy for several years. He assumed responsibility for processing, analysing and publishing the extensive echo-sounding records. Koczy developed a unique method for obtaining water samples close to the ocean floor, which could be used even at great depths; the samples obtained in this manner yielded results which established him as a pioneer in the study of the properties of deep oceanic waters. Most important, however, was

that Koczy was able to formulate his ideas about the geochemical balance of the radioactive elements in the hydrosphere on the basis of his measurements of radionuclides in oceans and sediments.

In 1957 Koczy accepted an invitation from Dr F. G. Walton Smith, director of the Institute of Marine Sciences, University of Miami, to go to Miami to develop a division of physical sciences. Here he assembled a large group of world famous research scientists engaged in activities ranging from underwater acoustics to the study of radionuclides produced in sediments by cosmic rays. His efforts were very important in making the institute one of the world's leading oceanographic research centres. Koczy felt a strong obligation to establish a good teaching programme and he was instrumental in making the institute one of the best in the training of graduate students.

While at the University of Miami, Koczy continued his investigations of natural radionuclides in sea water and sediments. He became a leading authority on age determination of ocean sediments. Under his direction a dating method was developed which was based on the ratios of the activities of protactinium-231 (a daughter nuclide of uranium-235) and thorium-230 (a daughter of uranium-239); capable of dating sediments up to 200,000 years old, it is the most commonly used and reliable of the various geochronological methods. Koczy also showed that the concentration of radium-226 in the oceans was too great to be derived from land sources. He proposed that the radium diffused from the ocean sediments (this was a particularly important discovery, for radium-226 is the only natural radioactive tracer released in quantity at the bottom of the ocean). On the basis of the vertical distribution of concentrations of radium, Koczy calculated vertical mixing rates for deep ocean waters and the time of residence of these water masses.

Koczy never dismissed the practical side of his work because of his profound understanding of the social functions of science. He became concerned with, for example, the relationship of oceanography to fisheries and radioactive waste disposal at sea. His capabilities and his concern for the development of science naturally led to an involvement with scientific policy on a national level. Koczy was appointed early a member of the National Academy of Sciences Committee on Oceanography and for four years was one of its most active and enthusiastic members. In the same period, he sat with the Earth Sciences Advisory Panel of the National Science Foundation. He also held advisory positions with the Atomic Energy Commission, the Environmental Sciences Service Administration and Bureau of Commercial Fisheries. In addition, he was on the editorial boards of many scientific publications. Koczy was involved in one of oceanography's most imaginative investigations of the sea, the Joint Oceanographic Institutes Deep-Earth Sampling, the purpose of which was to drill into the ocean floor to obtain long core samples in an effort to expand the understanding of the origin and history of the ocean basins. Koczy was also chairman of the Gulf Universities Research Corporation, an organization devoted to oceanographic investigations of the Gulf of Mexico and the Caribbean through the co-operative efforts of universities in the area.

Although a dedicated scientist, Koczy took a great interest in cultural activities throughout his life. His colleagues in Vienna recall that he was a voracious reader and frequently visited the theatres and museums; he is reputed to have attended almost every concert of the Vienna Philharmonic Orchestra in his student days. At the time of his death he was on leave from the University of Miami and was a distinguished visiting professor at the University of Hawaii.

RICHARD G. BADER  
NILS JERLOV  
BERTA KARLIK  
ANITA THORHAUG

## University News:

### Leeds

DR MARTIN LÖB has been awarded the title of professor of mathematical logic and Dr David Clayson has been awarded the title of professor in the Department of Experimental Pathology and Cancer Research.

### London

DR J. S. WILKIE, reader in the history and philosophy of science, has been appointed to the chair of history and philosophy of science at University College, and the title of professor of internal combustion engineering has been conferred on Dr Wei-Tze Lyn in respect of his post at King's College.

### Reading

DR H. H. HOPKINS, at present a reader in applied optics in the Imperial College of Science and Technology, has been appointed first holder of the chair of applied optics in the Department of Applied Physical Sciences.

### Welsh College of Advanced Technology

DR R. W. EDWARDS, head of the Pollution Division at the Ministry of Technology Water Pollution Research Laboratory, has been appointed professor and head of the Department of Applied Biology, Welsh College of Advanced Technology (University of Wales Institute of Science and Technology (Designate)).

## Appointments

PROFESSOR R. HIDE, professor of geophysics and physics at the Massachusetts Institute of Technology, has been appointed to a senior post in the Meteorological Office where he will direct a new laboratory of geophysical fluid dynamics.

PROFESSOR A. W. KAY, University Department of Surgery, Western Infirmary, Glasgow, and Professor R. A. Gregory, Physiological Laboratory, Liverpool, have been appointed to the Medical Research Council in succession to Sir Hedley Atkins and Professor W. D. M. Paton.

## Announcements

DR J. NAPIER of the University of London has been appointed by the Smithsonian Institution to examine the feasibility of establishing an International Centre for the Study of Primate Animals.

THE newly instituted Royal Society Mullard Award, consisting of a gold medal and a prize of £1,000, is to be presented to Dr G. D. H. Bell, director of the Plant Breeding Institute, Cambridge, for his contributions to agricultural production in breeding the Proctor barley.

AN International Society for Developmental Psychobiology is being formed to facilitate communication among research workers interested in relating biological processes to behaviour in the developing organism. Further information can be obtained from Dr B. J. Key, Department of Neuropharmacology, Medical School, Birmingham 15.

MR RONALD W. CLARK of 10 Campden Street, London, W.8, is writing a biography of Sir Edward Appleton and wishes to hear from persons who have material or information to contribute.

## Meetings

PURE and Applied Chemistry, September 4-10, Prague (Organizing Committee, XXIst International Congress of Pure and Applied Chemistry, POB 139, Praha 6-Dejvice).

MOLECULAR Structure and Spectroscopy, September 5-9, The Ohio State University (Professor K. Narahari Rao, Molecular Spectroscopy Symposium, Department of Physics, The Ohio State University, 174 West 18th Avenue, Columbus, Ohio).

NUCLEAR Structure, September 7-13, Tokyo (Professor M. Sakai, Institute for Nuclear Study, University of Tokyo, Tanashi-machi, Kitatama-gun, Tokyo).

SYMPOSIUM of the British Society of Scientific Glassblowers, September 8-9, University of Reading (J. A. Frost, 3 Grass Hill, Caversham, Reading, Berks.).

WAVES in Plasma, September 11-13, Culham Laboratory (The Meetings Officer, the Institute of Physics and the Physical Society, 47 Belgrave Square, London, SW1).

GROWTH Hormone, September 11-13, Milan (Dr. A. Pecile, Secretary of International Symposium on Growth Hormone, Via A del Sarto, 21, Milan).

THIN Walled Steel Structures Symposium, September 11-14, University College of Swansea (Symposium Office, c/o SSIDA, Albany House, Petty France, London, SW1).

HIGH Voltage Insulation in Vacuum, September 13-14, Institution of Electrical Engineers (The Meetings Officer, the Institute of Physics and the Physical Society, 47 Belgrave Square, London, SW1).

ELECTRON Optics, Instrumentation and Quantitative Electron Microscopy, September 19-21, University of St. Andrews (The Meetings Officer, the Institute of Physics and the Physical Society, 47 Belgrave Square, London, SW1).

INDUSTRIAL Production of Plants, September 20-22, Vienna (Ruthner Industrieanlagen für Pflanzenbau, Gesellschaft m.b.H., Wien II, Obere Donaustrasse 49-51).

USE of Radioactive Isotopes in Pharmacology, September 20-23, Geneva (Secrétariat général de la Conférence, 20 bd. d'Yvoy, 1211 Geneva 4).

INTERNATIONAL Spectrometry Conference, September 25-29, Technical University, Berlin (Dr W. Fritzsche, Gesellschaft Deutscher Chemiker, 6000 Frankfurt (M) Postfach 9075).

CARBOHYDRATE Discussion Group (a Chemical Society Subject Group), September 26, London (Dr R. D. Guthrie, The Chemical Laboratory, University of Sussex, Brighton).

VERTEBRATE Palaeontology and Comparative Morphology, September 27-28, Queen Elizabeth College, University of London (Dr C. B. Cox, Department of Zoology, King's College, Strand, London, WC2).

Low Energy Nuclear Physics, September 27-29, University of Manchester (The Meetings Officer, the Institute of Physics and the Physical Society, 47 Belgrave Square, London, SW1).

PROBLEMS of Birds as Pests, September 28-29, Royal Geographical Society, Kensington Gore, London (The Institute of Biology, 41 Queen's Gate, London, SW7).

ERRATUM. In the article "The Flow Behaviour of Blood in the Circulation" by R. L. Whitmore (*Nature*, 215, 123; 1967) equation 5 should read

$$\eta_{\text{eff}} = \frac{\eta_p}{1 - \left(\frac{R-4}{R}\right)^4 \left(1 - \eta_{rc}\right)}$$

Line 1 in the right-hand column on page 126 should read "... the asymptotic values should be 25 per cent", and the last author in reference 19 should be spelt Salzman.

## APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

**TECHNICAL ASSISTANT** to take part in a research project into the structure and function of invertebrate nervous systems using various modern techniques—Prof. G. M. Hughes, Department of Zoology, The University, Bristol, 8 (August 1).

**RESEARCH ASSISTANT** in the DEPARTMENT OF SOCIOLOGY—The Registrar, University of Essex, Wivenhoe Park, Colchester, Essex (August 4).

**RESEARCH ASSISTANT** in the CHEMISTRY DEPARTMENT for an investigation of the synthesis of new unsaturated carbohydrate derivatives for testing as anti-tumour agents—Prof. L. Hough, Queen Elizabeth College (University of London), Campden Hill Road, London, W.8 (August 5).

**EDITORIAL ASSISTANTS** (with a good degree in chemistry and for some duties a working knowledge of languages would be an asset) for various duties with the Society's scientific publications—The Editor, The Chemical Society, Burlington House, Piccadilly, London, W.1 (August 10).

**LECTURERS or ASSISTANT LECTURERS** (2) in the DEPARTMENT OF ELECTRICAL ENGINEERING and ELECTRONICS (candidates for one of the posts should be specialists in the field of control engineering)—The Registrar, The University, Liverpool, quoting Ref. RV/87 (August 10).

**ASSISTANT EXPERIMENTAL OFFICER** (with a degree in botany, chemistry or a related subject) in the ENTOMOLOGY SECTION to participate in studies on the behaviour of dipterous pests; and an **ASSISTANT EXPERIMENTAL OFFICER** (with a degree in botany, chemistry or a related subject) in the PLANT PHYSIOLOGY SECTION to assist with work on the extraction and purification of plant hormones and the study of their physiological properties—The Secretary, National Vegetable Research Station, Wellesbourne, Warwick (August 11).

**RESEARCH ASSISTANT** (with a degree in botany, biochemistry, microbiology or genetics, and a special interest in biochemical genetics, particularly enzymology) in the DEPARTMENT OF BOTANY—The Secretary, University College London, Gower Street, London, W.C.1 (August 11).

**COMPUTING ASSISTANT** to assist members of staff and postgraduate students in the DEPARTMENT OF MECHANICAL ENGINEERING in the development and operation of ALGOL programmes for the University KDF9 computer—The Registrar and Secretary, The University, Leeds, 2 (August 12).

**ASSISTANT EXPERIMENTAL OFFICER** (graduate with an interest in the physiological aspect of host-parasite relations would be suitable) in the MYCOLOGY SECTION for work on the grey mould diseases of soft fruit and tomatoes—The Secretary, Scottish Horticultural Research Institute, Invergowrie, Dundee (August 14).

**ASSISTANT KEEPER** (with an appropriate degree which should include botany and/or the Diploma of the Museums Association) of NATURAL HISTORY—The Director, Castle Museum, Norwich, NOR 65B (August 14).

**LECTURER (Clinical)** in the DEPARTMENT OF PATHOLOGY—The Assistant Registrar, The Medical School, University of Birmingham, Birmingham, 15 (August 14).

**LECTURER** (preferably with an interest in any aspect of fungi) in the DEPARTMENT OF BOTANY—Head of the DEPARTMENT OF BOTANY, The University, Newcastle upon Tyne 1 (August 14).

**RESEARCH ASSISTANT** (with a Ph.D. degree or equivalent) in the DEPARTMENT OF CHEMISTRY for work on the synthesis of a naturally occurring polypeptide—The Secretary, University of Exeter, Northcote House, The Queen's Drive, Exeter, Devonshire (August 14).

**RESEARCH FELLOW** (medically qualified and preferably holding the D.P.M.) in PSYCHIATRY—Prof. W. H. Trethowan, Department of Psychiatry, Queen Elizabeth Hospital, Birmingham, 15 (August 14).

**S.R.C. POSTDOCTORAL RESEARCH FELLOW** (with considerable experimental experience in either physical gas dynamics including shock tube research, atomic and molecular beams, measurements of collision cross sections or ion detection and analysing systems) in the DEPARTMENT OF PURE AND APPLIED PHYSICS—Dr R. M. Hobson, Department of Pure and Applied Physics, University of Belfast, Northern Ireland (August 14).

**ASSISTANT LECTURER in PHILOSOPHY**—The Registrar, The University, Canterbury, Kent, quoting Ref. A/69 (August 15).

**RESEARCH ASSISTANT** in the DEPARTMENT OF STATISTICS—The Registrar, University College of Wales, Aberystwyth (August 16).

**LECTURER in BIOLOGY** in the DEPARTMENT OF APPLIED MICROBIOLOGY AND BIOLOGY—The Registrar, University of Strathclyde, George Street, Glasgow, C.1, quoting Ref. 66/67 (August 18).

**SENIOR RESEARCH OFFICER** (with research experience in some branch of engineering) in the DEPARTMENT OF APPLIED MATHEMATICS for research in rheology—The Registrar, University College of Wales, Aberystwyth (August 19).

**ASSISTANT LIBRARIAN** (with an honours degree, and preferably some experience in dealing with scientific material)—The Secretary, University of Exeter, Northcote House, The Queen's Drive, Exeter, Devonshire (August 21).

**CHAIR OF PHYSIOLOGY** at St. Mary's Hospital Medical School—The Academic Registrar, University of London, Senate House, London, W.C.1 (August 21).

**SENIOR LECTURER** and a **LECTURER in MATHEMATICS** at the University College of Rhodesia—The Inter-University Council, 33 Bedford Place, London, W.C.1 (August 21).

**ASSISTANT LECTURER** in the DEPARTMENT OF MEDICINE, Royal Infirmary—The Professor of Medicine, Department of Medicine (University of Edinburgh), The Royal Infirmary, Edinburgh (August 25).

**LECTURER** (preferably with a medical qualification) in PHARMACOLOGY—The Secretary, The Middlesex Hospital Medical School, London, W.1 (August 25).

**LECTURERS or ASSISTANT LECTURERS** (2) (with research interests in any branch of applied mathematics) in the DEPARTMENT OF APPLIED MATHEMATICS, St. Salvator's College—The Secretary, University of St. Andrews, College Gate, St. Andrews, Fife, Scotland (August 25).

**SENIOR LECTURER in VETERINARY SCIENCE** at the University of Queensland, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, August 25).

**LECTURER or ASSISTANT LECTURER** (preferably with a research interest in topology) in the DEPARTMENT OF MATHEMATICS—The Registrar, University College of Wales, Aberystwyth (August 26).

**SENIOR LECTURER** (with research and/or industrial experience in fluid dynamics and preferably holding a higher degree) in FLUID MECHANICS in the DEPARTMENT OF MECHANICAL ENGINEERING—The Registrar, University of Strathclyde, George Street, Glasgow, C.1 (August 30).

**FELLOW in CELL BIOLOGY** to study the biochemical self sufficiency of chloroplasts—The Registrar, The University of York, Heslington, York (August 31).

**LECTURER in COMPARATIVE EDUCATION**—The Secretary, University of London Institute of Education, Malet Street, London, W.C.1 (August 31).

**LECTURER or ASSISTANT LECTURER in APPLIED MATHEMATICS**—The Registrar, The University, Senate House, Bristol, 2 (August 31).

**LECTURER** (with a good honours degree in physics, electronics or electrical engineering and research or teaching experience in some aspect of the electrical properties of materials) in MATERIALS SCIENCE—The Registrar, University College of North Wales, Bangor, North Wales (August 31).

**LECTURERS/SENIOR LECTURER in PURE MATHEMATICS** at the University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 31).

**SENIOR LECTURER in INSECT PHYSIOLOGY** in the DEPARTMENT OF ENTOMOLOGY, University of Queensland, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, August 31).

**CHAIR of MATERIALS** in the FACULTY OF ENGINEERING at Queen Mary College—The Academic Registrar, University of London, Senate House, London, W.C.1 (September 7).

**CHAIR of NUCLEAR ENGINEERING** at Queen Mary College—The Academic Registrar, University of London, Senate House, London, W.C.1 (September 7).

**LECTURER or ASSISTANT LECTURER** (registered member of the veterinary profession) in the DEPARTMENT OF VETERINARY PATHOLOGY—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh (September 16).

**LECTURER or ASSISTANT LECTURER** (with interests in any of the major economic fields of geology) in the DEPARTMENT OF GEOLOGY—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh (September 16).

**ASSISTANT LECTURER** (with a good honours science degree, preferably in biochemistry, and preferably some postgraduate research experience) in the DEPARTMENT OF CHEMICAL PATHOLOGY—The Secretary, The University, Aberdeen (September 30).

**CHAIR and HEADSHIP of the DEPARTMENT of APPLIED MINERAL SCIENCES**, Houldsworth School of Applied Science—The Secretary, The University, Leeds, 2 (September 30).

**ENTOMOLOGISTS** (2) (with an honours degree in biology with emphasis on entomology and practical postgraduate experience, preferably in biocontrol) at the COMMONWEALTH INSTITUTE OF BIOLOGICAL CONTROL for field work on biocontrol projects—The Secretary, Commonwealth Agricultural Bureaux, Farnham House, Farnham Royal, Buckinghamshire (October 15).

**HEAD TECHNICIAN** (with wide experience in the techniques of normal or pathological histology and preferably F.I.M.L.T. or equivalent qualifications) for the Laboratory of Cytopathology—The Personnel Officer, National Institute for Medical Research, Mill Hill, London, N.W.7.

**HEAD** (virologist of wide experience with a knowledge of immunology, and preferably with a medical qualification) of the VIRUS VACCINES DEPARTMENT, Lister Institute of Preventive Medicine, Elstree, Herts.—The Secretary, Lister Institute of Preventive Medicine, Chelsea Bridge Road, London, S.W.1.

**LECTURER in GEOGRAPHY** to specialize in human geography and in the regional geography of either Africa or Asia in the Department of Arts, Science and Education—The Registrar, University of London, Goldsmiths' College, New Cross, London, S.E.14.

**POSTDOCTORAL FELLOW** (preferably with some experience of the chemistry of phenols and/or quinones) in the DEPARTMENT OF ORGANIC CHEMISTRY, for research into the preparation, properties and uses of quinone-methides—The Registrar, The University, Liverpool, 3, quoting Ref. RV/88.

**RESEARCH ASSISTANT (Biochemist)** (preferably graduate in chemistry, biochemistry or physiology) for an investigation of oestrogens in relation to infertility—The Group Secretary, Romford H.M.C., Oldchurch Hospital, Romford, Essex, quoting Ref. No. 25/103.

**RESEARCH ASSISTANT** (preferably honours graduate chemist) to join in chemical studies of human arterial disease (atheroma)—Dr C. J. W. Brooks, Chemistry Department, University of Glasgow, Glasgow, W.2.

**RESEARCH ASSISTANT** (with a good honours degree in mathematics) in the DEPARTMENT OF APPLIED MATHEMATICS and MATHEMATICAL PHYSICS to carry out theoretical research, which could lead to the degree of Ph.D., on "The Effects of High Electric Fields of Semiconductors"—Dr Alan R. Beattie, Department of Applied Mathematics and Mathematical Physics, University College, Cardiff.

**RESEARCH ASSISTANTS** in a wide range of projects in the following departments: Computing and Cybernetics; Management and Business Studies (particularly in the fields of multi-access systems and the theory of automata); Applied Physics; Applied Chemistry; Pharmacy; Mechanical and Production Engineering; Electrical and Electronic Engineering; Civil Engineering and Building—The Registrar, Brighton College of Technology, Moulsecomb, Brighton 7, Sussex.

**TECHNICIAN (O.N.C. or equivalent qualification, and preferably some experience of bacteriology)** in the DEPARTMENT OF GENETICS—Prof. R. H. Pritchard, The University, Leicester.

## REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

### Great Britain and Ireland

- Malnutrition in the 1960s? Pp. 32. (London: Office of Health Economics, 1967.) 2s. 6d.
- Association of the British Pharmaceutical Industry. Annual Report and Year Book 1966-67. Pp. 39. (London: Association of the British Pharmaceutical Industry, 1967.)
- International Nickel. Mechanical and Physical Properties of the Austenitic Chromium-Nickel Stainless Steels at Subzero Temperatures. Pp. 26. (London: International Nickel, Ltd., 1967.)
- Department of Education and Science: Ministry of Technology. Statistics of Science and Technology. Pp. vi + 80. (London: H.M. Stationery Office, 1967.) 15s. net.
- Women and Top Jobs: an Interim Report. Pp. 81. (London: Political and Economic Planning, 1967.) 8s.
- Science Museum. Science Library Bibliographical Series No. 792: Some References to Pearl Essence 1959-1966. (Supplement to No. 772.) Pp. 4. (London: Science Museum, 1967.)
- Gas Goes Natural: The Prospects and Plans of the Gas Industry. Pp. 16. (London: The Gas Council, 1967.)



- Royal Observatory Bulletins, No. 129: Studies of the Globular Cluster  $\omega$  Centauri. 6: Photometry of Cepheids with Periods Greater than One Day. By R. J. Dickens and J. V. Carey. Pp. E335-E351. (London: H.M. Stationery Office, 1967.) 3s. 6d. net. [76]
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#### NATIONAL INSTITUTE FOR RESEARCH IN DAIRYING (UNIVERSITY OF READING) BACTERIOLOGY DEPARTMENT

Biochemist or microbiologist, preferably with experience of immunology, required for a group working on natural defence mechanisms of the udder against mastitic pathogens, in particular staphylococci. The group works in close association with a team working on the control of mastitis. The natural inhibitors and antibodies present in bovine blood stream, milk and the mammary gland are under study. The department is well equipped and facilities are available for the use of conventional and germ-free animals. Arrangements exist for registration for a Higher Degree. Appointment to grade of Scientific Officer (£926 to £1,574) or Senior Scientific Officer (£1,744 to £2,155) according to age and experience.

Apply to Secretary, N.I.R.D., Shinfield, Reading, naming two referees and quoting reference 67/25. (268)

#### BRAIN WAVES

There is a vacancy for a

#### TRAINEE ELECTRO- ENCEPHALOGRAPHY TECHNICIAN

in a regional centre based on Goodmayes Hospital, Ilford, Essex. The work involves recording of brain activity by means of electrodes on the head, as an aid to diagnosis, and requires an ability to deal with people as well as some interest in electronic apparatus. Salary during training is £260 to £520 according to age (16 to 24) plus London weighting. (Furnished accommodation may be available.) Educational requirements are G.C.E. "O" levels in English Language, Mathematics, and a Science subject.

For details apply to:  
The Secretary,  
Goodmayes Hospital,  
Barley Lane,  
Goodmayes,  
Ilford, Essex. (421)

#### ROWETT RESEARCH INSTITUTE

BUCKSBURN, ABERDEEN

#### MICROBIOLOGY DEPARTMENT

Applications are invited for a post on the **Scientific Officer/Senior Scientific Officer** scale. The post is concerned with extension of work on the nitrogen metabolism of micro-organisms in rumen contents using stable isotopes of N and <sup>14</sup>C and <sup>3</sup>H. Applicants should have a degree in microbiology or in biochemistry or chemistry with experience in microbiology. Salary according to experience within the range S.O. £926 to £1,574 or S.S.O. £1,744 to £2,155. Superannuation under F.S.S.U. Further particulars are available.

Applications, including the names of three referees, should be lodged with the Secretary of the Institute within one month. (329)



## Scientific Civil Service

### DEPARTMENT OF AGRICULTURE AND FISHERIES FOR SCOTLAND

MARINE LABORATORY, Aberdeen

**MICROBIOLOGIST/BIOLOGIST/BIOCHEMIST** (graded S.S.O./S.O./Research Fellow (Junior or Senior)) required for programme on fish diseases and pathology (initially concerning the diseases of salmonid fish). Candidates should possess experience in bacteriology or virology. Post-graduate experience preferred but not essential.

**MARINE ECOLOGIST** (graded E.O./A.E.O., for man only) required to join a team working on the ecology and population dynamics of herring and other pelagic fishes. In addition to laboratory work the post will involve periods of duty at sea aboard research vessels and commercial fishing vessels.

#### QUALIFICATIONS

Research Fellows: Applicants should have the qualifications required for S.O. and must have reached a high standard of research ability. In addition the following are required: Junior R.F.—2 years' post-graduate research. Senior R.F.—3 years' post-graduate research.

#### SALARY

Junior R.F. fixed in range £1,180 to £1,575;

Senior R.F. fixed in range £1,745 to £2,155.

Except in the case of Research Fellowships, which are tenable for three years, the above posts carry prospects of permanent pensionable appointments.

**APPLICATION FORMS** and further particulars from Establishment Officer, Department of Agriculture and Fisheries for Scotland, Room 172, St. Andrew's House, Edinburgh 1. Closing date for receipt of applications August 21, 1967.

## MINISTRY OF TECHNOLOGY

### TORRY RESEARCH STATION

P.O. Box 31, 135 Abbey Road, Aberdeen

**MICROBIOLOGIST** (graded E.O./A.E.O.) to work on microbiology of fish and fishery products and associated projects such as biochemical aspects of bacterial toxonomy.

Prospect of permanent pensionable appointment.

**APPLICATION FORMS** from the Director at the above address, quoting E/AT/043. Closing date August 14, 1967.

#### SALARIES, QUALIFICATIONS and AGE LIMITS

##### SCIENTIFIC OFFICER/SENIOR SCIENTIFIC OFFICER

**SALARIES:** S.O.: £926–£1,574. (Increments and special £120 award for approved post-graduate experience.)

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#### QUALIFICATIONS:

S.O.: 1st or 2nd class hon. degree or equivalent or higher qualifications in appropriate subject. Age under 29.

S.S.O.: As above plus at least 3 years' post-graduate research. Age normally 26–31.

##### ASSISTANT EXPERIMENTAL OFFICER/EXPERIMENTAL OFFICER

**SALARIES:** A.E.O.: £568 (age 18)–£803 (age 22)–£1,017 (age 26)–£1,243. E.O.: £1,365–£1,734. Most Assistant Experimental Officers become Experimental Officers before reaching A.E.O. scale maximum. Promotion prospects to Senior Experimental Officer: £1,977–£2,411. Salaries are supplemented in the London area.

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A.E.O.: age 18–27, E.O.: normally 26–30.

A free booklet entitled "THE SCIENTIFIC CIVIL SERVICE" is available on request from Civil Service Commission, Savile Row, London, W.1. (379)

**MONASH UNIVERSITY**

MELBOURNE, AUSTRALIA

CHEMICAL ENGINEERS, PHYSICAL  
CHEMISTS, BIOCHEMISTS

Applications are invited for three posts as Lecturer/Senior Lecturer in the Department of Chemical Engineering. A previous current advertisement for one of these posts indicated the preferred field to be either FOOD AND BIO-CHEMICAL ENGINEERING or THERMODYNAMICS AND EXTRACTIVE METALLURGY. Additional candidates are now sought with ability in DIFFUSIONAL SEPARATION PROCESSES, or in TRANSPORT PHENOMENA or in EXPERIMENTAL CHEMICAL KINETICS. Good candidates with interests in other areas, including mathematics with a strong interest in chemical engineering, will also be considered. The Department is well housed in a new building. Good computing facilities are available.

Current salary scales are: Senior Lecturer, \$A6,500 to \$A7,600 per annum; Lecturer, \$A4,800 to \$A6,300 per annum, but salaries are at present under review and it is expected that the maximum of the Senior Lecturer scale will be increased to \$A8,750 per annum, with relative increases through the scale, and the Lecturer's range will become \$A5,400 to \$7,300. Superannuation on the F.S.S.U. basis is provided.

Benefits: Full travelling expenses for an appointee and family; removal allowance; repatriation after three years if desired; initial subsidized housing; availability of loans for home purchase; STUDY LEAVE is granted at the rate of one term's leave for six months' service, with provision for financial assistance.

Further general information is available from the Academic Registrar, Monash University, Clayton, Victoria, Australia, or the Secretary-General, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1. Technical inquiries to Professor O. E. Potter, Chairman, Department of Chemical Engineering of the University. Closing date: September 15, 1967. The University reserves the right to make no appointment, or to appoint by invitation. (418)

**UNIVERSITY OF SHEFFIELD**DEPARTMENT OF METALLURGY  
APPOINTMENT OF AN ELECTRON  
MICROSCOPIST (EXPERIMENTAL  
OFFICER)

Applications are invited for the post of Electron Microscopist (Experimental Officer) in the Department of Metallurgy. Candidates must possess a university degree or equivalent and must have experience in the maintenance and use of modern electron microscopes. The successful candidate will have a special responsibility for two high resolution electron microscopes; he will be expected to develop improved techniques for the study of complex metallic structures and to apply them to researches in progress in the Department. Initial salary according to qualifications and experience in the range: Experimental Officer, £915 to £1,415; Senior Experimental Officer, £1,470 to £2,410, with F.S.S.U. provision.

Further particulars from the Registrar, to whom applications (4 copies) should be sent not later than August 31, 1967. (373)

**UNIVERSITY OF LEEDS**DEPARTMENT OF MECHANICAL  
ENGINEERING  
COMPUTING ASSISTANT

Applications are invited for the post of Computing Assistant in the Department of Mechanical Engineering. The successful applicant will be required to assist members of staff and postgraduate students in the Department of Mechanical Engineering in the development and operation of ALGOL programmes for the University KDF9 Computer. Full participation in a wide range of research projects will be encouraged. Previous experience of programming and/or numerical analysis applicable to engineering would be an advantage. Salary according to age, qualifications and experience, in the range of £915 to £2,050 per annum.

Applications (three copies), stating age, qualifications and experience, and naming three referees, should reach the Registrar and Secretary, The University, Leeds, 2 (from whom further particulars may be obtained), not later than August 12, 1967. (372)

**WATER RESOURCES BOARD****Freshwater Biological Association  
UNIVERSITY OF LANCASTER**

The Water Resources Board in conjunction with the Freshwater Biological Association and the University of Lancaster invite applications from those who are appropriately qualified for the following appointments:

**1. A PRINCIPAL SCIENTIFIC OFFICER  
(£2,250 to £3,107)****or SENIOR SCIENTIFIC OFFICER**

**(£1,744 to £2,155)** who would be responsible for the biological aspects of the Morecambe Bay Barrage Feasibility Study. He would be stationed at the Freshwater Biological Association's Windermere laboratory. His work would be complementary to that of the consulting engineers, whose study will take about three years and is estimated to cost about £500,000. He would investigate and advise on the effects of proposed engineering works on the ecology of the area with particular reference to:

- (a) the effects on migratory fish in the rivers flowing into the Bay;
- (b) the changes in the animal and plant communities likely to result from the conversion of a salt-water estuary into a freshwater lake or lagoons, and any practical problems to which these changes might give rise;
- (c) the existing sea water fisheries.

**2. A RESEARCH STUDENT**

to work with the above who would be based, if eligible, and may register for a higher degree at Lancaster University.

The appointments to these two posts will be considered together. Thus if a zoologist who had experience in the ecology of fresh or estuarial waters, or an interest in migratory fish, were appointed to post no. 1, the junior post might be held by an honours graduate wishing to pursue research for a higher degree on the higher plant or algal ecology of the area. Alternatively, a primarily botanical ecologist with sufficient qualifications and a suitable breadth of experience would be considered for post no. 1; such a person might be assisted by a research student working on a particular problem within the wider field, for example, the effects on migratory fish. A certain amount of travelling will probably be involved.

The appointments will commence from September or October 1967. The higher post will be for three years and the research studentship will be tenable for two to three years.

Applications (three copies) together with the names of two referees, should be sent by Saturday, August 19, 1967, for post no. 1 to The Director, Freshwater Biological Association, The Ferry House, Ambleside, Westmorland, and for post no. 2, to Biology Department, University of Lancaster, St. Leonard's House, Lancaster.

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**UNIVERSITY OF LONDON**CHAIR OF NUCLEAR ENGINEERING AT  
QUEEN MARY COLLEGE

The Senate invite applications for the Chair of Nuclear Engineering tenable at Queen Mary College (salary not less than £3,570 a year plus £100 London Allowance).

Applications (10 copies) must be received not later than September 7, 1967, by the Academic Registrar, University of London, Senate House, W.C.1, from whom further particulars may be obtained. (412)

**ST. BARTHOLOMEW'S HOSPITAL  
LONDON, E.C.1**

**SENIOR RESEARCH TECHNICIAN** required for interesting and varied work on immunity in reticuloses and other malignant diseases. Associates of the Institute of Medical Laboratory Technology with a diploma in haematology would find ample opportunity and material for a thesis. Whitley Council Salary Scales.

Write, naming two referees, to the Clerk to the Governors, quoting Project No. 863, Ref. ASC/1089. (406)



## TECHNICIANS

"Shell" Research Limited have vacancies for **TECHNICIANS** in the Product Evaluation and Plant Pathology Divisions of their Woodstock Agricultural Research Centre, Sittingbourne, Kent.

Candidates should have reached H.N.C. level in a biological subject. An additional asset would be a qualification in the field or agriculture, while some experience of Parasitology, Malacology, or of Pesticides generally would also be advantageous. Candidates should be prepared to carry out both laboratory and field work and, occasionally, to travel in the U.K. or abroad.

Fringe benefits include an excellent pension scheme, staff restaurant, and a sports/social club.

*Apply in writing, giving brief details of age, qualifications, and experience, to:*



**Personnel Division,  
"Shell" Research Limited,  
Woodstock Agricultural Research Centre,  
SITTINGBOURNE, Kent.**

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## INTERNATIONAL FLAME RESEARCH FOUNDATION

### RESEARCH INVESTIGATORS

Applications are invited for the posts of Research Investigators to work at the Central Research Station of the Foundation at Ijmuiden in Holland. The successful candidates who will preferably have a Ph.D. Degree in Physics or Engineering, are expected to join a small team of postdoctoral investigators to work on problems of combustion physics, in particular aerodynamics and heat transfer aspects of turbulent flames. The work of the Foundation involves frequent contacts on an international level with research scientists working in similar fields, and the Foundation encourages publication of high quality original work.

Starting Salary: £1,600 per annum. There are additional emoluments payable, including pension contribution, holiday bonus, costs of removal, etc. There is assistance with housing.

(390)

Applicants should write in the first place to:  
**Professor J. M. Beer,  
Department of Fuel Technology and  
Chemical Engineering,  
University of Sheffield,  
Mappin Street, Sheffield 1.**

## NORTHERN POLYTECHNIC HOLLOWAY, LONDON, N.7

Applications are invited for appointments as **RESEARCH ASSISTANTS** in **MATHEMATICS**. Candidates should have a First or Second Class Honours Degree in Mathematics or Statistics. The successful applicants will be expected to read for an M.Phil. or Ph.D. Degree in the field of either Statistics or Hydrodynamics. They will also be required to assist with the teaching in the Department for six hours per week.

**Salary scale, £810 by £30 to £870.**

Apply to the Head of the Department of Mathematics.

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## UNIVERSITY OF SYDNEY

### LECTURERS/SENIOR LECTURERS IN PURE MATHEMATICS

Applications are invited for the above-mentioned positions. The teaching programme of the Department of Mathematics (Pure Mathematics) covers undergraduate pass and honours courses followed by courses leading to the M.Sc. and supervised research for the Ph.D. Currently research is in progress in group theory, representation theory, analysis and theory of numbers. For two of the vacancies preference will be given to applicants with research experience in topology or the use of computers in pure mathematics. The Department has ready access to the computers in the University's Basser Computing Laboratory. Salary for a Lecturer is within the range \$A4,800 by \$A220 to \$A6,340 per annum, and for a Senior Lecturer within the range \$A6,600 by \$A200 to \$A7,600 per annum. Academic salaries are at present under review.

Information concerning superannuation, housing scheme, sabbatical leave, etc., and method of application is obtainable from the Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1. Applications close in Australia and London on August 31, 1967. (416)

## ROYAL COLLEGE OF ART, S.W.7

### EXPERIMENTAL CARTOGRAPHY PROJECT

Applications are invited for the following two full-time posts as Research Fellows to form part of a small team concerned with the second stage of this project financed by the Natural Environment Research Council.

#### COMPUTER SCIENTIST PERCEPTION PSYCHOLOGIST

Salary within University lecturers' scale £1,530 to £2,690 per annum according to age and experience.

Further details and application form can be obtained from the Registrar, Royal College of Art, Kensington Gore, London, S.W.7. (420)

## UNIVERSITY OF QUEENSLAND

### SENIOR LECTURER IN VETERINARY MEDICINE

The University invites applications for the position of Senior Lecturer in Veterinary Medicine in the Department of Veterinary Clinical Studies. Applicants should hold a degree in veterinary science registrable by the Veterinary Surgeons' Board of Queensland and must have had experience in dairy cattle practice and/or swine practice. Higher degrees, publications, and teaching and administrative experience will be taken into account. The appointee will give courses of lectures on the diseases of cattle and of swine to final year students, will participate in dairy cattle and swine practice and in research studies on herd health. The salary range for a Senior Lecturer is \$A6,400 by \$A200 (6) to \$A7,600 per annum; however, academic salaries are presently under review. The University provides Superannuation similar to F.S.S.U., Housing Assistance, Study Leave and Travel Grants.

Additional information and application forms will be supplied upon request to the Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1. Applications close in London and Brisbane on August 25, 1967. (415)

## UNIVERSITY OF BELFAST

### EXPERIMENTAL OFFICER IN THE DEPARTMENT OF BOTANY

The Senate of The Queen's University of Belfast invites applications for the post of Experimental Officer. The salary scale is £1,000, rising by £50 per annum for 2 years to £1,100 by £55 per annum for 4 years to £1,320, and by £50 per annum for 2 years to £1,420. Initial placing on this scale will depend on qualifications and experience. The duties will include assistance in the teaching of techniques in microscopy, including electron microscopy and in research.

Letters of application (one copy), giving the names of two referees, should reach the Secretary, Academic Council, by August 18, 1967, from whom further details may be obtained. (426)

**UNIVERSITY OF WAIKATO**  
HAMILTON, NEW ZEALAND  
SENIOR LECTURESHIPS AND  
LECTURESHIPS

The University of Waikato invites applications for positions in the subjects listed below. Appointments may be made at the grade of Senior Lecturer, Lecturer, or Junior Lecturer.

GEOGRAPHY  
PSYCHOLOGY/SOCIOLOGY  
PURE MATHEMATICS

The salary scales are: Senior Lecturers, \$NZ4,600 to \$NZ5,400; Lecturers, \$NZ3,000 to \$NZ4,400; Junior Lecturers, \$NZ2,400 to \$NZ2,800. (\$NZ2=£1.) An applicant who is exceptionally well qualified in economics, geography, philosophy, psychology, sociology, or pure mathematics may be considered for an appointment as Reader at a salary between \$NZ5,800 and \$NZ6,600.

The University of Waikato began last year teaching full-time co-ordinated courses of study in the Schools of Humanities, Social Sciences and Education. It occupies a site of about 125 acres on the outskirts of Hamilton. The academic organization is based on schools of study rather than on faculties and departments. The development of postgraduate work and research is encouraged and conditions for study leave are favourable.

Conditions of appointment are available from the Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1. Applications close in New Zealand and London on August 28, 1967.

(424)

**ENGINEER or PHYSICIST**  
for

Medical Research Council

Additional members of scientific and technical staff are required for work on advanced electro-optical "input" devices. Salary between £1200 to £2200 depending on qualifications and experience.

For further information please write or telephone:

The Director,  
Clinical Effects of Radiation Research Unit,  
c/o 107 Sydney Street,  
London, S.W.3.

(380)

**UNIVERSITY OF PAPUA AND  
NEW GUINEA**

Staff will be required in 1968 for new posts at the levels of Tutor/Senior Tutor/Lecturer/Senior Lecturer in:

BIOLOGY, GEOGRAPHY, HISTORY OF  
SCIENCE AND TECHNOLOGY,  
MATHEMATICS

The university was established in 1965 and the first matriculated students started work this year. Appointees will have the opportunity to contribute to the development of courses appropriate to a new university in an emergent community; there will be opportunities for research and class groups will be small.

Salaries: Tutor, \$A3,820 to \$A4,300 per annum; Senior Tutor, \$A4,700 to \$A4,950 per annum; Lecturer, \$A5,300 to \$A6,840 per annum; Senior Lecturer, \$A7,100 to \$A8,100 per annum; plus \$A360 per annum for married appointees. F.S.S.U. type superannuation is available and accommodation will be provided at reasonable rental. Taxation rates are very substantially below those operating in Australia and the United Kingdom. There are special financial provisions for children attending secondary schools outside the territory.

Further details, including conditions of employment, travel and removal expenses provided, a general policy, statement on the purposes of the University, and information on the method of application may be obtained from the Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1, or from the Registrar of the University, P.O. Box 1144, Boroko, T.P.N.G., Closing date for applications: August 15, 1967.

(413)

**SIMON FRASER UNIVERSITY**  
**Research Opportunities**  
**in Chemistry**

The Department of Chemistry of this rapidly growing university, which is situated on the outskirts of the city of Vancouver, has openings for suitably qualified students in the graduate programme leading to the degrees of M.Sc. and Ph.D. Qualified students will be expected to have an honours degree in Chemistry, or A.R.I.C. Financial assistance in the form of teaching assistantships and research assistantships, to the value of \$3,100 per annum in the first year, will be available to suitable candidates. The trimester system in operation at Simon Fraser University allows students to enter the graduate programme in September or January.

*Interested persons should write in the first place to*

**The Chairman, Graduate Admissions Committee,  
Department of Chemistry,  
Simon Fraser University,  
Burnaby 2, B.C.,  
CANADA.**

(289)



**MICROBIOLOGIST**

with Ph.D. or equivalent, required for Senior Graduate Staff of the Group Research Laboratories. Candidates should have research experience in microbiological aspects of food technology, or of continuous culture and growth kinetics of yeasts and moulds and bacteria.

The successful candidate will be concerned with bacteriological research in food processes and will be expected to maintain contact with other research centres.

Salary and conditions are those of a progressive unit.

*Apply in first instance to:*

**Group Technologist,  
BOVRIL GROUP RESEARCH LABORATORIES,  
148/166 Old Street, London, E.C.1.**

(367)

**UNIVERSITY OF LIVERPOOL**

**BIOCHEMIST OR VETERINARY  
PATHOLOGIST**

Applications are invited for the post of Research Assistant in the Immunology Cancer Research Unit. Postgraduate research experience and/or higher degree desirable, but not essential. Experience in immunological techniques, protein and enzyme chemistry and/or tumour transplantation in pure strain animals an advantage, but not essential.

The successful candidate will be a member of a team researching into fundamental immunological aspects of cancer in animals and man. Excellent research opportunities in well equipped new laboratories. Salary range £1,100 to £1,500 per annum (plus F.S.S.U.) according to qualifications and experience.

Applications, with curriculum vitae, and names of three referees, to the Registrar, University of Liverpool, Liverpool 3. Please quote Ref.: RV/100/N.

(405)

**UNIVERSITY OF EXETER**  
**DEPARTMENT OF CHEMISTRY**

Applications are invited for a Research Assistantship in Chemistry, tenable for one year from October 1, 1967, for work on the synthesis of a naturally occurring polypeptide under the direction of Professor H. N. Rydon. Applicants should have a Ph.D. degree or equivalent and experience in peptide synthesis. The salary will be £1,200 per annum with F.S.S.U. benefits.

Applications (three copies), including the names of two referees, should reach the Secretary of the University, Northcote House, The Queen's Drive, Exeter, by August 14, 1967.

(384)

**UNIVERSITY OF BRISTOL**

Applications are invited for the post of LECTURER or ASSISTANT LECTURER in APPLIED MATHEMATICS within the Department of Mathematics tenable from October 1, 1967, or as soon after this date as possible. Salary scales: Lecturer, £1,470 to £2,630; Assistant Lecturer, £1,105 to £1,340 per annum with superannuation benefits.

Further particulars may be obtained from the Registrar, the University, Senate House, Bristol, 2, to whom applications should be sent by August 31, 1967.

(316)

## NEW DRUG RESEARCH

Have you had Experience  
or are you interested in any  
of these topics?

### SYNTHESIS

*Amino Acids  
Peptides  
Heterocyclics  
Sulphur Chemistry*

### ANALYSIS

*Analysis of Drugs  
Autoanalytical Techniques  
Elemental Analysis  
Chromatographic Techniques*

### BIOLOGICAL EVALUATION

*Bioassay Techniques  
Toxicology and Pharmacology of Drugs  
Histology*

**OUR AIMS:** We are interested in synthesising new compounds that might be usefully employed in the control and correction of metabolic disorders.

**OUR NEEDS:** An expansion of our research activities has created several vacancies for *organic chemists and analysts, biologists, physiologists, and pharmacologists*, within the new research laboratories of Horlicks Pharmaceuticals Limited.

**OUR LOCATION:** The laboratories are situated in the delightful countryside of rural Berkshire adjacent to Windsor Great Park. They offer facilities and a working environment that are compatible with creative research work.

**WE WELCOME:** Application for these posts both from graduates with 1-2 years' experience and those recently qualified with a first or second-class Honours. Applicants are invited to write to:

**The Personnel Manager,  
Horlicks Pharmaceuticals Limited,  
Orchard Lea, Winkfield, Windsor,  
Berkshire.  
Telephone Number: Winkfield Row 2963**

(301)

## Algologist/ Plant Physiologist

to study primary production and factors limiting the production of phyto plankton at Loch Leven as part of an International Biological Programme study.

**Qualifications:** a first class or good second class honours degree in Botany with special interest in primary production or plant physiology. Candidates with a degree in biochemistry or an allied subject with interest in or knowledge of fresh water algae will also be considered.

**Location:** the successful applicant will be based at Edinburgh but may spend part of his time at the Freshwater Fisheries Laboratory, Pitlochry.

**Starting salary:** according to qualifications £926 to £1,744 rising to £2,155.

Further information and application forms, to be returned by August 18, may be obtained from the Nature Conservancy, 12 Hope Terrace, Edinburgh 9.

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### NEW ZEALAND

#### MARINE DEPARTMENT

Applications are invited for the under-mentioned vacancies:

Vacancy B 13/13/17/2512

#### SCIENTIFIC OFFICERS

Fisheries Research Division

Fisheries Research Division of the Marine Department require two Scientific Officers for research on Mollusca and Demersal Fish.

**Salary:** Appointments will be made in the range \$NZ2,635 (for Honours \$NZ2,730) to \$NZ3,140 for a new qualified Ph.D. and up to \$NZ5,330 for suitably experienced scientists.

**Qualifications desired:** Applicants should have a degree with 1 or 2A honours in Zoology.

**Passages:** Fares for appointee and his wife and family, if married, will be paid.

**Incidental expenses:** Up to \$NZ70 for a single man and \$NZ200 for a married man can be claimed to cover the cost of taking personal effects to New Zealand.

Application forms and general information are available from the High Commissioner for New Zealand, New Zealand House, Haymarket, London, S.W.1, to whom applications will close on August 18, 1967.

Please quote reference B 13/13/17/2512 when inquiring. (410)

### UNIVERSITY OF BRISTOL

#### STATISTICIAN

Honours graduate in Mathematics, with qualifications in Statistics and an interest in biological problems, is required by the Department of Agriculture and Horticulture, Long Ashton Research Station, for the post of Head of the Statistics Section. Appointment as Scientific Officer or Senior Scientific Officer, according to qualifications and experience.

Further particulars from: The Registrar, Senate House, Bristol 2, to whom applications should be sent by the end of August. (376)

### HACKNEY & QUEEN ELIZABETH GROUP HOSPITAL MANAGEMENT

#### COMMITTEE

QUEEN ELIZABETH HOSPITAL  
FOR CHILDREN,

HACKNEY ROAD, LONDON, E.2  
(INSTITUTE OF CHILD HEALTH)

Applications are invited from graduates with good honours degree in chemistry or equivalent qualifications to assist in research on mucopolysaccharides in disease. The post is for three years and suitable for those wishing to study for a higher degree. Salary according to experience and qualifications on the scale to £963 to £1,658 per annum plus London Weighting allowance.

Apply immediately for application form obtainable from Group Secretary, Administrative Offices, Hackney Hospital, London, E.9. (427)

### MACAULAY INSTITUTE FOR SOIL RESEARCH

Applications are invited from graduates with first- or upper second-class honours in mathematics with statistics, or a Diploma in Statistics, for a vacancy in the Statistics Section. The duties are mainly concerned with the design and analysis of field and laboratory experiments, providing a statistical and data processing service. There is access to electronic computers. The appointment will be made in the Scientific Officer Grade, starting salary within the range £926 to £1,574 per annum, according to age, qualifications and experience. Superannuation under F.S.S.U.

Forms of application may be obtained from the Secretary, The Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen, AB9 2QJ, to whom they should be returned before August 28, 1967. (425)

## GROWTH LIKE TOPSY'S

THE Medical Research Council is accustomed to ask a lot of its wellwishers. Its annual report is traditionally one of the most laconic of public documents, more an advertisement of recent achievements than an explanation of how and why money has been spent in particular ways. The latest instalment (see page 570) is no exception to this rule, even though on this occasion the council has made a passable attempt to explain how certain items in its budget have been increased as a result of circumstances outside its own control. But nothing in the 317 pages of text which make up the annual report for the year ending last March could be mistaken for a proof that the council is possessed of a coherent strategy for medical research. No doubt there are many among the members of the council who will point out that it has managed well enough without a strategy for 54 years, and that it need not at this late stage embrace the doctrines of conscious decision making which younger organizations find helpful and even essential. But the truth is quite the opposite. If the council carries on like this, it could find itself in serious trouble before many years have passed.

That said, it is only fair to recognize that the council's own smugness probably derives from its quite extraordinary record of successes in the past 20 years. It has quite rigorously backed good people, not theoretical schemes. One result is that it now has on its payroll a distinguished company of people whose output of original research is an important part of all the science practised in Britain. And it will be a long time before people forget that it was the council which had the foresight to provide funds and an assured future for what is now the Laboratory of Molecular Biology at a time when the University of Cambridge was discredibly indifferent to an urgent need and a splendid opportunity. But this is only one example of what the council has done to provide a setting in which talented people can function efficiently. The council, devoted to the principle of direct labour as the saying goes, has more than 3,000 people on its books of whom 900 are scientists and most of whom are employed in the council's own laboratories and in the 77 research units scattered through the country. For all the scale of this operation, however, the council has hardly ever put a foot wrong—the proposed Clinical Research Centre may yet prove one of the exceptions. So who can blame the council if at times it seems like a kleptomaniac baseball promoter anxious to see that every player of distinction is properly signed up? Who can complain at the successful pursuit of excellence?

The first thing to be said is that the criteria by which the council determines its own success may not be the

ones it should use. The policy of supporting academic research by the direct employment of research units which often work separately from the universities, and which are sometimes not even loosely connected with them, is at least open to question. And since the most immediate and tangible benefit of academic research is usually the benefit it provides for university teaching, there is something in the view that much of what the council has spent on its research units could have brought greater if less spectacular benefits if it had been spent on the direct support of university research. As things are, there is some evidence that the council's policy of supporting research units so handsomely draws talented academics away from teaching. Nobody can be cheerful about the gibe that Dr F. H. C. Crick, an MRC employee among other things, lectures more often in Cambridge, Massachusetts, than in Cambridge, England, even if the University of Cambridge is more to blame for that than the MRC. But then there are other problems or potential problems as well. Thus MRC staffs do not have the same freedom as their academic colleagues. Although directors of research units are usually far-sighted enough to interpret the regulations liberally, their restriction is open to abuse.

For the council as a whole, however, the most serious problem occasioned by the existence of the research units is that the budget as a whole is made inflexible. After all, setting up a unit implies a long term commitment extending beyond the usual span of a research grant. It is no wonder that the council this year finds itself confessing that of its total expenditure of more than £12 million last year, forward commitments were such that only £900,000 could be reckoned "freely deployable". It is only natural to wonder what plight the council would have been in if the Council for Scientific Policy had been unable to win a handsome increase of expenditure for the research councils in the financial year now ended. Would the council have gone round sacking people, or would it have had to put up the shutters in the informal financing of research? And what will happen if the Treasury turns nasty this year, or next? Although there is nothing in the annual report to explain what has been happening, the council's expenditure on informal research grants in university departments is fortunately growing more quickly than any other item in its budget, but it would be good to know whether the council is really guarding against what seems to be an obvious danger.

Because these questions about the council's conduct of its operations suggest themselves naturally, it does not follow that there are no answers to be made. Indeed, it is conceivable that everything has been thought out properly in advance. At this stage the



only complaint that can be made is that the council keeps its reasoning to itself. The most immediate hazard is that in the process wrongheaded policies may go unrecognized, even by the council. But there is possibly a greater cause of loss than that. Because the council occupies a pre-eminent position in the pattern of research in Britain, it is natural to look to it not merely for the conduct of research but for a lead in the evolution of research policy. If the council is ever to shake off its reputation for gifted—or perhaps just lucky—empiricism, it will have to cultivate the skill of stating its objectives publicly.

## FUSION BY HALVES

THE decision that the scale of operations at the Culham Laboratory should be cut in half is painful for everybody concerned. Even Mr Wedgwood Benn had no relish for the task of cutting down the budget, which is plain enough from what he had to say in the House of Commons on July 26. But the sense of regret which may haunt the Minister of Technology will be nothing compared with the regret at the laboratory itself. People will start looking around for jobs elsewhere and will quite understandably ask why it is they who should be the victims of such cruel circumstances.

Unfortunately, of course, there is very little doubt that the decision now taken has been necessary. For several years now the work at Culham, as at the other thermonuclear laboratories scattered around the world, has been directed at a receding target. It is a long time since people used to make calculations about the detailed operating characteristics of thermonuclear power stations—the optimum output of electricity, for example, or the cost of the concrete shielding necessary to keep the installations safe. Instead, thermonuclear research has become a synonym for the careful study of instabilities in electrical discharges through highly ionized plasmas. Those engaged on it have been cheered up when from time to time it has become clear that their work could be useful in other fields—in astrophysics, for example. Although it would be foolish now to say that the commercial exploitation of thermonuclear power is not feasible—indeed, the opposite is probably the truth—those who work in fusion have been forced to take the long view. This is one reason why they have been quick to point out that the experiments with the large pieces of equipment which they use may bring much more immediate benefits in electrical engineering, for thermonuclear equipment is a notorious consumer of high density current. The trouble here is that it is not possible, in Britain at least, to justify the cost of research programmes by the putative value of the spin-off they may provide. In the circumstances there is no doubt that Culham has grown too big.

But if not fusion why not something else? In other words, why shouldn't Culham take a leaf out of the Harwell book and attempt to diversify its activities?

Reading between the lines of Mr Benn's statement in the House of Commons, the ministry is not keen to encourage this possibility. That, too, is also right. For one thing, there is no assurance that Culham would be any more successful at finding self-financing work to do than the other public laboratories now touting for industrial contracts. Evidently there are limits to the extent to which industrial research can be subcontracted. But it is also important to acknowledge that it would be intolerable if the long-term pattern of applied research in Britain were determined by the historical accidents of where laboratories were established in the forties and fifties. Indeed, long-term economic interests may even require that laboratories should be regarded as expendable. Certainly they are much less durable capital assets than, say, steel works. The cost of building them and equipping them is often less than the cost of running them for five or ten years. In other words, if the present scale of operations at Culham is really too lavish, there is no point in supporting it at an artificial level. To let things run down to half over five years seems sensible in the circumstances.

Whether it is wise to let Culham continue as a dependant of the Atomic Energy Authority is another matter. In previous years there seems no doubt that Culham has profited enormously from rubbing shoulders with those engaged on reactor design at Harwell and elsewhere. For some years to come, however, there will be much less profit to be derived that way. A question which needs asking is what setting will best allow a £2 million a year laboratory like Culham to keep going productively. There is a strong case for thinking that the laboratory would be better off under the Science Research Council than as a continuing dependant of the AEA. (Another less satisfactory possibility would be an autonomous laboratory within the Ministry of Technology.) For one thing, there would then be a chance that it could flourish as the magnet laboratory on which the SRC seems to have set its heart. It might also then be able to contribute more effectively than at present to the development of university interests in plasma physics. As things are, the universities do not participate in the design of the biggest pieces of equipment. And in the long run, of course, it would be possible to arrange that the scale of expenditure at Culham could be determined by a sensible matching against other demands for funds for long-term research.

There remains the question of whether the experience with the Culham laboratory will persuade scientists that working for the Government is as hazardous as working for industry. However much advantage may be taken of what is called natural wastage at the laboratory, the chances are high that many people will find themselves moving to other places against their wishes, possibly because opportunities of promotion are not easily offered. It would be sensible if the Government would recognize that this is as much a social problem as the difficulty caused in coal fields throughout the country by the running down of fuel production. In other words, the Government has a

duty to ensure that people whose jobs come to an end or whose prospects are unreasonably dimmed must be given some kind of help to find other outlets for their energies. No doubt there will be some talk of compensation payments. No doubt attempts will be made to fit people in elsewhere within government establishments. But it would be better in the long run, and a good precedent as well, if the Government were to recognize that what the circumstances require is a thorough-going programme of retraining and resettlement backed up by generous schemes for helping people move from place to place. One of the ironies of the two decades since the war is the growth of the assumption that scientists working in some narrow field cannot be expected to transfer their interests to some other. It seems to have been forgotten that during the Second World War every other biologist was trained to be a radar engineer almost overnight. If the country is really short of talent, and if the Ministry of Technology is anxious somehow to increase the effectiveness of what there is, it should put its energies into making full use of the people who will no longer be fully occupied at Culham.

## SPEAKING FOR UNIVERSITIES

THERE is something entirely appropriate about the election of the new chairman of the Committee of Vice-Chancellors and, in the same week, the announcement that the Department of Education and Science will, after all, assume direct responsibility for seeing that universities spend public money in a manner which is seemly but as yet undefined (see page 576). The universities have never been as much in need of somebody who can act on behalf of them all. With one thing and another it has been a bad year for the universities. For one thing, it is becoming ominously to seem that the first bite at the Robbins cherry may also be the last. Gone already is the heady talk of how the university system might grow and grow so as to provide a substantial part of the adult education on which such hopes as may survive for British economic prosperity must rest. The gloom which has been accentuated by the tactless handling of the equipment grants by the University Grants Committee may lift when the funds for the five years from 1968 are announced later in the year, but nobody can be cheerful at this stage. And then there is the galling incident of the Government's decision to increase fees for university students from overseas without properly consulting the universities. Yet the universities themselves have done very little to face up to the problems which confront them. Such talk as there may have been of more efficient operation seems to have been largely ineffectual. The Standing Conference on University Entrance will probably come out with more liberal rules for admission in the next few months, but the universities as a whole have done

little more than Oxford after Franks to protect themselves from criticism.

This is why the new chairman of the Committee of Vice-Chancellors can play a decisive role. It has been plain for the past five years that the UGC is less and less effective as a buffer between the universities and the Government. That this should have happened is inevitable. The growth of the scale on which the universities are supported by public funds is less important than the now open abandonment of the whole convention that universities should be protected from the knowledge that they are living on taxpayers' money. Indeed, it is no longer possible for a government to allow that universities acting for themselves should make important decisions affecting public policy. And it is, of course, entirely sensible that the Government should have the last word about questions such as the proportion of the British GNP to be consumed by higher education. A good many in the universities will agree with that. Yet the universities must be able to retain freedom over academic policy and self-respect as well. Even if the UGC can keep for itself something more than the role of being Sir Herbert Andrew's auditing department, there is now no chance that a body of civil servants like the UGC can set itself up as a means of keeping an effective dialogue with the Government on university policy and, when necessary, as over fees for overseas students, to defy the Government. For a long time it has been obvious that only the Committee of Vice-Chancellors could fill that role.

But what needs to be done? The most immediate task which Dr Christopherson should undertake is to let it be known that the Committee of Vice-Chancellors is not some kind of quasi-judicial body, a pillar of the establishment, but an institution which must be reckoned a political force. For too long the committee has sought to win respect by making itself almost inaudible on matters of public policy. The trouble, of course, is that if it is to carry university opinion, the committee must function publicly, making statements, publishing newsletters and sometimes even taking steps to improve its standing with the non-academic world. If on some occasion it finds that the Government has not consulted it on important issues of principle, it should feel free and strong enough to take unilateral action on its own account. (A public appeal for funds to make up overseas fees would have been an interesting exercise.) And then, of course, the committee must somehow capture the respect and the loyalty of the separate universities, so that it can act quickly on their collective behalf. This in turn will imply that universities must delegate some of their freedom of action to the committee and, lower down the hierarchy, that university departments must give up some of their present autonomy for the greater good. How far things can go in these directions, only time will tell. But clearly one of the most obvious needs is that the chairman of the committee should command respect. Fortunately, Dr Christopherson is the sort of man who will appreciate what needs to be done.

## NEWS AND VIEWS

### Cuts at Culham

By deciding to reduce by half the expenditure on thermonuclear fusion research at Culham Laboratory, the Atomic Energy Authority has risked offending its friends without much pleasing its enemies. The work of the laboratory has been devoted to the production of a commercial reactor using thermonuclear fusion reactions, instead of the fission reactions used in current reactors. Although a committee set up by the authority is believed to have recommended that the laboratory be shut altogether, the authority finally decided that expenditure should be reduced by 10 per cent annually for five years, at the end of which time there should be a review of the situation. Although the problems of plasma containment are particularly elegant and interesting, nobody would deny that they are difficult in the extreme, and the axe has been hanging over Culham for several months.

The cost of the laboratory last year was about £4 million. It has been higher than this; in 1964-65 it cost £5 million to run. It is not a particularly old laboratory—construction did not begin until 1960. The staff of 800 includes 190 qualified people and a number of visitors from abroad on short or long term contracts. Although the great majority of the effort has been directed at controlling artificial plasmas, the laboratory has also allowed itself to be involved in the study of natural plasmas by space soundings. Recently it won the contract to supply the telescope for the ESRO astronomical satellite. Despite doubts about the satellite project, which will not get the final go-ahead for some months, there does not seem much room for economy in this side of the work. Since only a quarter of the budget this year is intended for capital expenditure, most of the cuts will have to be made on current expenditure, which is likely to mean reductions in staff. Despite this, the authority hopes that there will be no formal redundancies and that the staff can be reduced by natural wastage; there is also an unexplained optimism that the wastage will not be directed overseas.

At Culham itself the staff are naturally upset by the decision, although it was not unexpected. Much of the rancour is directed at the cloak of secrecy with which the AEA has surrounded the decision, and the fact that only two years after opening a laboratory which cost £6 million to build, the AEA can close half of it without even explaining publicly the reasons for the decision. Mr Benn's explanations in the House of Commons cut no ice at Culham, and his rejection of suggestions that the programme at Culham was inflated by the staff there have merely served to confirm the feeling that the authority alone is responsible for the mess. There is also doubt about what the half of the laboratory that will be left will be used for; above all the staff at Culham feel the need for something challenging to do. Unless the authority or the minister can produce ideas soon, the argument runs, the remarkable esprit de corps which has been a feature of Culham may be lost.

### New Broom for ESRO

It can be stated with confidence that the election of a theoretician, Professor Hermann Bondi, as director-general of ESRO does not imply a decision to abandon experimental work. This would be to belittle both ESRO's power of survival and Professor Bondi's experience of the hardware of space research. Professor Bondi, 48 and a familiar figure in astronomy since he graduated from Trinity College, Cambridge, in 1940, is Professor of Mathematics at King's College, London. He was elected director-general at a meeting of the council of ESRO on July 27, and succeeds Professor Pierre Auger, who has been director-general since ESRO started. The appointment is for three years, and after an initial period when Professor Bondi will be arranging for his commitments at King's College to be taken over, he will be working full-time for ESRO.

ESRO has some tricky decisions to make during the next year—whether, for instance, to remain a coherent entity or to merge with ELDO. The ambitious astronomical satellite which ESRO hopes to launch still awaits final approval, although contracts have been provisionally placed, one with the Culham laboratory of the AEA. Professor Bondi is familiar with the mechanism of decision-making—he is chairman of the Astronomy Policy and Grants Committee of the SRC, and a member of the Astronomy, Space and Radio Board. He is also chairman of the British National Committee for Astronomy. ESRO is doubtless already bracing itself for the shock of his arrival—cynics have been heard to comment that whether or not Professor Bondi needs ESRO, ESRO certainly needs him.

### Effortless Progress

THE Medical Research Council has survived another unruffled year, to judge by its annual report (HMSO, £1 6s. 6d.). Of all the research councils, the MRC is surely the most machine-like. This year even a new constitution, which provides for an increase in membership of the council from 12 to 16, and for the Secretary of State for Education and Science to take over the responsibilities of the Committee of Privy Council for Medical Research, has failed to put a spanner in the works.

For the past few years the MRC has been batting on a gentle wicket. Grants have been steadily rising at a rate of around 20 per cent per year (11 per cent in real terms, the council points out), and last year the council spent £11.8 million, more than double the figure for 1962-63. The council now supports no less than 77 units, the National Institute for Medical Research at Mill Hill, and research at universities and hospitals. Short term grants, though, are taking an increasing share of the budget—17 per cent, or £2.2 million this year.

The report for 1966-67 includes a 60 page section describing progress in some of the fields of research which the council supports. These well written and useful reviews include descriptions of the work which has shown that men with two Y chromosomes may be predisposed to crime. Although XYY males are very rare indeed in the population at large, a survey in a special security hospital has shown 9 such men in a sample of 315 (Jacobs *et al.*, *Nature*, **208**, 1351; 1965).

Other reviews cover the scientific aspects of ageing, the control of protein synthesis in living cells, and pulmonary hypersensitivity caused by organic dusts. Two main types of allergic reaction have been distinguished, called Type I and Type III, and they may be present in lung disease either separately or together. Type I allergy is induced by exposure to everyday dust, and as many as 10 per cent of the population are at risk, but it leaves no tissue damage. Type III is induced by constant exposure to dusts, and the reaction, which involves tissue damage, develops more slowly. Farmers inhaling the dust from damp and mouldy hay, pigeon fanciers and granary and sugarcane workers are all found to suffer from this disease. Tests now make it possible to diagnose these different diseases, and farmer's lung is already registered as an industrial disease.

## Science in Belgium

THE Belgian National Council for Scientific Policy has just published its report for 1967. It fears that the fact that Belgium lags behind other European states in science and technology may be prejudicial to her economic integration into Europe. Belgium cannot reasonably expect to compare in scientific output with the greater European countries; this would require a disproportionate percentage of the gross national product to be spent on research. It is the aim of the council to bring Belgium up to the level of the smaller European countries, such as Sweden or Holland. To this end, the council recommends that by 1972 expenditure on science should have risen to 2.15 per cent of the GNP. Government spending must rise from 8.2 million francs in 1966 to 17.8 million in 1972, when it is expected to comprise 3.42 per cent of the budget.

The council has appointed commissions to prepare the budgets for research establishments and deal with the expansion of the university system, and to study the means by which research programmes are directed and financed. A good deal of administrative reorganization will be necessary, especially that concerned with technological as opposed to basic research. A greater knowledge of the research facilities and trends in Belgian industry is needed, and the council has already begun collecting this kind of information.

An interesting proposal is that the council should recommend the salaries of research workers attached to projects subsidized by the State.

## New Man for Universities

THE fuss about university accountability has already revealed one unexpected fact. The Committee of Vice-Chancellors and Principals, a conspicuously modest and self-effacing body which represents universities at times like these—and tends to disappear in the interim—has a new chairman. He is Dr D. G. Christopherson, Vice-Chancellor and Warden of the University of Durham. He took over as chairman early in July, and although nominally elected for one year, it will be breaking all the traditions of a particularly traditional organization if he is not re-elected on the nod for a further two terms. This was what happened to his predecessor, Sir Charles Wilson.

Dr Christopherson has experience in a number of posts, and, perhaps fortunately in his new job, of a great variety of universities. After graduating at University College, Oxford, he was Henry Fellow at Harvard University in 1938, and a scientific officer in the Ministry of Home Security during the war. Since the war he has been a demonstrator and lecturer at Cambridge, professor of mechanical engineering at Leeds and of applied science at Imperial College, before going to the University of Durham in 1960. In 1961 he was chairman of the academic planning board for the University of Kent, and has been a member of the Science Research Council since 1965. In the present atmosphere of uncertainty, universities may be glad that there is a new man at the helm, although Dr Christopherson might well have preferred to have been pitched in at a less controversial moment.

## Golden Gates

CONSIDERING California's wealth in laboratories and scientific institutes, it is not surprising to learn that she receives the lion's share of American federal funds for research and development. In fact, California received 31.7 per cent of the \$14,400 million spent in 1965; the second largest share went to New York with nearly 9 per cent; Maryland received 6.1 per cent; Massachusetts and Texas each received 5.1 per cent. These figures come from the National Science Foundation's report on the "Geographical Distribution of Federal Funds for Research and Development".

Between 1963 and 1965, Federal spending on research and development rose by 17 per cent, from \$12,250 million to \$14,400 million. In this period, fourteen States showed a decrease in spending and thirty-seven an increase in spending. The change in distribution was largely caused by the phasing out of the Gemini programme and the building up of the Apollo programme, and the completion by the Air Force of several large weapon systems.

On a prime contract basis, 63 per cent of the Federal research and development spending went to industry, 21 per cent to Government laboratories, 8 per cent to educational institutions, 4 per cent to federal contract research centres administered by educational institutions, and 4 per cent to other non-profit-making institutions. As might be expected, a large proportion of the Government laboratories are in or around Washington, and so three States—Maryland, the District of Columbia, and Virginia—received almost a third of the spending on Government laboratories. California received a sixth of the spending in this sector.

The universities have had a greater increase in their share of federal research and development spending than either industry or the Government laboratories; in 1963 they received \$851 million and in 1965 they received \$1,200 million. Although the universities receive only 8 per cent of the total federal research and development spending, their influence is far more extensive because they train scientific manpower and provide consulting services and technical expertise to both industry and Government. New York, California and Massachusetts received 37 per cent of this educational institution spending, thanks to the influence of seven universities: Columbia and Cornell in New York, California (Los Angeles and Berkeley) and Stanford in California; and MIT and Harvard in Massachusetts.



## Concord Still Going Up

THE Public Accounts Committee of the House of Commons is in particularly good form at the moment. Not content with its recent report on Bristol-Siddeley engine contracts, it has now published a report detailing other examples of overcharging. Almost inevitably, one of the things which the report reveals is that the cost of Concord has gone up again, despite the fact that the last estimate of £500 million contained no less than £50 million for "contingencies". The extra figure this time is £28 million, which the Ministry of Technology had apparently forgotten to include—it represents an allowance for spending at the ministry's own laboratories. The committee also criticizes the agreement between France and Britain on intramural expenses, which was "ambiguous".

The committee also suggests that other contracts have been agreed at prices favourable to the companies involved. In particular, the price of Buccaneer aircraft was too high, and the committee notes that Hawker-Siddeley have now agreed to repay any profits over the figure of 17.5 per cent on costs; it does not, however, reveal how much this is.

## Organizing Aid

WHAT is the best way of distributing aid given for the general economic development of the poorer countries of the world? The problem of a strategy for aid-giving is dealt with by a recent publication of the Overseas Development Institute, called *Pledged to Development*, and written by John White, a research member of the Institute.

Previous studies by the institute have revealed wide divergences in the motivation, administration and financial characteristics of the different aid programmes, as well as in the approach of the aid-giving countries to the fundamental questions of the nature of economic aid. A further study of a single aid-receiving country—Uganda—showed that these divergences can give rise to practical problems. Not only is there a loss in efficiency when donors are dealing with different problems in the same undeveloped country—more fundamentally, a successful aid programme must deal with the whole economy, over a long period of time. Since very few donors are prepared to take on this kind of project, even for a small underdeveloped country, consortia or consultative groups of donors must be formed. In 1958 the Indian Consortium was formed, and in the next few years similar groups were formed for Pakistan, Greece and Turkey. For a number of other countries consultative groups were set up, which differ from consortia in that the countries contributing are asked to make no specific financial pledge to the development programme.

It is with these groups and the strategy of aid that the book deals. The word "strategy" is one used by the author. It reflects his view that future operations of the group will require more than co-operation between the various members; a joint command is needed, delegating authority to the countries concerned. For this an international organization with some degree of operational autonomy is required.

This point, and other questions of the mechanism of the group, are discussed in the light of two earlier chapters which are case histories of the Pakistan

Consortium and the Turkish Consortium, respectively the most and the least successful of the consortia so far formed. Mr White's conclusions as to the nature of the groups and how they should work differ in some respects to the views of the World Bank, the agency responsible for the largest number of aid groups.

As he points out, the original aim of the aid-giving countries in the late 1950s was to raise the standard of living of all the poorer parts of the world to a tolerable level. For various reasons this soon declined into a situation in which aid was given to only a few countries. Perhaps the groups envisaged by Mr White will be more successful in dealing with the critically important problem of world hunger.

## More for NRDC

THE National Research Development Corporation has nearly spent the £25 million that it has been entitled to borrow from the Ministry of Technology. Because of this, the Minister, Mr Anthony Wedgwood Benn, will introduce legislation to increase the limit of the advances that may be made by the ministry to the corporation. The ceiling of the corporation's borrowing powers was last raised, in June 1965, from £10 million to £25 million. Mr Benn's legislation, which will double the amount to £50 million, can be expected to pass through Parliament early next year.

Though the corporation's income from licence receipts has been increasing consistently since 1962, it will continue to depend on loans from the ministry for a long time. The loan of 1966 alone far exceeded the earnings of the corporation over the previous 31 months. But increasing the power to borrow is the clearest way to solvency for the corporation. Backing research projects for profit is, like any other stochastic process, more likely to be successful the greater the scale of operations.

## Instruments under Pressure

SUPERFICIALLY, the annual report of the Scientific Instrument Manufacturers' Association for 1966-67 makes moderately happy reading—overall production is up by 4.7 per cent, above the chancellor's target growth rate of 3 per cent, and export sales are up by 9.1 per cent. When, however, the figures are compared with those for the previous five years, 1960-65, things do not look so bright—in that period there was an annual increase in production of 12 per cent. This setback can be attributed mainly to the "freeze", because production in the first half of 1966 rose by 11 per cent compared with 1965, while in the third quarter it rose by only 3 per cent and in the last quarter it fell by 5 per cent.

Although exports were up 9.1 per cent, imports were also up, and by a greater percentage. At the present moment, there is a favourable balance of payments in this industry, but unless imports slow down or exports speed up, imports can be expected to overtake exports within the next four or five years. The present drop in overall world trade and the tariff cuts agreed in the Kennedy Round which will slowly come into force over the next five years will do nothing to help the industry's position.

The scientific instrument industry in Britain is well protected behind some relatively high tariff barriers,

averaging about 30 per cent; these will be cut by half, to about 15 per cent. On the export side, tariff barriers are also being cut: the EEC tariffs drop from an average of about 15 per cent to 10 per cent; Japan's tariffs drop from an average of 15 per cent to 7.5 per cent; and almost all USA tariffs are being cut by half. This will make British products more competitive with those of the home country but it will also have some side effects. In EFTA, for instance, we can export instruments to Sweden free of charge to compete with those of Sweden, but the tariff cuts mean that instruments from outside EFTA gain competitiveness while ours remain the same. Similar arguments apply to Commonwealth countries, where Commonwealth preference has aided Britain in the past.

Some idea of the effect of the tariff cuts can be gained by considering one particular instrument such as a balance with a sensitivity of five centigrams or better: to import one into Britain a tariff of 33½ per cent has to be paid, and this will now drop to 17 per cent; the Japanese tariff drops from 15 to 7.5 per cent; the USA tariff from 25 to 12.5 per cent; the EEC tariff from 18 to 9 per cent; and the Swedish tariff for balances from outside EFTA from 10 to 5 per cent. The figures for a thermometer are about the same: Britain's tariff drops from 25 to 12 per cent; Japan's from 15 to 7.5 per cent; the USA tariff on clinical thermometers remains the same at 42.5 per cent, but drops from 42.5 to 21 per cent on all other thermometers; the EEC tariff drops from 17 to 12 per cent; and the Swedish tariff for all thermometers from outside EFTA drops from 10 to 5 per cent.

The report states that not all segments of the industry were equally affected by the "freeze". Optical instruments, which slumped badly in 1965, recorded increased production throughout the year and almost regained their 1964 position. Electrical and electronic instruments made a marked improvement in their export figures, but industrial process measuring and control instruments were badly affected and production for export dropped by 21 per cent. The production of measuring and scientific instruments showed an increase of only 3 per cent.

## Patent Law Reform

THE Membership of the Board of Trade Departmental Committee was announced recently by the President of the Board, Mr Douglas Jay. The chairman will be Mr Maurice Banks, who is at present chairman of the Ministry of Technology's Advisory Council on Calibration and Measurement. The other members will be Mr P. J. Stuart Bevan (Deputy Chairman), Mr J. C. Duckworth, Mr G. D. Everington, Mr D. Gladwin, Mr A. W. Mallinson, Mr H. R. Mathys, Mr C. W. Morle, Mr A. Silberston and Professor F. C. Williams. The committee will act with the assistance of the Comptroller-General of the Patent Office.

The formation of the committee comes at a time when revision of patents and patent law is being undertaken in both Europe and America. Indeed, one of the tasks of the committee will be to facilitate the interchange of patent information along the lines agreed by the recent Council of Europe Convention. A standing committee, headed by Mr Mathys, will advise the board on these matters.

What hampers the passage of patent information is, in part, the fact that the establishment of patents involves legal and other problems peculiar to particular countries. In America, the Sherwin Committee (many of whose recommendations are embodied in the Patent Bill now before Congress) suggested the setting up of information centres, in which interlinked computers stored patent information in a standardized system. Such ideas may come to fruition slowly. In the meantime, there may be a case for transferring responsibility for patents from the Board of Trade to the Ministry of Technology. This would be to recognize that patents are as much pieces of scientific information as commercial assets.

## All Done by Numbers

ANY effort to take the guesswork out of engineering is to be commended. To this end, the Institution of Chemical Engineers and the Institution of Mechanical Engineers, with financial backing from the Ministry of Technology, are producing a series of Design Data Memoranda for publication. The first four—approximate data on the viscosity of some common liquids; heat transfer in tubes; thermal conductivity of liquid alcohols; and thermal conductivity of water substances—were launched at a press conference on Tuesday, July 25.

Data sheets were originally pioneered by the Royal Aeronautical Society twenty-six years ago. The Fielden Committee stated in 1963 that they were a way of improving standards of engineering design, and proposed that the work be extended to other fields, and that other professional engineering institutions should participate. It is hoped that the issue of up to date engineering design data will aid engineers in design problem and will save industry from maintaining costly non-specialist data.

## Anniversary Conference

FORTY years ago this year the first announcements of the diffraction of electrons by crystals were made, in letters to *Nature* by C. Davisson and L. H. Germer, then of Bell Telephone Laboratories, New York, and by G. P. Thomson and A. Reid, of Aberdeen University (*Nature*, 119, 558 and 890; 1927). In commemoration of this anniversary, a conference was held last month at Imperial College, London, under the auspices of the Institute of Physics and the Physical Society (Electron and Microscopy Analysis Group). The conference was opened by Professor C. C. Butler, F.R.S. The two survivors of the four original discoverers, Sir George Thomson, F.R.S., and Professor Germer, each spoke on the history of electron diffraction. Professor Germer reviewing low energy electron diffraction, a field in which he was particularly active until his recent retirement.

## No Fundamental Length

from a Correspondent in High Energy Physics

A RECENT experiment at the Brookhaven National Laboratory (S. J. Lindenbaum *et al.*, *Phys. Rev. Letters*, 19, 193; 1967) has provided for the first time a critical test of the current concepts of space and time

at really short distances. The conclusion drawn from this experiment is that for distances down to the order of  $10^{-15}$  cm there is no evidence whatsoever for a primitive acausal (in the sense of special relativity) region or fundamental length.

It has been known for some years (R. Oehme, *Phys. Rev.*, **100**, 1503; 1955) that the existence of an acausal region of space time (across which signals might be propagated at velocities exceeding that of light) leads to a failure of forward dispersion relations for elastic scattering. These relations, which are based only on very general theoretical considerations, state that apart from various subtraction constants and kinematical terms the real part of the elastic scattering amplitude (say, for pion-proton scattering) is given by an integral over energy of the total cross section for the process multiplied by well defined functions of this energy. Thus if the real part of the scattering amplitude can be measured (and this is the difficult part) for some simple elastic process where the total cross sections are well known, a critical test of our space-time concepts becomes available.

Lindenbaum and his colleagues have carried out elastic pion-proton scattering experiments in the momentum region 8–26 BeV/c using an impressive array of counter equipment linked directly to computers to provide both permanent record and immediate on-line analysis of data. Although two amplitudes are required to describe this process in general (corresponding to the possibility of reversing the spin of the proton) only one is of significant magnitude at small scattering angles and the experiment is correspondingly performed at angles less than 25 mrad. The real and imaginary parts of this amplitude can be separated by examining in detail the small interference effect (in the differential cross section) between the dominant strong interaction and the much weaker electromagnetic one. It is the very small magnitude of this effect coupled with both theoretical and experimental uncertainties as to form factor and multiple scattering corrections which make this such a hard experiment.

The interference has been found to be destructive for negative pion scattering and constructive when positive pions are used. In the former case the ratio of the real to imaginary parts of the amplitude is almost constant at  $-0.13$  throughout the measured range, and in the latter case rises smoothly from  $-0.22$  at 8 BeV/c to  $-0.14$  at 20 BeV/c. By comparing these results with the measured total cross sections (the authors have also remeasured these, but have not yet published their results) integrated in the dispersion relations, it has been established that good agreement exists at least up to values of 20 BeV/c for the pion momenta.

Experiments which test such basic concepts are quite rare, and ones which combine such powerful techniques in both theory and experiment are rarer still. It is a welcome change in these days of the mass production of resonances to see one of the largest machines used in this thoughtful manner.

## Cytochrome c

from a Correspondent in Molecular Biology

CYTOCHROME *c* is an interesting protein for a number of reasons, not least because it offers an unrivalled opportunity for observing biochemical evolution. Cyto-

chrome *cs* have been isolated from vertebrates, invertebrates, micro-organisms and plants, and through the efforts of E. L. Smith, Margoliash and their associates, the amino-acid sequences of a wide range of these have been determined. The most recent study, by Stevens, Glazer and Smith (*J. Biol. Chem.*, **242**, 2764; 1967), gives the first sequence of a plant cytochrome *c*—that from wheat germ—and therefore represents a particularly interesting addition to the series.

The polypeptide chain consists of 112 residues, compared with 104 for all vertebrate pigments. It differs from the other cytochrome *cs* of known sequence (considering only the first 104 residues) in 35–46 amino-acids, and actually shows fewer differences from the human heart protein than that from a fungus. It is extraordinary to discover that between all the known sequences the number of positions in the chain which remain invariant is now reduced to 35. It is true that in many of the variable positions the substitutions are limited to amino-acids of similar kind, for example, that of one non-polar residue for another, but this is by no means always the case. Only one uninterrupted sequence of more than two residues is present in all cytochrome *cs*, namely, the eleven residues, 70–80. In addition, two of the constant residues are cysteines 14 and 17, which are covalently linked to the haem group, and the two residues now generally supposed to act as ligands for the iron on either side of the haem plane, histidine-18 and methionine-80. All known cytochrome *cs* appear to be capable of interacting with mammalian cytochrome oxidase, and the steric requirements for this recognition process evidently therefore involve only a minority of the residues. One may perhaps speculate that the long invariant sequence is in some manner involved in this aspect of the function.

It is a happy circumstance that at the same time the first report has appeared from Dickerson's laboratory (*J. Biol. Chem.*, **242**, 3015; 1967) of the X-ray structure of horse heart cytochrome *c* at 4 Å resolution. This is sufficient to define many of the structural features of the molecule. Cytochrome *c* appears to possess little or no  $\alpha$ -helix. From the distribution of electron density it appears that the molecule conforms well with the principle that the hydrophobic side chains are packed into the interior of the molecule, while the polar side chains coat the exterior. The two cysteine residues attached to the haem, and one of the ligands, histidine-18, can be identified with certainty. The haem group lies in a long crevice down the long axis of the molecule, with one edge only exposed. The chain appears to be in a largely extended state, and the structure is very different from those of the other haem proteins, myoglobin and haemoglobin.

It is to be hoped that as more details become available it will soon be possible to identify the invariant positions in the chain, which must be supposed necessary to define the structure as that of a functional cytochrome *c*.

## Fungal Morphogenesis

from a Correspondent in Microbiology

ONE of the most exciting challenges of contemporary biology is the interpretation in biochemical terms of morphological change. Studies of biochemical differen-

tiation, as this subject is known, have been notably rewarded where microbial systems have been examined; in particular, the diverse processes of sporulation found in micro-organisms have interested many workers. A convenient approach to the study of sporulation involves the use of metabolic inhibitors which interfere with protein or nucleic acid syntheses. Experiments of this type can provide precise information on the process of induction and define the temporal parameters of the morphogenetic sequence. The investigations of Young and Fitz-James and Aronson exemplify this stratagem and have indicated the major changes in protein and nucleic acid metabolism which occur during endospore formation in *Bacillus*.

In the imperfect fungus *Trichoderma* conidia are light-induced, a fact familiar to mycologists for many years. This fungus has been taken by Galun and Gressel as a model system for analysing the involvement of nucleic acids in morphogenesis. Galun and Gressel observed a minimum growth period of 16 h before this mould was capable of responding to light induction and that conidiation was prevented by fluorouracil (FU) at concentrations which did not inhibit growth (*Science*, **151**, 696; 1966). Subsequently, these workers found that an hour's treatment with FU would completely suppress sporulation up to 7 h after light induction (*Developmental Biol.*, **15**, 575; 1967). Once conidiophore branching was completed, however, after about 11 h, sporulation always proceeded although at a reduced level. This aspect of *Trichoderma* differentiation is very reminiscent of the "point of no return" concept in the water mould *Blastocladiella* and the phase of "spore commitment" in *Bacillus* species.

Suppression of sporulation was reversed by uracil, though the uracil appeared to exert an additive rather than the expected competitive effect on fluorouracil action. Uridine also affected reversal of FU inhibition, but, while the tritium label from uridine appeared both in uridylic and cytidylic acid peaks, the carbon-14 label from the analogue was found only in the uridylic-fluorouridylic acid peaks. Fluorouracil suppresses sporulation by its specific action on RNA and it is noteworthy that indefinite growth can continue with a selective inhibition of this morphogenetic pathway. All species of RNA were shown to contain FU and additionally a new sRNA was eluted from methylated albumin columns between the 4S and 5S species. Pulse labelling with FU or uracil yielded a similar RNA elution profile suggesting that the analogue was incorporated into mRNA. Of a large number of other RNA antimetabolites tested, only 8-azaguanine produced results similar to fluorouracil and induced synthesis of the exotic sRNA species.

At the present time the only substantial conclusion to be drawn from these experiments is that the photo-induced sporulation in *Trichoderma* is RNA mediated. The suppression of this specific morphogenetic sequence could be via FU-sRNA, FU-mRNA, via both, or via yet another mechanism. Extended studies of these fraudulent nucleic acids will doubtless lead to important findings on the molecular control of growth and morphogenesis, and future reports from this group will be awaited with considerable anticipation. In particular, the fact that pre-induced cultures can be partially suppressed by FU encourages the hope that significant data may be forthcoming on the nature of the light induction mechanism.

## Mouse Satellite DNA

from a Correspondent in Cell Biology

DENSITY gradient centrifugation separates mouse nuclear DNA into two components: a major component, about 90 per cent of the total, and a minor component, satellite DNA, with a slightly lower buoyant density. This satellite DNA has some very remarkable properties. It is unusually homogeneous in G+C content (Flamm *et al.*, 1966) and undergoes very rapid renaturation at low DNA concentrations (Walker and MacLaren, 1965). Waring and Britten (*Science*, **154**, 799; 1966) found heat denatured satellite DNA renatures faster than any of the other animal, bacterial and viral DNAs that they examined. It even renatures fifteen times faster than simian virus 40 DNA which only has 6,000 base pairs, so satellite DNA must form a homogeneous population of molecules with a repeated sequence of only about 300-400 base pairs. Waring and Britten suggested each mouse genome probably contains about one million copies of this short sequence.

Flamm, McCallum and Walker at Edinburgh have recently achieved the separation of the two complementary strands of the satellite DNA duplex and report on their properties (*Proc. Nat. Acad. Sci.*, **57**, 1729; 1967). After isolating the satellite DNA from the major component by gradient centrifugation they re-ran the satellite band in a caesium chloride gradient at pH 12.5. At this high pH the DNA duplex is separated into its two strands and these have different buoyant densities. The heavy strand is rich in pyrimidines C+T, and the light strand in the purines A+G. The two strands have complementary base compositions and the renaturation characteristics of equimolar mixtures are very similar to those of native satellite DNA after heat denaturation. Neither heavy nor light strands alone will renature.

Interestingly, although some sequence homologies exist between the total DNAs of mice, rats and guinea-pigs, mouse satellite DNA does not renature with total guinea-pig or rat DNA which includes the satellite DNA of these species. It appears that the nucleotide sequences of satellite DNA are completely species specific.

This work is the first in which the complementary strands of DNA from a higher organism have been isolated and the very fact that this can be done implies that satellite DNA is homogeneous and has only a short base sequence. This does not mean, however, that satellite DNA is of low molecular weight. Flamm and his collaborators have isolated molecules with a molecular weight of 40 million, indicating at least 200 repeating sequences of 300-400 base pairs strung end to end. This makes it highly unlikely that satellite DNA is viral or proviral in origin, for it seems inconceivable that a virus could exist with such a genome. Other properties of mouse satellite DNA, its presence in all the strains of mice and tissues investigated, its association with metaphase chromosomes (Maio and Schildkraut, *J. Mol. Biol.*, **24**, 29; 1967) and its metabolic stability, also argue against a viral origin.

What is the function of satellite DNA? It is unlikely to code for protein and yet it forms 10 per cent of the cell's total DNA. What possible purpose is served by having so many, apparently identical, short sequences within the same genome?



## Parliament in Britain

### University Grants

IN the House of Lords, Baroness Phillips, answering for the Government on university expenditure and government grants, said Parliament had voted £22.5 million for 1967-68 for the purpose of meeting drawings on university furniture and equipment. The UGC, when issuing the initial drawing limits, decided to work within a total of £20 million, keeping £2.5 million in reserve. Universities were invited to submit a claim if they wanted a higher limit, and in the event the claims amounted to £21.5 million over and above the total of the limits notified earlier. The UGC saw no possibility of justifying this increase and had proceeded to distribute the remaining £2.5 million.

She said that it was certainly true that the limit which had been assigned to some universities might prove to be less than they could legitimately claim that they needed and less than they would have drawn if the drawing of grants had been left to take their course as in the past. But it was also true overall that more money was being provided for furniture and equipment than in any year in the past. The UGC was making a careful study of the situation, and particularly that of the comparatively small number of universities where, because of particular circumstances, the impact of drawing units had been particularly hard. (Debate, July 25.)

### Co-operation

IN a debate on Science and Technology (Anglo-European Co-operation), Mr Eric Moonman, M.P. for Billericay, said that for many years science and technology had failed to make the impact in Western Europe which might have been expected. People holding key positions in government and industry had failed to pay science and technology due attention. Decisions had been taken about short- and long-term planning without reference to those sections of Government that were encouraging scientific projects, while in

industry large numbers of companies in Europe felt it undesirable or unnecessary to appoint scientists or technologists to key positions or on boards of executives. As a result, their voice was not heard or was not loud enough. Collaboration among European nations and firms was important, he thought, but needed to be established on a meaningful basis.

Mr Anthony Wedgwood Benn, Minister of Technology, agreed with almost all Mr Moonman had said. The high cost of research made it necessary to establish companies large enough to afford it, and the high cost of marketing made it necessary to have large markets to sustain this research. Britain was handicapped by the fragmentation of firms. Although some of Britain's corporations were of a world size they were operating on too small a scale. It was one of the principal objects of the Ministry of Technology to try to bring about larger units in the British engineering industry. (Debate, July 24.)

### Booms

IN a written answer, Mr Anthony Wedgwood Benn, Minister of Technology, said that the sonic boom heard in the London area on July 17 was not comparable in noise, effect or decibel measurement to that which could be expected from Concord. (Written answer, July 21.)

### Doctors

IN a written reply, Mr K. Robinson, the Minister of Health, said that an interview team of five, headed by the Principal Medical Officer from his department, was being sent to visit the USA and Canada early in the autumn to meet British doctors there and offer them appointments in the hospital service or introductions to general practice or to hospital boards. The project had been publicized in the medical press and a large number of inquiries had already been received from British doctors interested in returning to Britain. (Written answer, July 24.)

## Keeping an Eye on Universities

by our Special Correspondent

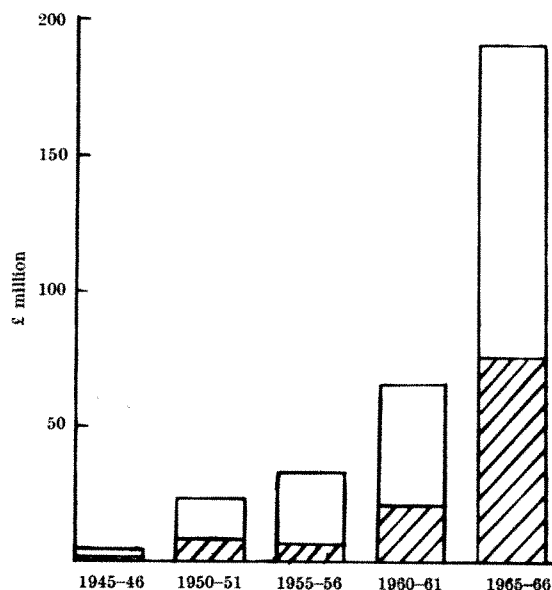
Universities in Britain are soon to have another intermediary in their increasingly uneasy relationship with the Government. From the beginning of next year the financial affairs of the universities will be open to audit by the Comptroller and Auditor General, the Government financial watchdog. Most of the universities are unenthusiastic about the idea, but need more details of how the scrutiny will operate before committing themselves.

MR ANTHONY CROSLAND has an uncanny talent for timing. His most unpopular announcements are always made a week or so before Parliament goes into recess—before Christmas it was the increase of fees for overseas students, last week the confirmation that university accounts are to be audited by the Comptroller and Auditor General. To the surprise of his department, this latest decision has so far raised far less steam than the fees issue. It comes, of course, as less of a surprise, as the Committee of Public Accounts of the House of Commons recommended it in a report published early this year

(*Nature*, 213, 438; 1967). In addition, it may be that universities, traditionally slow to react, are still embroiled in arguments with the University Grants Committee about equipment grants.

The Public Accounts Committee announced its recommendations in January this year. They were short and to the point; from August 1, 1967, the comptroller should be given access to the books and records of the UGC and the universities, and in the meantime steps should be taken both to work out suitable conventions as to how the scrutiny should be handled and to ensure that

the universities are fully informed about the nature and purposes of the scrutiny, and what it would in practice involve. Opposition—unexpectedly bitter and determined—centred on the fear that financial judgments would inevitably encroach on academic preserves. Lord Butler muttered darkly about dictatorship, the Committee of Vice-Chancellors and Principals about academic freedom. Nobody denied, though, that since the war the financial load of the universities on the Exchequer has gone up by leaps and bounds (see chart). This was the mainspring behind the committee's drive to make sure money was not being wasted.



Growth of support for universities in Britain from the public purse. Open areas represent recurrent grants, hatched areas capital grants. The estimate for 1966-7 is £211 million, £134.5 million in recurrent grants and £76.5 million in capital grants.

How will the new system work? Mr Crosland's statement left much unsaid. What it did make clear is that the UGC will continue to support the universities by way of block grants, and that the universities will be responsible for seeing how the money is spent. It will not be part of the comptroller's duty to question policy decisions or decisions reached on academic grounds. The department is going out of its way to make these points clear, and referring inquiries to the proceedings of the public accounts committee, in which almost every page contains an assurance that the comptroller will not intervene in matters falling outside his exact terms of reference. It is also known that the accounting officer for the universities will be the senior civil servant at the Department of Education and Science, Principal Secretary Sir Herbert Andrew. The department's attitude is to play down the importance of the change; really, it says, nothing at all has changed. It does not yet know exact details of how the scrutiny will be organized, and much of the time before the system comes into operation on January 1, 1968 will be taken up with working out the conventions, in discussion with the Committee of Vice-Chancellors and Principals and the UGC.

Until more information emerges, the interested parties are reserving their positions. As yet it is neither clear how the comptroller will go about his job, nor what he will do if he discovers anything discreditable in the university accounts. In the proceedings of the public accounts committee, Sir Bruce Fraser, the comptroller, gave some idea of how he would tackle the job. Asked how he would regard an experiment in teaching methods which had turned out to be expensive, he said that if on auditing the books he found an unexplained increase of this type,

he would ask why. If he were told that the increase had come about as a deliberate decision of academic policy, that is all he would wish to know. "I would say no more about it. It would be ridiculous for me to substitute my judgment for the academic judgment of the people who know about it."

A central figure in the new arrangements seems likely to be the accounting officer, Sir Herbert Andrew. As he is responsible for answering criticisms directed at the universities, he will probably act as the go-between in the relations between the universities and the comptroller. It is unlikely that anything so tasteless as a direct confrontation between the comptroller and the vice-chancellors would be tolerated, and Sir Herbert may well be thrust willy-nilly into the disputed ground between the two parties, as a sort of referee. As he will be expected to answer any criticisms which the comptroller has, he will have to be very well informed indeed about the activities of the universities. Talking to the committee of public accounts, Lord Murray of Newhaven, Chancellor of the University of Southampton, saw this as a danger: "The accounting officer would have to protect himself to meet his obligations . . . I think it would be difficult for him to stop at asking why as well as what expenditure is incurred".

The comptroller has for many years audited the books of one college, Cranfield College of Aeronautics. During the PAC hearings, it was suggested that the audit had had the effect of limiting the research and development at Cranfield. These suggestions, made by witnesses from the Association of University Teachers, were later withdrawn after representations by the college. It is fair to say that the college was embarrassed by the allegations, which did not correspond with its own experience. Although it does not feel that public statements by the college would be helpful at this stage, its experience of the comptroller has inevitably attracted interest.

Each year officials from the comptroller's department—the Exchequer and Audit Department—visit the college, look at the books, and ask questions. Their report then goes before the comptroller. If the comptroller is dissatisfied, or has further questions to ask, he approaches the DES, which will—if necessary—ask the college for further information. This, according to the Exchequer and Audit Department, is the normal, accepted form of audit; if the comptroller is not satisfied with the answers given, or if he can see any other reason for mentioning the matter, he may include a reference to it in his annual report to the Public Accounts Committee. Further discussion is then exclusively between the DES and the PAC. If this system is adopted with the universities, it will clearly increase the work load of the Exchequer and Audit Department—the present staff stands at about 450, of whom 400 are auditors. Although it is hard to see how an effective audit can be carried out without visiting the universities, some preliminary screening procedures may be adopted, in which the universities would send audited books to the DES. It is hard to believe, however, that the comptroller would be willing to protect universities' sensibilities by dispensing with visits altogether.

Where the UGC fits into the picture is even more obscure. Its traditional role as buffer between department and universities is beginning to look bedraggled, and these latest moves will do nothing to reassure either party. By a stroke of fortune, though, Mr Crosland has been supplied with an issue in which the intervention of a third party could perform a valuable public service. The argument between the universities and the UGC over equipment grants has been unusually bitter. The universities feel let down, while the UGC plaintively says that it only did its job. If the comptroller could sort out responsibility for the mess—and give some idea how it could have been avoided—the universities might begin to feel that he could perform a useful function.

# The East African Rift System

by

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Dr McConnell joined the Geological Survey of Tanganyika in 1937 and worked there and in other parts of the African continent during the following eighteen years. He reviews here geological aspects of the East African rift structure which have a bearing on problems of the upper mantle and continental drift.

THE study of rift valleys has assumed a new importance in the past 10 years because of the discovery by Heezen<sup>1</sup> of comparable features on the world-wide system of mid-ocean ridges, and the International Upper Mantle Project<sup>2,3</sup> has shown considerable interest in the East African rift system. Previous reviews of this system have been published<sup>4-7</sup>, but it is now possible to give a clearer picture of the entire field because of the recent accumulation of detailed mapping since the post-war expansion of the Overseas Geological Surveys<sup>8</sup> and the establishment of the Research Institute of African Geology at the University of Leeds<sup>9</sup>, and it may be of value to outline the geological characteristics of the system in view of the latest work.

A rift valley may be defined as an elongated sunken area, with a characteristic width<sup>6</sup> of 30-60 km, which has

descended between parallel faults. The term was introduced by Gregory<sup>10</sup> to designate the grabens (down-faulted strips) of the great fracture (*Brüche*) system of eastern Africa, first described by Suess<sup>11</sup>. In the East African rift system, as now described, true rift valleys are numerous, but the rift faults, although forming a continuous system, are not always in pairs determining a graben, but may take the form of block faults or be replaced by flexures. The rift system was believed by Suess and Gregory to include the Red Sea and the Dead Sea-Jordan Valley complex, but only the portion within the African continent will be considered here.

The classical East African rift system, as shown in Fig. 1, is more than 4,000 km in length from Asmara, near the Red Sea, to the Zambezi River, and reaches a maximum

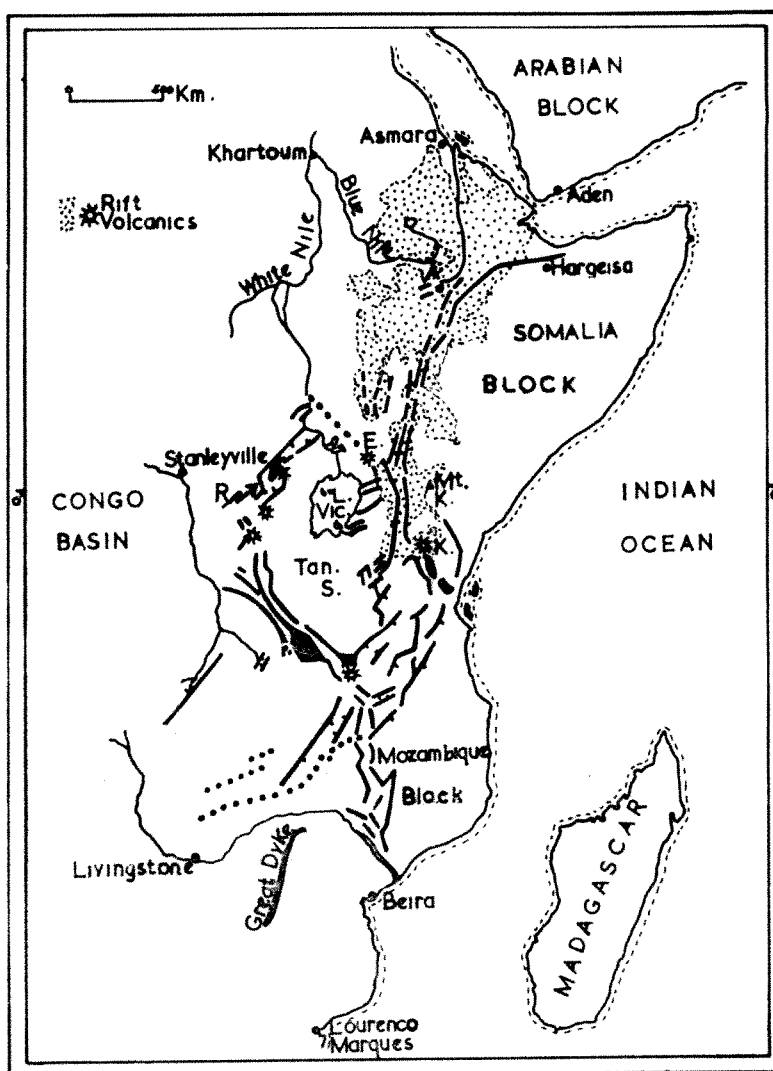


Fig. 1. Structural map of the East African rift system showing major rift scarps (schematized) and allied features. Solid black denotes raised blocks. Heavy dots denote dislocation zones. A, Addis Ababa; R, Ruwenzori Mountains; E, Mt. Elgon; Mt. K, Mt. Kenya; Tan. S., Tanganyika Shield; K Kilimanjaro.

width of 1,000 km in Tanzania. This immense system is much quoted in the literature of world tectonics, but its complexity is little understood because of the difficulty of synthesizing the many detailed investigations which are necessarily restricted to limited map sheets. Enough geological mapping is now available, however, to give a preliminary view of many important aspects of the rift system which concern: (1) the geological formations involved; (2) the pattern of faulting; and (3) the age of faulting.

Geological evidence<sup>5,12,13</sup> now appears to confirm that the pattern of the rift system originated in Pre-Cambrian times<sup>7,14,15</sup> as a line of deep faults centring on a N.N.E.-S.S.W. axis, diverted locally around pre-existing cratons, and that movements have occurred during major orogenic cycles down to the present. Dixey<sup>16</sup> has given evidence for the existence of actual rift valleys as early as the Jurassic, but during periods of stability the fault scarps and grabens were generally planed down by erosion<sup>17,18</sup> and even crossed by river systems.

It is a fact of outstanding geological importance that the formations principally affected are of Pre-Cambrian age; the pattern of rift faulting has been found to reflect the Pre-Cambrian structures of the basement and formations of later date are merely relatively thin cover rocks which conceal these structures. To attain an objective view of the history and geological structure of the rift system as a whole it is therefore necessary to give special consideration to those few areas, particularly in Malawi, Tanzania and Uganda, in which the remarkable Pre-Cambrian structures within the rifts are clearly visible, and not, as is the general rule, concealed by young volcanics and sediments, or by lake waters.

In view of the inherent difficulties of Pre-Cambrian geology, however, the study of the very significant association between rift faulting and Tertiary-Recent volcanism<sup>19-23</sup> which is so well exposed in the Kenyan and Ethiopian sectors is also most important for the unravelling of the history of the rift system. This volcanism<sup>24,25</sup> is characteristically alkaline and of great interest petrologically; the associated post-Miocene rift structures are also particularly clearly seen, but it must be remembered that these phenomena are typical only of the northern part of the system and that elsewhere Cainozoic volcanism is rare, being either completely absent or limited to intersections in the fault system.

A map, Fig. 1, showing the pattern of faulting compiled from the latest information<sup>26-31</sup> illustrates the difficulty, which results from the curvature of the component faults and grabens, of explaining the formation of the rift system by invoking either tension<sup>6,19</sup> or compression<sup>32-34</sup> alone. The strike of the components of the rift system varies between N.W.-S.E. and E.N.E.-W.S.W., but the map clearly indicates an overall N.N.E.-S.S.W. axis which is represented in the north by the strike of the Gregory and Ethiopian rift valleys. This axis is split into eastern and western branches by the resistance of a central craton termed the Tanganyika Shield<sup>7</sup>, thus giving rise to the great width of the system in its equatorial sector.

In the Ubende area of Tanzania, adjacent to Lake Tanganyika, a portion of the Pre-Cambrian floor of the western branch of the rift system is exceptionally well exposed. In 1943-44 I mapped the region and showed<sup>14</sup> that the N.W.-S.E. rift fault scarps in this area follow Pre-Cambrian movement zones identified by blastomylonites, phyllonites, migmatites and elongated anatectic granites whose origin was assigned to an Ubendian Diastrophism<sup>7</sup>. These metamorphic zones were later studied by Sutton and Watson<sup>35,36</sup> and the presence of Pre-Cambrian movement zones was confirmed. Similar associations and parallelism of Pre-Cambrian mylonites and migmatites with rift faulting have also been described from many different sectors of the rift system<sup>7</sup>, irrespective of strike.

At the southern termination of the rift system, in the Zambezi River sector, the fault pattern appears to split again around the Rhodesian Shield, and Vail<sup>37</sup> has described elongated troughs of Karroo sediments, to the east and west of this shield, which he believes to be the southern continuation of the rift system manifested by faulting in late Karroo and Cretaceous times. It has been pointed out elsewhere<sup>7</sup> that the line of the Great Dyke of Rhodesia continues exactly the strike of the Gregory rift system, and this line also bisects the Bushveld Igneous Complex. Cousins<sup>38</sup> has since shown that a chain of igneous complexes of Bushveld type follows this same line as far south as Trompsburg<sup>39</sup> (lat. 30° S.), a nearly straight line between the Zambezi and Orange rivers of some 1,500 km. The main axis of the rift system (Fig. 2) appears therefore, from present geological indications, to be a major N.N.E.-S.S.W. lineament (deep fault) of great age extending over a strike of some 5,500 km, diverted and split where penetrating pre-existing cratons representing the ancient core of Africa, and outlined by intrusive bodies which can be dated. This feature may be compared in strike with the great N.N.E.-S.S.W. fracture zones in the floor of the Indian Ocean, independent of the mid-ocean ridges, which have recently been described by Heezen and Tharp<sup>40,41</sup> and are schematically shown in Fig. 2.

The question of the age of the rift system can now be approached with more confidence in view of the recent synthesis of the geochronology of equatorial Africa by Cahen and Snelling<sup>42</sup> in which five geological cycles comprising syntectonic and post-tectonic episodes are described. An early cycle, sparsely represented by dates older than  $3,000 \times 10^6$  yr, is followed by the Shamvaian Cycle (compare Superior Cycle<sup>43,44</sup>) dated  $2,700-2,300 \times 10^6$  yr and recognized in East Africa. The term Ubendian is now firmly attached to a widely recorded cycle dated  $2,150-1,650 \times 10^6$  yr (compare the Svecofennid-Hudsonian

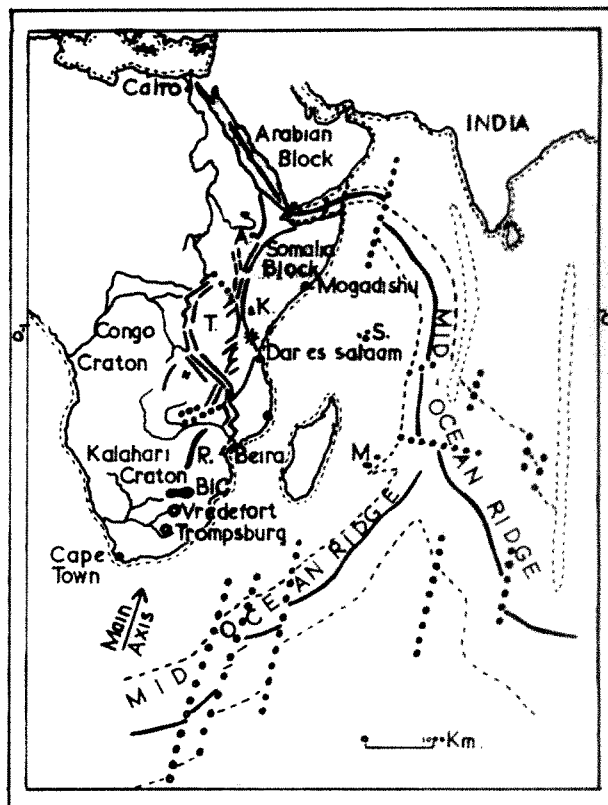


Fig. 2. Structural map illustrating main axis of rift system features of eastern Africa. Structure of Indian Ocean outlined after Heezen and Tharp. Black lines denote major fault scarps. Heavy dots denote dislocation zones. A, Addis Ababa; K, Mt. Kenya; T, Tanganyika Shield; S, Seychelles; R, Rhodesian Shield; M, Mauritius; BIC, Bushveld Igneous Complex.



cycle) in Tanzania (compare also ref. 54) and Uganda, where it coincides with the Buganda-Toro-Kibalian movements and granitization in the Western Rift (for example the Ruwenzori Mountains<sup>45,46</sup>). Table 1 shows that the chain of Bushveld type igneous complexes has given ages comparable with these early cycles. The latest investigation<sup>50</sup> of the Great Dyke of Rhodesia gives an age of  $2,530\text{--}2,800 \times 10^6$  yr, corresponding to the Shamvaian Cycle, and two independent and very thorough studies<sup>47,48</sup> of the Bushveld Complex have given Ubendian ages. Evidence from the Ruwenzori Mountains (ref. 45, p. 263) placed the age of the earliest rift faulting tentatively between  $2,500 \times 10^6$  and  $3,000 \times 10^6$  yr, and so, relating this result with the age of the Great Dyke, it is here suggested that an extensive dislocation of Africa, originating the East African rift system, took place in the Shamvaian Cycle. This original disruption was followed by the event now termed Ubendian, which imposed its isotopic age pattern on many of the migmatites and granites associated with the older event.

I have proposed<sup>7,45</sup> that the rift system arose in these early Pre-Cambrian times from a right-lateral wrench as a result of a tendency for the African continent to drift to the north-north-east while its eastern margin (Somalia, Mozambique) either remained fixed or drifted to the south-south-west. This hypothesis was based on the movement pattern observed around the Tanganyika Shield, which was postulated to be a pre-Ubendian craton around which the rift system had split. Later work has produced supporting evidence for early movements of this nature, but it must be emphasized that recent mapping has confirmed that no appreciable horizontal displacement has occurred along the rift faults since Ubendian times. If indeed the original movements were of lateral slip nature, then it must be supposed either that the strain pattern varied during the many later revivals, or that strike slip was impeded by the complication of the fault system along N.W.-S.E. and N.E.-S.W. lines, following the regmatic pattern<sup>55,56</sup>, and also by widespread granitization leading to a sort of "grouting" along fault zones. Renewed activity was, in any event, manifested by vertical<sup>12,21,57</sup> tectonics, perhaps accompanied in depth by metamorphic or magmatic activity as a result of the conversion of dynamic to thermal energy.

Later Pre-Cambrian cycles (including syntectonic and post-tectonic phases) are defined by Cahen and Snelling<sup>42</sup> as Kibaran (Karagwe-Ankolean) from  $1,290 \times 10^6$  to  $850 \times 10^6$  yr, and Katangan-Mozambiquian from  $1,100$  to  $450 \times 10^6$  yr. These episodes began with extensive deposition in basins which locally followed the pattern of the rift system<sup>9,42</sup>, although details are still under consideration, and were accompanied by extensive migmatization and granitization along the rift zones which are reflected in the

radiometric updating of previously consolidated areas. Evidence of sedimentation in basins along the rift system is clear in Karroo times<sup>4,18,37</sup> and this cycle was closed by extensive vertical movements in the Jurassic which appear to have been manifested by arching on an immense scale (compare cymatogeny<sup>58</sup>) followed by the formation of grabens, perhaps on the lines proposed by Cloos<sup>59</sup>. After a period of planation the same pattern of events appears to have been followed in time and in space during the Alpine cycle, which culminated in the formation of the present rift valleys and single scarps in post-Miocene times<sup>23</sup>.

At the present time the East African rift system is represented by rift valleys and fault scarps on a wide arch, elongated meridionally, forming the eastern high plateaus of Africa. This structure has been compared with the world-wide system of mid-ocean ridges with their central rift valleys<sup>60,61</sup>, but King<sup>22</sup> has pointed out fundamental geophysical and geological differences between these two systems, and it must be emphasized that the mid-ocean ridges are exclusively in oceanic crust and associated with geologically rapid spreading accompanied by the formation of new oceanic crust by the upwelling of basic magma<sup>62-65</sup>. The East African system, on the contrary, is confined to continental crust, and shows no spreading or formation of new crust. Any distension arising from graben faulting is slight because of the steepness and commonly antithetic nature of the fault planes: although many faults dip  $50^\circ\text{--}70^\circ$  towards the rift, vertical faulting is more widespread than has been generally assumed following Dixey<sup>6</sup>, but reverse faults are exceptional. There is a sharp contrast between the dyke-filled extension fractures of Iceland<sup>66</sup>, regarded as characteristic of a mid-ocean ridge, and the structure of a typical continental rift valley. The marked decoupling between structures in the oceanic crust and on the adjacent continent which is seen on the Pacific coast of North America<sup>68</sup> is also significant.

The physiographic and seismic characters of the East African rift system and the Red Sea-Gulf of Aden sectors of the world-wide mid-ocean ridges<sup>63,67</sup> are remarkably similar, however, and the close conjunction of the two systems in Ethiopia is a point of great importance and invites further detailed investigation.

It is intended to exclude theoretical considerations from this article so far as possible, but it may be suggested that the repeated rejuvenation along similar lines of movements which originated in early Pre-Cambrian times is of some significance and suggests that Africa has remained frozen to the higher layers of the upper mantle, and that the main N.N.E.-S.S.W. axis of the rift system reflects a deep-seated mantle lineament of great age. Belousov<sup>69,70</sup> has pointed out that the survival of regional structural characteristics over many geological periods reflects a permanent relationship between crust and upper mantle. In view of

Table 1. RADIOMETRIC DATES ALONG THE EAST AFRICAN RIFT SYSTEM

Formation	Reference	Rock and stratigraphic position	Locality	Sample	Method	Age ( $10^6$ yr)
Rusizi-Ubendian Belt	42	Kate Granite emplaced in Ubendian gneisses	Abercorn Dist., Zambia. $9^\circ$ S.; $31\text{--}5^\circ$ E.	Biotite	K : Ar	$1,725 \pm 70$
	42	Quartz-muscovite vein cutting metamorphosed sediments	Ditto	Muscovite	K : Ar	$1,800 \pm 70$
	42	Greisen associated with post-orogenic granite intruding Ubendian gneisses	Chunya Dist., Tanzania. $8^\circ$ S.; $33^\circ$ E.	Muscovite	K : Ar	$1,800 \pm 70$
	42	Post-Ubendian mineralization. Average of three galenas	Mpanda Dist., Tanzania. $6\text{--}5^\circ$ S.; $31^\circ$ E.	Galena	Common lead	1,658
	42	Pegmatite cutting Rusizian	Uvira, Kivu Prov., Rep. of Congo. $3^\circ$ S.; $29^\circ$ E.	Microcline	Rb : Sr	$1,820 \pm 240$
Buganda-Toro Belt	42	Andalusite-mica schist, Stuhlmann Pass Series, Buganda-Toro-Kibalian belt	Luzilubu R. Valley, Ruwenzori Mtns. Uganda. $0^\circ$ ; $30^\circ$ E.	Biotite	K : Ar	$1,820 \pm 60$
Tanganyika Shield	54	Dolerite from N.E.-S.W. dyke swarm intruding gneisses of Tanganyika Shield near Gregory Rift Valley	Near Lake Evasi, Tanzania. $3^\circ 58'$ S.; $35^\circ 10'$ E.	Pvroxene Plagioclase	K : Ar K : Ar	$1,630 \pm 80$ $1,730 \pm 80$
Bushveld Igneous Complex	48	Age assigned on basis of four independent investigations on minerals from granites, mafic rocks and alluvials	Bushveld Igneous Complex, South Africa, between $25\text{--}29^\circ$ S. and $23\text{--}25^\circ$ E.	Micas Monazite Zircon Galena Whole rock	Rb : Sr (U : Pb) (Th : Pb) U : Pb Common lead Rb : Sr	$1,950 \pm 150$
	47	Red granite intruding mafic rocks of Bushveld Igneous Complex. Average of four samples	Four localities north of Pretoria, South Africa	Whole rock Biotite Plagioclase	K : Ar Rb : Sr	$1,800^*$
Great Dyke of Rhodesia	50	Pierite from Wedza, Selukwe and Hartley igneous complexes	Three localities on Great Dyke, Rhodesia	Whole rock Biotite Plagioclase	K : Ar Rb : Sr	Min. $2,530 \pm 30$ Max. 2,800

\* Value adjusted following Faure *et al.*, ref. 49.

the recent confirmation of spreading from the centre of the mid-ocean ridges<sup>64,65</sup>, the reality of continental drift becomes more and more widely accepted, but the historical persistence of structural features in the crust would support the opinion of Heezen<sup>71</sup> that this continental separation takes place by expansion of the earth<sup>6,44</sup>, combined with the formation of new oceanic crust, rather than by the drift of the crust over the mantle. The extent of Cainozoic volcanism in the northern sector of the system may be allied to the development of the Gulf of Aden-Red Sea oceanic-type rifts during the Alpine orogenic cycle<sup>67</sup>.

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## Perception of Emotionally Toned Words

by

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Some theorists believe that the way in which words are perceived by the hearer depends on two different mechanisms. One mechanism may prevent the operation of the other to an extent depending on whether the words are pleasant, neutral or bad. The number of correct responses is found to be greatest for neutral words and least for bad words. Some features of the data support the theory of dual mechanisms.

If an obscene word is presented to a listener over a noisy telephone channel, he is less likely to report it correctly than he is to report other, socially acceptable, words at the same signal noise ratio. This fact has been the source of considerable activity among experimental psychologists over the past two decades, because of its obvious connexion with clinical difficulties, and because it seems at first sight to require a mechanism that is curiously inefficient. That is, it seems to suggest that a complete recognition mechanism for words exists in parallel with the mechanism

with which we can report consciously, and that the former can prevent the operation of the latter. The original experiments have therefore been subjected to a good deal of technical criticism. One line of attack has been that experimental subjects could well perceive the word and yet as a subsequent and perfectly conscious process be unwilling to say it or write it down in an academic setting; a second that it is well known that the probability of a word affects the efficiency of its perception, and that the probability of obscene words is not accurately known.

A great deal of experimentation directed at these and other points has therefore taken place, and has been adequately reviewed elsewhere<sup>1,2</sup>. In general, it would probably be fair to say that most investigators now believe that the effect is genuine, but many of the techniques used to report it seem to depend considerably on the personality of the particular subject and on the precise degree of the emotionality of the words. It is perhaps worth noticing that many experiments use, as a criterion of the emotionality of a word, the time taken by the subject to produce an association to it: and this may be unreliable. It appears to have been rare to use a technique such as that of Johnson, Thomson and Frincke<sup>3</sup>, namely, to get the emotional quality of the words assessed directly by samples of subjects. In any event, there remains the problem of the mechanism of the effect.

Recently, however, Broadbent<sup>4</sup> has had some success in applying a particular mathematical model to the related problem of the perception of words of different probability. The model used was that the perceptual mechanism contains a number of random variables each corresponding to one of the words in the language, and that perception corresponds to the selection of that variable which has the largest value on a particular occasion. Evidence from the senses is supposed to shift the average value of the correct variable, without eliminating its variability, and pre-existing biases of the mechanism are reflected by an increase, even in the absence of stimulation, of the mean values for corresponding variables. Such a process could be represented by a mechanism of the type suggested by Treisman<sup>5</sup> and Morton<sup>6</sup>, and it will be obvious that changes in the bias towards or against certain words do not involve any biologically unlikely mechanism which recognizes a word first and then decides not to admit sensory information. The bias applies whether or not sensory information is present.

Table 1

Stimulus of class I class II	Probability of		
	Correct answers	Wrong answer of class I	Wrong answer of class II
A	B	C	
D	E	F	

Unfortunately, the mathematical analysis of multiple alternative decisions of this kind is not sufficiently complete for application to experimental data, and Broadbent accordingly made use of an approximation developed by Luce<sup>7</sup>. The details need not be reproduced, as they are available elsewhere<sup>4</sup>, but it is worth noticing that it involves the assumption that the variances of the variables are equal. Given this approximation, one can test the model by gathering data in a table. When this was done (Table 1) the model appeared to be more plausible than traditional theories. That is, decision theory is as applicable to the perception of words as it has been shown to be for simpler signals<sup>8</sup>. In addition, however, and perhaps more importantly, in a table of this sort, certain relationships will hold if the difference between correct perceptions of two classes of word results purely from the bias factors in the mechanism. In that event, and if  $N_1$  is the number of words in the language in class 1 and  $N_2$  is the number of words in the language in class 2, then

$$\frac{A}{B} \times \frac{F}{D} = \frac{N_2}{N_1}$$

If, on the other hand, a difference in correct perceptions results entirely from a change in the amount of sensory information received, and there is no difference in bias

$$\frac{B}{C} = \frac{E}{F} = \frac{N_1}{N_2}$$

The former of these results did indeed hold for words of different probability in Broadbent's results, and it appears therefore that the classic effect of word probability arises entirely from pre-existing biases in the mechanism, and does not require any system of control by one recognition mechanism over another.

It seems obvious that the same technique should be applied to the perception of words of different degrees of emotionality, and we report here two experiments in which this has been done. The technique is relatively complex, and it is probably best therefore to state the method in a series of logical steps, even although some of them occurred in a different chronological order when the experiment was actually carried out.

**Step 1.** Sixty monosyllabic words were chosen from the word frequency count of Thorndike and Lorge<sup>9</sup>, all in the AA class: that is, having more than a hundred occurrences per million words. They were chosen to include twenty words of apparently pleasant emotional quality, twenty apparently unpleasant ones, and twenty neutral ones<sup>10</sup>. This assessment was confirmed by giving twenty-two Cambridge housewives a list of all the sixty stimulus words, and asking them to rate each word on a seven point scale for pleasantness/unpleasantness.

**Step 2.** The sixty words were then ordered randomly in a list, and recorded on one track of a tape, using a 'Vortexion' twin-channel recorder. On the other track of the tape, electronically generated wide-band noise was recorded, and the two channels were mixed to a single external speaker.

**Step 3.** Because of the dynamic range of speech, and the distinct possibility that the vocal level of the speaker might unconsciously vary with the emotional quality of the word, one cannot by physical control guarantee sufficiently identical stimuli. It is known, however, that word probability and emotionality effects are greatly reduced by a "forced-choice" technique, where the listener is told a small set of alternatives and asked to pick the one which he has heard. Consequently, the stimulus tape with its accompanying noise was played to a group of six subjects who were provided with the list of stimulus words. A forced-choice score for each word was thus available, obtained on the actual tape and sample of noise to be used in the principal experiment, so that it should accurately reflect the quality of the physical stimulus. By eliminating five words from each group of words, three groups each of fifteen were produced (see Table 2) which were equal in score on the forced-choice procedure, and only these stimulus words were scored in the remainder of the experiment.

**Step 4.** The tape was then presented to twenty-two housewives, aged between 20 and 50 years, who had no knowledge of the vocabulary and were instructed simply to write down whatever word they thought they had

Table 2. HIGH FREQUENCY WORDS

Class	Good		Neutral		Bad	
Stimulus words	bed	6.36	deep	3.73	bad	1.77
	clean	6.36	far	3.95	blood	2.86
	dream	5.36	fast	4.27	death	2.04
	eat	5.68	foot	4.04	fail	2.18
	good	6.36	long	4.14	fat	2.73
	health	6.00	mind	4.41	fear	1.36
	light	5.73	pay	4.94	hang	2.23
	nice	5.41	plain	3.59	hard	2.59
	peace	6.86	red	5.14	kill	1.23
	rose	6.68	round	4.45	late	2.82
	see	6.00	run	4.36	loss	1.69
	sleep	6.64	short	3.68	pain	1.45
	wash	5.54	square	4.00	poor	1.77
	wise	5.86	stop	3.77	sick	1.41
	wish	5.32	taste	5.27	war	1.18
	Mean rating	6.01		4.25		1.95
	Class boundaries	7.00-5.30		5.29-3.10		3.09-1.00

heard after each stimulus; they were encouraged to put down a word even if they felt very uncertain about its being correct. They were told that all the words were monosyllabic.

**Step 5.** From the answer sheets in this experiment, there were collected together those erroneous responses which were made to the forty-five selected words, and which lay in the same frequency class as the stimuli: that is, which were *AA* words. There were 125 such words, and these were administered to a fresh group of thirty-two housewives who were asked to rate them for pleasantness and unpleasantness by the same procedure as the original stimuli. This step therefore established how many errors were made in each class of emotional quality, to each class of stimulus, and ignoring words in the wrong frequency class. (An advantage of the mathematics used is that any responses outside the classes of interest can be ignored without affecting the argument.)

**Step 6.** In order to determine the relative numbers of words in each class in the language, a random sample of 103 words was obtained from the Thorndike-Lorge frequency count by taking all *AA* monosyllables on every fifth page of the book. These again were rated for pleasantness and unpleasantness by the same group of thirty-two housewives as was mentioned in step 5. This then allows the relative numbers of words of this frequency class in each emotional class to be decided using the same cutting points already set up for the stimuli and the error words.

**Step 7.** The entire procedure was then repeated for a different frequency class of words, namely, monosyllables occurring between ten and forty-nine times per million in the Thorndike-Lorge count. There were the following minor differences in the procedure: nine subjects were used for the forced-choice responses, and only four words were eliminated from each group of twenty to produce balance of the tape recordings. The stimulus words used are shown in Table 3. Thirty-two housewives were used as listeners in the chief experiment: they produced ninety-two error words of the same frequency class as the stimuli, and these were rated by another group of thirty-one housewives. Intermingled with the error words in this rating were 121 words from a random sample from the Thorndike-Lorge count including all monosyllables of the appropriate frequency on every seventh page of the book. (A lower proportionate sample is necessary to produce the same number of words for rating, because by Zipf's law there are more words in the language of low frequency than of high.) Because low frequency words are on the whole slightly less pleasant<sup>2</sup>, the boundaries between the three classes of emotional quality had to be rather different from those of the high frequency words, but, just as in the earlier experiment, the same boundaries

were used for the stimuli, for the erroneous responses, and for the sample from the Thorndike-Lorge count.

One fairly important difference from the earlier experiment was that the subjects listening to the tape recording were asked after each response to write whether they felt sure or unsure that it was correct. This attempt to get additional data was an afterthought, which will be seen to be of possible importance.

The chief features of the results are set out in Table 4. It will be seen, first, that there is undoubtedly an effect of the emotionality of the stimulus on the number of correct responses, even with the precautions that had been taken, and with words that are completely acceptable socially. For the high frequency words, neutral words are significantly better than good words,  $P < 0.01$  by the Wilcoxon test, and than bad words,  $P < 0.05$ . For the low frequency words, the neutral stimuli are only significantly better than the bad words,  $P < 0.001$ , and are no better than the good words. The bad words are, however, also significantly worse than the good words,  $P < 0.01$ .

Table 4

		Good	Neutral	Bad
High frequency words	Correct responses	19.39	30.91	23.63
	Errors averaged for all types of stimulus	10.45	27.78	2.53
	Sample from T-L count	23.30	71.84	4.86
	Ratio of errors	4.13	10.98	1
	Ratio of sample	4.80	14.80	1
Low frequency words	Correct responses	21.09	22.07	12.30
	Errors averaged for all types of stimulus	2.02	7.10	5.28
	Sample from T-L count	19.82	47.12	33.06
	Ratio of errors	0.38	1.34	1
	Ratio of sample	0.60	1.43	1
	Proportion of wrong responses labelled "sure"	0.36	0.34	0.53

Turning now to the number of errors of each emotional class, no systematic differences between different types of stimuli appeared, and in particular there was no tendency for errors to be of the same class of emotionality as the stimulus. The data for all kinds of stimulus have therefore been averaged, and at first sight the ratio of errors of different types appears to follow the numbers of correct responses and therefore to show a bias in favour of the use of neutral rather than good or bad words. This is not the case, however, as we can see from the comparison between the ratio of errors produced and of words sampled at random from the language. It is quite clear that the ratios are closely similar and differences between them are quite insignificant. Indeed, the differences actually obtained are in some cases in the wrong direction to explain the effect on correct responses. The only reason why people mostly use words of neutral emotional tone as their misperceptions is that there are more words of that type available for them to produce.

On the model used in this analysis therefore we have to suppose that emotional words do not behave like words of low probability: there is not a constant bias against them in the perceptual mechanism, but there appears actually to be a failure of the stimulus information to reach the perceptual mechanism. In terms of the familiar parameters of the theory of signal detection by human operators<sup>8,11</sup>, the effect is on  $d'$  and not on  $\beta$ , whereas the effect of probability is on  $\beta$  and not on  $d'$ . The difference is consistent with certain discrepancies between the probability and emotionality effects found by Newbigger<sup>12,13</sup>.

Frankly we were not expecting these results, and have been described at an informal level as showing "that Freud was right after all". Two chief possibilities suggest themselves as mechanisms by which the effect could

Table 3. LOW FREQUENCY WORDS

Class	Good	Neutral	Bad
Stimulus words	bath 6.42	barn 4.16	ache 1.90
	breeze 5.35	brass 4.71	bleed 1.97
	bride 6.39	brick 4.13	brute 1.29
	cash 6.03	chant 4.32	crash 1.32
	cruise 6.45	crow 3.29	cruel 1.03
	feast 6.19	flock 4.42	dirt 1.55
	hug 6.03	halt 3.74	dread 1.42
	lawn 5.90	hut 3.97	fog 1.77
	mirth 6.06	mode 4.00	fright 1.35
	neat 5.52	moist 3.26	grief 1.03
	peach 6.19	plough 4.77	guilt 1.23
	ripe 5.77	puff 3.96	mock 2.16
	soup 5.13	purse 4.77	scream 1.61
	spice 4.84	scarf 4.35	stale 2.06
	thrill 6.39	sixth 4.13	starve 1.19
	tune 5.64	starch 3.93	whip 1.74
Mean rating	5.89	4.11	1.54
Class boundaries	7.00-4.80	4.79-3.10	3.09-1.00



occur without requiring a complete duplication of the perceptual mechanism. First, it would be possible for those random variables in the model which correspond to emotional words to have in the absence of stimuli the same average value as those for neutral words. When rises in the value of such a variable occur, however caused, they produce some physiological change which reduces the level of sensory input. This would not prevent emotional words from occurring as wrong responses, but would make it less likely that they would occur as correct ones. This theory would have some difficulty, not perhaps completely fatal, with the fact that emotional words are no more common as errors to emotional stimuli than they are to neutral stimuli. If the information has been sufficiently extracted to have an effect on the sensory input, one would expect it to show in the nature of the error made.

The second possible theory is that which at present attracts us; and this is that our calculations have misrepresented the true state of affairs by supposing equal variances for each word. If the emotional words possessed a higher variance in the absence of stimulation, but the same mean value as neutral words, then the effect we have obtained would have been expected. That is, if emotional words were sometimes receiving a positive bias and sometimes a negative one, because of passing states of mood, and did this to a greater extent than neutral words but received the same change in their mean value from sensory information, then we would expect the results that have actually been obtained. Unfortunately, there is no way of checking this conjecture, except through the judgments of "sure" and "unsure" which were obtained in the experiment on low frequency words. Not only is there no established way of analysing experimental data if the underlying decision process is of unequal variance, but there is even less possibility of assigning a meaning to ratings of confidence in a multi-alternative situation. The following argument, however, seems intuitively clear.

Let us ignore the ratings of confidence on correct responses, and consider only those on errors. Whatever the mechanism for assigning confidence to an erroneous response, it would seem plausible that a higher proportion of responses would receive a high rating of confidence if the variable underlying them has a larger variance. The upper tail of the distribution of such a variable will stand out above the upper tails of variables with a smaller variance. In fact the proportions of error responses labelled "sure" rather than "unsure" are given in the last line of Table 4, and it will be seen that bad words tend to be produced as errors very confidently, as compared with neutral words. There is therefore some evidence, albeit lacking in rigour, that the resting bias on bad words varies more seriously than that on neutral words, and this might conceivably explain the difficulty of perceiving them. As things stand, however, it is clear only that the emotionality of words has an effect on their perception which is different from that of probability, and that it is not to be explained by a steady bias against such words in the perceptual mechanism before sensory evidence is received.

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## Significance of the Binding of Iron by Transferrin

by

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Iron is carried in blood plasma by the protein transferrin, which is able to carry two atoms of iron. The availability of bound iron to immature red blood cells seems to vary according to the way in which the iron is distributed on the molecule of transferrin.

THE fact that the plasma iron is carried on a single protein, transferrin, does not necessarily mean that it forms a uniform pool<sup>1</sup>. Each molecule of the protein can carry two atoms of iron, and because complexing with iron produces changes in the conformation or charge of the protein<sup>2,3</sup> there may be differences between molecules carrying one or two atoms of iron. There may also be differences in the behaviour of iron atoms attached to one of the two binding sites rather than the other.

The first set of experiments was devised to test whether or not iron bound to transferrin in the plasma is uniformly available for uptake by immature red cells. Iron free plasma was labelled with a mixture of iron-59 and carrier iron approximately to half saturate the total iron binding capacity (level A). The iron free plasma was prepared by dialysis against acetate buffer pH 6.0, containing 0.01

molar sodium EDTA followed by dialysis to pH 7.4 in a buffer containing Krebs-Ringer bicarbonate. Complete removal of EDTA was ensured by dialysis against several changes of the latter buffer. The radioactivity present in the plasma was then proportional to its total content of iron. This plasma was incubated with reticulocytes until approximately half of the iron had been removed (level B). A control plasma was prepared by adding the same mixture of iron-59 and carrier iron to a further sample of iron free plasma until the percentage saturation of the two samples was equal at level B. The uptake of iron into fresh reticulocytes from both samples was then measured during incubation at 37° C (Fig. 1). It will be noted that in both plasmas the amounts of radioactivity, total iron and transferrin, as well as reticulocytes, were equal. If some iron bound to transferrin, however, is preferentially available to the reticulocyte most of this will have been removed from the sample of plasma which was pre-

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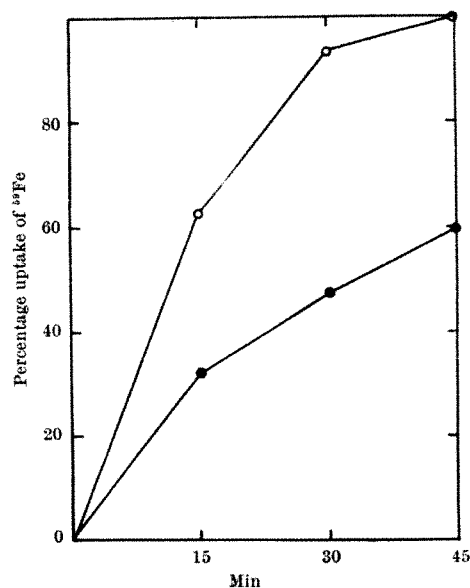


Fig. 1. Comparison of rate of uptake of  $^{59}\text{Fe}$  by reticulocytes.  $\circ$ , Control plasma;  $\bullet$ , plasma preincubated with reticulocytes.

incubated. The reduced uptake of iron by fresh reticulocytes from the preincubated plasma is therefore consistent with the idea that the iron in the plasma is not a uniform pool but that some is more readily available than the rest. As a control experiment, a mixture of iron-59 and carrier iron was added to both samples to restore the total iron from level B to level A, the level in the test sample before any treatment with reticulocytes. As expected, the difference was markedly reduced, falling from 61 per cent to 12 per cent. Similar results were obtained when plasma from a patient with severe iron deficiency was used instead of dialysed plasma. This result excluded the possibility that the observed differences in uptake were due to denaturation of the transferrin during the dialysis procedure. It is therefore concluded that all iron bound to transferrin is not equally available to immature red cells.

The cause of this could be either preferential uptake of iron from saturated transferrin,  $\text{Tf}(\text{Fe})_2$ , rather than from half saturated molecules,  $\text{Tf}(\text{Fe})_1$ , or the preferential uptake from some binding sites. When unlabelled iron is added to both the preincubated and the control samples of plasma to saturate the iron binding capacity, the difference in uptake persists. In these conditions only  $\text{Tf}(\text{Fe})_2$  molecules are present, and so the difference must be caused by different rates of uptake from some of the binding sites.

The factors that determine which iron is removed first by reticulocytes were further investigated by a different type of experiment. In this, the rates of uptake of iron-59 by reticulocytes added at different percentage saturations of transferrin were directly compared. In order to get a valid comparison, the total iron, transferrin and radioactivity in each sample must be identical and this can be achieved by a method developed from that used by Schade to test the integrity of purified transferrin<sup>4</sup>. A tracer amount of iron-59 is added to equal amounts of two plasmas, A and B, and allowed to combine with the transferrin. To plasma A an equal amount of unlabelled plasma B is added and to plasma B an equal amount of

Plasma A	Plasma B
+	+
Tracer $^{59}\text{Fe}$	Tracer $^{59}\text{Fe}$
Allow to stand for 1 h for combination to take place	
+	+
Plasma B	Plasma A
Follow uptake of $^{59}\text{Fe}$ in reticulocytes	

Fig. 2. Scheme for the comparison of  $^{59}\text{Fe}$  uptake by reticulocytes from labelled plasmas. The method ensured that samples are identical in all respects except that  $^{59}\text{Fe}$  is attached to the normal plasma in one case and the abnormal plasma in the other.

unlabelled plasma A is added (Fig. 2). The two samples are now identical except that in one case the radioactive label is on plasma A while in the second it is on plasma B. The uptake of radioactivity by reticulocytes from each sample is then measured. In the first experiment of this type a fresh plasma at 25 per cent saturation was divided into two parts and the saturation of one increased to 50 per cent by adding cold iron. The uptake of iron-59 from each sample by reticulocytes was then measured as described here. Iron-59 added at 50 per cent saturation was taken up by the reticulocytes more rapidly than that added at 25 per cent saturation (Fig. 3). In contrast to this, total iron uptake from both samples was identical. The difference in uptake of iron-59 disappeared when both mixtures were saturated with cold iron before uptake was measured. In interpreting these results it has to be remembered that the added iron-59 reflects the binding sites on transferrin originally not occupied by iron. Iron added to plasma *in vitro* combines with the binding sites at random<sup>5</sup> and therefore according to their availability. At low percentage saturation there will be a large pool of iron free transferrin, some  $\text{Tf}(\text{Fe})_1$ , and very little  $\text{Tf}(\text{Fe})_2$ , so that a tracer amount of iron-59 will combine mainly with iron free transferrin to produce labelled  $\text{Tf}(\text{Fe})_1$ . At greater saturations the pool of iron free transferrin will be smaller, whereas the amount of  $\text{Tf}(\text{Fe})_1$  and of  $\text{Tf}(\text{Fe})_2$  will increase, and added tracer iron-59 will combine more frequently with  $\text{Tf}(\text{Fe})_1$  to produce labelled  $\text{Tf}(\text{Fe})_2$ , that is  $\text{Tf}(\text{Fe})_2$  ( $^{59}\text{Fe}$ ). The results of this experiment therefore show that there is a difference in behaviour between half saturated,  $\text{Tf}(\text{Fe})_1$ , and saturated,  $\text{Tf}(\text{Fe})_2$ , forms of transferrin, for at 50 per cent saturation many more saturated molecules are labelled than at 25 per cent. Final saturation with cold iron converts all molecular species to the saturated form,  $\text{Tf}(\text{Fe})_2$ , and so the difference in uptake of the label disappears. At any given saturation the relatively small quantity of iron added as iron-59 will produce very few doubly labelled molecules  $\text{Tf}(\text{Fe})_2$  at either level of saturation. The difference observed is therefore caused by a specific effect of carrying two iron atoms rather than one iron atom and not merely that reticulocytes remove both iron atoms from one molecule of transferrin. Indeed, if the difference were caused by doubly labelled molecules it would not disappear when the mixture is finally saturated with cold iron.

The following experiments show that the effect of percentage saturation is not the only factor, but that there is

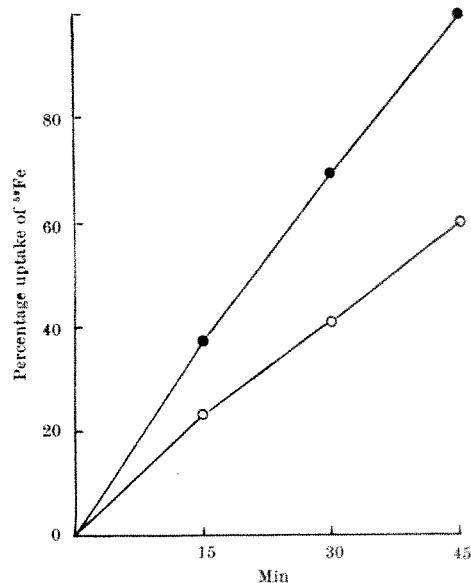


Fig. 3. Comparison of uptake by reticulocytes of  $^{59}\text{Fe}$  from plasma at different percentage saturations using the labelling method given in Fig. 2.  $\bullet$ , Tracer  $^{59}\text{Fe}$  added at 50 per cent saturation;  $\circ$ , tracer  $^{59}\text{Fe}$  added at 25 per cent saturation.

also a functional difference between some of the binding sites, the iron attached to some being preferred by reticulocytes. Plasma was divided into two parts and the saturation of one part was reduced by incubation with reticulocytes before performing the experiments described earlier (Fig. 2). Again, the uptake of the label was faster from the plasma with the greater percentage saturation (Fig. 4a). When both mixtures were saturated with cold iron, however, the uptake became faster from the mixture in which the label had been added to plasma at a smaller percentage saturation, that is the plasma preincubated with reticulocytes (Fig. 4b). This suggests that some binding sites preferentially deliver their iron to the reticulocytes.

Having defined these two effects it is possible to confirm that they occur *in vivo* by examining plasma from patients with inactive and overactive erythropoiesis. The same technique (Fig. 2) is used with normal plasma and plasma from suitable patients. The results are shown in Table 1. When the labelled iron is attached to plasma from patients with aplastic anaemia it is taken up by reticulocytes more slowly than would be expected from the percentage saturation and this behaviour is accentuated by saturation of the final mixtures with cold iron. By contrast, uptake from the plasma of patients with overactive erythropoiesis (haemolytic anaemias) is faster than from normal plasma with comparable percentage saturation of iron. This is in keeping with the idea that overactive erythropoiesis clears iron from some sites in preference to others. The difference, however, between the control plasma and the test plasma on saturation was only maintained in some instances. The interpretation of this result is at present not clear.

The evidence presented supports the view that the plasma iron is not a single pool equally available for uptake by immature red cells. The availability varies according

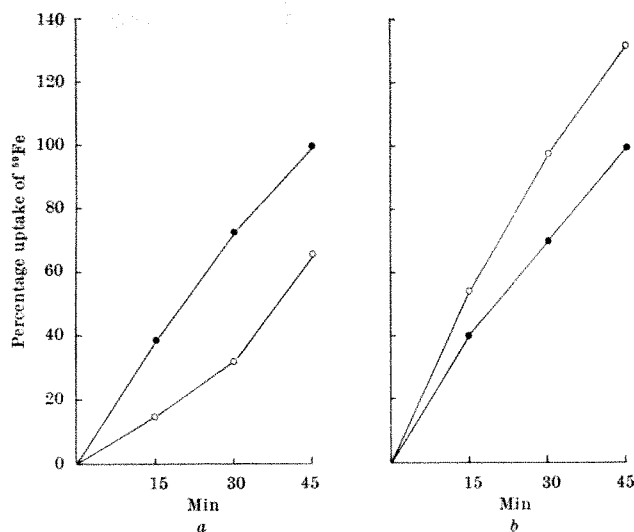


Fig. 4. Comparison of uptake by reticulocytes of  $^{59}\text{Fe}$  from plasma, one sample having been preincubated with reticulocytes to reduce the percentage saturation. The labelling method is shown in Fig. 2. a, Not saturated; b, finally saturated with cold iron. ●, Tracer  $^{59}\text{Fe}$  added at 34 per cent saturation; ○, tracer  $^{59}\text{Fe}$  added at 16 per cent saturation.

Table 1. COMPARISON OF UPTAKE BY RETICULOCYTES FROM VARIOUS PLASMAS USING THE METHOD OUTLINED IN FIG. 2

Source of plasma	Percentage saturation of transferrin	Percentage difference in uptake of $^{59}\text{Fe}$ at 15 min compared with normal male plasma	
		Not saturated	Finally saturated with cold iron
Normal male	25	100	100
Normal menstruating female	23	110	—
Inactive erythropoiesis (aplastic anaemia)			
Case 1	25	93	82
Case 2	59	90	53
Overactive erythropoiesis due to			
Pernicious anaemia	33	135	129
Thalassaemia major			
Case 1	22	112	102
Case 2	54	213	101

to the way in which the iron is distributed on the carrier protein, transferrin. There appear to be two distinct effects and these may be important at different stages in the process of iron uptake by reticulocytes. The first step in this process is the combination of transferrin with a receptor on the cell surface. The work of Jandl and Katz<sup>6</sup> suggests that a difference between saturated and half saturated transferrin would be important at this stage probably because the saturated molecular species competes more successfully for the receptor than the half saturated species. The difference between binding sites will presumably affect the next stage when iron is taken up into immature red cells, the iron on some sites being preferred. It is not suggested that the other sites are unable to deliver iron to these cells, for reticulocytes can clear transferrin almost completely of iron.

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## Gravitational and Cosmological Red-shift for Quasars

by

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Barbieri has shown that it is possible to differentiate between the gravitational and cosmological components of the red-shift of quasars by observational means. On the other hand, the interpretation of the results depends to a considerable extent on whether the source is considered to be static or expanding.

Most of the work on the nature of quasi-stellar sources, following the principal line of the basic paper by Greenstein and Schmidt<sup>1</sup>, has been based on a somewhat *a priori* assumption about the physical cause of the red-shift  $z$ . Recently, however, Barbieri<sup>2</sup> has shown that the relative importance of both its possible cosmological and gravita-

tional parts  $z_c$  and  $z_g$  can be obtained from purely experimental data, if the quasar is assumed to be constructed according to a model of the Greenstein-Schmidt type. Barbieri's procedure is based on six equations. These include the three relations 1, 2 and  $3b + 4a$  of Greenstein and Schmidt, expressing the gravitational red-shift  $z_g$ , the width  $w_g$  of emission lines due to the gravitational

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effect and the relation between observed brightness  $F$  of  $H_\beta$  and emission from the volume of ionized hydrogen where the lines are produced.

$$z_g = \frac{\Delta\lambda_g}{\lambda_0} = \frac{GM}{c^2 R} = 1.47 \times 10^5 \frac{M/M_\odot}{R} \quad (1)$$

$$w_g = \frac{\Delta R}{R} \Delta\lambda_g = \frac{\Delta R}{R} \lambda_0 z_g \quad (2)$$

$$4\pi r^2 F = 4\pi R^2 \Delta R E \quad (3)$$

where  $M$  and  $R$  are the mass and radius of the quasar,  $G$  the gravitational constant,  $c$  the velocity of light,  $\Delta R$  the depth of the atmosphere surrounding the dense core in which the emission lines are formed,  $r$  the distance of the quasar and  $E$  the volume emissivity in  $H_\beta$ .

To these are added three further relations, giving the total red-shift, the connexion (for a euclidian metric) of the cosmological red-shift with distance, and the geometrical relation between distance and the diameter of the quasar

$$z = z_g + z_c \quad (4)$$

$$r = \frac{cz_c}{H} \quad (5)$$

$$\frac{2R}{r} = \frac{\theta}{K} \quad (6)$$

$H$  is the Hubble constant,  $\theta$  the angular diameter in seconds of arc and  $K = 2.06 \times 10^5$ .

These six equations make it possible to determine the six unknown characteristics of the quasar,  $M$ ,  $R$ ,  $\Delta R$ ,  $r$ ,  $z_g$ ,  $z_c$ , for all the other quantities necessary can be obtained experimentally, at least in principle; and, using the results so far available for 3C 273, Barbieri obtains as the chief feature of this quasar a high ratio for  $z_g/z_c$ , of the order of 10–100, depending on the magnitude of the line width  $w$ , and a distance  $r$  reduced by a factor between 4 and 10 in respect of the purely cosmological one.

This procedure, of course, hinges on the assumptions fixing some of the quantities necessary to solve equations (1)–(6), and mainly on the line width  $w$ , which is taken to be the sum of the gravitational width  $w_g$  plus the width  $w_s$  due to the scattering of the line in the emitting atmosphere. This latter quantity can be evaluated theoretically<sup>3</sup> and has in fact been estimated for several lines of different quasars, including 3C 273, by Burbidge *et al.*<sup>4</sup>;  $w_g$  is then obtained by subtracting  $w_s$  from the total measured width  $w$ .

The model for a quasar underlying these considerations thus turns out to be static, in the sense that possible changes occurring in its structure are considered to be sufficiently slow not to produce any detectable effect. Most of the phenomenology which is actually being observed on several quasars, however, seems rather to suggest a dynamical situation, usually described as a state of expansion of the inner core, either continuous or in separate outbursts. Barbieri is induced by these facts to go somewhat beyond his own assumption by completing it on the following lines: the higher red-shifts of quasars in respect to radio galaxies may be due to their excess gravitational red-shift; and it might be expected that, because of expansion, these highly concentrated structures could gradually transform to normal radiogalaxies, readjusting their red-shift in the process to its natural cosmological proportionality with distance.

It thus appears that a logical extension of Barbieri's procedure might consist in dropping his static assumption, and completing his set of equations by a further group expressing the dynamical consequences of the expansion of the quasar. We have then to consider an expansion width  $w_e$  of the emission lines to be added to the other

widths, while the expansion red-shift  $z_e$  can be completely neglected in respect to the cosmological red-shift, because the maximum expansion velocity, when all the line width is attributed to emission, could at most be of the order of 1,000 km/sec.

The aim of this note is to present a very simple consequence which follows immediately from the introduction of this new term.

Let us in fact add the following equations to equations (1)–(6)

$$w_g = w - \Delta s - w_e \quad (7)$$

$$w_e = \frac{2v}{c} \lambda_0 \quad (8)$$

where  $\Delta s$  is an appropriate correction for the broadening due to scattering, and  $v$  is the expansion velocity of the atmosphere emitting the lines.

Although  $w_e$  cannot be evaluated because we are unable actually to measure  $v$ , we can, however, assuming that the system is expanding, already put as a lower limit for  $v$ , the value  $v_e$  of the escape velocity

$$v_e = \sqrt{2GM/R}$$

By introducing this expression into equation (8), equations (1)–(8) can be solved in respect to the six unknowns already mentioned and  $w_e$  and  $w_g$ , yielding the minimum values of  $w_e$  and  $z_c$ , and therefore the maximum values of  $w_g$  and  $z_g$ , compatible with expansion. We thus get for the ratio  $z_g/z_c = 1/y^2$

$$\frac{1}{A} = (y^2 - 1) \left[ 1 - \frac{B}{y} \right] \quad (9)$$

where

$$A = \frac{cE\theta^3(w - \Delta s)}{8K^3 H \lambda_0 F} \quad B = \frac{2\sqrt{2z} \lambda_0}{w - \Delta s}$$

It is immediately apparent from the expression for  $B$  that in all known cases it will be larger than unity. On the other hand, the right-hand side is negative when  $y < B$ , and therefore no solutions exist. For  $y > B$ , the right-hand side is positive and increases steadily from 0 to  $\infty$  as  $y$  increases from  $B$  to  $\infty$ . There is therefore a solution  $y$  for any value of  $A$ ;  $y = B$  for  $A = \infty$ , and increases steadily as  $A$  decreases.  $y = B$  is then the minimum value which can be obtained for any  $A$  and corresponds to the highest percentage of gravitational red-shift. Let us apply these considerations to the case of 3C 273  $B$ , and take  $z = 0.158$ ;  $\lambda_0 = 4861 \text{ \AA}$  ( $H_\beta$ ),  $w = 50 \text{ \AA}$ . Even neglecting the scattering correction  $\Delta s$  in order to get the smallest possible value, we obtain

$$B = 1.1 \times 10^2$$

From this we get the upper limit for the gravitational red-shift

$$\frac{z_g}{z} = 0.83 \times 10^{-4}$$

quite irrespective of any other determination of the remaining parameters of the source.

The argument can be easily extended even to distant quasars, for which the correlation between distance and red-shift may be non-linear and usually expressed as

$$r = \frac{c}{H} f(z_c) \quad (5a)$$

where  $f(z_c)$  may be any function always increasing with  $z_c$ , depending on the special cosmological model. Then equation (9) must be replaced by

$$\frac{1}{A} = \frac{y^2}{z} f \left[ z \left( 1 - \frac{1}{y^2} \right) \right] \left( 1 - \frac{B}{y} \right) \quad (9a)$$



and, as the right-hand side is again positive and increases steadily with increasing  $y$ , when  $y > B$  and  $z$  is kept constant for any quasar, the proof follows in exactly the same way, and  $y = B$  is again the solution corresponding to the highest possible gravitational red-shift. This conclusion still holds when the exact expression for the gravitational red-shift is used instead of equation (1). Barbieri's type of approach to the analysis of quasars, although apparently phenomenological and a straightforward way for gathering objective information, is still strongly dependent on the fundamental assumptions concerning the nature of the source. In fact, should we assume that quasars are in a static phase, a rather important percentage of the red-shift should be gravitational. If, on the other hand, quasars are expanding, then we are compelled to set an upper limit to the gravitational red-

shift which is practically negligible. Should quasars be structures alternatively quiescent and active as the phenomenological distinction between quasi-stellar objects and quasi-stellar sources might indicate, we should expect to find substantial differences in the emission lines between these two phases; QSS lines should be broader than QSO lines, because the expansion width is added to the basic gravitational contribution; this does not appear to be the case according to the present data.

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## In vitro Synthesis of Bacteriophage Lysozyme

by

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Active lysozyme of bacteriophage  $T_4$  has been synthesized in a cell free system programmed by RNA from cells infected with the phage. The appearance of this lysozyme activity has the characteristics of *de novo* synthesis.

THE advantages of achieving the *in vitro* synthesis of active enzymes in a well characterized system are many. Not only would it allow a more precise definition of the components and conditions necessary for protein synthesis, but it should also provide a useful method for the study of factors controlling the rate of protein synthesis, at the level of translation or transcription. Until now, however, there have been few studies of *in vitro* synthesis of specific proteins, and only in the case of haemoglobin and of proteins programmed by genomes of RNA viruses<sup>1-4</sup> have peptide analyses shown good correspondence between the *in vitro* and natural products. Unfortunately, none of these products has enzyme activity, which makes it difficult to assess the fidelity of the *in vitro* translation.

Of particular importance will be the development of systems where DNA from organisms with well defined operator-repressor control is the source of genetic information. Thus the findings by DeVries and Zubay<sup>5a</sup>, Schweiger, Traub and Zillig (personal communication), and Imai *et al.*<sup>5b</sup>, on the DNA dependent synthesis of a fragment of  $\beta$ -galactosidase, of  $T_4$  deoxycytidylate deaminase and tryptophane synthetase, respectively, are of great interest. In this direction we have been looking for synthesis of  $T_4$  specific proteins using RNA extracted from phage infected cells. Initially we feared that the extracted RNA might be partially degraded so that it could not direct the synthesis of complete proteins, but this is not the case. Our first series of experiments showed the efficient synthesis of at least a fragment of the head protein of  $T_4$  in extracts from uninfected cells. This suggested that modifications of the translation machinery caused by  $T_4$  infection<sup>6-9</sup> are not essential for reading the head protein messenger.

Soon afterwards we found that active  $T_4$  lysozyme is made in the same system. This enzyme was chosen in particular because the assay is very sensitive and because it is a relatively small protein (molecular weight 18,000) with a single well characterized polypeptide chain<sup>10</sup>. Moreover, extracts of uninfected *E. coli* lack lysozyme, so that the use of  $T_4$  mRNA in these extracts precludes

possible artefacts such as chain completion or activation of pre-existing enzyme.

We present here the results of our experiments with lysozyme, and those concerning the head protein will be reported elsewhere.

The RNA used to programme the *in vitro* lysozyme synthesis was prepared as follows. *E. coli* RNase  $I_{10}$  (ref. 11) was grown in *M-9* medium and infected with  $T_4$  D as described in method III of Bolle, Epstein, Salser and Geiduschek (submitted to *J. Mol. Biol.*). Cultures containing about  $3 \times 10^{12}$  cells were poured on ice and collected in a Sharples continuous flow centrifuge. To extract the RNA, the cells were resuspended in 40 ml. of 0.01 molar potassium chloride, 0.005 molar magnesium chloride, and 0.01 molar *tris* buffer, pH 7.3. Egg-white lysozyme was then added to a final concentration of 300  $\mu$ g/ml., and the suspension was frozen in liquid air. The cells were thawed and sodium dodecyl sulphate was added to a final concentration of 1 per cent. The viscous solution was briefly warmed in a bath at 64° C until a decrease in opacity of the suspension indicated cell lysis. Sodium acetate buffer, pH 5.2, was then added to a final concentration of 0.1 molar, followed by an equal volume of phenol saturated with water. The phenol extraction lasted 4 min and was carried out in a New Brunswick rotary shaking water bath at top speed and 64° C. The temperature of the extraction mixture reached about 61° C during this time. The aqueous phase was extracted with phenol a second time in the same way and the RNA was precipitated from 2 molar sodium chloride with 1.5 volumes of ethanol. The precipitated RNA was washed three times with 70 per cent ethanol plus 0.01 molar *tris*, 0.01 molar sodium chloride, dissolved in water and clarified by centrifugation at 27,000*g* for 20 min. After dialysis against deionized water for 4 h the concentration of RNA was adjusted to 8 mg/ml., and the solution was frozen in small samples and stored at -20° C. More vigorous techniques, such as five phenol extractions lasting 8 min each at 68° C, have also given RNA that is active for *in vitro* synthesis of lysozyme, but the result is

less dependable and considerable degradation of  $^{23}\text{S}$  RNA has been observed in such cases.

$S\text{-}30$  extracts from *E. coli* strain RNase  $I_{10}^-$  were prepared by the method of Nirenberg and Matthaei<sup>12</sup> as used by Capecchi<sup>3</sup>. The cell free extracts were dialysed for 6 h against 10 mmolar magnesium acetate, 10 mmolar *tris* buffer, pH 7.5, 30 mmolar potassium chloride, and 6 mmolar mercaptoethanol, frozen in small samples and stored at  $-20^\circ\text{C}$ . Before each experiment the  $S\text{-}30$  extract was pre-incubated for 30 min at  $36^\circ\text{C}$  to reduce endogenous protein synthesis. The pre-incubation mixture contained in each ml. 0.5 ml. of  $S\text{-}30$  extract; 50  $\mu\text{moles}$  of *tris* buffer, pH 7.8; 60  $\mu\text{moles}$  of ammonium chloride; 1  $\mu\text{mole}$  of dithiothreitol (Cleland's reagent); 0.1  $\mu\text{mole}$  of each of nineteen amino-acids (minus leucine); 0.02–0.04  $\mu\text{mole}$  of leucine (as indicated); and 5  $\mu\text{mole}$  of magnesium acetate in addition to the 5  $\mu\text{mole}$  from the  $S\text{-}30$  extract. It was found that the addition of an energy generating system during the pre-incubation does not give a further reduction in the endogenous activity.

The incubation mixture for *in vitro* protein synthesis contained in each ml. (including the contribution of the pre-incubation mixture): 0.25 ml. of pre-incubated  $S\text{-}30$  extract (final protein concentration of about 3 mg/ml. and 70S ribosome concentration of about 1 mg/ml.); 63  $\mu\text{moles}$  of *tris* buffer, pH 7.8; 50  $\mu\text{moles}$  of ammonium chloride; 8  $\mu\text{moles}$  of magnesium acetate (unless stated otherwise); 0.14  $\mu\text{moles}$  each of nineteen amino-acids (minus leucine), tritiated leucine (4 mc./ $\mu\text{mole}$ ) to give the specific activities indicated; 3  $\mu\text{mole}$  of ATP; 0.2  $\mu\text{moles}$  of GTP, 5  $\mu\text{moles}$  of phosphoenol pyruvate; 20  $\mu\text{g}$  of pyruvate kinase; 5  $\mu\text{moles}$  of polyethylene glycol of average molecular weight 6,000, and RNA fractions as indicated. The polyethylene glycol was added because it caused a 1.2–3-fold stimulation of the rate of incorporation of amino-acids (unpublished work of Gesteland). After incubation of the mixture at  $36^\circ\text{C}$  for the times noted, 0.1 ml. samples were removed and frozen for later assays of lysozyme. Incorporation of tritiated leucine into material insoluble in hot trichloroacetic acid was determined on another sample.

Fig. 1 shows the stimulation of incorporation of tritiated leucine into polypeptide, *in vitro*, by various concentrations of the RNA preparations. Bacteriophage  $R_{17}$

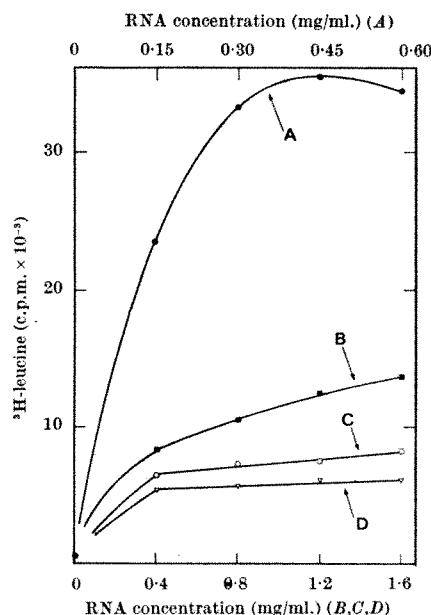


Fig. 1. RNA dependence of incorporation of amino-acids. Each point represents the hot trichloroacetic acid insoluble material in 0.1 ml. of reaction mixture. Incubation was for 20 min and the specific activity of the leucine was about 160 c.p.m./ $\mu\text{mole}$ , assuming no dilution of the 0.005  $\mu\text{moles/ml.}$  of added leucine. (A)  $R_{17}$  RNA; (B)  $T_4$  20 min RNA; (C)  $T_4$  5 min RNA; (D) RNA from uninfected *E. coli*.

RNA (refs. 4 and 13), which is used as a standard messenger for comparison, normally produces a thirty- to eighty-fold stimulation over the background with no added RNA. The RNA extracted from uninfected *E. coli*, or from cells infected for 5 or 20 min with  $T_4$  ( $T_4$  5 min RNA or  $T_4$  20 min RNA), is three to seven times less active on a weight basis than  $R_{17}$  RNA. Because, however, only 3 per cent of the *E. coli* RNA (Salser, Janin and Levinthal, *J. Mol. Biol.*, in the press) and 5 per cent of the  $T_4$  RNA (ref. 14) is messenger, this mRNA fraction is, if anything, more active than  $R_{17}$  RNA. The *E. coli* and  $T_4$  RNAs tend to saturate amino-acid incorporation at lower levels than does  $R_{17}$  RNA.

Typical results of lysozyme assays are shown in Fig. 2, where turbidity of the lysozyme substrate is plotted as a function of time. If a sample from an *in vitro* protein synthesis mixture, incubated with no added RNA, is mixed with the substrate, there is a slow background decrease in the turbidity (curve A). Curve B shows the result obtained when the *in vitro* system is supplemented with RNA extracted from cells 20 min after infection with  $T_4$ . The rapid decrease in turbidity indicates the presence of active lysozyme. Table 1 gives the data for incorporation of tritiated leucine and for lysozyme activity calculated from the maximum slope of curves such as these.

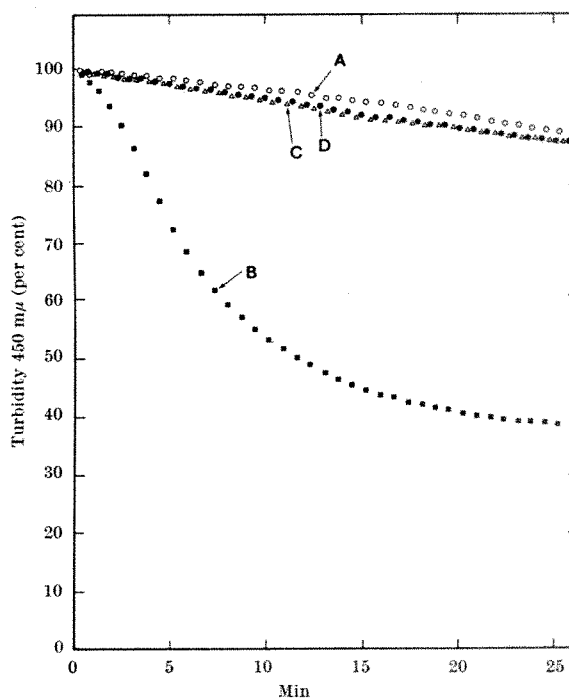


Fig. 2. Lysozyme assay. The substrate consists of *E. coli* B cells treated with chloroform and washed by the method of Sekiguchi and Cohen<sup>15</sup> but containing in addition to 0.05 molar *tris* buffer, pH 7.5, 1 mmolar magnesium acetate and 0.6 mmolar mercaptoethanol. The substrate is pre-incubated at  $25^\circ\text{C}$  for 10 min, then 0.1 ml. of an *in vitro* incubation mixture is added to each cuvette containing 1.0 ml. of the substrate. Lysis of the cells is followed at 450 mμ and  $25^\circ\text{C}$  in a Gilford recording spectrophotometer. The initial optical density is 0.4. The *in vitro* system was supplemented with: (A) no added RNA; (B)  $T_4$  20 min RNA (400  $\mu\text{g/ml.}$ ); (C)  $T_4$  5 min RNA (400  $\mu\text{g/ml.}$ ); (D) RNA from uninfected cells (400  $\mu\text{g/ml.}$ ).

In spite of the stimulation of incorporation of tritiated leucine by RNA from uninfected cells or from cells infected for only 5 min with  $T_4$ , neither RNA stimulates the production of lysozyme activity, as is shown in curves C and D of Fig. 2, and in Table 1. The lack of activity of the 5 min RNA is expected because *in vivo* synthesis of phage lysozyme does not begin until after this time<sup>16</sup>.

To further support the view that the lysozyme synthesis is *de novo*, RNA was extracted from cells (RNase  $I_{10}^-$ , *su*<sup>-</sup>) infected with the phage mutant *am* H-26 which contains an amber mutation in the structural gene for the phage lyso-

zyme. This RNA, while active for the incorporation of amino-acids, does not stimulate the synthesis of lysozyme (Table 1). The mutant phenotype of *am* H-26 does not seem to include pleiotropic effects on other late messengers, for a normal yield of mature phage can be released from the cells with egg-white lysozyme. Consequently, this result shows that the enzyme activity found in the products of *in vitro* synthesis is specific for the phage lysozyme messenger. RNA extracted after 20 min of infection with mutants in another late gene (*am* H-11 and *am* A-489, both in the structural gene for  $T_4$  head protein, results not shown) is fully active in programming the *in vitro* synthesis of lysozyme activity as expected.

Table 1. AMINO-ACID INCORPORATION AND LYSOZYME ACTIVITY IN PRODUCTS OF *in vitro* PROTEIN SYNTHESIS

RNA added to complete system	Lysozyme activity	$\mu$ Moles of tritiated leucine incorporated/ml./20 min
$T_4$ 20 min RNA	39.3	722
RNA from uninfected cells	3.7	427
$T_4$ 5 min RNA	3.9	625
$T_4$ 20 min RNA from <i>am</i> H-26	2.0	698
$T_4$ 20 min RNA extracted without egg-white lysozyme	30.0	792
$T_4$ 20 min RNA—zero time	3.1	1
No added RNA	2.9	59
$R_{17}$ RNA (300 $\mu$ g/ml.)	2.8	1,420

The final concentration of RNA in the incubation mixture in each case was 400  $\mu$ g/ml. (except  $R_{17}$  RNA). Lysozyme activity was determined from tracings as shown in Fig. 2 by measuring the maximum slope of the decrease in turbidity, and is expressed as the decrease in optical density/min  $\times 1,000$ . The total optical density at time zero of the assay was near 0.4 in each case. The lysozyme activities are from 0.1 ml. of *in vitro* reaction mixture added to 1 ml. of the substrate. Assuming no dilution of the 0.01  $\mu$ mole/ml. of added leucine, 1  $\mu$ mole represents 55 c.p.m. (Leucine in the reaction mixture dilutes this 1.2–1.4-fold.)

Because all the RNA samples (including those from uninfected cells or cells infected for 5 min) used here were extracted with egg-white lysozyme to help open the cells, and because the active RNA samples come from cells making lysozyme, it is possible that the mRNA fractions could be contaminated with lysozyme activity. This is ruled out by the following control experiments. If the  $T_4$  20 min RNA is prepared with the omission of egg-white lysozyme (cells infected for 20 min are sufficiently opened without the enzyme), normal incorporation of amino-acids and lysozyme activity are observed in the *in vitro* system (Table 1). Negligible activity is found if the 20 min RNA preparations are assayed directly for lysozyme (results not shown). At zero time samples for *in vitro* synthesis with  $T_4$  20 min RNA show no activity (Table 1 and Fig. 3).

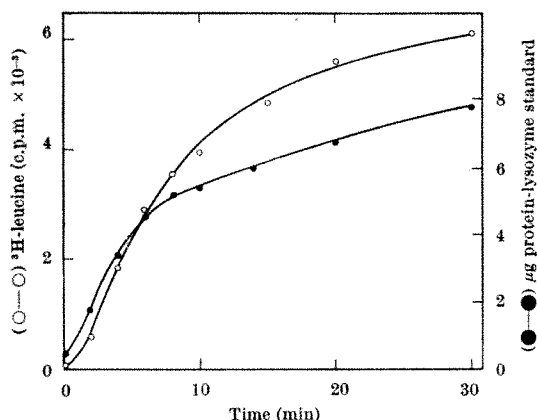


Fig. 3. Time course of synthesis. The *in vitro* protein synthesis is with  $T_4$  20 min RNA (400  $\mu$ g/ml.). Duplicate 0.1 ml. samples were withdrawn at intervals during the incubation and were assayed for lysozyme activity as in Fig. 2, and for incorporation of tritiated leucine (specific activity 55 c.p.m./ $\mu$ mole, as in Table 1). The lysozyme activity is expressed in terms of the protein in a crude  $T_4$  lysozyme standard used throughout these experiments. This standard was made by infecting *E. coli* RNase  $I_{17}$  with  $T_4$  for 30 min. The cells were collected, ground with alumina and centrifuged for 20 min at 18,000g. The supernatant was frozen in small samples and used to prepare standard curves relating the maximum rate of lysis of the substrate to  $\mu$ g of protein in the extract. The substrate varies from day to day and so a new standard curve is made each day.

Table 2. INHIBITORS OF PROTEIN SYNTHESIS

Additions	Lysozyme activity	$\mu$ Moles of tritiated leucine incorporated/ml./20 min
$T_4$ 20 min RNA +:		
Chloramphenicol, 0.5 mmolar		
Added after protein synthesis	36.3	737
Added before protein synthesis	2.5	3
Puromycin, 1 mmolar		
Added after protein synthesis	35.9	718
Added before protein synthesis	1.6	2
$T_4$ RNase, 5 units		
Added after protein synthesis	39.9	767
Added before protein synthesis	2.2	2
No RNA added	2.9	59

See legend to Table 1. Similar experiments with pancreatic RNase are complicated by the fact that RNase itself causes a decrease in the substrate turbidity (a similar effect has been noted by Brenner<sup>14</sup>). This decrease is small and essentially complete by 5 min in the conditions used. Control experiments showed that lysozyme could be effectively detected by following the drop in turbidity after this time. The results agree with those above: when pancreatic RNase is added before the *in vitro* incubation it prevents synthesis of lysozyme.

Finally, the following experiments using inhibitors of protein synthesis also rule out the possibility of lysozyme activity contaminating the mRNA fractions or the cell free system in general.

Synthesis *de novo* of enzyme activity in a cell free system should be inhibited by any conditions that block protein synthesis. Table 2 shows the results of experiments using chloramphenicol, puromycin and RNase  $T_1$ . Inhibition of incorporation of amino-acids is accompanied by abolition of lysozyme synthesis in each case.

Fig. 3 shows a comparison of the time courses of incorporation of amino-acids and the appearance of lysozyme activity. The kinetics are similar except for a relative decrease in lysozyme synthesis in the later portion of the reaction.

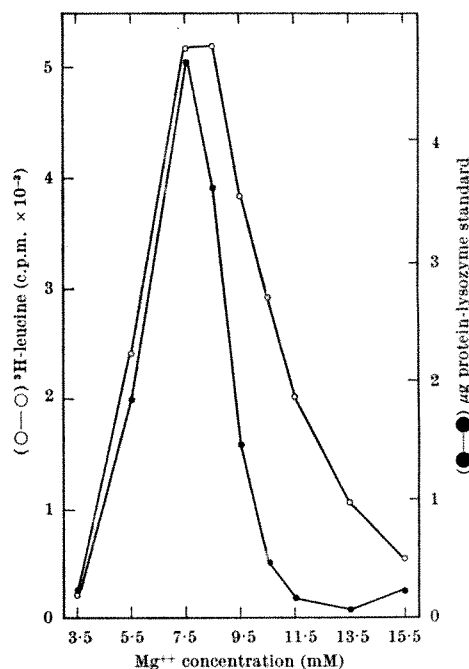


Fig. 4. Dependence on concentration of magnesium. Each point represents the lysozyme activity or radioactivity in a 0.1 ml. sample (see Fig. 3) from an incubation mixture containing  $T_4$  20 min RNA (400  $\mu$ g/ml.) and the indicated concentration of magnesium acetate. Incubation was for 20 min. The specific activity of the leucine was 55 c.p.m./ $\mu$ mole. The 30 min extract used as a standard in these experiments contained, per  $\mu$ g of total protein, lysozyme activity equivalent to 0.131  $\mu$ g of egg-white lysozyme. Purified  $T_4$  lysozyme has 165 times greater specific activity than egg-white lysozyme, as calculated from the data of Terzaghi (PhD thesis, University of Oregon, 1965), using different assay conditions. With this ratio we compute the fraction of protein synthesized *in vitro* that is active  $T_4$  lysozyme. For example, at the magnesium optimum in Fig. 3 (7.5 mmolar) the incorporation of leucine represents 920  $\mu$ mole or about 0.1  $\mu$ g of total amino-acids incorporated/0.1 ml. of reaction mixture (assuming that leucine makes up 10 per cent of the total amino-acids). The lysozyme activity in this 0.1 ml. corresponds to about 0.004  $\mu$ g of  $T_4$  lysozyme (assuming the foregoing ratio of specific activities of  $T_4$  and egg-white lysozymes, and assuming equal specific activities of the *in vitro* and *in vivo* made lysozyme).

The dependence of *in vitro* protein synthesis on the concentration of magnesium is very characteristic and can be used as another criterion for correlating protein and lysozyme synthesis. Magnesium response curves are shown in Fig. 4. Lysozyme synthesis is completely inhibited by concentrations of magnesium which are either too high or too low for efficient incorporation of tritiated leucine. The optimal concentrations of magnesium for total protein synthesis and lysozyme synthesis are the same, but at concentrations greater than optimal, relatively less lysozyme is synthesized. At 11 mmolar magnesium, where incorporation of amino-acids is 50 per cent of the maximum, less than 10 per cent of the maximal lysozyme activity is observed. This decrease in the enzyme activity of the *in vitro* product at high concentrations of magnesium suggests that incorrect initiation or some other decrease in fidelity is occurring. This is consistent with results obtained using synthetic polynucleotides<sup>17-19</sup>.

Finally, there seems to be a good correlation between the relative amounts of lysozyme made *in vitro* and *in vivo*. An approximate calculation (shown in the legend of Fig. 4) suggests that 4 per cent of the total protein made *in vitro* is lysozyme. This is similar to the fraction of late phage specific protein synthesis devoted to lysozyme *in vivo*.

In conclusion, all our evidence suggests that the appearance of lysozyme activity observed in the *in vitro* system has the characteristics of *de novo* synthesis. That is, the synthesis is mRNA specific and is inhibited by chloramphenicol, puromycin and RNase. The incorporation of amino-acids and the appearance of lysozyme activity have similar kinetics and dependence on magnesium. Finally, the inability of RNA from a *T<sub>4</sub>* mutant with an amber mutation in the lysozyme structural gene to stimulate synthesis of lysozyme shows that the activity we measure is that of phage lysozyme.

Perhaps the chief contribution of the work discussed here is that the *in vitro* translation in a well characterized RNA dependent system can be sufficiently accurate to yield active proteins, and that this can be done using

RNA extracted by standard techniques from cells infected by a DNA phage. The assay is dependable and easy, and routinely gives a stimulation at least ten-fold over background, and so it will provide an accurate and convenient assay for biologically meaningful translation.

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## Effect of Antilymphocytic Antibody and Antibody Fragments on Human Lymphocytes *in vitro*

by

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The mode of action of horse anti-lymphocyte serum (IgG) and its pepsin fragments has been investigated by lymphocyte agglutination tests, cytotoxic tests and the uptake of uridine and thymidine. It is found that its divalent pepsin fragment (F(ab')<sub>2</sub>) agglutinates lymphocytes and stimulates the uptake of uridine and thymidine whereas the univalent fragment (Fab') is inactive in all these tests.

THERE have been numerous reports from this<sup>1</sup> and other laboratories describing the immunosuppressive properties of antilymphocyte serum\* (ALS) and its effects on lymphocytes *in vitro*; its mode of action is, however, still obscure, although various theories have been proposed. The present

\* Serum denotes not only serum separated in the usual way from clotted blood but also plasma from which the fibrinogen has been removed by heating (56° C for 30 min) and filtration. ALS denotes any serum having demonstrable effects on lymphocytes *in vivo* or *in vitro* whether raised with injections of intact lymphoid cells (as in the present experiments), intact cells of other types, or cellular fractions.

experiments, which are concerned with the effects of horse-anti-human ALS, serum fractions and antibody fragments on human blood lymphocytes *in vitro*, form part of a systematic study designed to elucidate this question.

ALS in the form of filtered inactivated plasma was prepared from the last two collections of blood obtained from a horse which had received nine injections of intact cells from human infant thymus or adult spleen over a period of about 10 months (see Table 1). Previous investigations<sup>2</sup> showed that IgG and 7S 'Sephadex' fractions



Table 1. SCHEDULE OF IMMUNIZATION OF HORSE

Date Day-mth-yr	Cells used for immunization Source*	Total No.	Per cent viable	Route of injection
7-3-1966	Infant thymus	5.4 × 10 <sup>8</sup>	20	Intravenous
18-3-1966	"	4.1 × 10 <sup>8</sup>	90	"
29-3-1966	"	5.4 × 10 <sup>8</sup>	76	"
4-5-1966	"	16.6 × 10 <sup>8</sup>	Nil	"
24-6-1966	"	10.0 × 10 <sup>8</sup>	"	"
18-10-1966	Adult spleen	8.5 × 10 <sup>8</sup>	80	"
19-10-1966	"	16.5 × 10 <sup>8</sup>	82	Subcutaneous
27-10-1966	"	62.2 × 10 <sup>8</sup>	84	"
13-12-1966	"	25.3 × 10 <sup>8</sup>	62	"

Bleeding, 10 l. on 23-12-1966; 10 l. on 30-12-1966.

\* The thymuses were obtained at routine autopsy (by courtesy of Dr A. D. Bain). One spleen was obtained within 30 min of death and the others were removed in the course of radical gastrectomy for carcinoma of the stomach.

from earlier batches of serum from the same animal contained most of the material responsible for lymphocyte agglutination and cytotoxicity, and for the stimulation of uridine and thymidine uptake by lymphocytes in culture, whereas much of the erythrocyte agglutinin was in the 19S 'Sephadex' fractions and could be absorbed out of whole serum without significantly reducing its lymphocyte agglutinin and cytotoxic titres or its stimulating effect on lymphocytes in culture. In the present experiments, therefore, all preparations in which the erythrocyte agglutinin reciprocal titre exceeded four were absorbed with human erythrocytes to bring the titre below this level before they were tested against lymphocytes. Normal horse and antilymphocytic IgG globulins were prepared by diethylaminoethyl (DEAE) cellulose batch chromatography on Whatman 'DE11' exchanger with an ion exchange capacity of 1.0 m.equiv/g. The products were concentrated by lyophilization and reconstituted to 1 g per cent (w/v) in 0.06 molar phosphate buffer (pH 7.2) containing 0.15 molar sodium chloride. Their purity was checked by immunoelectrophoresis using a rabbit-anti-horse serum and polyacrylamide gel electrophoresis. The antibody preparation contained only small amounts of contaminating protein, probably IgA (T). The F(ab')<sub>2</sub> portion of the IgG molecule, which contains both antigen combining sites, was prepared by digestion in pepsin<sup>3</sup>. The digestion was performed at 37°C for 48 h in 0.1 molar acetate buffer pH 4.0 (using one part enzyme to fifty parts protein by weight). The

products were finally dialysed against the above phosphate buffered saline. Degradation of the IgG preparations to the F(ab')<sub>2</sub> fragment was shown to be complete by immuno-diffusion and, in the case of the antilymphocytic preparation, by subsequent cytotoxic analysis. The univalent Fab' fragment was prepared by reduction of the divalent F(ab')<sub>2</sub> fragment with 0.1 molar cysteine in 0.06 molar phosphate buffer (pH 7.2, containing 0.15 molar sodium chloride) and then alkylated. This was achieved by dialysing the reduced preparations against large volumes of 0.02 molar iodoacetate in the same buffer. The excess iodoacetamide was removed by dialysis for 24 h against three changes of large volumes of phosphate buffer (100 volumes) through which nitrogen was continuously bubbled. This treatment was found to be essential because trace amounts of iodoacetamide (less than 0.0002 molar) were extremely toxic to lymphocytes in culture. Extensive dialysis procedures (greater than 2 days) in the absence of nitrogen resulted in a significant amount of recombination of the Fab' units to the F(ab')<sub>2</sub> fragment. All these preparations were sterilized by 'Millipore' filtration and stored at -20°C.

The IgG globulin content of the sera was determined by quantitative cellulose acetate electrophoresis and by the antibody-agar radial diffusion procedure<sup>4</sup> using a rabbit-anti-horse IgG serum and horse IgG standards. The protein content of the samples was determined by the Folin phenol procedure<sup>5</sup> and in the case of the purified preparations and fragments it was also determined by measuring the extinction coefficient at 280 mμ in a 1 cm cell.

Agglutination and cytotoxic tests were performed as described by Abaza and Woodruff<sup>6</sup> with the following modifications. (a) Lymphocytes were obtained from 400 ml. fresh heparinized human blood by filtering it through a Fenwal 'Leuko-Pak', to remove the polymorphs, and then sedimenting the red cells by adding 120 ml. of 3 per cent gelatine and allowing it to stand for 45 min at 37°C and then for 30 min at room temperature. (b) In both tests the number of viable lymphocytes in each tube or well was four million. (c) Rabbit complement was used instead of guinea-pig complement in the cytotoxic test. With rabbit complement a higher value is

Table 2. EFFECT OF ANTILYMPHOCYTIC AND NORMAL HORSE SERUM AND DERIVATIVES, AND PHYTOHAEMAGGLUTININ, ON THE UPTAKE OF TRITIATED URIDINE AND TRITIATED THYMIDINE BY HUMAN LYMPHOCYTES IN CULTURE

Preparation tested*	Cell donor	C.p.m.† (thousands)	Uridine test		C.p.m.† (thousands)	Thymidine test	
			Relative counts Observed†	Mean		Relative counts Observed†	Mean
ALS and derivatives							
ALS	B	1,000, 834, 811	12.3, 10.2, 9.9	10.8	652, 882, 600 608, 656	14.7, 19.9, 13.5 84.6, 91.3	16.0 88.0
	C	200, 236	11.5, 13.4	12.5			
	D	310, 297, 294	4.59, 4.41, 4.36	4.45	150, 294, 195	18.2, 32.0, 23.6	24.6
	G	629, 429	11.0, 7.50	9.27			
	N	141, 158, 119	4.27, 4.78, 3.64	4.23			
	O	253, 266	2.71, 2.86	2.79			
IgG	C	118, 138	6.69, 7.95	7.32	415, 266	57.8, 37.0	47.4
	J	146, 143, 107	3.24, 3.18, 2.38	2.94			
	K	407, 379, 435	6.72, 6.24, 7.17	6.71	130, 211 129, 169	20.4, 31.4 3.13, 4.09	25.9 3.61
	N	146, 160, 155	4.33, 4.87, 4.71	4.64			
	O	196, 206, 169	2.10, 2.21, 1.81	2.04			
	F(ab') <sub>2</sub>	J	177, 181, 155	3.94, 4.02, 3.43			
N		289, 156, 146	8.79, 4.75, 4.34	5.96			
O		526, 476, 385	5.64, 5.10, 4.12	4.95			
Fab'	G	68.4, 57.5	1.20, 1.01	1.10			
PHA	G	629, 508	11.0, 8.90	9.95			
	L	447, 271	11.3, 6.85	9.08			
	M	89.7, 74.3	6.76, 5.59	6.18			
	O	476, 541	5.10, 5.79	5.45			
NHS and derivatives							
NHS	A	43.0, 42.7	1.14, 1.13	1.14	56.5, 37.9, 67.2 85.1, 29.8, 35.3	2.62, 1.76, 3.12 1.91, 0.80, 0.67	2.50 1.12
	B	62.0, 82.4, 78.9	0.76, 1.01, 0.97	0.91			
	J	51.6, 54.0	1.13, 1.20	1.16			
	L	46.9, 19.1	1.31, 0.54	0.93			
Fab'	G	65.3, 41.1	1.15, 0.72	0.93			

\* The dose per culture of serum and serum derivatives contained 2.0 mg IgG or fraction. The dose per culture of PHA was 0.1 ml. reconstituted dried phytohaemagglutinin (Wellcome).

† Figures on the same horizontal line relate to replicate cultures set up at the same time with cells from the same donor.

obtained for the titre and, what is more important, the end point is sharper. (d) The final serum dilutions were adjusted according to the concentration of IgG or antibody fragment in the preparation so that the first tube or well contained approximately 1 mg IgG or fragment in a total volume of 0.4 ml. This is referred to as a dilution of 1:4 because our standard preparation of IgG contains 1 mg/0.1 ml. On this basis the reciprocal lymphocyte agglutinin titres of antilymphocytic serum, IgG and F(ab')<sub>2</sub> were essentially the same and ranged from 1024 to 4096 in tests performed with lymphocytes from various donors. The Fab', on the other hand, had a negligible titre (titre<sup>-1</sup> ≤ 4). The cytotoxic titre<sup>-1</sup> (with rabbit complement) was 1024 for antilymphocytic serum and IgG, and negligible for both fragments (≤ 4). The agglutinin and cytotoxic titres of normal horse serum, and of IgG and fragments derived from it, were negligible (titre<sup>-1</sup> < 4).

Lymphocyte cultures were set up in Bijoux bottles and incubated at 37°C. Each culture initially contained about 10 million viable lymphocytes and 10–20 million erythrocytes in 2 ml. medium 199 plus 0.5 ml. autologous plasma. Usually the plasma was inactivated by heating (56°C for 30 min), but in experiments designed to study the effect of complement unheated plasma was used. The various preparations to be tested for stimulating activity were added as described here. Some cultures were set aside for morphological study, but the majority were used to study the incorporation of tritiated uridine or thymidine. Tritiated uridine (specific activity 2.73 c./mmole) was added when the cultures were set up, and tritiated thymidine (specific activity 5 c./mmole) was added to cultures which had been incubated for 72 h. In each case the final concentration of isotope was 1.67 µc./ml. Eighteen hours after the addition of tritiated uridine, or 24 h after the addition of tritiated thymidine, the cells were spun down and washed three times in phosphate buffered saline and then successively in 5 per cent trichloroacetic acid, phosphate buffered saline, and absolute methanol. After the final spin the supernatant was discarded and the precipitate was taken up in the minimum possible quantity of hyamine hydrogen chloride and heated for a few minutes in a water bath at 60°C. After cooling, 10 ml. of scintillant (NE 213, Nuclear Enterprises, Edinburgh) was added and counting was performed with a Packard 'Tricarb' scintillometer. Cultures were set up usually in triplicate (occasionally duplicate or quadruplicate). The counts per minute in control cultures which contained only cells, medium 199 and autologous plasma showed not more than 25 per cent variation from the mean value (and often much less than this) when each contained cells from the same donor, but the mean c.p.m. in control cultures of cells from different donors ranged from 17,520 to 81,600 (fourteen donors; mean 46,250 ± 21,800) in cultures incorporating tritiated uridine, and from 6,400 to 44,430 (four donors; mean 19,930 ± 12,790) in cultures incorporating tritiated thymidine. It has seemed desirable therefore to calculate for each treated culture the relative count, that is, the ratio of

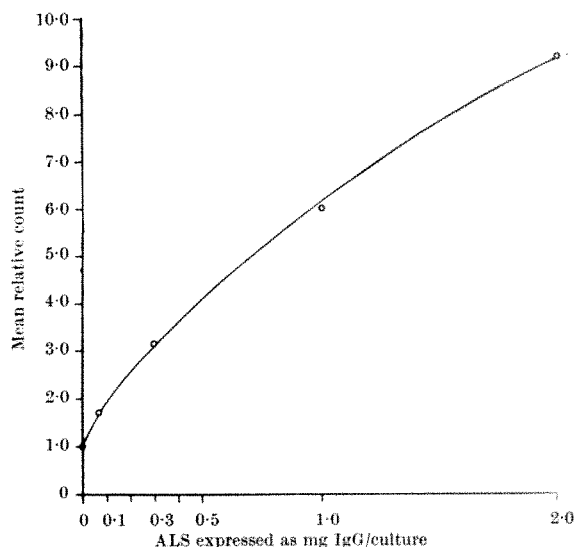


Fig. 1. Curve showing relationship between the degree of stimulation of uridine uptake (expressed as mean relative count) and the amount of ALS added to the culture. Based on experiments using the same serum but lymphocytes from six different donors.

c.p.m. in that culture to the mean c.p.m. of the control cultures set up at the same time with cells from the same donor.

The results of tests with a standard dose of each of the various preparations are summarized in Table 2, from which it will be seen that antilymphocytic serum, IgG and F(ab')<sub>2</sub> were all highly active in stimulating uptake of both uridine and thymidine, and when given in comparable dosage to the same donor all produced approximately the same degree of stimulation. Initial experiments with Fab' were invalid because the preparation contained toxic amounts of iodoacetamide, but the preparation referred to in Table 2, which had no stimulating effect, was non-toxic as shown by the trypan blue test and by the fact that a preparation made in the same way from normal horse serum did not depress the uptake of uridine.

In the absence of complement, stimulation of the uptake of uridine is, within the limits of the experiment, an increasing function of the amount of antilymphocytic antibody added to the culture. The dose response curve is shown in Fig. 1. The effect of complement is shown in Fig. 2. When the lymphocytes were suspended in medium 199, and ALS and complement (in the form of raw autologous plasma) were added later, there was extensive cytolysis, and stimulation of uridine uptake was completely inhibited (Fig. 2a). When, on the other hand, the cells were suspended in a mixture of medium 199 and autologous plasma, and ALS was added 30 min or so after the suspension had been prepared, the degree of inhibition was very much less (Fig. 2b). These results confirm in a slightly different system some recent observa-

Table 3. EFFECT OF TIME OF EXPOSURE TO STIMULATING AGENT AND PRE-TREATMENT WITH Fab' ON UPTAKE OF <sup>3</sup>H URIDINE BY HUMAN LYMPHOCYTE CULTURES

Stimulating agent	Pre-treatment				Relative count	Mean
Agent and dosage	Duration of exposure	Agent and dosage	Duration of exposure	Cell donor	Observed	
ALS (0.2 mg IgG)	18 h	Nil		G	11.0, 7.50	9.27
		Fab' from ALS	15 min	G	4.65, 7.59, 3.04	5.09
	1 h	Fab' from NHS	15 min	G	4.62, 5.85, 10.2	6.89
		Nil		H	4.91, 4.96, 5.65	5.17
		Fab' from ALS	15 min	H	10.8, 11.2, 17.4	13.1
		Fab' from NHS	15 min	H	14.4, 13.3, 14.9	14.2
	15 min	Nil		H	1.94, 1.91, 1.75	1.87
		Fab' from ALS	15 min	H	2.70, 2.89, 2.29	2.62
		Fab' from NHS	15 min	H	2.52, 2.27	2.40
PHA (0.1 ml.)	18 h	Nil		G	11.0, 8.90	9.95
		Fab' from ALS	15 min	G	8.86, 8.76	8.80
	15 min	Fab' from NHS	15 min	G	17.0, 10.5	13.7
		Nil		H	13.0, 14.7, 13.5	13.7
		Fab' from ALS	15 min	H	8.06, 5.25, 5.92	6.41
		Fab' from NHS	15 min	H		11.0

tions of Holt, Ling and Stanworth<sup>7</sup>. As would be expected, complement caused little, if any inhibition of the stimulating effect of  $F(ab')_2$ . Thus the ratio

Mean relative count in cultures with complement

Mean relative count in cultures without complement

for cultures containing  $0.2 \mu\text{g } F(ab')_2$  was 0.80 and 0.92 in two separate experiments, and these figures do not differ significantly from the ratios observed in untreated control cultures which, for some unknown reason, consistently showed a somewhat lower count when raw autologous plasma was used instead of inactivated plasma (Fig. 2).

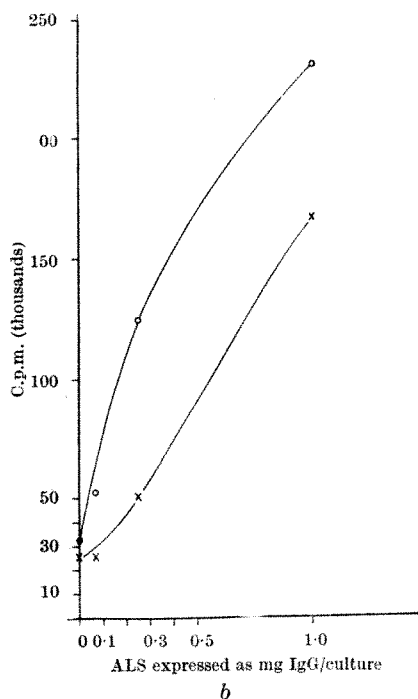
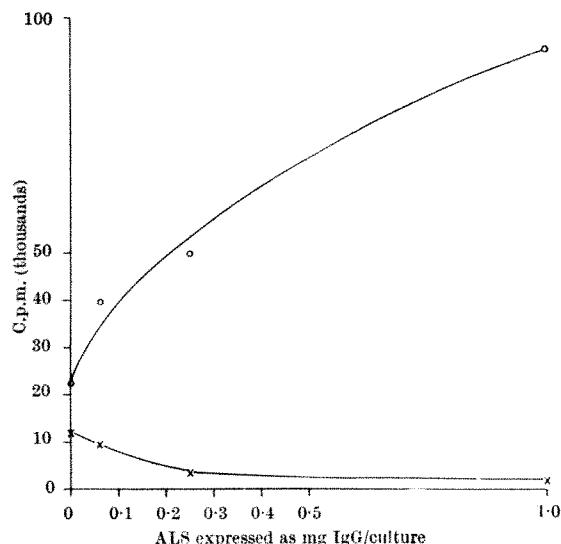


Fig. 2. Effect of complement on uridine uptake at different ALS concentrations. ○, Without complement; ×, with human complement. (a) 2.0 ml. of a suspension of lymphocytes (donor E) in medium 199 was put in each culture bottle. ALS and either raw or inactivated autologous plasma was added later, followed by tritiated uridine. Counting was done after 18 h. (b) 2.5 ml. of a suspension of lymphocytes (donor I) in a mixture of medium 199 (four parts) and raw or inactivated autologous plasma (one part) was placed in each culture bottle. About 30 min after the suspension had been prepared ALS and tritiated uridine were added. Counting was done after 18 h.

Experiments in which lymphocytes were exposed to stimulating agents for short periods of time and then twice washed before being put up in culture (Table 3) showed that exposure to ALS for 15 min results in only very slight stimulation, and exposure for 60 min to less than maximal stimulation, whereas exposure to phytohaemagglutinin (PHA) for 15 min is as effective as exposure throughout the 18 h of culture.

Pre-treatment of lymphocytes by exposing them for 15 min to  $Fab'$  prepared from ALS, followed by two washes, did not significantly block the stimulation of the uptake of uridine by subsequent exposure to ALS (Table 3); indeed when the exposure to ALS was limited to 60 min, uptake appeared to be increased by pre-treatment with  $Fab'$  from either ALS or normal serum. On the other hand, there is a suggestion that the stimulating effect of PHA is to some extent inhibited by pre-treatment with antilymphocytic  $Fab'$  though not with  $Fab'$  from normal serum. The significance of these observations is difficult to assess because we do not know for how long  $Fab'$  remains attached to the surface of the lymphocyte. It should be possible to answer this question by using either the Coombs test or immunofluorescence, and experiments of this kind are being undertaken.

As we have seen, antilymphocytic serum and IgG stimulate the uptake of uridine and thymidine by lymphocytes in the absence of complement, but when complement is present they fail to do so, and at high concentration cause cell lysis. This suggests that the action of ALS *in vivo* may depend on such factors as the local concentration of antibody and complement, and the degree of anti-complementary activity, in lymphoid tissue and also in the blood stream.

It is noteworthy that  $F(ab')_2$  is as effective as IgG not only in respect of lymphocyte agglutination but also in respect of the stimulation of uptake of uridine and thymidine.

Clearly it will be of great interest to determine the effect of  $F(ab')_2$  on the blood lymphocyte count and also on immunological responsiveness *in vivo*. This cannot readily be determined in man, but experiments with  $F(ab')_2$  from horse-anti-rat ALS are in progress. The most likely explanation of the failure of  $Fab'$  to block subsequent stimulation by ALS or IgG would seem to be that the  $Fab'$  does not become firmly attached to combining sites on the surface of the lymphocyte. Alternatively, it is possible that a "homoreactant" phenomenon<sup>8</sup> is operative. If this is so, intact antilymphocytic IgG or normal IgG would be capable of agglutinating  $Fab'$  coated cells and thus possibly causing transformation. The question of whether ALS and PHA attach to the same receptors, which we had hoped to be able to answer, remains open.

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# Vision in Monkeys after Removal of the Striate Cortex

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Monkeys deprived of their visual cortex (area 17) have been thought to be unable to discriminate much more than "total luminous energy". In particular, they have been considered incapable of localizing visual events in space. It has now been shown that they can be trained to detect and accurately reach out for objects of certain kinds presented visually.

TOTAL removal of the striate cortex in monkeys results in a loss of vision so severe that in their ordinary behaviour the animals seem blind. In formal testing situations, however, it has been possible to demonstrate a certain degree of visual capacity: pupil responses to light are still present; opto-kinetic nystagmus elicited by a large field of moving stripes is present, at least some months after surgery<sup>1,2</sup>; in addition, performance may be good on certain kinds of visual discrimination. Kluver, in a series of classic papers<sup>3</sup>, analysed the visual discrimination capacity of the de-striate monkey as an ability to discriminate "total luminous energy". More recently Weiskrantz<sup>4</sup> has shown that some discriminations are possible even when the discriminanda are matched for luminous flux and has suggested that there is an ability to discriminate "total retinal activity"—contour length, movement and flicker contributing besides luminous flux. There has been general agreement, however, that spatial information is no longer available: the lesion eliminates "visual space with its dimensions"<sup>5</sup>. We wish to report here new observations on two de-striate monkeys which show that they can not only detect but accurately locate certain objects by vision.

The subjects were two adolescent rhesus monkeys. Bilateral total removal of striate cortex was carried out under 'Nembutal' anaesthesia by occipital lobectomy followed by aspiration of the striate tissue remaining on the lateral surface and in the depths of the calcarine fissure. Histological verification of the lesions has not yet been made because the animals are still alive awaiting the completion of subsequent experiments.

The observations were made about 19 months and 5 months, respectively, after surgery. During the previous months both monkeys had been tested on various kinds of visual discrimination<sup>4</sup> and had reached a good level of performance on some of the problems. These discriminations included brightness problems, for example, black versus white, and elementary pattern problems, for example, black and white stripes versus homogeneous grey (matched for total luminous flux), but the visual capacity revealed could be interpreted in terms of discrimination of total retinal activity and required no supposition of spatial discrimination. In addition, the monkeys had been tested in a nystagmus drum and showed clear opto-kinetic nystagmus; they also showed good pupil responses. Apart from this evidence they had given almost no sign of visually guided behaviour.

A surprising fact had, however, been noticed: the monkeys occasionally turned their heads or eyes in the direction of a moving object. We were inclined at first to attribute this to an acute sound sense or an ability to detect movements of air. Further observation convinced us, however, that the response was genuinely based on vision and we were led to examine it more carefully. Within a few days we were able to demonstrate a quite unexpected visual capacity.

Both monkeys showed similar development of visually guided performance and a case history of one (monkey Hln, 19 months post-surgery) can serve to describe the testing method and results. Testing was carried out

in the home living cage, fitted with a 2 ft. × 2.5 ft. front of 2.5-in. wire mesh. The first stage was to train the monkey to reach through the mesh to find a morsel of food (a nut or piece of fruit) which was held out to him by the experimenter. The requirement was that he should search the field by reaching from one place to another until he made contact with the experimenter's hand holding the food. This training was accomplished by a simple process of shaping in which at first the experimenter deliberately moved his hand to whatever position the monkey happened to reach and then progressively required him to reach closer and closer to his hand before rewarding him. Within a short time the monkey learned to find the experimenter's hand in any part of the field by a series of exploratory reaches. The next stage was to try to help him in his search by visual cues.

It was soon clear that when the experimenter held his hand stationary the monkey had no idea where it was and reached at random. If, however, the hand was moved, by waving it gently or wagging a finger, he did have some idea of its location and reached at once in roughly the right direction. It seemed at first that his ability to localize was very poor, but with practice he got better and better. Eventually he could reach for the hand promptly and accurately as soon as it moved; but while it was stationary he still appeared quite blind. To ensure that his ability to locate movement was not based on non-visual cues, he was tested in darkness, whereon his performance dropped to a chance level.

A man's hand at monkey's arm's length is a rather big target and the accuracy of reaching had not yet been tested to its limit; moreover, the effective stimuli had not been well defined. Further testing was therefore undertaken more systematically. Observation so far had shown that the response of reaching to touch a perceived object was a simple and seemingly natural response for the "blind" monkey; it had the advantage also that it was self-correcting because success or error in locating the object was immediately signalled by touch. This response was therefore retained as the index of vision. Instead of the experimenter's hand, the objects to be detected were 1-in. square wooden blocks held by the experimenter on the ends of wire sticks. Different visual stimuli could be attached to or painted on these blocks and they could be held stationary or moved. The monkey was required to reach out and grasp the block. Success was rewarded by a morsel of food put directly into his hand which he held out expectantly after letting go of the block. With this procedure, the following visual ability has been demonstrated.

*Detection of a moving object.* The monkey is extremely sensitive to moving objects of all kinds. In a typical case, he will reach out to grasp with a sure hand a 1-in. black cube which is waved very slightly at arm's length from him. His sensitivity is so acute that it is hard to hold objects still enough to prevent him detecting their tremor. Speed of movement, over a wide range, seems not to matter. All sizes and shapes of objects from a 0.25 in. cube to a long straight bar are detectable, although with large objects he tends to reach for one edge. The objects can be lighter or darker than the background. The absolute level of illumination may be varied over the

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whole range over which a human observer can easily see; he has, for instance, performed well both in a very dimly lit room and under very bright illumination while a film was being made.

For most routine testing the stimulus object has been a 1 in. cube painted with black and white stripes. In order to avoid ambiguity concerning where the monkey should reach to, the movement given to it is rotation about its centre rather than change in position. When the monkey sees the object he usually shows an obvious expression of recognition (an "aha!" reaction) and turns his eyes towards it before reaching. He is best at detecting the object if it is presented near the centre of his visual field and will often miss it in the peripheral field; rough observation suggests that he gets very inefficient about 40° out from the centre. He is equally good in all quadrants of the field. He does not appear to be able to discriminate distance and if the object is presented nearer or farther away than the standard arm's length he over- or under-reaches; he will, for instance, reach forlornly for a moving object 6 ft. away. It is not necessary for movement of the object to be kept up until he reaches for it or even until he has had time to fixate it; he can detect and locate a sudden sharp movement. He can remember the location of an object after it has stopped moving and if, for instance, he has his hands full of food or is otherwise occupied when the object is moved he may defer reaching for several seconds but then reach accurately. If translational movement is used rather than simple rotation, he can often reach accurately for an object which has moved and come to a stop, although he may sometimes reach to a point along the object's path rather than its terminal position. He does not, however, seem to be able to track a moving object and is unlikely to reach accurately for it while it is still moving along; this inability is paralleled by his apparent failure to follow a moving object with his eyes.

*Detection of a stationary object.* The monkey never detects a stationary black or white painted cube. This is so even though he may search actively with his eyes and thus cause the image of the object to move over his retina. He cannot reliably detect a stationary neon light in ordinary room illumination, although occasionally he does reach for it with surprising accuracy. If the background illumination is reduced, however, he does become able to detect the light, and in darkness can locate it well. With a light of 1.5 log foot lamberts his performance becomes good below about 2.7 log foot lamberts background illumination. No systematic observations have been made on eye movements or the relative efficiency of different parts of his visual field here because it is difficult for the experimenter to see.

*Detection of light flashes.* The monkey can detect and locate a flashing neon light in either room illumination or darkness. He is very sensitive to flash rates of about 10 per sec (flash duration 5 msec). As the flash rate is increased he remains good until about 45 per sec, but is unable to detect rates beyond 55 per sec. This threshold corresponds very closely to the critical flicker fusion frequency for a human observer. If the flash rate is initially high, say 100 per sec, so that the light appears stable, and is then progressively lowered, he detects the light at about the point where it begins to flicker for a human observer. He can detect slow flash rates down to single isolated flashes, although he does have more trouble in locating the latter. With slow rates of about 1 flash per 4 sec he does not reach after the first flash but turns his eyes towards it and waits for the next flash to occur in nearly central vision before reaching. With single isolated flashes where he can get no further information by waiting he tends to reach rather tentatively, although often quite accurately, unless the flash happens to occur close to where he is already looking, in which case he is more confident. As with detection of movement, he is much less efficient in the peripheral than in the central field.

*Detection of two objects presented together.* To test further the monkey's ability to localize objects in space, an attempt was made to train him to respond to two objects presented simultaneously in different positions. The objects were held out as before and he was required to grasp one and then the other in sequence to get a reward. Pairs of rotating 1-in. cubes or flashing neon lights were used. They were presented in random spatial relationship to each other.

The monkey very quickly caught on to the procedure. He can perform well with both kinds of stimuli. He is best when the objects are fairly widely spaced, about 10 in. apart, but can also do well with them closer together; when they are less than about 3 in. apart, however, he sometimes reaches for the same object twice or occasionally reaches between them. The relative spatial orientation of the two objects does not matter and he is just as good at locating two objects one above the other as two objects side by side. This capacity provides him with the basis for simple spatial pattern discrimination: for instance, he clearly discriminates a horizontal from a vertical relationship when he responds appropriately to objects presented in the two ways. He nearly always fixates each object before reaching for it, although he does not have to. With moving objects, the movement does not have to be kept up until he reaches and he can accurately locate two objects which are given only a sudden rotational jerk; he has not, however, shown much ability to locate two single light flashes.

Two general features of the monkey's performance are of interest. One is that he performs well only when he attends to what he is doing. If he is over-excited or distracted by some commotion outside his cage his performance breaks down and he tends to reach wildly. Although this may seem obvious, it would not in fact be typical of a normal monkey performing the same task. The other feature is that he sometimes shows "false positive" responses, reaching apparently purposively to an empty part of the field. Together, these observations suggest that he is working not far beyond the threshold of his capacity and that he may mistake internal "noise" for an external stimulus.

The other monkey in the group (monkey Hmr, 5 months post-surgery) has showed a similar ability to detect both moving objects and stable and flashing lights. His performance at best is as good as that of the monkey just described. He has, however, developed a bad habit of swiping for the object instead of reaching straight to it. He also shows rather more false positives than the other monkey. In general he appears less eager to be tested and tries less hard.

The most conservative explanation of these results would be that the striate cortex has not been removed completely in either monkey. The removal of the last vestige of striate tissue from the depths of the calcarine fissure is surgically difficult and the possibility of incomplete removal cannot be discounted until histological verification of the lesions is made. Allowing, however, that this may be so, there is good reason to doubt that it would be an adequate explanation of the results. First, any remaining striate tissue must lie in an area serving a small part of the far periphery of the visual field; but the monkeys are consistently better at locating objects near the centre of the field; they fixate objects with central vision, they can locate two objects at once in different parts, and they show no bias towards any quadrant of the field. Second, Cowey and Weiskrantz<sup>5</sup>, using a method of perimetry with monkeys that accurately controls for eye movements, have shown that after striate lesions there is an ability to detect light flashes over the whole of the affected area of the field which certainly does not depend on remaining striate tissue. Although their study did not bear on the ability to localize flashes, the clear demonstration of the possibility of extra-striate vision makes it easier to accept the present results without special pleading.

The most striking feature of the present study is the evidence for spatial localization by the de-striate monkey. Similar findings have never been reported in detail, although Pasik, Pasik and Krieger<sup>6</sup> say that, some months after surgery, their de-striate monkeys "followed large white targets and grasped at a source of light". When compared with evidence for other species, however, the results may be thought less surprising. In lower mammals there are several reports of the existence of considerable visual capacity after removal of striate cortex, in particular an ability to discriminate moving from stationary objects (reviewed by Weiskrantz<sup>7</sup>). Especially notable is the recent study of the tree shrew<sup>8</sup> where striate ablation was found to have no obvious effect on visually guided behaviour. In the cat it has been reported that striate ablation soon after birth may have little effect on the subsequent development of vision<sup>9</sup>. But in man also there are reports in the clinical literature of cases of cortical "blindness" after damage to the striate area where residual vision existed in the affected fields. Riddoch<sup>10</sup> describes several cases showing ability to perceive movement within perimetrically blind fields. He says, "The patients have great difficulty in describing the nature of the movement they see: it is so vague and shadowy. But they are quite sure that neither shape nor colour can be attributed to it, and that it can be detected in a field which is entirely blind to stationary objects". Describing a similar case, Holmes<sup>11</sup> says, "only large white moving objects may be recognized . . . when they cease to move he sees them no longer; they disappear". Bender and Krieger<sup>12</sup> report cases showing an ability to perceive and localize movement and also an ability to see a flickering light or a stable light under conditions of otherwise total darkness. They mention that the perceptions were often unstable and that sometimes illusory movement was seen. Denny-Brown<sup>13</sup> reports a patient with complete hemianopia who could perceive small moving objects in the blind half of the field. He comments that self-induced head movements did not help the patient to see an object if it was stationary. In few of these clinical cases, however, has there been adequate post-mortem verification of the extent of damage to striate cortex. Perhaps for this reason, the possibility of extra-striate vision in man has received little acknowledgment.

The question remains as to what nervous structure may mediate the residual vision after striate lesions. The obvious candidate is the superior colliculus. We have recently made electrophysiological recordings from single

units in the superior colliculus of monkeys and the response properties of these units are in fact well suited to underlie the visual behaviour described in this study. It would, however, be disingenuous to press an analogy between single unit responses and behaviour when a direct method for testing the hypothesis is available. We hope, therefore, soon to study the effects of making collicular lesions in our two de-striate monkeys.

*Note added in proof* (July 4, 1967). Since this paper was submitted, further work with the same animals has revealed a degree of visual capacity beyond that described. With more extensive training there has plainly been a progressive change in the limits to what the animals can see. Monkey Hln has developed an ability to locate stationary lights at much lower contrast levels than those given above: he can now respond to even a dim pea-bulb in room illumination, although he remains apparently quite unable to detect objects darker than the background. The other monkey, Hmr, who has become more willing to be tested, has shown a still more surprising development: he can now respond reliably to almost any small stationary object in room illumination, whether lighter or darker than the background. Both monkeys still apparently find the task of detecting stationary objects relatively hard compared with, say, moving objects. The impression is that they have central tunnel vision for stationary objects. They look from one place to another until, as though by chance, the object falls near the central field and then they may stare fixedly at it before reaching; when they do reach, their accuracy is as good as with moving objects. In view of these new results we should say that we cannot be confident that we have even yet demonstrated the full potential for vision of these animals.

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## Cutaneous Receptive Fields of Single Nerve Cells in the Thalamus of the Cat

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This article describes some new receptive properties of cells in the nucleus ventralis posterolateralis. It is shown that the nucleus contains mechanoreceptive cells with excitatory or inhibitory thermoreceptive influences on them, that the observation of surround inhibition in this nucleus depends in part on the type of anaesthesia used, and that some of the cells have directional sensitivity.

THE study of the receptive fields of single cells in the various nuclei of sensory systems has been valuable in understanding the transformations used by these nuclei in the analysis of sensory information. In the sensory systems of the skin, receptive field is usually defined as the area of skin from which natural stimuli, thermal or mechanical, cause excitation of the cell. In anaesthetized cats, we have investigated cells in that part of the thalamus

(the main core of the nucleus ventralis posterolateralis) which receives the main output of the dorsal column nuclei, where analytical transformation defined in a semi-quantitative way by previous experiments<sup>1</sup> has already occurred. This region projects in turn to the cerebral cortex. The position of the tungsten recording electrodes was checked histologically, and the position of many cells marked by electrolytic lesions.

The cells are arranged in a somatotopic fashion; and the region studied was chiefly that for the forelimb. Their receptive fields were almost all on the opposite forelimb. Of some 150 cells of which the fields were adequately investigated, only three had fields on the ipsilateral forelimb: these lay among cells with contralateral forelimb fields, and two of them were shown to lie rather rostrally in the nucleus. The great majority of cells had purely mechanoreceptive fields, responding to bending hairs, to sustained light pressure on the skin or to light mechanical stimuli applied to the pads; some showed a combination of hair sensitivity with pad or skin pressure sensitivity. This agrees with previous investigations of this region of the thalamus<sup>2,3</sup>.

Thermal stimuli were regularly used in testing receptive fields, usually by applying radiant heat and sometimes by warming or cooling with thermoelectric devices in contact with the skin, temperature of the contact element being determined with thermocouples. No cells were found which were sensitive to small changes of temperature within the range 15°–45° C in which peripheral thermoreceptors are known to respond<sup>4</sup>. Cells with sensitive thermoreceptive properties have been found in n. ventralis posteromedialis responding to stimuli applied to the tongue<sup>5</sup>; but the thermoreceptive limb afferents of the cat are unmyelinated<sup>4</sup> and may project in a more complex system of small fibres and cells not resolved by present methods. Two types of cell were regularly found, however, which responded to more intense thermal stimulation: they formed a small proportion (about 10 per cent) of cells of which the fields were adequately studied. These cells had sensitive mechanoreceptive fields on one or more of the pads. In the more common of the two types, characterized in all but one case by an additional hair-sensitive region adjacent to the pads, a thermal stimulus above a reproducible threshold in the range 45°–61° C caused a prolonged repetitive discharge outlasting the stimulus by some seconds. Such cells differ in several ways from those described by Perl and Whitlock<sup>6</sup> as sensitive to noxious stimuli, particularly in their low mechanical threshold. In the other type, characterized by lack of any hair-sensitive region adjacent to the pads, removal of a thermal stimulus of similar magnitude, if above a critical thermal threshold, was followed by an intense discharge of impulses. A later application of heat during the period of intense discharge consistently reduced or stopped this discharge, removal of this subsequent stimulus causing a further surge of intense discharge. Although the functional significance of this sort of thermoreceptive property in cells which also have sensitive mechanoreceptive properties is not clear, these findings suggest that this part of the nucleus has other inputs than from the dorsal column nuclei, in which this kind of thermoreceptive response has not so far been recognized. There are reasons for believing that these properties are not simply due to the two sorts of stimulus exciting the same type of receptor. For example, it was found that a small additional dose of anaesthetic completely abolished the thermal response of one cell of the first type, the cell retaining unimpaired its original slowly adapting mechanoreceptive properties. We also found that the mechanoreceptive properties of the cells which gave thermal responses were not apparently changed by repeated applications of a thermal stimulus which reached threshold for excitation, each mechanical test being made immediately after a thermal stimulus.

The mechanoreceptive fields of a large proportion of the cells projecting to the thalamus from the dorsal column nuclei have been shown to have an inner excitatory zone more or less surrounded by an outer inhibitory zone<sup>1</sup>, and it is thought that this mutual antagonism increases spatial contrast in this system as in the visual system. These fields with surround inhibition seem to be fairly stable under experimental conditions and this pattern is not abolished under moderately deep anaesthesia, for ex-

ample with barbiturates. In n. ventralis posterolateralis, on the other hand, to which these cells project, surround inhibition has been observed less regularly: Poggio and Mountcastle<sup>7</sup>, for example, found it in only 5 per cent of cutaneous cells in this region of the thalamus of the unanaesthetized monkey. The experiments of Nakahama *et al.*<sup>3</sup> suggest a higher incidence of surround inhibition in this nucleus in the cat anaesthetized with pentobarbitone sodium (see their Table 1). With this anaesthetic we found surround inhibition in only 10 per cent of thalamic cells of which the skin fields were adequately studied, averaged over all experiments. But in particular experiments a much higher incidence was found (up to 35 per cent), whereas in many experiments it was not seen at all. One possible reason for simple surround effects not being seen is that the receptive fields have become more complex than those of cells in the dorsal column nuclei. We are inclined to discard this explanation, because under conditions where inhibition was not observed, clear-cut static excitatory fields were seen which gave no indication of specificity with respect to orientation or direction of movement of the stimulus. A second and more general possibility is that in many experimental animals, because of the type or level of anaesthesia, a disturbance in the balance of excitatory and inhibitory actions in the various mechanisms acting on the thalamus leads to the surround inhibition transferred from the dorsal column nuclei being concealed. Some support is given to this by the fact that we have seen surround inhibition consistently with chloralose anaesthesia, in 47 per cent of all cells studied under this anaesthetic. Lighter anaesthesia with short-acting barbiturates (hexobarbitone or thiopentone) did not provide favourable conditions.

Most of the cells in the thalamus which had an inhibitory surround to an excitatory cutaneous field had receptive characteristics similar to those found for such cells in the dorsal column nuclei<sup>1</sup>. Most of those studied were hair-sensitive, usually with small excitatory fields particularly on the forepaw. Such cells did not show any preference for stimuli of specific orientation or direction of movement; though the summation occurring when the stimulus moves does cause rapidly adapting hair-sensitive or pad-sensitive cells to discharge more impulses, giving a preference for moving stimuli which is directionally unspecific, within the confines of the field defined with static stimuli. One pad-sensitive cell, for example, often fired only a single impulse on application of a static stimulus, but fired a high-frequency train of impulses for as long as the stimulating instrument was drawn lightly across the pad in any direction.

Some cells responding to light pressure on the skin with a slowly adapting discharge do, however, have more complex receptive fields. Cells with these characteristics ('touch-pressure' cells) are known to project to the thalamus from the dorsal column nuclei<sup>1</sup> and, in much smaller numbers, from the lateral cervical nucleus<sup>8</sup>; but no inhibitory surround effects have been seen on cells of this type in these nuclei<sup>1,9</sup>. We have found, in agreement with Nakahama *et al.*<sup>3</sup>, that an inhibitory surround, also activated by sustained light pressure, may occur in the thalamus; and this presumably depends on an intrathalamic mechanism. With a number of touch-pressure cells, we have suspected that the field for moving stimuli differed from that for static stimuli, with a preference for one direction of movement; but there was often uncertainty, either because the cell was lost after too short a period of observation, or because of the possibility of artefacts introduced by the method of testing—for example, the situation may be complicated by a moving pressure stimulus exciting receptors at a distance by stretching the skin. Only two such cells were observed for long enough to allow definite conclusions about their fields. The use of moving stimuli elicited responses which could not have been predicted merely from a knowledge of the static field. The field (meaning here the whole area

from which either excitatory or inhibitory effects were produced) became larger, and the regions which were purely excitatory or inhibitory for static stimuli were no longer specific in this way, the borders of the static excitatory zone having to a large extent lost their identity. With one cell, for example, very light pressure applied by a finger moving at about 3 cm/sec in a proximal direction, and starting in the static excitatory zone, caused excitation in the static inhibitory zone further up the arm, whereas stimuli moving distally through this distance into and across the static excitatory field caused inhibition of resting discharge. Both cells that were well investigated showed this type of directional sensitivity with stimuli moving in the long axis of the limb. The dynamic fields tended to vary in extent and sensitivity with time. It appears that these more complex fields represent a stage in the analysis of the direction of movement of stimuli in light contact with the skin.

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## Experimental Lepromatous Leprosy

by

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Injection of *Mycobacterium leprae* has produced leprosy in mice previously thymectomized and given 900 r. of whole body irradiation to depress their immunological capacity.

LEPROSY is the result of infection by *Mycobacterium leprae*—bacilli which are closely related to those responsible for tuberculosis. *Myco. leprae*, however, unlike *Myco. tuberculosis*, cannot as yet be grown *in vitro*, and to such a limited extent *in vivo* that progress in the aetiology and treatment of the disease is making little headway. This is frustrating, because *Myco. leprae* was one of the first bacteria to have been linked with specific disease in man and yet leprosy has remained a serious world health problem because it gives rise to disabilities and deformities which place a heavy financial burden on society.

An infection can be produced in the foot pads and ears of mice and some other rodents inoculated locally with *Myco. leprae* from patients with leprosy<sup>1-4</sup>. The infection remains localized and multiplication is very small; for example, if  $10^4$  organisms are injected about  $10^6$  can be recovered in 8 months, but if  $10^6$  or more are injected no more than the equivalent of the initial number can be recovered in 8 months. The number of bacilli which can be recovered declines after 8 months and the proportion of degenerate (non-viable) forms increases, but the yield increases again and reaches a second peak at about 13 months, which may be greater than that at 8 months but very rarely exceeds  $10^7$ . After this the number which can be recovered again declines, with a proportionate increase in the number of non-viable forms. Needless to say, the lesion is microscopic and serial sections have to be cut from the ear or foot pad before the colonized zone is found. Its appearance is quite different from any seen in patients with leprosy. The animals, as might be expected, appear to be unaffected as the result of this infection throughout their life span.

Patients with leprosy show many symptoms and signs ranging between two extremes: self limiting tuberculoid leprosy with few bacilli and diffuse lepromatous leprosy with many bacilli. The clinical form which the disease presents seems, therefore, to depend either on the number of organisms which have invaded (size of dose) or on the

patient's capacity (immunological) to combat the infection. The infection in mice is certainly not related to the number of organisms injected so that it seemed reasonable to investigate the effects of reducing their immunological capacity. Two series of mice were thymectomized and given total body irradiation, 900 r., because this is known to have a strong depressive effect on other immunological processes, such as the homograft reaction and antibody production. This resulted in a dramatically greater yield of bacilli from the foot pads in both series<sup>5,6</sup>. Further large scale experiments have confirmed that thymectomy and irradiation with 900 r. results in a greatly increased yield of bacilli after injection of suspensions of  $10^4$  into the hind foot pads and ears of mice. Moreover, inocula of  $10^6$  and  $10^8$  also multiply freely.

These observations were encouraging. Even more encouraging was the unexpected observation that 12 months after inoculation, not only had the local infection increased in intensity but specific sites elsewhere in the body had become infected—for example, the nose and forepaws. Furthermore, the infected foot pads in some of the animals became swollen and the overlying skin was covered with small nodules. In short, the animals appeared to be presenting the image of human leprosy. The spread of infection to specific body sites was particularly noteworthy. This phenomenon prompted us to see whether a systemic infection would follow the intravenous injection of a suspension of bacilli. This route had never led to infections in normal mice; but in the thymectomized and irradiated mice 10 months after injection, bacilli were found in the ears and foot pad, and 19 months after injection nearly a thousand times more organisms could be recovered than had been injected and 95 per cent of these were in the ears, nose and foot pads.

The bacteriological changes already described were paralleled by histological changes in the thymectomized mice given 900 r. These were very interesting, for colonies first started to grow in voluntary muscle fibres as in the



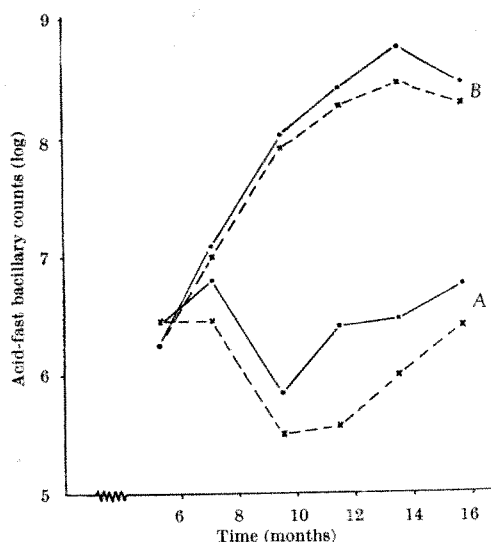


Fig. 1. Growth curves of *Myco. leprae* in skin sites of normal mice (A) and thymectomized and irradiated (with 900 r.) mice (B) injected with  $10^4$  bacilli. Each point represents the mean of counts from both ears and both hind foot pads. ●—●, Total count; ×—×, "viable" count.

case of normal mice<sup>7</sup>, but they gradually disseminated and colonized other cells until months after infection the histopathological picture in the ears, foot pads and cutaneous nerves was comparable in all respects with that in skin and cutaneous nerves of patients with lepromatous leprosy.

The form and nature of the experiments in support of these claims are outlined in Table 1. A brief description of the techniques, together with the quantitative data from a selection of completed bacteriological experiments and an account of the salient histological changes, will follow.

Female CBA mice were used; thymectomy was performed at the age of 6 weeks by the method of Taylor<sup>8</sup> and 2-4 weeks later the mice were given whole body irradiation, of either 600 r. or 900 r. Mice receiving 900 r. were immediately injected with bone marrow from female CBA mice, one donor supplying sufficient cells for three recipients.

Four separate suspensions of *Myco. leprae* were obtained by homogenizing skin biopsies from four unrelated

patients with active lepromatous leprosy. The methods of inoculating and collecting bacilli from foot pads and ears have already been described<sup>2,4</sup> and a similar method was used for collecting bacilli from the other tissues investigated.

Normal and T/r. animals, which had been injected with *Myco. leprae* into the four skin sites, were killed usually after 5 or 6 months and thereafter at 2 monthly intervals. Homogenates of each of the foot pads and ears were counted separately to obtain the total number of bacilli, and the number of non-viable organisms present, the number of viable organisms being determined by subtraction<sup>9</sup>. The animals injected intravenously were killed as follows: normal animals, 7, 10, 12, 16 and 22 months, and thymectomized and irradiated (with 900 r.) animals 6, 12, 14, 16 and 19 months, after inoculation.

Fig. 1 shows the counts obtained in the early stages after the injection of  $10^4$  *Myco. leprae* into each site in one of the four experiments. Thymectomy and 900 r. significantly enhanced the infection and multiplication was continued for 13 months with a slight fall off at 16 months, whereas multiplication fluctuated in untreated animals. Fig. 2 illustrates the effect of replacing the equivalent of total mouse lymphoid tissue immediately after thymectomy and irradiation with 900 r. and thereafter at 3 monthly intervals, in mice inoculated with  $10^4$  bacilli into the four skin sites. It shows that the severity of the infection was significantly reduced by this means but not to the level it assumed in normal animals.

In another experiment we showed that total body irradiation of 600 r. was less effective than a lethal dose of 900 r. This means that doses less than those necessitating bone marrow transfusions will not permit maximum yields of bacilli to be obtained in thymectomized mice.

Although inocula of  $10^6$  *Myco. leprae* failed to multiply in the four skin sites of normal mice, such inocula multiplied as freely in thymectomized animals irradiated with 900 r. as did inocula of  $10^4$  (Fig. 3). The count was still increasing after 16 months and it was in animals from this experiment that a systemic spread of the infection to the nose, forepaws, tail skin and leg muscles occurred

Table 1. FORM AND NATURE OF THE FOUR EXPERIMENTS

Experiment	Treatment	Injection of <i>Myco. leprae</i> No.	Site	No. of mice
Experiment 1	Untreated	$10^4$	4 skin sites*	6
	600 r.	$10^4$	" " "	6
	T/600 r.	$10^4$	" " "	6
	T/900 r.	$10^4$	" " "	6
Experiment 2	Untreated	$10^4$	" " "	6
	T/900 r.	$10^4$	" " "	6
	T/900 r. and lymph. T	$10^4$	" " "	10
	Untreated	$10^6$	" " "	6
	T/900 r.	$10^6$	" " "	11
	Untreated	$3 \times 10^7$	Intravenous	6
Experiment 3	T/900 r.	$3 \times 10^7$	"	12
	Untreated	$10^4$	4 skin sites	6
	T/600 r.	$10^4$	" " "	7
	T/900 r.	$10^4$	" " "	13
	Untreated + DDS	$10^4$	" " "	6
Experiment 4	T/900 r. + DDS	$10^4$	" " "	6
	Untreated	$10^4$	" " "	6
	T/900 r.	$10^4$	" " "	6
	Untreated	$10^7$	" " "	6
	T/900 r.	$10^7$	" " "	12
	Untreated	$10^8$	2 skin sites†	6
	T/900 r.	$10^8$	" " "	12

*Myco. leprae* was obtained from individual Malaysian patients for experiments (1), (3) and (4) and from an English patient for experiment (2).

T, Thymectomized.

Lymph. T, lymphoid tissue replacement from normal mouse.

DDS, fed 0.01 per cent diaminodiphenyl sulphone in diet.

\* Both ears and both hind foot pads.

† Right ear and right foot pad.

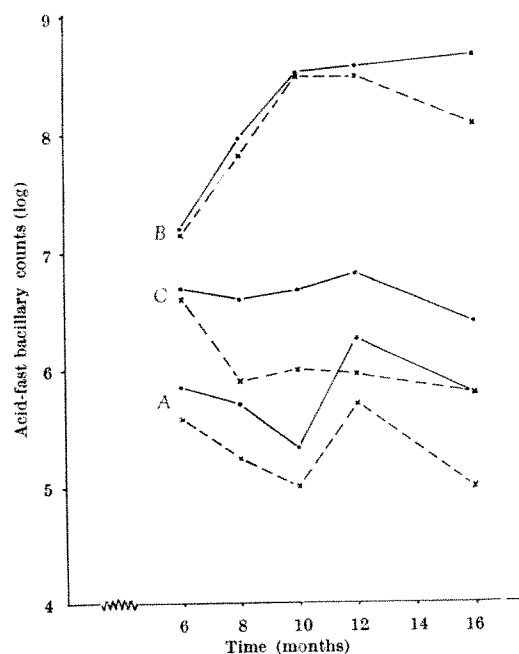


Fig. 2. Growth curves of *Myco. leprae* in skin sites of normal mice (A) and thymectomized and irradiated mice (B) and the effect of lymphoid tissue replacement (C). Inoculum was  $10^4$  bacilli. Each point represents the mean counts from both ears and both hind foot pads. ●—●, Total count; ×—×, "viable" count.

Table 2. LOCALIZATION AND YIELD OF *Myco. leprae* IN THYMECTOMIZED AND IRRADIATED (900 r.) AND NORMAL CBA MICE INJECTED INTRAVENOUSLY WITH  $3 \times 10^7$  BACILLI

Organ or tissue	Normal mice					Thymectomized-irradiated mice						
	7 months	10 months	12 months	16 months	22 months	6 months	10 months	12 months	14 months	16 months	19 months	
Foot pad	0	$10^5$	0	0	$10^5$	0	$10^7$	$6 \times 10^7$	$2 \times 10^7$	$5 \times 10^7$	$7 \times 10^8$	
Ear	0	0	0	0	0	0	$4 \times 10^7$	$3 \times 10^8$	$6 \times 10^8$	$2 \times 10^8$	$2 \times 10^8$	
Nose				$2 \times 10^5$	$5 \times 10^5$			$10^8$	$10^8$	$2 \times 10^8$	$2 \times 10^8$	
Skin, body	0	0	0	0	0	0	0	$10^8$	$3 \times 10^8$	0	$5 \times 10^8$	
Skin, tail			0	0	0			$5 \times 10^7$	$4 \times 10^7$	$2 \times 10^8$	$2 \times 10^7$	
Muscle, leg	0	0	0	0	0	0	$2 \times 10^5$	$2 \times 10^7$	$9 \times 10^8$	$4 \times 10^7$	$8 \times 10^7$	
Muscle, body			0	0	0			$3 \times 10^8$	$3 \times 10^8$	$10^8$	$5 \times 10^7$	
Liver	+	+	+	$3 \times 10^8$	0	+	+	+	$7 \times 10^8$	$2 \times 10^8$	$10^8$	
Spleen	+	+	+	0	0	+	+	+	$2 \times 10^8$	$6 \times 10^7$	$5 \times 10^7$	
Lung			0	0	0				$10^8$	$4 \times 10^7$	$10^7$	
L. nodes	±	+	+	+	+	±	+	+	$10^8$	+	+	
Brain				0	+				0	0	+	
Heart				0	0				0	0	+	

0 =  $< 10^3$ .

consistently 12 months after injection. In about half the animals in one experiment, swelling of the inoculated hind feet developed, and the skin covering the pad contained nodular swellings comparable with those in patients with lepromatous leprosy (Fig. 4). So far, no nodular swellings have affected the ears or sites of dissemination in animals from this experiment. Normal mice in this or any other experiment have never exhibited swellings.

In another experiment, mice were injected intravenously with  $3 \times 10^7$  *Myco. leprae* and the results show that a heavy systemic infection occurs only in the thymectomized animals irradiated with 900 r. (Table 2). The full significance of these findings is shown in Table 3, which shows that at 19 months, for example, 95 per cent of all the organisms recovered were in the nose, pinnae, fore and hind paws. Furthermore, the proportion of viable organisms from these sites was large, although not as great as from body and leg muscles. To test the significance of these observations more stringently, another thymectomized and irradiated mouse was killed at 19 months. The total yield of organisms from a homogenate of the whole animal was  $1.8 \times 10^{10}$  as against the summed total yield from its litter mate of  $9.8 \times 10^9$  (Table 3).

To test that the organisms from enhanced infections behave in the same way as *Myco. leprae* from patients, bacilli collected from different tissues in thymectomized and irradiated mice are being injected into the foot pads of normal mice. The behaviour of these organisms has

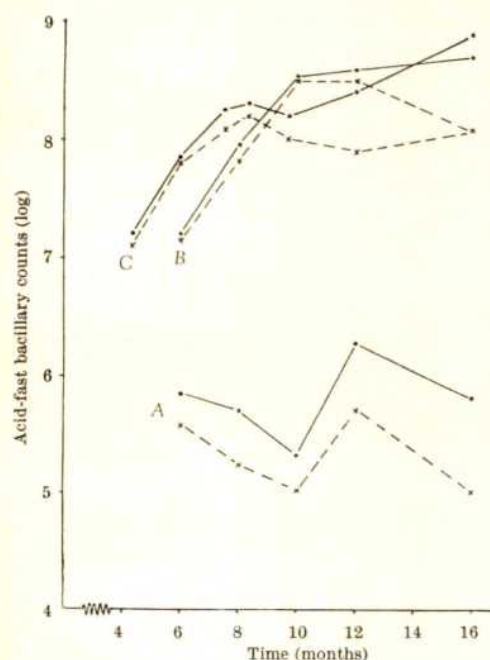


Fig. 3. Growth curves of *Myco. leprae* in skin sites of normal mice (A) injected with  $10^4$  bacilli and in thymectomized and irradiated mice injected with  $10^4$  (B) or  $10^5$  (C) bacilli. No growth was detected in normal mice injected with  $10^4$  bacilli. Each point represents the mean counts from both ears and both hind foot pads. ●—●, Total count; ×—×, "viable" count.



Fig. 4. Nodular swelling of hind foot pad in a thymectomized and irradiated mouse injected with  $10^4$  *Myco. leprae* 9 months earlier.

been bacteriologically indistinguishable from those derived from patients with lepromatous leprosy. To test further that the enhanced growth of bacilli in these mice was caused primarily by a reduction in immunity and not by any selection or change in the behaviour of the bacilli themselves, their growth was shown to be completely inhibited by feeding 0.01 per cent diaminodiphenyl sulphone, as it is in normal mice infected with bacilli from patients. There is thus substantial evidence that the

Table 3. LOCALIZATION AND YIELD OF *Myco. leprae* IN A THYMECTOMIZED AND IRRADIATED (900 r.) MOUSE 19 MONTHS AFTER INTRAVENOUS INJECTION OF  $3 \times 10^7$  BACILLI

Organ or tissue	Estimated bacillary yield		Degenerate bacilli (per cent)
	Per site ( $\times 10^6$ )	Per cent of total yield	
Hind foot pads	1,740	18	56
Fore foot pads	960	10	60
Foot pads, total		28	59
Ears	4,800	49	50
Nose	1,800	18	61
Muscle, leg	76	0.8	30
Muscle, body	160	1.6	31
Skin, tail	21	0.2	89
Skin, body	5	0.05	55
Liver	130	1.3	82
Spleen	53	5.0	87
Lung	10	0.1	70
Total	9,755		



enhanced mycobacterial infection induced in these mice has the bacteriological characteristics of *Myco. leprae* as distinct from other mycobacteria.

The tissues were sub-divided so that they could be prepared for light, fluorescent and electron microscopy. Sections stained with haematoxylin and carbol fuchsin were scored for the number and viability of the organisms present in each unit volume of tissue before the actual number present on the contralateral side had been disclosed. There was complete agreement between these arbitrary scores and the actual number of organisms.

In the skin sites, the counts of which are recorded in Fig. 1, 5 months after injection there were scattered microcolonies of various sizes in voluntary muscle fibres in the normal foot pads<sup>7</sup>. The smaller colonies contained viable organisms, but some of the larger ones had ruptured and macrophages nearby contained non-viable organisms. By contrast, no non-viable organisms were found in the foot pads of thymectomized and irradiated animals and microcolonies in muscle fibres were more numerous, more widespread but smaller than in normal animals. There were a few viable organisms in macrophages and perineurial cells. No bacilli were seen in the skin of animals from either group. After 9 months there was no substantial change in foot pads from normal mice, but in the treated animals there were many more (2 plus) organisms. These bacilli were now more equally distributed in muscle fibres, in macrophages and in perineurial cells and most were still viable. Thirteen months after infection the number of organisms in the normal foot pads was about the same, but the proportion of non-viable forms had increased and were contained in both macrophages and in vacuoles in muscle fibres. In thymectomized animals irradiated with 900 r. the number of bacilli present had increased still further (3 plus) but a number of non-viable bacilli was found in macrophages and perineurial cells. Viable bacilli were seen for the first time among the sole plate nuclei of motor nerves and in neighbouring Schwann cells.

The histological situation in tissues from sites in which counts are recorded in Fig. 2 after lymphoid replacement was similar after 9 months in the three foot pads examined. There were numerous but very small microcolonies filled with closely packed viable organisms in voluntary muscle fibres. There was also a large number of empty bacillar cell walls in the cytoplasm of Schwann cells of motor nerve fibres, among sole plate nuclei in perineurial cells and a few in macrophages. The total number of organisms was scored as 1 plus.



Fig. 5. Electron micrograph showing globus and foam (Virchow) cell in forepaw dermis of a thymectomized and irradiated (900 r.) mouse injected intravenously with  $3 \times 10^7$  *Myco. leprae* 17.5 months earlier. G, Globus; F, foam cell.

From other experiments, four samples have been selected to demonstrate the histological changes in increasingly severe infections. (a) In a foot pad from a thymectomized and irradiated mouse which had been injected with  $10^4$  *Myco. leprae* 6 months previously, the infection was severe (3 plus). There were numerous microcolonies of viable bacilli in muscle fibres but almost as many in perineurial cells and macrophages. Microcolonies were no longer confined to perineurial cells around nerve bundles in muscles but had spread to similar cells in the dermis. Bacilli were also disseminating from perineurial cells to neighbouring cells which were clustered around neuromuscular bundles. There were also organisms in motor nerve Schwann cells and in pericytes surrounding blood capillaries. The number of non-viable organisms, particularly those in macrophages, had increased sharply. (b) The second sample was from one of the enlarged foot pads. The skin was several mm thick and sections from it, stained with haematoxylin and carbol fuchsin, were indistinguishable from those of skin covering nodules in patients with lepromatous leprosy. Its bacillary content was 6 plus. There were numerous globi in the dermis filled with viable bacilli as well as foam (Virchow) cells in all stages of development. There were viable organisms in the endothelial lining of capillaries, as well as in perineurial and Schwann cells of cutaneous nerves. The dermis which contained the majority of intracellular bacilli was separated from an unusually flattened epidermis by a zone containing very few cells (similar to the "clear zone" in the skin of patients with lepromatous leprosy). At intervals there were chains of bacilli passing across this zone from the dermis into and through the epidermis as far as the keratinized layer from which they were being shed. The bacilli lay in the cytoplasm of cells with long thin processes which connected nerve bundles in the dermis to the epidermis. The cytoplasm of the cells forming the terminal links of these chains also passed into the epidermis along with the bacilli. The medial plantar nerve was also very heavily infected with numerous globi in perineurial and Schwann cells, and there were also foam cells in all stages of development. (c) The third sample was cartilage from the nose of a thymectomized and irradiated animal injected intravenously with *Myco. leprae* 14 months previously. It was covered by a very heavily infected mucoperichondrium which was stuffed full of globi containing viable bacilli. Some of the globi had ruptured through the overlying mucous membrane. The bacilli which had left, and were still leaving them, had become embedded in mucus lying within and also lining the walls of the nasal cavity. (d) The fourth sample was the pad of a forepaw from a thymectomized and irradiated animal which had also been injected intravenously 17.5 months earlier. The skin was comparable in all respects with that from a patient with lepromatous leprosy. Fig. 5 is an electron micrograph showing a small globus containing viable organisms, together with a foam (Virchow) cell lying in the dermis of the skin of the forepaw.

Such infected animals are now being used as a model to analyse the disease process induced by *Myco. leprae* and the source and nature of the inflammatory cells involved.

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## LETTERS TO THE EDITOR

## ASTRONOMY

## Radio Emission from the Sources 3C 225 and 3C 267

THE possibility of a relationship between radio galaxies and quasi-stellar sources is of great importance not only as a means for distinguishing between a local and cosmological theory of the latter sources, but also because if such a relationship does exist it would provide important evidence on the physical processes occurring<sup>1</sup>.

The term quasi-stellar sources as used by Schmidt<sup>2</sup> and E. M. Burbidge<sup>3</sup> refers to compact, blue, optical objects of stellar appearance with appreciable red-shifts; not all these objects are powerful sources of radio emission. Of those that are, some show a low frequency cut-off in their spectrum<sup>4</sup> or extremely small angular diameter<sup>5-9</sup>, but these features are by no means uniquely characteristic of this class of object. It is likely that intense optical and radio emission occurs for only a limited period and, because their lifetimes may be different, such characteristics may appear at different periods during the evolution of the same object.

Examples of blue stellar objects, associated with extensive radio sources without any compact radio component, are now known (for example, 3C 47), as well as extensive radio sources which contain a very compact radio component associated with a blue stellar object (for example, 3C 273).

Here we shall describe observations of two radio sources of large angular extent each containing a compact component without any apparent associated blue object despite the fact that the large angular extent implies a moderate distance. Unless the two components of each source represent a chance association, the observations

suggest that the intense optical emission has been of limited intensity or duration.

The observations were made with the one-mile radio telescope at Cambridge at frequencies of 408 and 1,407 Mc/s. The method of observation was the same as that already described<sup>10</sup>. The maps of the two sources are presented in co-ordinates in which the beam is circular; this involves the compression of the declination scale by a factor of about four in both cases.

3C 225. A map of the source obtained at 1,407 Mc/s is shown in Fig. 1. The source comprises two components separated by 374 sec arc in position angle (P.A.) 165°. Both are unresolved with upper limits to the angular diameters of 10 sec arc in right ascension and 40 sec arc in declination. The components are of unequal intensity, component B contributing about 70 per cent of the total emission. Positions, flux densities at both frequencies and derived spectral indices are given in Table 1.

Source	Right ascension	Declination	Flux density ( $\times 10^{-26}$ w/m <sup>2</sup> (c/s))		Spectral index
			1,407 Mc/s	408 Mc/s	
3C 225 A	09h 39m 25.3s	14° 05' 36"	1.50	3.4	0.65
	09 39 32.3	13 59 32	3.30	8.6	0.77
3C 267 A	11 47 20.8	13 04 00	1.77	7.2	0.73
	11 47 23.2	13 04 11	1.11		

There is good evidence for believing that a component of very small angular size exists. Observations with an interferometer of large baseline<sup>5,6</sup> at frequencies of 159 and 408 Mc/s have indicated a component having a diameter of less than 0.6 sec arc, and recent observations at 178 Mc/s by Little and Hewish<sup>9</sup> of the scintillation caused by the interplanetary medium have indicated that 30–40 per cent of the emission at this frequency must originate in a region less than 0.2 sec arc in extent.

The simplest interpretation is to identify the compact source with the weaker of the two components of Fig. 1. Support for this is provided by the 178 Mc/s observations, which were re-examined for us by Mr Little; they were made with a fan-beam transit instrument and show the maximum of scintillation  $4 \pm 2$  sec earlier than the maximum response—a figure which may be compared with the value 5.1 sec by which the weaker component occurs earlier than the centroid at this frequency.

The presence of such a compact source suggests that at low frequencies the effects of synchrotron self-absorption might be apparent. Williams<sup>4</sup> has shown that sources of diameter 0.2 sec arc and spectra as in Table 1 become optically thick at frequencies of about 50 Mc/s—a figure which is comparatively insensitive to the value of  $10^{-4}$  gauss assumed for the magnetic field. The total flux density at frequencies well below 50 Mc/s would therefore be entirely that from component B and would be about 30 per cent less than that expected by simple extrapolation from higher frequencies.

Observations of the total flux density of 3C 225 over a wide range of frequency<sup>11-17</sup> are plotted in Fig. 2; these include values given to us by Costain and Roger at 22.25 Mc/s and by Bridle at 10 Mc/s. It also shows as open circles the separate contributions from the two components derived from the present observations. It can be seen that the measured values are fully consistent with the adopted model in which the spectrum of component B is straight over the entire frequency range while that of component A has a cut-off at about 50 Mc/s.

A search for related optical objects on the *Sky Survey* prints shows no conclusive identifications, and certainly none as intense as might be expected for a source of such large angular extent. Using the model for extragalactic sources proposed recently<sup>1</sup>, the distance of 3C 225 is unlikely to exceed 500 Mparsec and an object as bright intrinsically as other quasi-stellar sources would have an m<sub>pg</sub> of about 13.

3C 267. The 1,407 Mc/s map of 3C 267 is shown in Fig. 3. The source consists of two components of unequal

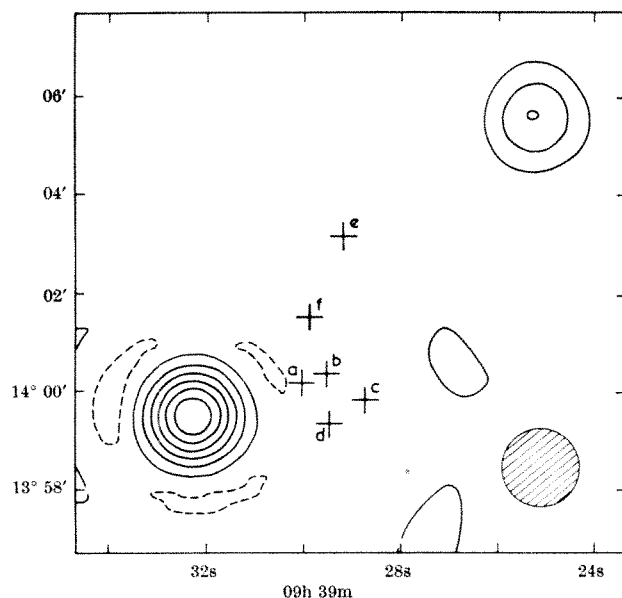


Fig. 1. The map of 3C 225 at 1,407 Mc/s. The declination scale has been compressed by  $\sin \delta$ ; the beam is shown by the shaded circle. The optical objects marked are (a) 17.2<sup>m</sup> blue stellar object; (b) 18.0<sup>m</sup> red galaxy; (c) 19.0<sup>m</sup> slightly blue galaxy; (d) 19.5<sup>m</sup> red galaxy; (e) 14.0<sup>m</sup> slightly red stellar object; (f) 20<sup>m</sup> red object. D. Willis has provided a spectrum which shows it to be a star.



flux density separated by 37 sec arc in P.A.  $73^\circ$ . Positions and flux densities of the components are given in Table 1. Both components are unresolved, with upper limits to the angular size of 10 sec arc in right ascension and 40 sec arc in declination. Observations of the source by Cohen<sup>18</sup> at 430 Mc/s showed scintillation due to the interplanetary medium, suggesting that about 30 per cent of the radiation must originate in a region less than 0.2 sec

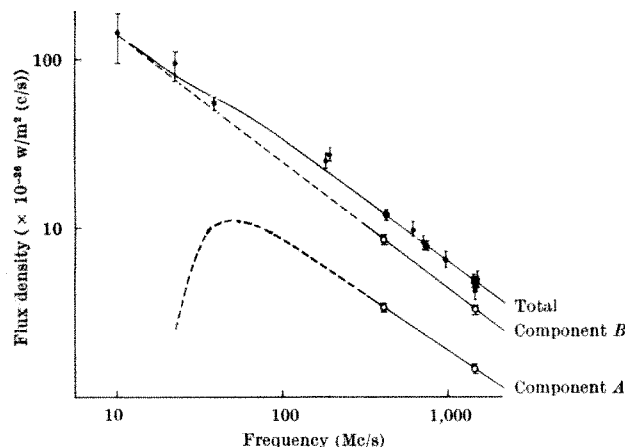


Fig. 2. The present observations of flux density ( $\circ$ ) for the two components can account for the observed spectrum if the weaker has angular size less than 0.2 sec arc and shows synchrotron self-absorption below about 50 Mc/s. This would also account for the observed degree of scintillation and the results of long baseline interferometry.  $\bullet$ , Total flux density.

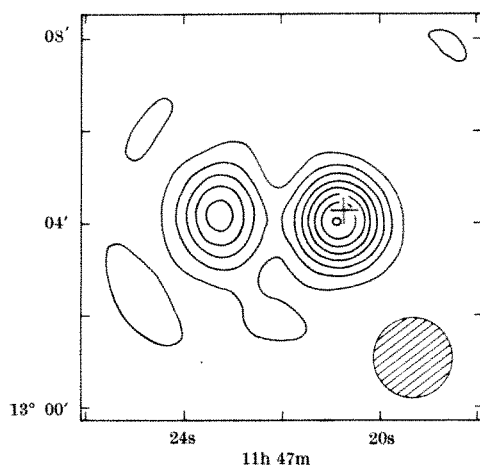


Fig. 3. The map of 3C 267 at 1,407 Mc/s. The declination scale has been compressed by  $\sin \delta$ ; the beam is shown by the shaded circle. The cross marks a 20<sup>m</sup> red object.

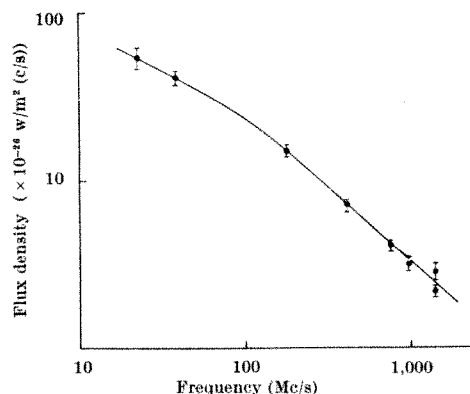


Fig. 4. The spectrum of the total flux density of 3C 267 shows marked curvature similar to that in the total spectrum of 3C 225.

arc. The compact source is expected to show synchrotron self-absorption and the spectrum of the total flux density (Fig. 4)<sup>11-14</sup> shows a curvature at low frequency very similar to that of 3C 225.

An extremely faint red object at the limit of the 48 in. *Sky Survey* plates ( $\sim 20^m$ ) found by Véron<sup>19</sup> is marked with a cross. There are no other objects brighter than 20<sup>m</sup> within 30 sec arc of the components or the line joining them.

These sources, while being of very considerable total extent, nevertheless contain an extremely compact component which shows low frequency self-absorption. The absence of any blue object which can reasonably be associated with either radio source may imply a difference in lifetime of the optical and radio emitting regions.

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## Stonehenge 56 Year Cycle

Colton and Martin<sup>1</sup> are to be congratulated on the independent discovery of eclipse cycles based on a fixed number of lunations. The cycles that they list are among the long list of cycles which have been exhaustively studied and described in a rare book by Professor G. van den Bergh<sup>2</sup> that is seldom found outside astronomical libraries. The particular cycles tabulated by Colton and Martin are named, in order of increasing length, *Hepton*, *Octon*, *Inex*, etc.

Colton and Martin's conclusions, however, have no direct bearing on the Stonehenge eclipse cycle that I have proposed<sup>3</sup>. I did not, as they claim, attempt "to find an eclipse cycle which gives eclipses on the same calendar date". Indeed, I would hesitate to suggest that the inhabitants of Stonehenge recognized calendar dates based on a full solar year. In my opinion they were interested in eclipses during the midwinter and midsummer lunation, and possibly the lunations of the equinox. In contemporary usage it would be similar to asking when the "Harvest moon" or "Easter moon" will be eclipsed.

It is interesting to note in passing that the calendar-date cycle of 65 years, preferred by Colton and Martin, is one full Stonehenge cycle<sup>3</sup> of 56 years plus the next sub-interval of 9 years. Elsewhere I have presented graphic illustration of this solstice cycle<sup>4</sup> and some interesting implications of it are considered by various authors in the issue of *Antiquity* for June 1967.

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### Is a Quasar Nucleus a Star Cluster or a Single Body?

It has been suggested<sup>1</sup> that the optical variability of the quasi-stellar source 3C 273 is compatible with a model of a quasar which is a complex of several sources of the same type; for example, a star cluster within which there are independent occasional collisions and possibly flash-like supernovae as well. This claim is incorrect. In fact, the statistical properties of the optical variability of 3C 273 are such as to suggest that the separate parts of a quasar nucleus interact with each other. In other words, a quasar nucleus is in the limit of a single body<sup>2</sup>.

The conflicting views that quasar radiation can<sup>1</sup> and cannot<sup>2</sup> be interpreted by the superposition of independent occasional events can be formulated from the point of view of mathematical statistics, respectively, as the hypotheses: (A) the one-dimensional distribution of the intensity of the quasar radiation  $x(t)$  is Gaussian; (B) the distribution  $x(t)$  differs significantly from the Gaussian form. It should be emphasized, first of all, that it is not possible to distinguish between the hypotheses A and B by the spectral density of the optical signal (the Fourier transform of an auto-correlation function), which is the assumption of ref. 1. This arises from a theorem, well known in the theory of noise<sup>3</sup>, that any spectral density is equivalent to an appropriate sequence of identical impulses occurring at independent instants of time. Moreover, sampling spectral density at low frequencies is not representative as a rule<sup>4</sup>. Indeed, the ratio of the standard deviation of the sampling spectral density to its mean value appears to be near to unity in the low frequency range ( $\sim 0.1 \text{ yr}^{-1}$ ) of the light variation of 3C 273, to which the authors of the work<sup>1</sup> attach determinant importance.

Direct verification of the validity of the Gaussian law for the one-dimensional distribution of the brightness record  $x(t)$  seems to be possible by making a constructive distinction between hypotheses A and B. For a quasi-periodical signal, it is more convenient from a practical point of view to consider its envelope  $R(t)$

$$x(t) = R(t) \cos [\omega t + \theta(t)]$$

where the envelope  $R(t)$  and the phase  $\theta(t)$  can always be chosen independently<sup>5,6</sup>. For the case in question such a choice can be realized as follows

$$R(t) = \sqrt{\left(x^2 + \frac{1}{\omega^2} \left(\frac{dx}{dt}\right)^2\right)}, \quad \omega^2 = (2\pi/T)^2$$

$\omega^2$  is taken to be  $0.5 \text{ years}^{-2}$  in accordance with  $T \simeq 9$  years (see ref. 2)\*.

\* In this case, direct calculations give  $r(R, \theta) \simeq 0.06$  for the sampling correlation coefficient, and  $\sigma_r \simeq 0.16$  for the standard deviation, which confirms the non-correlativity of  $R(t)$  and  $\theta(t)$ .

If hypothesis A was valid,  $R(t)$  would have the Rayleigh distribution<sup>6</sup>, the density of which is

$$f(R) = \frac{\pi}{2R_0^2} \exp(-\pi R^2/4R_0^2)$$

where  $R_0 = \varepsilon(R)$  is a mathematical expectation, and  $\sigma_R = R_0 (4/\pi - 1)^{1/2}$  is a standard deviation. The significance test can be made using the variability coefficient of the sampling envelope<sup>7</sup>  $v = s/\bar{R}$ , where  $s$  is a sampling standard deviation and  $\bar{R}$  is a sample mean. It is possible to show that for the Rayleigh distribution

$$\varepsilon_v = \left(\frac{4}{\pi} - 1\right)^{1/2} = 0.523$$

$$\sigma_v = 0.372 n^{-1/2} + O(n^{-3/2})$$

To refer to the same statistical data<sup>2</sup> on the light variability of 3C 273 that were used in ref. 1, there are seventy-one values of  $R$ , correlated (due to smoothing) within 2 years, that is,  $n = 35$  independent values, so that  $\sigma_v = 0.063$ . The Rayleigh distribution is rejected at the 1 per cent significance level, if

$$|v - \varepsilon_v| > 2.58 \sigma_v$$

if  $v < 0.36$  or  $v > 0.68$ . According to ref. 2,  $v = 0.34$ . Hence, the Rayleigh distribution of the brightness envelope of 3C 273, and consequently that of hypothesis A, is rejected with 99 per cent confidence, which is uncommonly high for astrophysical problems.

Thus the optical variability of the 3C 273 quasar cannot be interpreted as a result of the superposition of independent random flashes in a compact star cluster. On the other hand, is it possible that this model is valid for other quasars where there are not yet enough data available for the variability?

There are serious objections to any hypothesis of a quasar nucleus as a compact star cluster. Some of these are based on considerations of energy, as has already been pointed out elsewhere<sup>2,8</sup>. In addition, in a detailed model of stellar collisions<sup>9</sup> it was shown that for the necessary quasar radiation power the energy release for each supernova (which is considered to have resulted from stellar collisions) should be  $10^3$ – $10^4$  times the energy release of the supernova observed in the optical region. If this rigid demand, which is likely to be unreal, be rejected, then the initial number of stars  $N$  may be much larger. In this case the time of formation of a compact cluster, proportional to  $N^2$ , is sharply increased, and it is not clear how this difficulty can be eliminated while retaining an element of catastrophe as a necessary requirement for a quasar formation. In this model some other serious difficulties of a cosmogonic nature are also encountered<sup>10</sup>.

The unacceptability of the model of a compact star cluster as an energy source can be shown by a far-reaching analogy between quasars, on the one hand, and radio galaxies, Seyfert galaxies, and the nuclei of common galaxies on the other.

The fact that the radio variability of the nucleus of the Seyfert galaxy NGC 1275 is similar to that of quasars<sup>11</sup> leaves little doubt about the fact that the nature of the physical processes in these objects is the same, but differs in the scope of its activity. This is also indicated by the discovery of strong infra-red radiation from the nucleus of the Seyfert galaxy NGC 1068, the spectrum of which is similar to the infra-red spectrum of 3C 273 (ref. 12). Meanwhile, an attempt to interpret these phenomena in the framework of a model of a compact star cluster encounters great difficulties on energy grounds, the more so because the reliability of the distances to these objects, unlike those to quasars, can no longer be disputed. The insurmountable nature of these difficulties is particularly clear in the interpretation of the infra-red source in the nucleus of our galaxy, the size of which is less than 0.2 parsec and which has a complete power of more than  $10^{39}$  ergs/sec (ref. 13). In this case the

average density of stars appears to be more than  $10^8$  parsec $^{-3}$ , that is, such compact clusters cannot exist for long periods of time. Finally, a direct argument against the model of a star cluster is quasar 3C 446, in which a rapid variability is found, showing that the source sizes are less than  $3 \times 10^{14}$  cm (see ref. 14). It is quite inconceivable that stars could preserve their individuality in this situation.

Thus the idea of a quasar nucleus as a compact star cluster encounters great difficulties of an energetic and cosmogonic character and is in marked contrast with the observed optical variability of 3C 273. At the same time, the assumption that the radiation source of the quasar nucleus is, in the limiting case, a single body (that is, the whole complex of strongly interacting fields) finds its direct verification in the statistical analysis of light variation of 3C 273. A general theoretical study of a possible nature of this single body and the display of its properties in a variable radiation flux is given in refs. 8 and 10.

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### Subtractive Dispersion Spectrographs of Possible Use in Ultra-violet Stellar Research

THE relative intensity of the short wavelengths in stellar spectra can be increased by means of a partial achromatization device consisting of two reflexion gratings. Experiments at this observatory have given promising results, and suggest that devices like this may have applications in space research as well as in conventional astronomy.

*Methods using a prism and a grating.* This principle has already been described by Wood<sup>1</sup> as a "partial achromatization by means of a grating", but it does not seem to have been used in ultra-violet stellar research. By subtracting the dispersion of a prism from that produced by a grating, a very low dispersion may be obtained in the ultra-violet even though the dispersion for longer wavelengths may still be considerable. In order to obtain a partial achromatization (spectrum turning in the far ultra-violet), however, the prism must have a large refracting angle. This may be a drawback of this method for space research, particularly if an objective prism is used. We have nevertheless made promising experiments in the laboratory indicating that such devices may be very useful for special purposes.

*Methods using two gratings or one grating combined with a plane mirror.* Wood<sup>2</sup> has described the use of two crossed gratings for removing ghosts. A similar arrangement has been used at the US Naval Research Laboratory

for avoiding stray light in a spectrograph<sup>3</sup>. The use of two consecutive concave gratings for producing an image of the Sun by means of a narrow part of its spectrum has also been considered before<sup>4</sup>, but I have not been able to find in the literature a report of the use of two consecutive plane gratings as a subtractive dispersion device for decreasing the dispersion in the violet. According to experiments made recently at the Swedish Solar Observatory in Anacapri, this method looks perhaps the most promising one for this purpose, and a few details may be of interest.

Let the angle of incidence for the two gratings be  $i_1$  and  $i_2$  and the angle of emergence  $\vartheta_1$  and  $\vartheta_2$ , and let the angle between the two gratings be  $\gamma$ . If the grating constant  $\sigma$  is the same for the two gratings and if the wavelength  $\lambda$  is observed in the first orders, the combined effect may be described by the well known grating formulae as follows

$$\begin{aligned}\sin \vartheta_1 &= \sin i_1 + \frac{\lambda}{\sigma} \\ i_2 &= \gamma - \vartheta_1 \\ \sin \vartheta_2 &= \sin i_2 + \frac{\lambda}{\sigma}\end{aligned}\quad (1)$$

By using suitable values of  $i_1$  and  $\gamma$ , partial achromatization may be achieved. The phenomenon is described by the function  $\vartheta_2(\lambda)$ . Fig. 1 gives three examples of this function calculated according to the equations (1) and with the assumption that the gratings have 576 grooves per mm and that the angle  $\gamma$  is  $40^\circ$ . The three curves correspond to angles of incidence  $i_1$  of  $5^\circ$ ,  $7^\circ$  and  $9^\circ$  respectively, giving angles of emergence  $\vartheta_2$  of  $36.6^\circ$ ,  $34.2^\circ$  and  $31.9^\circ$  for the three wavelengths of about 4500 Å, 3800 Å and 3000 Å corresponding to the vertices of the dispersion curves. This means that in all three cases the spectrum is returning, which produces an overlap of long and short wavelengths. This overlapping can be avoided, however, if the two gratings are rotated through a small angle in opposite directions. A most spectacular phenomenon is displayed if the slit of the spectrograph is divided into intervals (Fig. 2), so that the two branches are separated. The following relations give the vertex directions

$$\begin{aligned}\vartheta_1 &= i_2 = \frac{\gamma}{2} \\ \sin \vartheta_2 &= \sin \frac{\gamma}{2} + \frac{\lambda}{\sigma} = 2 \sin \frac{\gamma}{2} - \sin i_1\end{aligned}\quad (2)$$

Fig. 2 shows two subtractive dispersion spectra which I have obtained at Anacapri with the use of two Siegbahn gratings and with the Sun as source. The long wavelengths are seen in the longer branches of the two images with the

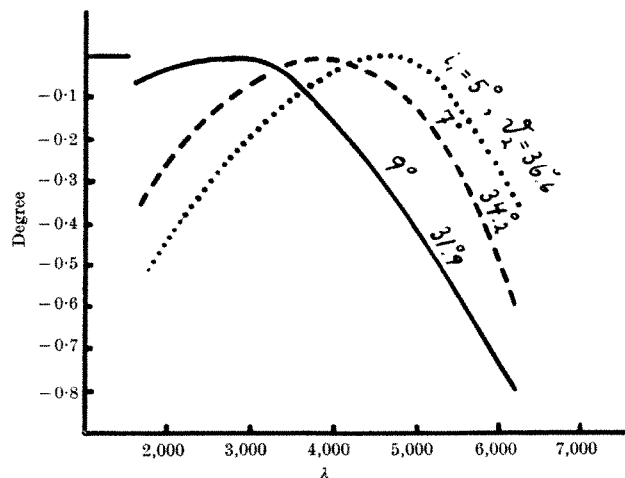


Fig. 1. Different dispersion curves for a combination of two plane gratings giving dispersions in opposite directions.





Fig. 2. Two solar spectra obtained by the use of two slightly inclined gratings and showing partial achromatization. The longer branches correspond to longer wavelengths, the shorter branches to shorter wavelengths.

H $\alpha$  line to the right. Despite the use of Eastman 'E IV' films, which have a low sensitivity in the green, the decrease in dispersion towards the vertex point produces a very uniform density on the film. The shorter branches show the violet and ultra-violet parts of the spectrum with the H and K lines slightly out of focus. In this branch the dispersion increases towards the shorter wavelengths, giving a rapid decrease in density with increasing distance from the vertex point. The phenomenon corresponds more or less to the curve valid for  $i_1 = 5^\circ$  in Fig. 1.

We have also carried out experiments with one grating combined with a plane mirror and have found effects similar to those with two gratings. It should be noted that diffraction gratings have previously been combined with plane mirrors<sup>5</sup>, not for the purpose of producing partial achromatization but for increasing resolving power. For our purpose, the use of two suitably blazed gratings seems to give greater intensity and less disturbing overlap. The gratings should have a good blaze in the first order and for the appropriate directions. In this way a very satisfactory intensity will be obtained, particularly at the vertex point, which may appear as a brilliant feature in the spectra.

The two grating device may be used in front of a telescope in the same way as an objective prism or in a spectrograph, with or without a slit. When used as an objective grating, the fairly rapid change in the spectrum with varying angle of incidence  $i_1$  may introduce some limitations. As may be inferred from Fig. 1, a change in  $i_1$  of one degree produces a change in the vertex position of slightly more than twice this amount. To some extent, photometric corrections for this effect seem possible, however. As far as can be judged from laboratory experiments, colorimetric work would seem possible, particularly by using the brilliant region at the vertex point. This may allow photometric measurements of the continuous spectrum far out in the ultra-violet. For somewhat longer wavelengths, real spectrophotometry will be possible.

By using two slightly inclined gratings (Fig. 2) and selecting a vertex point at about 4500 Å or so, the two branches of the spectrum of a nebula or similar object showing large Doppler shifts may be compared in order to determine radial velocities in spectra obtained without slit. If this is feasible, the method would have some traces in common with Fehrenbach's<sup>6</sup> method of measuring two reversed spectra obtained with two independent prisms.

Finally, the device may also have applications in solar physics. The brilliant vertex point may make it possible to obtain, for example, prominences at the limb in selected spectrum regions. When studying the prominences (or the disk) in the Balmer continuum or other continua, such a broad-band filter may be of value even if the solar image is elongated and not circular.

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## PLANETARY SCIENCE

### Lunar Variations in Auroral Zone

AN investigation of the frequency content of the geomagnetic record, collected during the IGY/IGC at several stations spread over the globe, has been carried out by high resolution spectrum analysis. I have already described the method<sup>1</sup> and a detailed discussion of the analysis will appear elsewhere. At most of the stations, besides the solar harmonics, several lunar tidal lines and luni-solar lines appeared significantly above the noise level of the spectrum. The amplitudes of these lines have been estimated from the power energy of the appropriate peak in the spectrum; in this note only the variation of the amplitude of the harmonic terms  $L_1$ ,  $L_2$  and  $L_3$  with respect to the dipole latitude is considered. The frequencies of these lines as calculated by Chapman's formula are 0.93227, 1.93227 and 2.93227 cycles per day respectively and the frequencies of the corresponding lunar tide lines  $O_1$ ,  $M_2$  and  $M'_3$  (corresponding to the term  $A_{84}$  of Schureman<sup>2</sup>) are respectively 0.92953, 1.93227 and 2.93427 cycles per day. With the frequency interval (0.00416666 cycles per day) allowed for in the spectrum analysis, the minor differences in the frequencies of the luni-solar and the tidal lines remain indistinguishable and the resultant effect appears as a peak in the spectrum.

Simple tidal theory indicates that the diurnal and semi-diurnal terms of the lunar tide vary with latitude  $\lambda$  as  $\sin 2\lambda$  and  $\cos^2\lambda$  respectively<sup>3</sup>. The important thing to notice is that because  $\cos^2\lambda$  approaches zero faster than  $\sin 2\lambda$ , at higher latitudes ( $> 45^\circ$ ) the dominance of the semi-diurnal tide decreases and the diurnal term becomes discernible. In the auroral zone, for example, near a latitude of  $65^\circ$ , the values of  $\sin 2\lambda$  and  $\cos^2\lambda$  are respectively 0.77 and 0.18 and thus the diurnal term has a favourably high coefficient in the auroral zone—a region of high conductivity in the overhead ionosphere because of the large charged particle precipitation.

A study of Fig. 1 indicates that the amplitudes of the resultant harmonic terms are considerably larger in the equatorial and in the auroral zones. It has been suggested by Matsushita<sup>4,5</sup> that the large amplitude of the lunar geomagnetic variation in the equatorial zone is due to a lunar equatorial electrojet. On either side of this equatorial electrojet belt the amplitudes of the lines ( $L_1$ ,  $L_2$ ,  $L_3$ ) as seen in Fig. 1 are small, but there is a sudden and considerable increase in their amplitudes within the auroral zone in either hemisphere, the amplitude of the line  $L_1$  being maximum. Beyond the auroral zone the amplitudes of these lines are smaller but still larger than those in mid and low latitudes and the diurnal effect predominates.



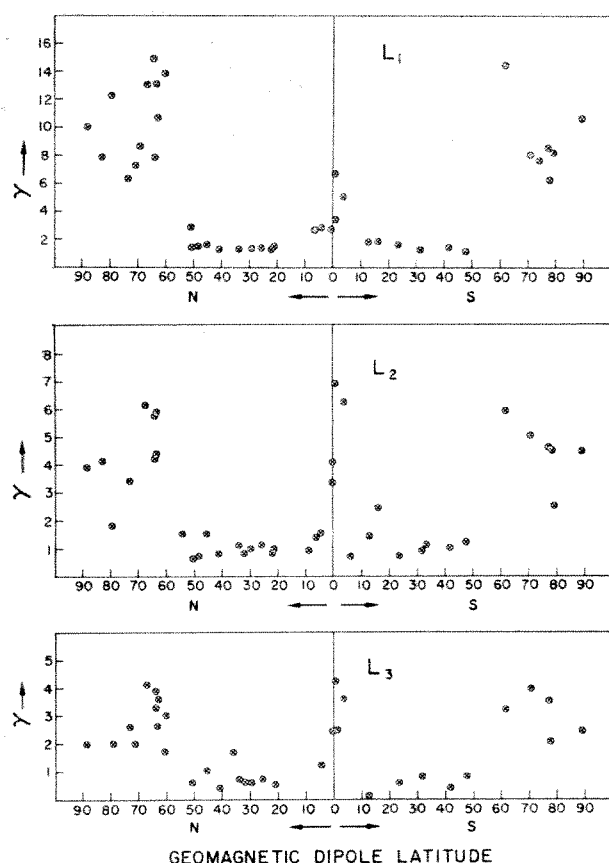


Fig. 1. Variation of the amplitude of the lines  $L_1$ ,  $L_2$  and  $L_3$  with the geomagnetic dipole latitude.

In the auroral zone, the diurnal tidal effect aided by the enormously large diurnal variation of conductivity due to particle precipitation seems to give rise to a lunar auroral electrojet which seems responsible for the large amplitudes of the lines  $L_1$ ,  $L_2$ ,  $L_3$ . Also, the phase differences computed in the cross spectrum analysis between the data from different stations, lying in the auroral zone, possess the same sign at luni-solar harmonics as at the solar harmonics. This indicates that the current system responsible for these large luni-solar variations in the auroral zone flows in the same direction as the well known auroral electrojet. Further studies of this luni-solar effect through geomagnetic and ionospheric record, taken in the auroral zone, are required to verify this conclusion.

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### Faunal Evidence bearing on the Age of Late Cambrian - Early Ordovician Metamorphism in Britain and Norway

It can no longer be stated categorically that metamorphism of the Dalradian Series in Connemara occurred before the *Didymograptus extensus* zone of the Arenig Series, because those sediments generally accepted as the

earliest post-tectonic sediments in the area have provided a *Didymograptus bifidus* zone (Lower Llanvirn) graptolite fauna. This means that the only stratigraphical younger age limit which can be placed on the Dalradian metamorphism in Connemara is some time plane at or near the base of the *bifidus* zone. This upward extension of the younger, minimum stratigraphical limit of the Dalradian metamorphism necessarily extends considerably that interval in Late Cambrian-Early Ordovician times during which an important phase of orogeny could have taken place. Furthermore, the statement by Leggo *et al.*<sup>1</sup> that "the base of the Arenig would seem to be younger than  $510 \pm 35$  m.y.", on the evidence of the rubidium/strontium age given by the post-metamorphic and (hitherto) pre-Arenig Oughterard granite, should be amended to refer to the base of the *bifidus* zone.

In Connemara, the slates and cherts in the Sruffaunduff, south-west of the summit of Bencraff, Co. Galway, have long been regarded as the oldest sediments of the Ordovician succession in that part of Ireland<sup>2-4</sup> and they have yielded the graptolites which have been used to give the younger age limit to the main metamorphism of the Dalradian Connemara Schists. Carruthers and Muff<sup>2</sup> attributed the Bencraff fauna to the *extensus* zone, and Theokritoff<sup>3</sup>, in his review of the Ordovician rocks near Leenane, did likewise. The fauna listed from this locality is more suggestive of the *bifidus* zone, however, and this age has been confirmed by recently collected material. The following forms have been identified:

*Dichograptid* stipe fragments, gen. et sp(p). indet.  
*Didymograptus* cf. *dubitalis* Harris and Thomas  
? *Tetragraptus* sp. indet.  
*Tylograptus geniculiformis* Mu  
*Tylograptus geniculiformis* var. *flexilis* Mu  
*Glossograptus acanthus* Elles and Wood  
*Hallograptus inutilis* (Hall)  
*Glyptograptus austrodentatus* Harris and Keble subsp. indet.  
*Pseudoclimacograptus* cf. *angulatus angulatus* (Bulman)  
? *Amplexograptus* sp. indet.

Reference to the *bifidus* zone is indicated particularly by the presence of *Tylograptus geniculiformis* and also by the rich and varied biserial element in the fauna. The absence of pendent species of *Didymograptus*, so distinctive a feature of the *bifidus* zone elsewhere in Britain, is not significant in this case because the affinities of the Bencraff graptolites are clearly with the Pacific region (Australia, China and western North America) where such forms are rare or absent.

Elsewhere<sup>5</sup>, it has been suggested that the graptolite fauna described by Blake<sup>6</sup> from the Bogo Shale of the Trondheim region probably belongs in the *Didymograptus hirundo* zone of the standard British Ordovician graptolite zonal sequence. The graptolites are important in giving a younger age limit to the Trondheim orogenic phase because the Bogo Shale lines near the base of the post-orogenic Hovin Group, though its exact position relative to the basal conglomerate (Stokkvola = Vella = Fjeldheim) of the group is uncertain<sup>6,7</sup>. An older age limit for the movements is provided by the occurrence of *Dictyonema flabelliforme*, indicative of the lower part of the Tremadoc Series, in the pre-orogenic Røros Group of the Trondheim region<sup>8</sup>. Moreover, there are indications that the movements were concentrated later rather than earlier in the interval between the occurrence of *D. flabelliforme* and the *hirundo* zone graptolite fauna in the Bogo Shale. First, the pre-orogenic Støren Group is stratigraphically intermediate between the Røros and Hovin Groups, so that the movements were not only post-Røros Group but also post-Støren Group. Second, in the Digermul Peninsula, in Finnmark, there is an apparently unbroken succession of 3,000 m of completely conformable clastic sediments, extending from the Eocambrian through the Cambrian to the Tremadoc<sup>9</sup>, which terminates against an over-thrust metamorphic complex; this situation implies the absence of a major orogenic event in the Lower Palaeozoic of Northern Norway at

least until late Tremadocian times. This movement phase in Northern Norway has been equated with the Trondheim orogeny in the Trondheim region of Central Norway by Sturt *et al.*<sup>10</sup>.

It is not unlikely that the Trondheim movements were still operative as late as early *hirundo* zone times because some elements of the Bogo Shale fauna are suggestive of a position high in the *hirundo* zone and such an upper stratigraphical age limit for the Trondheim movements is only slightly earlier than that for the Dalradian metamorphism in Connemara. Moreover, Sturt *et al.*<sup>10</sup> have suggested that a principal phase of the Caledonian orogeny occurred at or before  $490 \times 10^6$  yr in Northern Norway. This is based on the potassium/argon ages given by a group of alkaline rocks which were emplaced at a late stage in the deformation-intrusion-metamorphism sequence. Such an age of orogeny agrees well with the postulated radiometric age of the Dalradian metamorphism in Connemara.

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### Complete Late Pre-Cambrian to Early Palaeozoic Orogenic Cycle in Ghana, Togo and Dahomey

ISOTOPIC age determinations indicate the existence of a widespread  $500 \times 10^6$  yr thermal and metamorphic event, the "Pan African orogeny", which has affected most of the African shield outside three cratonic areas<sup>1</sup>. Black<sup>2</sup> has discussed the effects of this event on the West African craton and has concluded that it has the status of an orogeny. He drew attention to the way late Pre-Cambrian sediments lie partly on the craton and partly on the areas involved in the orogeny where they have been folded and metamorphosed.

I have distinguished sedimentary, structural and magmatic features in Ghana, Togo and Dahomey, which show that a complete orogenic cycle occurred in the late Pre-Cambrian and early Palaeozoic. Because in some parts of Africa the "Pan African orogeny" has been considered only as a thermal and metamorphic event, the recognition of a complete cycle in West Africa is of importance.

The understanding of the relationships within the sediments associated with the orogeny depends partly on abandoning the long-standing acceptance of uninformable relations between the Voltaian group and the Buem and Togo formations, and on recognizing that there is a measure of lateral equivalence between them.

The publications of the Ghana Geological Survey present a wealth of evidence supporting this view, of which I intend to present a detailed account elsewhere. For example, it has long been recognized that some sedimentary divisions—notably the Afram shales—lie in both the Buem formation and the Voltaian Group<sup>3-8</sup>.

Late Pre-Cambrian sedimentation began with the deposition of a series of fluvial and shallow water arenaceous sediments constituting the Lower Voltaian sandstones and the Togo quartzites. After this the orogenic cycle proper began and the tectonic differences between the stable craton and the mobile orogen started to influence the nature of the subsequent sedimentation. The overlying and laterally equivalent Voltaian Oti beds, Afram shales and Buem formation range from stable shelf or miogeosynclinal marine sediments, to sediments possessing eugeosynclinal characteristics. The Oti beds on the craton have a typical mudstone-shale-limestone association, with some of the limestones containing *Collenia*-like remains and sponge spicules. The Buem, on the other hand, includes greywackes, cherts, jaspers, a slide conglomerate, graded-bedding, slump-structures, and spilitic and pillow lavas<sup>9,11</sup>, and has clearly accumulated in a tectonically unstable environment. It is taken to represent a flysch deposit.

After the deposition of these sediments, orogenic folding affected the Buem and Togo formations and the uplift of this fold-belt constituted a source from which the molasse Obosum beds and Upper Voltaian sandstones were derived and deposited on the craton to the west. Palaeo-currents and grain-size variations in the Obosum beds clearly show their easterly source, and the intercalated conglomerates have a much higher proportion of plutonic rock fragments as compared with the earlier pre-orogenic Akroso conglomerate<sup>5</sup>.

The orogenic folding of the Buem and the Togo formations was accompanied by important thrust movements towards the west. Thrusts and mylonites often mark the contact between the Togo formation and the underlying crystalline Dahomeyan, and there are reasons for regarding the Buem and the Togo as tectonic units, frequently bounded by thrusts, and characterized by distinct sedimentary facies, tectonic style and metamorphic grade. Recent earthquake epicentres and posthumous fault movements mark the position of the orogenic belt at the present day<sup>8</sup>.

The associated igneous rocks range from the pre-orogenic Buem lavas which include both pillow and spilitic varieties, serpentinites and other ultra-basic rocks emplaced along thrust planes (refs. 12-14 and personal communication from K. C. A. Burke), and concordant and discordant granites. The granites are most abundant in the underlying crystalline Dahomeyan but also cut the Togo formation<sup>15</sup>. This igneous activity constitutes a normal orogenic igneous cycle.

The age of the sediments of the orogenic cycle is not precisely known, because no diagnostic fossils have been recorded from them. The small Accraian and Sekondian basins on the coast, which are likely to belong to the same sedimentary episode as the highest Voltaian, have yielded Devonian and Lower Carboniferous fossils. The recognition in better documented orogenic belts elsewhere that mineral isotopic ages may represent the time of the uplift of the belt and the onset of molasse sedimentation<sup>16,17</sup>, and not the time of the first crystallization, suggests that dates from the Dahomeyan<sup>18</sup> indicate that the deposition of the molasse Obosum beds began in the Upper Cambrian or the Lower Ordovician.

The existence of a complete late Pre-Cambrian to early Palaeozoic orogenic cycle in Ghana, Togo and Dahomey is of some importance to the hypothesis of continental drift. The recently reported discovery of  $2,000 \times 10^6$  yr and  $500 \times 10^6$  yr age provinces in the basement rocks of northern Brazil—which match the similar age provinces in West Africa when South America and Africa are fitted

together<sup>18</sup>—indicates that a similar complete orogenic cycle may also be present in northern Brazil.

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## New Zealand Region Volcanism and Structure

DURING 1959–65, five major New Zealand Oceanographic Institute cruises were completed, under the NZ Antarctic programme, in the subantarctic region south of New Zealand. Bathymetric data from these and other cruises dating back to 1950 have made it possible to define the morphology with reasonable accuracy over a 500,000 square mile area, between latitudes 48° and 57° S. and longitudes 157° and 180° E., the chief elements of which are Macquarie Ridge and the Campbell Plateau (Fig. 1). Fifty-two bedrock samples obtained on New Zealand Oceanographic Institute cruises add substantially to the previously limited geological data from this region, earlier obtainable only from the subantarctic islands sited on the ridge and plateau.

Until now, detailed analyses of the geology and structure of this region have not been possible because of the marked paucity of data. Seismic refraction studies show that Campbell Plateau is a section of continental crust with intermediate thickness<sup>1</sup>. Brodie<sup>2</sup> related the plateau to the Lord Howe Rise, suggesting that they might be formerly continuous structures dislocated by the Alpine Fault. Otherwise no attempt has been made to investigate the structure of the plateau.

From the seismicity of the Macquarie Ridge<sup>3,4</sup>, Ewing and Heezen<sup>5</sup> suggested that it was probably a branch of the mid-ocean ridge system. Continuity of the ridge from the New Zealand continental shelf to latitude 60° S. has been but recently demonstrated by Brodie and Dawson<sup>6</sup>. Hatherton has since implied that the ridge continues into southernmost New Zealand, where it is manifest as the isostatically undercompensated, upstanding Fiordland massif<sup>7</sup>.

Magnetic evidence from the South-western Pacific Basin south of the Campbell Plateau<sup>8</sup> demonstrates the existence of linear magnetic anomalies parallel both to the East Pacific Rise and the Antipodes Fracture Zone<sup>9</sup> forming the eastern margin of the Campbell Plateau.

All available data, including those most recently obtained by the New Zealand Oceanographic Institute, have been considered in an attempt to clarify the geology of structures on the sea floor south of New Zealand<sup>10</sup>. The narrow, arcuate, seismically active Macquarie Ridge, separated on its convex side from the Tasman Basin by a deep, narrow,

discontinuous trench, is most probably an island arc. Petrographic and chemical analyses of ridge basalts show them to be dominantly tholeiitic and similar in composition to Japanese tholeiitic basalts and dissimilar from mid-ocean ridge basalts. Between the ridge and the plateau is the seismically active Solander Trough in which Quaternary hornblende-andesites occur at Solander Island. The trough is part of a dilatational rift system comprising the Waiu Depression in the South Island, New Zealand, and the Emerald Basin, probably a newly formed ocean basin (Fig. 1). East of this rift occur the late Tertiary alkaline volcanics of the Auckland Islands<sup>11</sup>, Campbell Island, Dunedin<sup>12</sup>, and Banks Peninsula<sup>13</sup>. Such lateral transitions in magma type are typical of the transition zone between oceanic and continental crust in the circum-Pacific belt<sup>14</sup>. Here then is a continental margin similar to that recognized elsewhere in the circum-Pacific belt, fringing the Campbell Plateau for at least 500 km south of New Zealand. Recent geophysical investigations<sup>15</sup> show that earthquake foci increase in depth eastward away from Fiordland. This parallels the observed increase in alkalinity and once again is a feature typical of the circum-Pacific belt<sup>14</sup>. It supports the contention that the Macquarie–Solander island arc complex continues into southernmost New Zealand. The complex may have begun its development during the Oligocene, the age of oldest dated sediments in the Waiu Depression.

On the Campbell Plateau, the sparse geological data suggest Cretaceous planation followed by sinking of the plateau to about its present level<sup>16</sup>. Our recent data show tectonic development of broad east–west trending rises on the plateau (Fig. 1) culminating in late Tertiary volcanism. Some large-scale, simple geological process may relate the development of these Tertiary structures to the

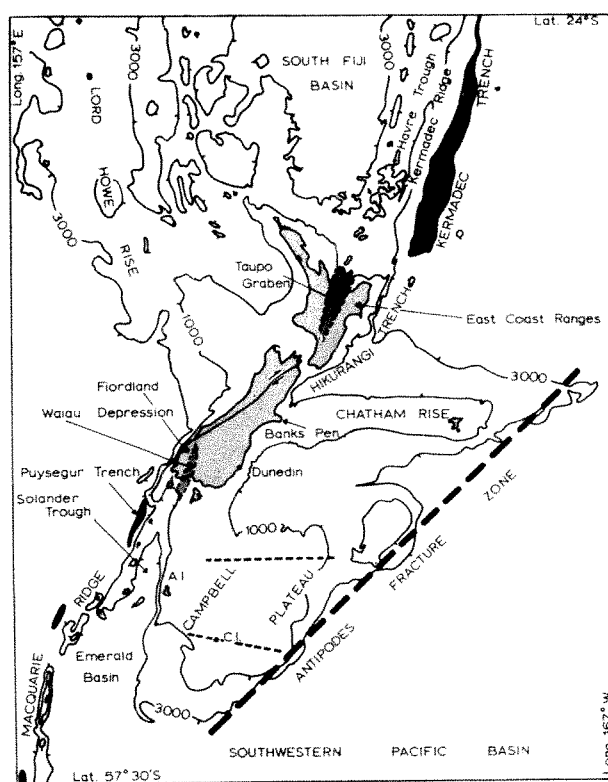


Fig. 1. The New Zealand Region. Southern trenches > 5,000 m deep and Kermadec Trench > 6,000 m deep, black. Land, light stipple; terrestrial and continental shelf extensions of offshore rift depressions, heavy stipple; Antipodes Fracture Zone, heavy dashed line; axes of uplift on Campbell Plateau, double dashed line; A.I., Auckland Islands; C.I., Campbell Island. Bathymetry from *Bathymetry of the New Zealand Region*, N.Z. Oceanogr. Inst. Chart., Misc. Ser. (in the press). Mercator projection; depths in metres.

Tertiary Macquarie-Solander complex, of markedly different trend and character.

The linear magnetic anomalies on the floor of the South-western Pacific Basin south of the Campbell Plateau<sup>8</sup> probably indicate movement of the crust away from the crest of the East Pacific Rise in the manner proposed elsewhere by Vine and Matthews<sup>17</sup>. Abrupt termination of the Chatham Rise by the Antipodes Fracture Zone suggests the rise structures were formerly continuous beyond the fault. Similarly curtailed structures are to be found in West Antarctica, and the Antipodes Fracture Zone thus corresponds to a rift margin fracture caused by movement of New Zealand away from West Antarctica consequent on Tertiary development of the East Pacific Rise. Menard<sup>18</sup> has suggested that the East Pacific Rise commenced its evolution in the North Pacific and spread southward. Because island arc development may be related to crustal movements centred on mid-ocean rises<sup>18,19</sup>, Macquarie Ridge could have formed in response to this southward spread. Continued southward and westward development of the East Pacific Rise now appears to have disrupted the Macquarie Ridge, of which a former southern continuation may be found in the ridge on which the Balleny Islands are sited.

In the North Island of New Zealand, continental margin associations typical of the circum-Pacific belt are found; in the Tertiary-Quaternary volcanic zones alkalinity increases westward from the Hikurangi Trench<sup>20</sup> as also do maximum depths of earthquake foci<sup>21</sup>. Northwards the volcanically active Taupo Graben is continuous offshore in the Havre Trough<sup>22</sup>; Tonga-Kermadec Ridge is continuous onshore in the East Coast Ranges of the North Island; Tonga-Kermadec Trench continues south as Hikurangi Trench (Fig. 1). Thus the North Island, New Zealand, is the southern extremity of an active island arc complex. Its structure and volcanism mirror that of southernmost New Zealand which is the northern extremity of the active Macquarie Arc. Fiordland and the East Coast Ranges, on continental crust, are non-volcanic in contrast to their offshore prolongations the Macquarie and Kermadec Ridges. Separation of the ends of oppositely directed island arcs by the Alpine Fault (Fig. 1) suggests that this is a transform fault of the type defined by Wilson<sup>23</sup>. The disposition and development of the Tonga-Kermadec arc have already been explained in terms of interaction between crustal movement systems centred on the East Pacific Rise and the Indian Ocean branch of the mid-ocean ridge system<sup>19,24</sup>. Macquarie Ridge may initially have been similarly sited along a line of convergence between oppositely directed forces centred on the Indian Ocean and Pacific mid-ocean ridge systems. This system today is evidently at a different stage of development from that governing the volcanically active Kermadec-Tonga system to the north.

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## Trend Surface Mapping of Raised Shorelines

THERE have been several attempts in recent months to illustrate and define the degree of tilting or deformation of the various raised shorelines in Scotland<sup>1</sup>. Considerable use has been made of shoreline distance diagrams or graphs, whereby heights of the raised beaches in a particular area are projected on to a baseline, usually drawn in the supposed direction of maximum tilt of the series of shorelines as a whole. Each separate raised beach height is shown as a point on the diagram, the position of which is defined by altitude (usually given above *O.D.*) and projected distance along the baseline: the shorelines are depicted by lines or zones on the diagram, which in some cases are calculated best-fit regression lines to the series of points representing a given shoreline; in other cases they have been fitted by eye.

In the sense that such diagrams are two-dimensional representations of height data which have areal distribution, they provide a partial picture and allow only limited analysis. A three-dimensional view may be obtained by fitting a simple plane to the data for each shoreline as has been done in the case of the main post-glacial shoreline in part of western Scotland<sup>2</sup>. More sophisticated analysis is possible, however, if higher order surfaces are considered. Techniques and applications of trend-surface mapping have been considered by Chorley and Haggett<sup>3</sup>, and the surface fitting programme described by Whitten<sup>4</sup> offers a convenient tool in the present context. As will be illustrated later, trend surface mapping provides a most useful method of presenting and analysing height data relating to raised shorelines. Regional trends may be precisely defined and an isobase map readily constructed. Local anomalies are revealed which raise further questions concerning the nature and causes of the deformation of the shorelines, and which in some cases may raise doubts as to whether the original assumption, that one is dealing with the same shoreline throughout, is correct.

Best-fit trend surfaces of linear, quadratic and cubic form have been fitted to the data recorded by McCann<sup>2</sup> for the main post-glacial shoreline in western Scotland, between the Firth of Lorne and Loch Broom, by the electronic computer programme described by Whitten<sup>4</sup>, using an 'IBM 7090' computer. The best-fit linear surface which had been fitted to the data previously, without the use of a computer, explains 60.27 per cent of the observed variation in height; the best-fit quadratic surface raises the level of "explanation" to 76.97 per cent, and the best-fit cubic surface to 78.23 per cent. In view of the small increase in "explanation" provided by the cubic as compared with the quadratic surface, the latter is considered most appropriate for further analysis.

This best-fit quadratic surface (Fig. 1) indicates that the simple trend of decreasing elevation of the shoreline in a north-westerly direction, illustrated by the linear surface<sup>2</sup>, is complicated by a zone of relatively high elevations in Skye, revealed in particular by the 21 ft. contour for the surface. The pattern of uniform decrease in elevation westwards and north-westwards, shown by the 45-25 ft. contours for the surface, holds true only for the south-western part of the area shown on the map. It may be suggested that the presence of the post-glacial shoreline in Skye at elevations somewhat greater than might be expected from considerations of the overall



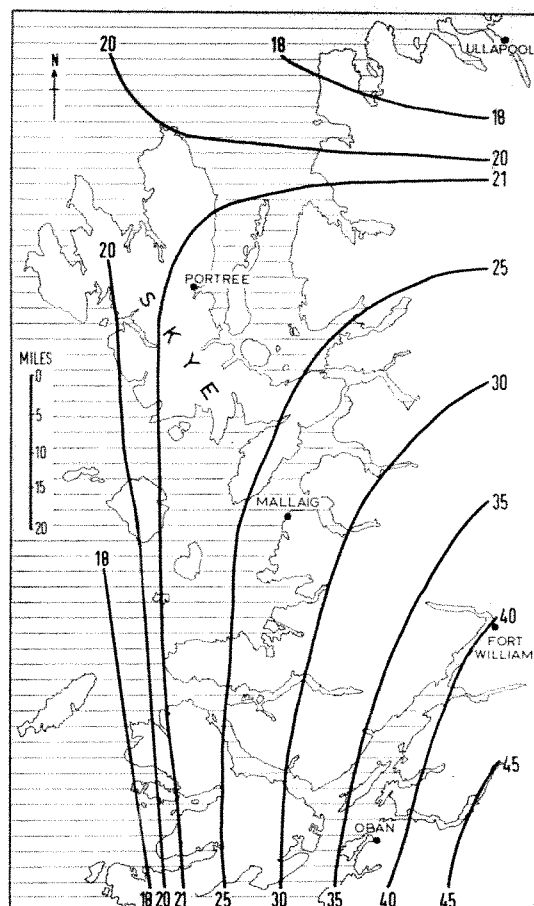


Fig. 1. Best-fit quadratic surface fitted to height data for the main post-glacial shoreline in western Scotland, between the Firth of Lorne and Loch Broom. Figures are given in feet above O.D.

pattern of deformation is a result of the greater isotatic depression of the land surface in that area due to the presence, at various stages in the glacial period, of a local Cuillins icecap. The trend surface offers no indication that one is dealing with more than one shoreline, and the map of the residuals from the surface, which may on occasion

provide additional information, appears to show little of significance in this case.

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### Intercontinental Correlation of Late Pleistocene Sea Levels

EUSTATIC variations in sea level are assumed to be world wide and simultaneous. During the Pleistocene, sea level fluctuated in response to the formation and melting of continental glaciers, and interglacial shorelines higher than the present sea level are found in several places. Coastal areas which have been stable since the development of the Pleistocene shorelines have shoreline features at similar elevations. Conversely, the establishment of shorelines at similar elevations in different areas implies stability. Because of the effects of sedimentation, erosion, water loading and tectonic adjustment, no coast is completely stable. The effects of water loading<sup>2</sup> on the elevation of the interglacial shorelines (and modern) along a particular coast, however, are usually similar so that the relative elevations are not seriously disturbed.

This investigation compares the late Pleistocene shorelines of coastal Georgia<sup>3-7</sup> with those of southern South West Africa<sup>8,9</sup>. The late Pleistocene shorelines of Georgia have elevations of approximately 7.5 m (Pamlico), 4 m (Princess Anne), and 1.4 m (Silver Bluff) (Fig. 1). I shall not consider the older, higher Pleistocene shorelines which are present. The Pleistocene shorelines are approximately parallel to the coast and are horizontal within the accuracies of present techniques of measurement. Along these shorelines are sedimentary accumulations characteristic of barrier islands and lagoons; the maximum elevation of the barrier islands above sea level is thought to have been about 10 m. Lagoonal salt marsh becomes filled in to the approximate level of the former high tide. Assuming a Pleistocene tidal range similar to the modern range, the high tide level would be approximately 1.1 m

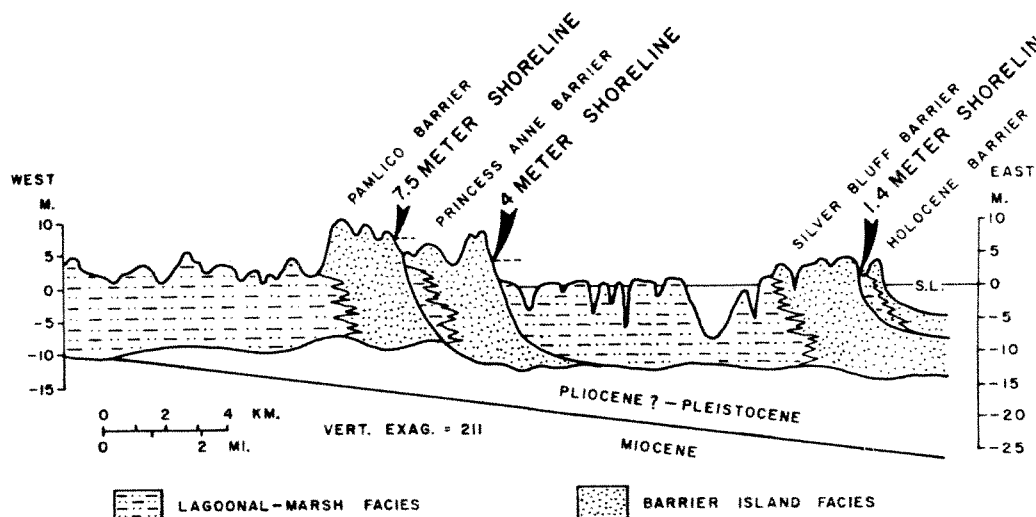


Fig. 1. Cross-section of central Georgia coast showing relation of Pleistocene and Holocene barrier island and lagoonal sediments to the Pamlico (7.5 m), Princess Anne (4 m), Silver Bluff (1.4 m) and Holocene shorelines. S.L. = Sea level.

above mean sea level. Despite erosion, compaction, and, in some areas, deposition, it is still possible to obtain a reasonable estimation of the former sea level.

A more precise method of estimating the mean sea level is based on the fossil burrows of the marine decapod *Callianassa* (*Ophiomorpha nodosa* Lungren)<sup>10</sup>. The modern form, *Callianassa major* Say, lives in the littoral and shallow neritic zones along the coast of the south-eastern United States. The animal is about 10 cm long and 1.2 cm in diameter and it constructs burrows in the soft sand usually 1.2–2 m long and about 1.6 cm in diameter except for the upper 20–25 cm which is constricted and not occupied by the animal. The principal burrow is approximately vertical; however, branches and bifurcations are common. It is maintained in the soft sand by a cementing agent produced by the animal. The cement penetrates the sand for a distance of 8–12 mm and it is the exterior of the cemented wall that is characteristic of the fossil burrow. Typically this external surface is covered with knobs 2–4 mm in height and 4–12 mm in diameter; however, the appearance of the knobs varies considerably depending on the permeability of the sand and other factors. Fossil burrows are known from Lower Cretaceous to present.

Modern *Callianassa major* lives in the beach as high as mean sea level. Using this criterion the elevations of the late Pleistocene seas have been determined at several places along 200 km of Georgia coast. Measurements for the Pamlico shoreline are 7 m, Princess Anne 3.7 m, and Silver Bluff 1.1 m (to these heights has been added an arbitrary 30 cm to compensate for erosion and the missing narrow part of the burrow). Thus it seems that if there has been movement it has affected the area as a whole.

Investigations along the coast of South West Africa cover an area from the Orange River north for a distance of about 100 km. During late Pleistocene time wave erosion produced sea cliffs as high as 3 m which were notched in places, and which had terraced surfaces seaward cut by deep gullies or surge channels. Zeuner<sup>11</sup> and Price<sup>12</sup> believe that the inner edge of such terraces represents sea level at the time of formation. On the coast of South West Africa there are four well-developed terraces complete with surge channels and sea cliffs. Despite minor irregularities, the three lower terraces are essentially horizontal along the coast. The fourth terrace, which is higher topographically as well as older than the other three, is warped from an elevation of about 40 m

near the Orange River to 8 m 100 km to the north-west.

The oldest of the three lower terraces has an elevation of about 8 m along the inner edge, the middle terrace an elevation of about 5 m, and the lower terrace an elevation of approximately 2 m (Fig. 2). These elevations are related to high stands of the sea at approximately these same levels. Deposits of marine sands and gravels overlie the terrace surfaces; in some cases where the cliff is low the gravels also overlap the cliff. The marine deposits overlying a terrace apparently result from a late phase of activity before the lowering of sea level which accompanied glacier formation and the succeeding submergences cut terraces at successively lower levels. The lowering of sea level after the formation of each terrace was sufficient to allow the development of stream channels in the terrace surface to below sea level<sup>9</sup>. This observation indicates fluctuations of sea level during the late Pleistocene rather than simply formation of successively lower terraces during stable periods in a general emergence.

The marine sands and gravels overlying the terraces are overlain by terrestrial sheet wash and aeolian deposits. The development of the Pleistocene shorelines might well have remained relatively unknown if it were not for the exceptional abundance of diamonds in the marine gravels overlying the terraces, which has made the complete removal of all sedimentary materials along these former shorelines economically feasible.

In spite of the contrast in mode of formation of the African and Georgian coasts, the elevations of the shorelines are essentially similar. The similarities of sequence and elevation suggest a general stability of the two areas during and since the development of these shorelines. The small differences in elevation can be explained in one or all of several ways. Regional tectonic movement in either or both areas may have resulted in slight elevation of the African area and/or a slight depression of the American area. The method establishing the mean sea levels may be slightly in error, because the inner edge of the eroded terrace and the base of the notched sea cliff may have formed, in this African area, 0.3–0.5 m above mean sea level. Also, hydrodynamic, meteorological, rotational or gravitational forces may have altered the shape of the hydrosphere and produced a slight variation in mean sea level during the late Pleistocene compared with the present time.

Uranium series dating of corals from the Key Largo limestone of southern Florida and the Bahama Islands

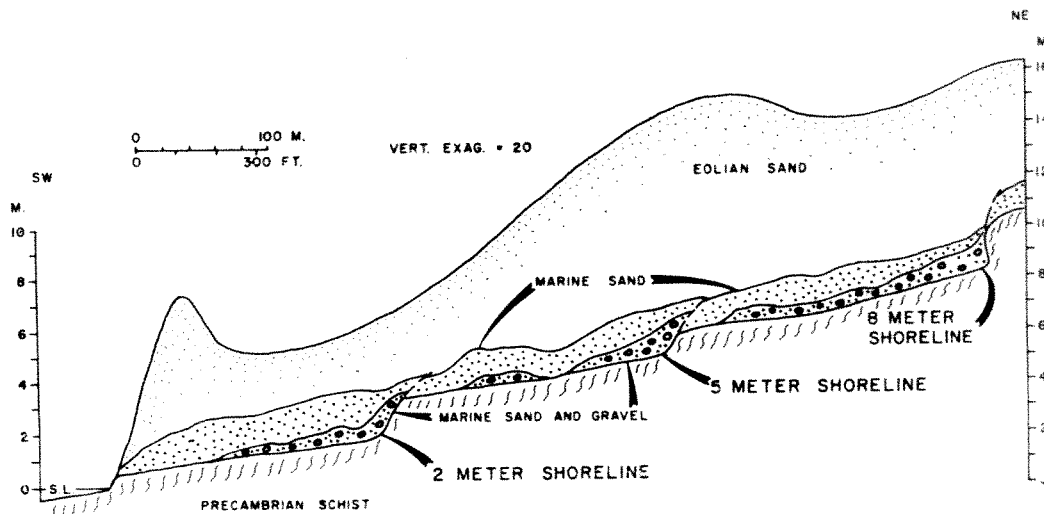


Fig. 2. Cross-section of coastal South West Africa 60 km north of the Orange River showing sea cliffs, terraces and beach deposits associated with the 8 m, 5 m and 2 m shorelines (after Stocken<sup>9</sup>). S.L. = Sea level.

suggest that the shorelines are late Pleistocene in age. The dates range from 100,000 to 170,000 yr B.P. and are thought to correspond to a high stand of the sea during the Sangamon (Riss-Würm) interglacial stages<sup>13</sup>. The Kay Largo limestone is correlated with the Pamlico (7.5 m) shoreline. Several radiocarbon dates of shells from Silver Bluff deposits suggest a mid-Wisconsin age (mid-Würm) of about 25,000–48,000 yr (ref. 14). Five dates within this range have been obtained from deposits along the Georgia coast. Additional dates from this interval have been reported<sup>15,16</sup> from marine or deltaic sediments above, or not far below, present sea level. A retreat of glaciers is also reported at this time<sup>17</sup>. Stone implements of the Chelles-Acheul culture, believed to be middle to late Pleistocene in age, have been found in the gravels of the 5 m terrace<sup>9</sup>.

In both the Georgian and African areas there is evidence that the late Pleistocene shorelines developed following a fluctuation of the sea to a level below the next lower shoreline and usually an emergence was followed by a submergence of lesser magnitude than the previous one. In Georgia the transgressive relation of barrier island sands to lagoonal salt marsh deposits suggests emergence followed by submergence. The formation of barrier islands is also thought to necessitate some submergence<sup>18</sup>. In the African area streams associated with each sea level have eroded deep channels across the terrace which pre-dates the next lower terrace and indicates a level of erosion which extended below the sea level associated with the next lower terrace. There is little indication of the magnitude of the Georgian and African emergences except for the one following the development of the Silver Bluff shoreline (1.4 m) for which evidence from several areas suggests a lowering of sea level of as much as 100–105 m below present sea level<sup>15,19</sup>. There is no evidence for a "post-glacial" high stand of sea level along either the Georgia or South West Africa coast.

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## Effect of Crude Oil on the Nuclear Magnetic Relaxation of Water Protons in Sandstone

Brown and Fatt<sup>1</sup> have found that the nuclear thermal relaxation time,  $T_1$ , for water protons in contact with sand is shorter than for bulk water and is increased by placing an organic film on the surface. They have suggested that the enhanced relaxation arises from strong local magnetic fields caused by paramagnetic centres on the surface. The film would then act as a physical shield. The KST model<sup>2</sup> describes the effect of such paramagnetic centres as

$$\frac{1}{T_1} - \frac{1}{T_B} = \frac{V_s}{V_B} r_s$$

where  $T_1$  is the observed relaxation time;  $T_B$ , the bulk relaxation time;  $V_s$ , the volume of fluid near the surface relaxing with rate  $r_s$ ; and  $V_B$ , the volume of fluid relaxing at the bulk rate<sup>3</sup>.

A typical measurement on distilled water in natural sandstone, made by free induction at 3 kG, is shown in Fig. 1. For a homogeneous system the curve is expected to follow

$$M = A e^{-t/T_1}$$

where  $M$  is the magnetization,  $A$  is proportional to the number of nuclei, and  $t$  is time. Fig. 1 has two such components, each representing about half the interstitial water. By the KST model this would be interpreted as a bimodal pore size distribution.

In nuclear magnetic logging of oil wells the relaxation rate of water which has infiltrated the formation from the drilling mud is measured. In water zones a signal like the long phase of Fig. 1 is seen, while in zones bearing oil a two-phase signal is seen, with a short phase like that of the water zone and a long phase resembling bulk water<sup>4</sup>.

This information suggested that the presence of crude oil in sandstone reduces the extent to which the surface can influence water which later infiltrates the rock, and that adsorption of surface-active components from the oil may be the mechanism responsible. Alternatively, it was possible that the paramagnetic centres had been reduced by the oil; however, treatment of clean sandstone with hydrogen peroxide and with hydrazine solutions did not alter its behaviour. Exposure to either oxygen or hydrogen at elevated temperatures resulted in no signal at all, presumably because trace surface iron species were irreversibly transformed to the ferromagnetic state, which caused the magnetization signal to decay too fast to be seen. R. J. S. Brown has informed us that in these circumstances he has noted an increase in the ferromagnetization of the rock.

The effect of various surfactants is shown in Table 1; however, only the long phase of the signal is tabulated.

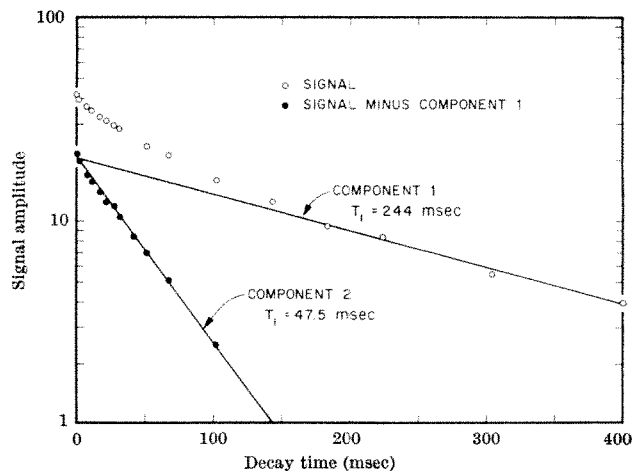


Fig. 1.

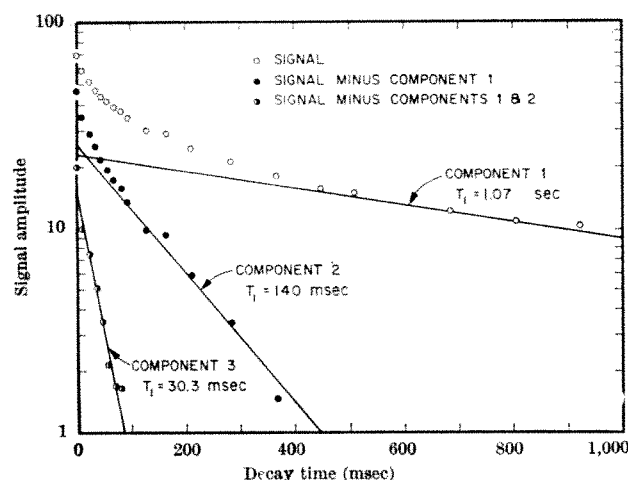


Fig. 2.

The surfactants were adsorbed from solution, and the solution was displaced with distilled water the relaxation of which was then measured. Approximate lengths for the surfactant molecules are shown. If, rather than zero, a molecular length of  $L = 3 \text{ \AA}$  is ascribed to the untreated system, these data are fairly well described by the expression

$$\frac{1}{T_1} - \frac{1}{T_B} = 6.3 L^{-1/2}$$

The value for the untreated rock corresponds to the thickness of a monolayer of water. A multitude of other expressions, such as  $T_1 = A + BL$  or  $T_1 = C - De^{-L}$ , are possible, but less appealing.

Table 1. EFFECT OF ADSORPTION ON  $T_1$ 

Adsorbate	$T_1$ (msec)	Molecule length ( $\text{\AA}$ )
Untreated	247	0
Methylene blue	316	4.5
Dodecylammonium acetate	470	15
Stearic acid	564	23

When clean sandstone was exposed to crude oil and then saturated with water, similar results were obtained. The magnitude of the effect depended on the exposure time; equilibrium required several days and yielded a  $T_1$  of about 1 sec (the  $T_1$  of bulk water  $\sim 2.3$  sec). This time was reduced if heat was applied during the exposure to oil. The bulk of the oil could be removed from the rock by thorough flushing with cyclohexane; this left only a tightly adherent residue that could be removed by washing with a chloroform-acetone solution and was analysed as asphaltene. Similar results were obtained whether the rock was water saturated or dry when the crude oil was introduced, which indicates either that the water was very effectively displaced, or that residual water was present in a thin film or in droplets that posed no barrier to asphaltene adsorption.

In one experiment the rock was only partially saturated with oil, the remainder being saturated with water. On flushing the rock with cyclohexane and saturating with water the relaxation curve in Fig. 2 was obtained. This is easily broken into the three indicated components.

The data show that the  $T_1$  of water in porous rock is increased by adsorption of large surfactant molecules on the surface and that this can occur when the rock is exposed to crude oil. The observed temperature effect is explained by the positive entropy change accompanying adsorption, as discussed by Moilliet *et al.*<sup>5</sup> Because of the viscosity of the crude oil, cyclohexane was sometimes used to remove the bulk oil from the rock before it was saturated with water. It was thought that this would not affect the distribution of adsorbed species as the washing

took place in minutes, and the time constant for adsorption was days.

The three components of the relaxation curve, Fig. 2, can be explained as follows: fast component, water in very fine pores; intermediate component, water in coarse pores; slow component, water in large pores that had been filled with oil and are thus coated with asphaltene. The experiment was quite analogous to the situation existing in an oil reservoir, in which some pores of the reservoir rock are largely filled with oil and some are largely filled with water. When the rock is flushed with water from the drilling mud a nuclear magnetic logging signal is seen from both types of pore, but the pores that have been exposed to oil have a long relaxation time. The very short signal, which represents fine pores, is not seen by the nuclear magnetic logging because of the limitations of the equipment used.

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## PHYSICS

### Analysis of X-ray and Neutron Diffracted Intensities calculated for a Random Network Model of Vitreous Silica

EXPERIMENTAL diffraction patterns of vitreous silica are characterized by low density at the centre and a small number of diffuse maxima at small angles, which indicates that only short range order exists. This leads to two different interpretations of the structure of vitreous silica—first, that it consists of an irregular non-crystalline network of silicon and oxygen atoms and, second, that it consists of an aggregate of extremely tiny crystals<sup>1,2</sup>. We now describe the preliminary results of calculating X-ray and neutron diffraction intensities from an irregular non-crystalline network model of vitreous silica, the construction of which was described earlier<sup>3</sup>.

The intensity of coherent X-ray scattering from an array of atoms which is allowed to take all possible orientations in space is given by Debye's equation<sup>4</sup>

$$I(s) = \sum_n \sum_m f_n(s) f_m(s) \frac{\sin 2\pi s r_{nm}}{2\pi s r_{nm}} \quad (1)$$

where  $I(s)$  is the intensity in electron units diffracted at an angle  $\theta$ ,  $s = 2 \sin \theta / \lambda$ ,  $f_n(s)$ ,  $f_m(s)$  are the atomic scattering factors for the  $n$ th and  $m$ th atoms respectively and  $r_{nm}$  is the magnitude of the vector separating these two atoms. The scattering from the random network model was calculated by a direct application of this equation, in which the double summation includes terms referring to each pair of atoms in the model and, in particular ( $n=m$ ), terms referring to all atoms singly. The individual contribution of the silicon and oxygen atoms to the coherent scattering intensity is then  $N_{\text{Si}} f_{\text{Si}}^2(s)$  and  $N_{\text{O}} f_{\text{O}}^2(s)$ , respectively, where  $N_{\text{Si}}$  and  $N_{\text{O}}$  are the numbers of silicon and oxygen atoms in the network and  $f_{\text{Si}}(s)$  and  $f_{\text{O}}(s)$  are the corresponding atomic scattering factors. Using this notation, the intensity pattern as a whole may be rewritten as



$$I(s) = N_{\text{Si}} f_{\text{Si}}^2(s) + N_{\text{O}} f_{\text{O}}^2(s) + f_{\text{Si}}^2(s) \sum_{n,m} \frac{\sin 2\pi s r_{nm}}{2\pi s r_{nm}} + f_{\text{O}}^2(s) \sum_{n,m} \frac{\sin 2\pi s r_{nm}}{2\pi s r_{nm}} + f_{\text{Si}}(s) f_{\text{O}}(s) \sum_{n,m} \frac{\sin 2\pi s r_{nm}}{2\pi s r_{nm}} \quad (2)$$

In this equation, the first sum represents the contribution of pairs of silicon atoms, the second, the contribution of oxygen atoms, and the third, the contribution of silicon-oxygen pairs.

Using equation (2), the intensity of coherent scattering was calculated on the University of London Institute of Computer Science 'Atlas' Computer for a range of values of  $s (= 2 \sin \theta / \lambda)$  up to 1.8.

To the values of the coherent scattering intensities deduced from Debye's equation should be added values for the independent incoherent scattering intensities. The incoherent scattering intensity for an atom is given in electron units by

$$I_{\text{inc}}(s) = R(Z - \sum_i g_i^2(s))$$

where  $g_i(s)$  is the scattering factor for the  $i$ th electron in the atom,  $Z$  is the atomic number and  $R$  is a recoil factor, which for the purposes of this calculation may be taken as unity without too much error. Values of  $G_i(s) (= I_{\text{inc}}(s)/R)$  were taken from the *International Tables*. The total incoherent scattering for the vitreous silica model is given by

$$I_{\text{inc}}(s) = N_{\text{Si}} G_{\text{Si}}(s) + N_{\text{O}} G_{\text{O}}(s)$$

The total X-ray scattering, expressed in electron units, is plotted in Fig. 1 as a function of  $\sin \theta / \lambda$ . In the same figure experimental scattering curves taken from papers by Warren<sup>5</sup> and Zarzycki<sup>6</sup> are shown. The calculated

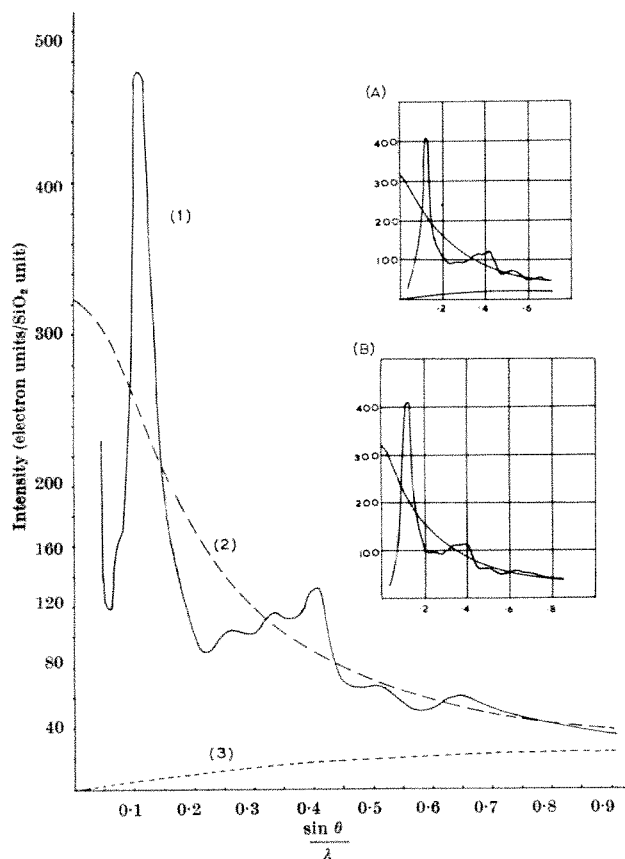


Fig. 1. X-ray scattering from a random network model of vitreous silica. (1) Total scattering (coherent plus incoherent); (2) total independent scattering; (3) incoherent scattering. Insets, experimental results from the work of A. Zarzycki<sup>6</sup>; B. Warren<sup>5</sup>.

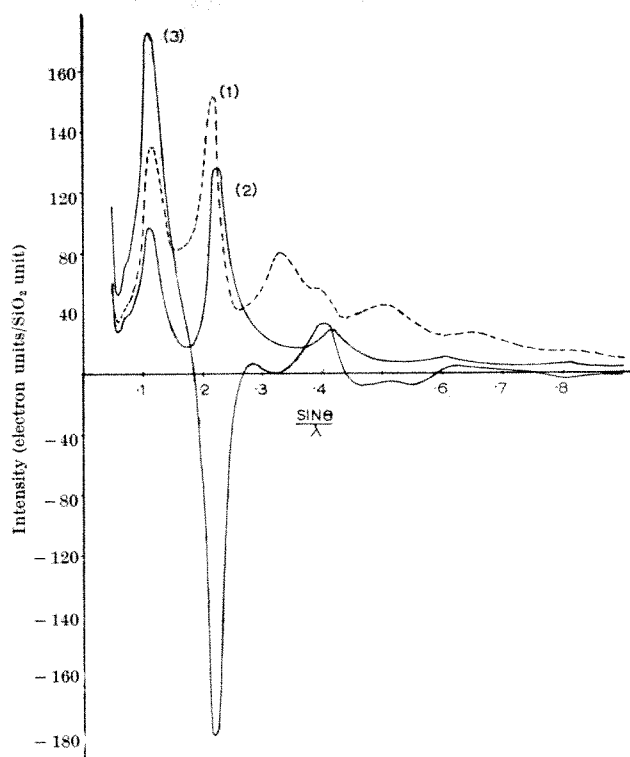


Fig. 2. Components of X-ray diffracted intensity: (1) due to silicon-silicon pairs; (2) due to oxygen-oxygen pairs; (3) due to silicon-oxygen pairs.

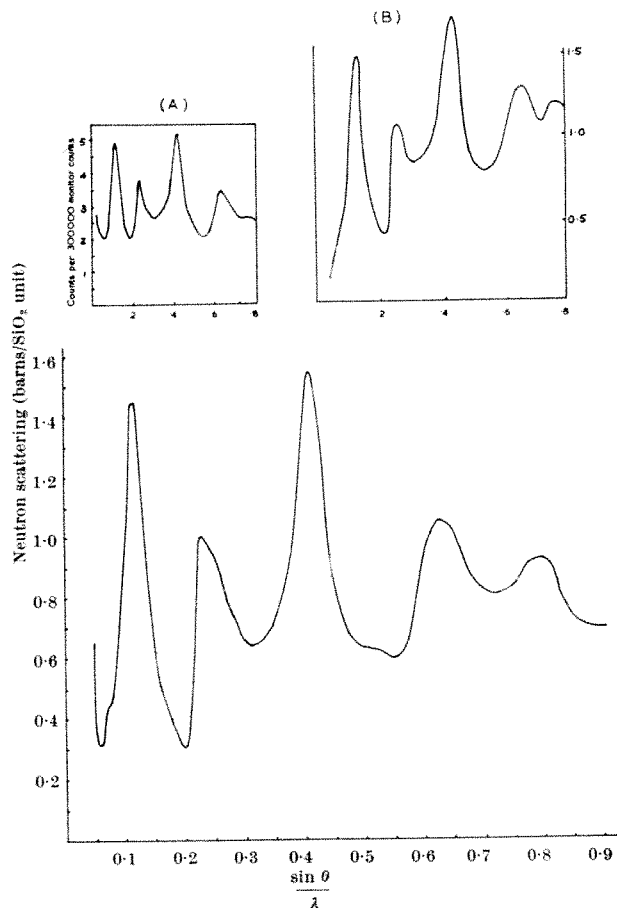


Fig. 3. Neutron diffracted intensity from a random network model of vitreous silica. Insets, experimental results from the work of A. Carraro<sup>7</sup>; B. Egelstaff<sup>8</sup>.

scattering curve agrees quite well with the experimental curves, the most noticeable difference being in the height of the first peak. If atomic scattering factors for  $\text{Si}^{4+}$  and  $\text{O}^{2-}$  are used in place of those for the neutral atom, the height of the first peak approximates to that given by Warren<sup>5</sup> and Zarzycki<sup>6</sup>. In vitreous silica, however, the Si—O bond is thought to be only about 40 per cent ionic and therefore these ionization states are unlikely. Further computations, using different values for atomic scattering factors and using different temperature factors, are in progress.

Fig. 2 shows, also in electron units, components of the coherent intensity as calculated for (1) silicon pairs, (2) oxygen pairs and (3) silicon-oxygen pairs. This clearly reveals that each type of interaction contributes significantly to the total scattering throughout the whole pattern.

Values for neutron diffracted intensities (in barns) are plotted in Fig. 3. These were calculated from equation (2) by substituting the scattering lengths  $b_{\text{Si}} = 0.42 \times 10^{-12}$  cm and  $b_{\text{O}} = 0.577 \times 10^{-12}$  cm for the atomic scattering factors  $f_{\text{Si}}(s)$  and  $f_{\text{O}}(s)$ . The same figure includes experimental curves taken from the work of Carraro<sup>7</sup> and Egelstaff<sup>8</sup>, which have been corrected for multiple and incoherent scattering.

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### Filtered Electron Diffraction Measurements from Thick Polycrystalline Metal Foils

DURING the past decade there have been considerable developments in high voltage electron microscopes and diffraction cameras which work reliably at voltages above 300 kV. These instruments are designed for studies of "thick" specimens—foils which it is practicable to prepare by cutting or thinning bulk materials. Metal specimens thinned from bulk are usually several thousand angstroms thick and, if examined at 100 kV, electrons which have been inelastically scattered fog the image or diffraction pattern. By working at much higher voltages the "contrast" of thick specimens is enhanced.

An alternative is to remove the loss electrons, and promising improvements of contrast in photographic instruments have been demonstrated<sup>1</sup>. Recently an automatic electron diffractometer designed by one of us has been applied to the study of thick polycrystalline films, with 50 kV electrons. Such instruments operate with filters capable of eliminating all electrons which have suffered more than a small energy loss—and are equipped with two-dimensional scanning, which is necessary for testing whether or not preferred orientation has been achieved.

Fig. 1 shows intensity profiles from an aluminium specimen 10,000 Å thick; the various profiles are obtained with different settings of the energy filter which gives

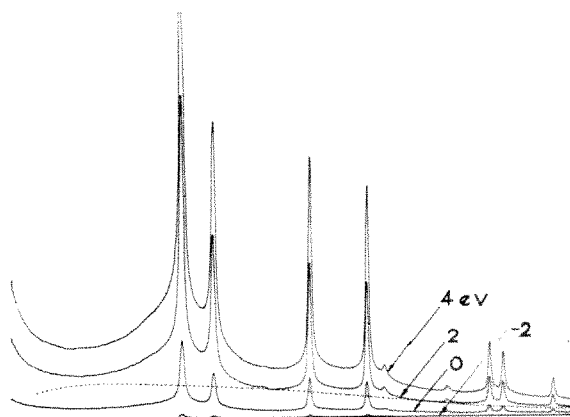


Fig. 1. Well-filtered intensity profiles from nearly randomly oriented polycrystalline aluminium foil of thickness 10,000 Å; 50 kV electrons.

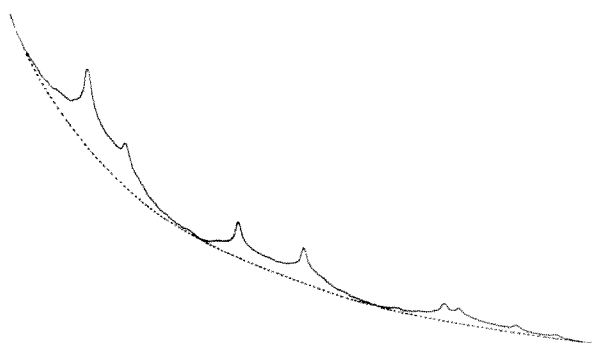


Fig. 2. An unfiltered intensity profile from 10,000 Å thick aluminium foil. The intensity gain is reduced by a factor of 17.5 compared with Fig. 1. 50 kV. (Zero is off-scale.)

nearly zero transmission when set at  $-2$  V with respect to the cathode. It is possible to make an approximate comparison between well-filtered and unfiltered profiles, and this is given, for a different 10,000 Å aluminium foil, in Table 1.

The enormous proportion of loss electrons is evident from Table 1, and the proportion is still enormous at large scattering angles. If the comparison were made for angular positions corresponding with background rather than at diffraction rings, the proportion of loss electrons would be greater.

Fig. 2 shows the unfiltered profile of 10,000 Å thick polycrystalline aluminium. A "smooth background" has been interpolated and broad humps can be seen above the background, with aluminium rings superimposed on them. There are signs of these humps in the well-filtered curves, but their proportions are quite different. Clearly no quantitative measure of aluminium ring intensity can be obtained from the unfiltered profile; nor can any conclusions be drawn about the relative intensity of the aluminium rings without filtering.

The profiles of Fig. 1 do not look very different from the nearly elastic profiles of a thin aluminium film randomly oriented and diffracting kinematically. Table 2 shows, however, a comparison with the quantity  $\sqrt{i_{\text{peak}}/(p/s^2a^2)}$  and the atomic scattering function (ref. 2)  $f$  for aluminium.  $i_{\text{peak}}$  is the intensity above the interpolated background of Fig. 1 and the 2 eV profile,  $p$  is the multiplicity,  $s = 2 \sin \frac{1}{2} \beta/\lambda$ , and  $a$  is the lattice spacing. The  $\{111\}$  and  $\{200\}$

Table 1. COMPARISON OF UNFILTERED AND WELL-FILTERED MEASUREMENTS OF INTENSITY, 10,000 Å ALUMINIUM AT 50 kV

Angular position ( $S\lambda$ )	$\sqrt{3}$	$\sqrt{4}$	$\sqrt{8}$	$\sqrt{11}$	$\sqrt{12}$	$\sqrt{16}$	$\sqrt{19}$	$\sqrt{20}$	$\sqrt{24}$	$\sqrt{27}$
Total unfiltered intensity, arbitrary units	1.00	0.785	0.573	0.503	—	—	0.34	0.33	0.27	0.25
Total filtered intensity, filter set at $+2$ eV	0.050	0.027	0.027	0.025	0.0076	0.0051	0.011	0.010	0.0075	0.0056
Ratio of total unfiltered to filtered intensity	21	29	21	20	—	—	30	33	38	45

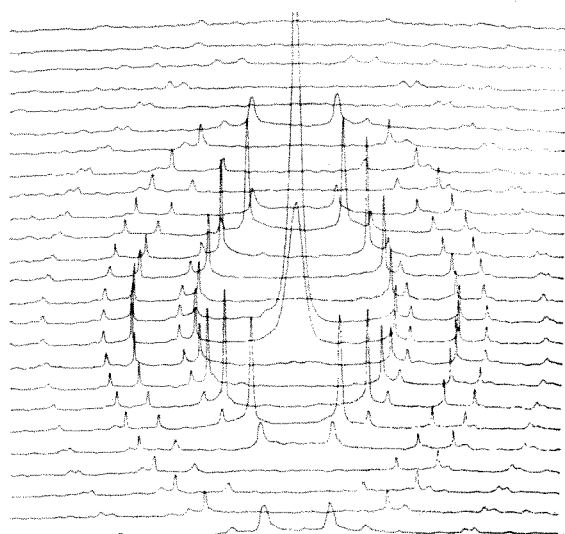


Fig. 3. 1360 Å thick silver film with preferred orientation. Filter bandwidth 5 eV, 50 kV.

peaks show evidence of dynamic effects; nevertheless it is remarkable that there should be so little evidence of dynamic scattering in so thick a film. Unfortunately it is not possible, with so thick a film, to use the half width of the peaks as a rough measure of crystallite size.

Table 2. TEST FOR KINEMATIC SCATTERING CONDITIONS IN 10,000 Å ALUMINIUM FOIL

Peak	111	200	220	311	222	400	331	420	422
$\sqrt{\left(\frac{I_{\text{peak}}}{(p/s^2a^2)}\right)}$									
relative to 220	1.125	1.125	100	0.778	0.754	0.737	0.520	0.484	0.468
$f$ relative to 220	1.95	1.58	100	0.81	0.77	0.64	0.57	0.56	0.50
Ratio	0.58	0.71	1.0	0.96	0.98	1.15	0.91	0.86	0.68

Similar measurements have been recorded with silver up to 1600 Å thick at 50 kV. The traces then show much quantum noise: this could, of course, be eliminated if a slower scan generator were available. All the silver films had considerable preferred orientation (as shown in Fig. 3) — a 1360 Å film with a 0.6 eV energy bandwidth. With silver films of this thickness the unfiltered profile shows very weak rings (invisible on the fluorescent screen) and the unfiltered background is about 100 times as intense as the 0.4 eV filtered one.

It has been claimed<sup>3</sup> that the great increase in transmission observed when silver specimens are cooled to 90° K is evidence of dynamic effects. The measurement supporting this statement was unfiltered and was from a 650 Å film at 30 kV. Our results indicate that unfiltered measurements from films of such a thickness mean little because there is a predominance of loss-electrons.

These nearly elastic measurements on thick films suggest that diffraction instruments, working at 100–150 kV, and equipped with efficient filters, would enable aluminium specimens 3–5 μ thick and steel foils thicker than 1 μ to be studied. The diffraction results also suggest that electron microscopes working between 100 and 150 kV, and using the successful filters which have been developed<sup>4</sup>, would be able to obtain filtered images of equally thick foils. With thick specimens the current elements may fall to 10<sup>-18</sup> amp on filtering, which would necessitate the use of image intensifiers to integrate quantum noise over periods of many seconds; improved stability and vacuum conditions would therefore be needed. The cost of these developments, however, would be small when compared with the cost of 1 MV instruments.

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### Electron Spin Resonance Transitions Involving Simultaneous Changes in Spin States of up to Three Neighbouring Protons

OCCASIONALLY an electron spin resonance absorption line is accompanied by satellite lines of weak intensity. These satellite lines, arising from a change in spin state of a nearby nucleus concurrent with the change in spin state of the electron, were first observed in irradiated frozen acids<sup>1</sup>. A theoretical explanation for these transitions has been given by Trammell *et al.*<sup>2</sup>, who also predicted a second set of satellites corresponding to two neighbouring protons concurrently changing their spin state. Recently Snipes and Bernhard<sup>3</sup> observed such satellite lines in γ-irradiated single crystals of barbituric acid dihydrate. We report here experimental evidence of a second and even a third set of satellites corresponding to two and three neighbouring protons simultaneously changing spin state.

Atomic hydrogen was produced in 6 molar sulphuric acid with cobalt-60 γ radiation at 77° K (ref. 4). In the course of examining the microwave power saturation of the atomic hydrogen doublet, it was found that at very low microwave power levels the atomic hydrogen line was always accompanied by one satellite line on each side (Fig. 1a). On increasing the microwave power, the intensity ratio of the satellite line to the main transition line also increased, and a new set of satellite lines became visible (Fig. 1b). At an even higher power level and higher spectrometer sensitivity, a further set of lines of weak intensity was obtained (Fig. 1c). This observation was made at both the high field and low field lines of the hydrogen doublet. According to the theoretical treatment<sup>2</sup>, the spacing of a satellite to the main line and the intensity ratio are field dependent. Because the splitting of the hydrogen doublet is 505 G, different values for the splitting and intensity ratio are expected, and we can very easily compare the theoretical predictions with the experimental results.

To obtain correct values of the splittings and intensities of the various lines, electron spin resonance spectra of atomic hydrogen were recorded at 85°–90° K over a wide range of microwave power levels. The spectra were analysed by setting up a computer programme under the following assumptions to reduce the number of free parameters.

(i) Each line is represented by the derivative of a Gaussian curve with three adjustable parameters, the amplitude, splitting and width.

(ii) A spectrum is composed of four sets of lines corresponding to zero, one, two, and three simultaneous proton transitions. The sets contain, respectively, one line, two lines of equal intensity, three lines with intensity ratios 1 : 2 : 1, and four lines with ratios 1 : 3 : 3 : 1.

(iii) The splitting from the main transition per proton flip is a constant, independent of whether other protons are making simultaneous flips.

(iv) The line width is the same for all lines within each set.

The computer was then programmed using the method of Powell<sup>5</sup>. An example of a spectrum fit in this way is shown in Fig. 2. The agreement between the experimental and calculated curves is inferior in this case to that obtained with most of the other spectra. From the experimental spectra analysed by the computer values of splitting and line intensities were obtained. The splittings observed experimentally, at magnetic field strengths of 2,954 and 3,459 G are 4.56 and 5.3 G respectively, and compare favourably with the theoretically predicted values of 4.4 and 5.15 G respectively.

By releasing the constraint provided by assumption (iii) no significant improvement in the fits to the data was obtained, thus justifying this assumption.

The saturation behaviour of the different groups of lines is similar, following first the linear relation between microwave power and signal amplitude and then bending off, indicating that saturation takes place. These findings differ somewhat from the results reported by Snipes *et al.*<sup>3</sup>, who could not find saturation for the satellite lines even at high power levels. After correcting for the effect of saturation we determined the intensities of the various satellites relative to that of the main transition.

It is now possible to make an estimate of the average distance of the flipping protons from the electron spin density by using the following relation given by Trammell *et al.*<sup>2</sup>

$$\frac{T_1}{2T_0} = \frac{3}{20} \cdot \left( \sum_i \frac{g_e^2 \beta_e^2}{H^2 r_i^6} \right) \quad (1)$$

where  $H$  represents the applied magnetic field,  $r_i$  the distance of the  $i$ th flipping proton from the proton on which the spin density is concentrated,  $T_1$  is the intensity of the first satellite relative to the intensity of the principal transition  $T_0$ . Because the simultaneous flipping of a second proton is independent of the flipping of the first

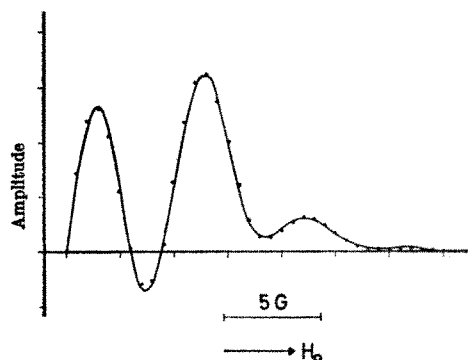


Fig. 2. One half of the first derivative electron spin resonance tracing of the high field hydrogen line with its satellites (—) compared with the calculated spectrum (●).

proton and to a certain extent dependent on the number of microwave quanta present in the cavity, the ratio of the transition probabilities is given by a squared expression of the following form

$$\frac{T_2}{2T_0} = \left( \frac{3}{20} \right)^2 \cdot \left( \sum_i \frac{g_e^2 \beta_e^2}{H^2 r_i^6} \right)^2 \quad (2)$$

where  $T_2$  is the total intensity of the triplet due to the double proton flip. The outer line of that triplet represents only a quarter of the total intensity.

Accordingly we obtain an expression for the transition probability ratio of the third set of satellites and the principal transition line

$$\frac{T_3}{2T_0} = \left( \frac{3}{20} \right)^3 \cdot \left( \sum_i \frac{g_e^2 \beta_e^2}{H^2 r_i^6} \right)^3 \quad (3)$$

Here  $T_3$  is the total transition probability of the triple proton flip. The outermost line of the quartet due to the triple proton flip represents only an eighth of the total intensity.

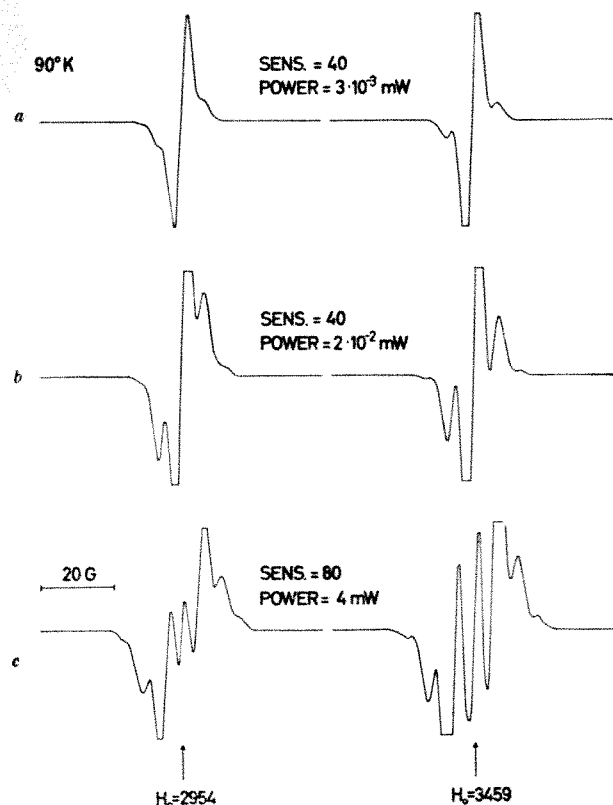


Fig. 1. First-derivative electron spin resonance tracing of the hydrogen doublet of sulphuric acid glasses irradiated at 77° K and recorded at 90° K. The relative spectrometer sensitivity and microwave power level are increasing from top to bottom as indicated. New satellite lines are appearing with increasing microwave power.

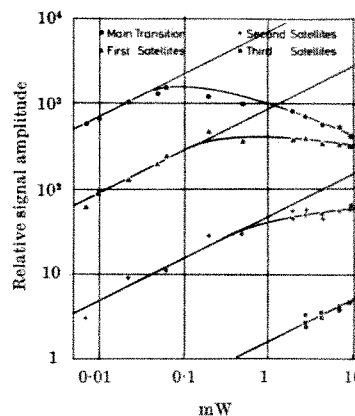


Fig. 3. Microwave power saturation of the main transition and the different satellites of the high field hydrogen line.

Using the equations given here, the average distance ( $r$ ) to the protons changing spin state simultaneously with the electron was found to be 1.79 Å, 1.80 Å and 1.85 Å for the single, double and triple proton flip respectively. The results of the single proton flip are in good agreement with the value of 1.73 Å given by Trammell *et al.*<sup>2</sup>. Because the satellite intensity is proportional to  $1/r^6$  or powers thereof, only the nearest protons are



contributing to it. From these results we conclude that there are at least three nearest protons surrounding a hydrogen atom in the glassy material investigated at distances ranging from 1.79 Å to 1.85 Å.

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### Hysteresis Experiments in Rheology

THE value of "normal" hysteresis experiments in rheology was recently discussed by Harris<sup>1</sup>, who observed that hysteresis loops could be obtained even with Newtonian fluids and that steady oscillatory experiments were therefore to be preferred for materials which exhibit the effect of thixotropy because of the simpler analysis. It should be remembered, however, that steady oscillatory experiments cannot yield information on thixotropic fluids when their resistance to flow is near maximum. A thixotropic fluid is defined here as a fluid which exhibits an isothermal, time dependent resistance to flow at constant rate of shear. For the case when the fluid is contained between concentric cylinders the condition offering near maximum resistance to flow can be studied by the method of Billington and Huxley<sup>2,3</sup>, in which a near instantaneous drive is applied to, for example, the outer cylinder, which is then maintained at constant angular velocity, and the resulting motion of the inner cylinder moving against the restoring couple of the measuring head is observed.

For a Newtonian fluid the governing equation for the motion of the inner cylinder, neglecting friction, is

$$I\ddot{\theta}_i + C\dot{\theta}_i + K\theta_i = C\Omega_0 \quad (1)$$

where  $I$  is the moment of inertia of the inner cylinder,  $K$  the restoring couple per unit angular displacement of the torsion head,  $\theta_i$  the angular displacement of the inner

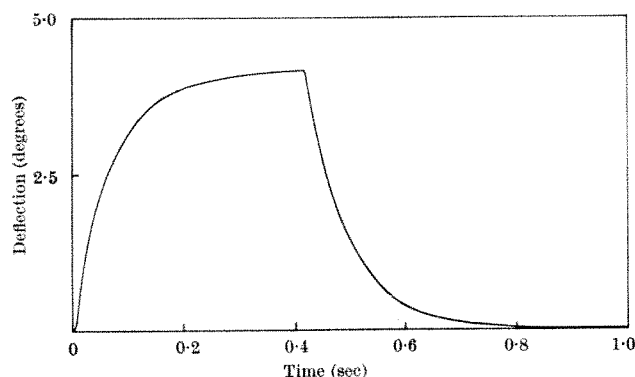


Fig. 1. Transient rise and decay of the deflection of the inner cylinder for a Newtonian oil.

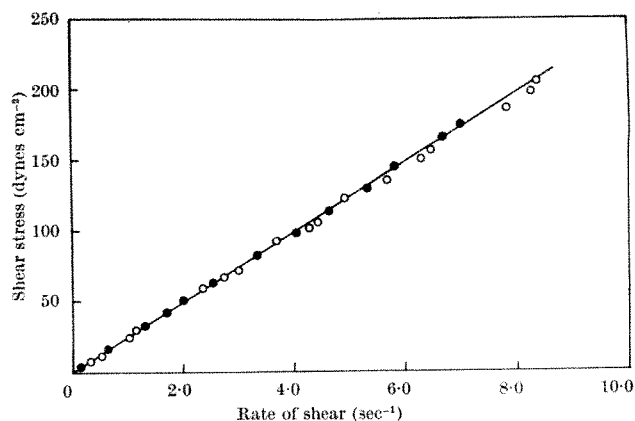


Fig. 2. Instantaneous shear stress—rate of shear graph for a Newtonian oil showing zero hysteresis. (Open symbols from rise; solid symbols from decay.)

cylinder,  $\Omega_0$  the speed of rotation of the outer cylinder and  $C$  is given by

$$C = \frac{4\pi\mu r_i^2 r_0^2 h}{r_0^2 - r_i^2} \quad (2)$$

where  $\mu$  is the viscosity,  $r_0$  the radius of the outer cylinder, and  $r_i$  and  $h$  are the radius and height of the inner cylinder respectively. Billington<sup>4</sup> considered the solution to equation (1) for the case when  $(C/2I)^2 \gg K/I$  and with the boundary conditions  $\theta_i = 0$  at  $t = 0$  and  $\dot{\theta}_i = \Omega_0$  at  $t = 0$ , which were verified by experiment, obtained the solution

$$\theta_i = \theta_i(\infty) \left( 1 - \exp \left[ -\frac{t}{\tau_\mu} \right] \right)$$

where

$$\theta_i(\infty) = \frac{C\Omega_0}{K} \quad (3)$$

and

$$\tau_\mu = \frac{C}{K}$$

If, after an infinite time, the motion of the outer cylinder is suddenly brought to rest, the corresponding solution to equation (1) with  $\Omega_0 = 0$  and the boundary conditions  $t = 0$ ,  $\theta_i = \theta_i(\infty)$  and  $\dot{\theta}_i = -\Omega_0$  is given by

$$\theta_i = \theta_i(\infty) \exp \left( -\frac{t}{\tau_\mu} \right) \quad (4)$$

Equations (3) and (4) for the transient rise and decay of the deflection of the inner cylinder, resulting from the near instantaneous application of the drive to the outer cylinder and then subsequently bringing this cylinder suddenly to rest, form quasi-steady solutions as can be verified by differentiation. Fig. 1 shows a trace of part of a transient rise and decay curve so obtained with a fluid of viscosity

$$\mu = 24.8 \text{ P. and } (C/2I)^2 \sim 10K/I$$

The quasi-steady solutions for the transient rise and decay can therefore be taken to form a "natural" cycle of shear which is distinguished from "normal" forced cyclic shearing in which the angular velocity of the outer cylinder is increased from zero to a given value at a uniform rate and then subsequently decreased to zero at the same rate. For the condition  $(C/2I)^2 \gg K/I$  both a forced shearing cycle and the natural shearing cycle, described here, give zero hysteresis loops to a sufficient approximation for Newtonian fluids, although complex phase relations arise when this condition is not satisfied. Fig. 2 shows a zero hysteresis loop of shear stress plotted against

rate of shear calculated from the relative angular velocity of the cylinders  $\Omega_0 - \dot{\theta}_t$  for a natural cycle of shear.

A distinction is made between the natural and forced cycles of shear because terms involving the rate at which the speed of the outer cylinder is changed do not arise in the former case. It will therefore assume importance for thixotropic fluids because it should be possible to study the flow properties in a condition closer to that offering maximum resistance to shear. Some results for lubricating greases have been obtained in this way<sup>5</sup>.

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## MOLECULAR STRUCTURE

### Sequence Similarities between Hen Egg-white and *T*<sub>4</sub> Phage Lysozymes

Inouye and Tsugita<sup>1</sup> have reported the sequence determination of *T*<sub>4</sub> phage lysozyme and remark that "no common first order structure is found between *T*<sub>4</sub> phage lysozyme and hen egg-white lysozyme, but the distribution of basic, acidic and hydrophobic amino-acids seems to be similar".

By employing the known structure of hen egg-white lysozyme<sup>2</sup> and the following guiding principles, a more definite correlation between the two sequences may be suggested. (a) The shorter egg-white lysozyme chain has evolved from a molecular species more like *T*<sub>4</sub> lysozyme by the deletion of about thirty-one residues (compare the deletion of residues in passing from myoglobin to haemoglobin). (b) The above principle implies that *T*<sub>4</sub> and egg-white lysozymes exert their catalytic function in a similar way so that residues implicated directly in the action of egg-white lysozyme must be present in broadly similar positions in *T*<sub>4</sub>. (c) The comparison of haemoglobin sequences<sup>3</sup> suggests that deletion from alpha-helical regions will be relatively uncommon, because it will usually disturb the environment of all later members of the helix to a serious degree. Where it does occur it should involve deletions corresponding to one or more turns of the helix. (d) The critical importance of hydrophobic groups in internal sites of proteins particularly indicated by the analysis of haemoglobins and myoglobin<sup>4</sup> suggests that hydrophobic internal residues of egg-white lysozyme, particularly at the *N*-terminal end where they may be required to act as a nucleus or "internal template" for folding<sup>5-7</sup>, will have their counterparts in *T*<sub>4</sub> phage lysozyme. (e) Common identical residues can be considered to be significant only if several can be brought into correspondence without the need for deletions between individual common residues. (f) Residues in *T*<sub>4</sub> and egg-white lysozymes related by a single base change of their codons can be regarded as less significant than those related by the above principles, for most amino-acids are removed in the code from others by only two base changes, and, in the long period of evolution separating the molecular species, reversals of base changes and even complete cycles of all three base changes may have occurred to confuse the issue. When, however, single base changes suggest one choice from several arrived at on these grounds, they may be useful. (g) In a similar way common charged residues, polar residues and small residues are regarded as of secondary importance, but may enhance the choice made on other grounds.

Table 1 shows a correlation between the egg-white and *T*<sub>4</sub> sequences made on the basis of these principles with the order of priorities indicated. It is not yet possible to weight each of the above factors in precise numerical terms and the analysis centres upon establishing qualitatively that one relationship between the chains uniquely satisfies them. Otherwise, despite a high index

Table 1. COMPARISON OF THE PRIMARY SEQUENCES OF HEN EGG-WHITE AND *T*<sub>4</sub> PHAGE LYSOZYMES

Hen egg-white lysozyme No. of residue	<i>T</i> <sub>4</sub> phage lysozyme No. of residue	Common character	Hen egg-white lysozyme No. of residue	<i>T</i> <sub>4</sub> phage lysozyme No. of residue	Common character
1 lys	1 met		57 gln	86 arg	p1
2 val	2 asn	p1	58 ile	81 asn	
3 phe	3 ile	H1	59 asn	82 ala	
4 gly	4 phe		60 ser	83 lys	p1
5 (arg)	5 glu	1	61 arg	84 leu	
6 (cysH)	6 met	1	62 try	85 lys	+1
7 (glu)	7 leu	H	63 try	86 pro	H
8 (leu)	8 arg	p	64 cysH	87 val	H
9 (ala)	9 ile	H1	65 asp	88 tyr	H1
10 (ala)	10 asp	1	66 asn	89 asp	-
11 (ala)	11 glu	1	67 gly	90 ser	p1
12 (met)	12 gly	s1	68 arg	91 leu	
13 (lys)	13 leu	H1	69 thr	92 asp	p
14 (arg)	14 arg	+1	70 pro	93 ala	1
15 (his)	15 leu	1	71 gly	94 val	H
	16 lys	+1	72 ser	95 arg	1
	17 ile		73 arg	96 arg	p1
	18 tyr		74 asn	97 cysH	1
	19 lys		75 leu	98 ala	
	20 asp		76 cysH	99 leu	H
	21 thr		77 asn	100 ile	H
	22 glu		78 ile	101 asn	p
	23 gly		79 pro	102 met	H1
	24 tyr		80 (cysH)	103 val	H
16 gly	25 tyr		81 (ser)	104 phe	H1
17 leu	26 thr		82 (ala)	105 gln	p
18 asp	27 ile	H1	83 (leu)	106 met	
19 asn	28 gly	1	84 (leu)	107 gly	
20 tyr	29 ile	1	85 (ser)	108 glu	
21 arg	30 gly		86 ser	109 thr	1
22 gly	31 his	+1		110 gly	s1
23 tyr	32 leu			111 val	
24 (ser)	33 leu	H		112 ala	
25 (leu)	34 thr	p1	87 asp	113 gly	1
26 (gly)	35 lys		88 (ile)	114 phe	H1
27 (asn)	36 ser	s1	89 (thr)	115 thr	p
28 (try)	37 pro		90 (ala)	116 asn	
29 (val)	38 ser	1	91 (ser)	117 ser	p
30 (cysH)	39 leu	H1	92 (val)	118 leu	H1
31 (ala)	40 asn		93 (asn)	119 arg	p
32 (ala)	41 ala	s	94 (cysH)	120 met	H
33 (lys)	42 ala	s	95 (ala)	121 leu	
	43 lys	+	96 (lys)	122 gln	p1
	44 ser		97 lys	123 gln	p1
	45 gln		98 ile	124 lys	1
34 (phe)	46 leu	H1	99 val	125 arg	
35 glu	47 asp	-1	100 ser	126 try	1
36 ser	48 lys	p	101 asp	127 asp	-
37 asn	49 ala		102 gly	128 glu	1
38 phe	50 ile	H1	103 asp	129 ala	1
39 asn	51 gly		104 gly	130 ala	s1
40 thr	52 arg	p1	105 met	131 val	H1
41 gln	53 asn	p1	106 asn	132 asn	p
42 ala	54 cysH		107 ala	133 leu	
43 thr	55 asn	p1	108 try	134 lys	
44 asn	56 gly		109 val	135 tyr	H
45 arg	57 val		110 ala	136 asn	
46 asn	58 ile	1	111 try	137 gln	
47 thr	59 thr	p	112 arg	138 thr	p1
48 asp	60 lys	p1		139 pro	
49 gly	61 asp	1	113 asn	140 asn	p
50 ser	62 glu	p	114 arg	141 arg	+
51 thr	63 ala	1	115 cysH	142 ala	
52 asp	64 glu	-1	116 lys	143 lys	+
53 tyr	65 lys		117 gly	144 arg	1
	66 leu			145 val	
	67 phe			146 ile	
	68 asn			147 thr	p
	69 gln		118 thr	148 thr	p
	70 asp		119 (asp)	149 phe	H1
	71 val		120 (val)	150 arg	p1
	72 asp		121 (gln)	151 thr	1
	73 ala		122 (ala)	152 gly	
	74 ala			153 thr	
	75 val			154 try	H
	76 arg		123 try	155 asp	
54 gly	77 gly	s	124 ile	156 ala	
55 ile	78 ile	H	125 arg	157 tyr	
56 leu	79 leu	H	126 gly	158 lys	
			127 cysH	159 asn	p
			128 arg	160 leu	H
			129 leu		

H denotes hydrophobic residues common to both; p, polar residues; s, small residues; - and +, residues which may be negatively charged and residues which may be positively charged; in each instance common to both sequences. 1 denotes a single base change separating the two residues and common residues are underlined.  $\alpha$ -Helix is indicated by vertical brackets.

of common features it may seem that some other sequence relationship would be equally favourable.

The most important restriction, that concerning the activity of lysozymes, is that residues analogous to egg-white lysozyme residue 35, a glutamic acid, and to residue 52, an aspartic acid, must be found because these residues are essential for activity according to the present evaluation of the binding of competitive inhibitors to crystalline lysozyme<sup>8</sup>. Only one carboxylic acid residue, aspartic acid 47, is present in the  $T_4$  chain between residues 25 and 50. There are five carboxylic acid residues between residues 50 and 75, but only the  $T_4$  residues 47 and 64 can be brought into correspondence with egg-white residues 35 and 52 without deletions between them. This choice reverses the position of aspartic and glutamic acids in the active site. It is strengthened by the triple correspondences of egg-white 31, 32, 33 with  $T_4$  41, 42, 43, and of egg-white 54, 55, 56 with  $T_4$  77, 78, 79, which suggest outside limits for the comparison of these regions of the two sequences. If deletion is permitted between the two active carboxyl groups it is possible to bring the aspartic acid residue 52 of egg-white into correspondence with the aspartic residues 70 or 72 of  $T_4$ . The latter possibility illustrates the limitation of the present comparison. The principles enunciated cannot always decide between minor variants within the broad overall correlation which does, however, appear to have a strong claim to being of the unique kind sought. Part of the interest in making a prediction before further lysozyme sequences are known is that, when they are established and the eye can immediately see straight-forward correspondences, it will be even more difficult to assess the real factors which must remain common to evolving structures.

The correspondence, particularly at the  $N$ -terminal end, is good so far as each of the stated principles is concerned, thus: internal residues of egg-white lysozyme are in general replaced by other hydrophobic groups in corresponding positions of  $T_4$ ;  $\alpha$  helix is not interfered with in the first  $\alpha$ -helix and only marginally in the second; single base changes account for any amino-acid changes in twenty of the first thirty residues.

The frame shift mutant of  $T_4$  phage examined by Terzaghi *et al.*<sup>9</sup> would involve the replacement of four residues in the second  $\alpha$ -helix: ser, pro, ser, leu, asp by val, his, his, leu, met. The rules which have been put forward to predict  $\alpha$ -helical sections by Guzzo<sup>10</sup> and Prothero<sup>11</sup> would suggest that  $\alpha$ -helicity need not be interfered with. The possession of an extra  $N$ -terminal methionine by  $T_4$  phage lysozyme, according to the sequence relationship

suggested, may be significant in terms of current theories of chain initiation.

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### Crystal Structures of the Lamellar Calcium Aluminate Hydrates

THE compound  $\text{Ca}_2\text{Al}(\text{OH})_7 \cdot 3\text{H}_2\text{O}$ , or  $4\text{CaO} \cdot \text{Al}_2\text{O}_3 \cdot 13\text{H}_2\text{O}$ , and several of the many related basic salts, are important because they are formed on hydration of cements<sup>1</sup>. These substances form platey crystals, which are hexagonal or pseudohexagonal, with (0001) cleavage. The unit cells of some are known, but no crystal structures have been determined. This can be attributed, at least in part, to the poor quality of the crystals, which are rarely more than  $100\mu$  across and a few microns thick, and which often show disorder in both orientation and stacking.

We have partially determined the structure of a crystal which approximated in composition to  $\text{Ca}_2\text{Al}(\text{OH})_7 \cdot 3\text{H}_2\text{O}$  with some structural  $\text{CO}_3^{2-}$ , and which may have been a polytype of hydrocalumite ( $\text{Ca}_{18}\text{Al}_8(\text{OH})_{54}(\text{CO}_3)_2 \cdot 21\text{H}_2\text{O}$  approximately).

X-ray photographs of single crystals indicated that the crystals were trigonal, with  $a_H$  5.73,  $c_H$  47.16 Å, space group  $R3c$  or  $R3c$  and  $Z=6$ . This is analogous to the cell found for  $\beta\text{-Ca}_2\text{Al}(\text{OH})_6\text{Cl} \cdot 2\text{H}_2\text{O}$  by Kuzel<sup>2</sup>. The intensities of 146 independent reflexions were determined using a Hilger automatic linear diffractometer; partial corrections for absorption were applied as mentioned by Arndt, North and Phillips<sup>3</sup>. Intensity statistics indicated a centre of symmetry, and the space group was therefore taken to be  $R3c$ . The unit cell is composed of six layers

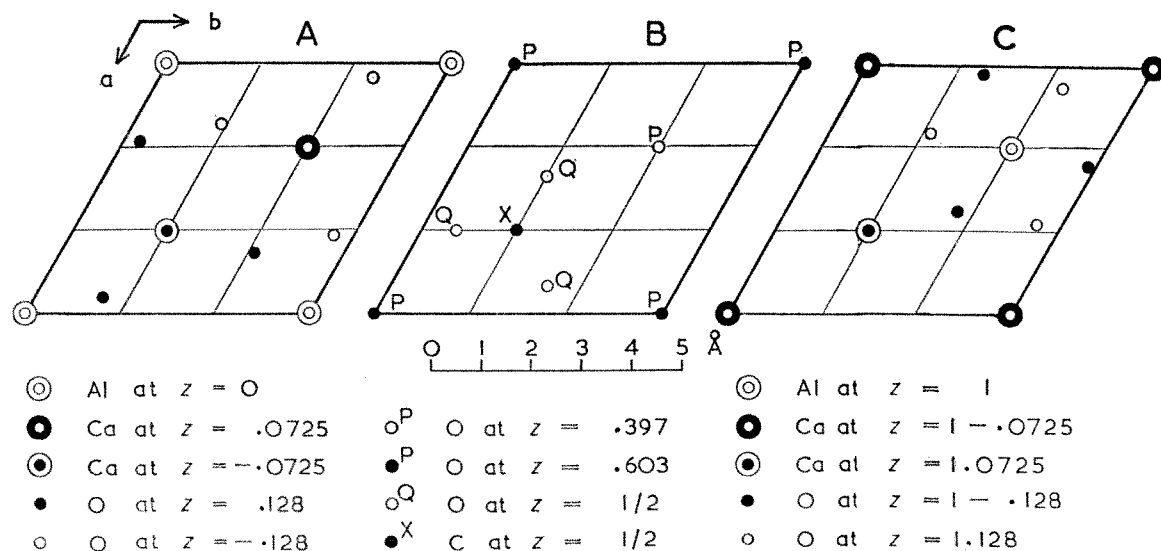


Fig. 1. Diagram of parts of the structure, seen in projection along the  $c$ -axis;  $z$  co-ordinates are given as fractions of the single layer thickness of 7.86 Å. A, Basic layer with Al at  $z=0$ ; B, interlayer region around  $z=1/3$ ; C, basic layer with Al at  $z=1$ .

lying perpendicular to *c*. Each layer is 7.86 Å thick in the *c*-direction and contains a single formula unit. If the structure of a single layer can be determined, the stacking of the layers, and thus all remaining features of the structure, follow from the space group.

The main features of the structure were determined from a three-dimensional Patterson synthesis, followed by Fourier and difference syntheses. The parameters have so far been refined to give  $R=0.16$ ; isotropic temperature factors were used, and unobserved reflexions were taken as having one half the structure amplitudes of the weakest ones observed. With some reservations, the results confirm the hypothesis of Buttler, Dent Glasser and Taylor<sup>4</sup> that these substances have structures based on mixed octahedral layers of composition  $\text{Ca}_2\text{Al}(\text{OH})_6$ , between which lie the water molecules and remaining anions.

Fig. 1 shows the essential features of the structure. The basic layers (Fig. 1A and C), which are composed of  $\text{Ca}_2\text{Al}(\text{OH})_6$ , lie in the *ab*-plane and occur at intervals of 7.86 Å in the *c*-direction. Comparison of Figs. 1A and C shows the stacking relation between adjacent layers. The basic layers are of ordered structure, and may be described either as distorted octahedral layers in which  $\text{Ca}^{2+}$  and  $\text{Al}^{3+}$  are regularly arranged, or alternatively as agglomerates of  $\text{Ca}^{2+}$  and  $\text{Al}(\text{OH})_6^{3-}$  ions. The interlayer regions (Fig. 1B) appear to be only partly ordered, with atoms statistically distributed among a number of sites; our results for these regions are tentative. The oxygen atoms at the sites marked *P* probably belong to water molecules, and raise the calcium co-ordination to seven. The remaining water molecules, hydroxide and carbonate ions, occupy the cavities centred on sites of the type marked *X*. The carbon atoms probably occur at *X*, and the oxygen atoms chiefly at or near sites of the type marked *Q*. A high proportion of the *P* sites appears to be occupied, and over half of the *Q* sites. Some oxygen atoms may also occur on the *X* sites.

The basic layers shown in Figs. 1A and C almost certainly occur in all members of the group, and the water molecules at the *P* sites probably occur in most of them. The essential differences between these substances lie therefore in the interlayer regions, and especially in the contents of the cavities previously mentioned. Variations in the type of layer stacking also occur, extremes of two and twelve layer arrangements having so far been reported<sup>1,2</sup>.

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### Actinide Chelates: Uranium (IV) *N,N*-Diethyldithiocarbamate

THERE is at present intense interest in the co-ordination of sulphur to metal ions, especially those of the *d*-transition series<sup>1</sup>. Many of these complexes have unusual physical, chemical and structural properties. Little is known, however, about the co-ordination of sulphur to the actinide metal ions, and we report here preliminary observations on the first uranium (IV) complex with a sulphur chelate. The ligand, *N,N*-diethyldithiocarbamate (dte), has itself been widely used in the earlier transition metals (many of the known complexes are listed in ref. 2) in which, for example, the apparently simple complexes  $M(\text{dte})_2$  ( $M(\text{II})$  = chromium, manganese, iron, copper and zinc) actually involve 5-co-ordination<sup>3</sup>.

A variety of uranium (VI) disubstituted dithiocarbamates are known<sup>4,5</sup> and are in fact the ultimate products when uranium (IV) chloride is reacted with the ligands in the presence of air. In such reactions, a green, light sensitive intermediate compound, postulated to be  $\text{U}(\text{S}_2\text{CNR}_2)_4$ , is observed<sup>5</sup>. The pure uranium (IV) compound of the diethyl derivative is made by reacting stoichiometric quantities of uranium (IV) chloride with the sodium salt of dte in dry ethanol in an inert atmosphere. The ethanol is quickly removed under vacuum at room temperature and the product recrystallized from dry benzene as yellow, air sensitive plates which are stable indefinitely when stored in argon. (Analysis: found, U, 28.4; required for  $\text{U}[\text{S}_2\text{CN}(\text{C}_2\text{H}_5)_2]_4$ , U, 28.64 per cent.) Addition of ether or isopentane to the benzene solution increases the yield to almost quantitative proportions. The complex decomposes if left in contact with alcohol and solutions in benzene or methylene chloride are particularly sensitive to traces of oxygen.

Ebullioscopic measurements in benzene gave an average molecular weight of 812 compared with the monomeric  $\text{U}(\text{dte})_4$  unit of 831. It appears likely therefore that the uranium atom is eight co-ordinated to sulphur, raising the question of the stereochemistry about the uranium. Single crystal X-ray diffraction photographs obtained using Precession and Weissenberg cameras established that the lattice belongs to the monoclinic system with  $a=19.05$  Å,  $b=11.67$  Å,  $c=16.03$  Å and  $\beta=63^\circ 35'$ . Simple flotation experiments pointed to a density of between 2 and 3 and therefore the cell probably contains six molecules, giving a calculated density of 2.58. Observed reflexions, for  $hkl$ ,  $h+k=2n$ , for  $h0l$ ,  $h=2n$ , and for  $0k0$ ,  $k=2n$ , showed the probable space group to be  $C2/m$  ( $C^{2h}$ ). It is anticipated that intensity data will be collected and the complete structure solved.

Magnetic and spectral properties of  $\text{U}(\text{dte})_4$ , together with the synthesis and properties of analogous tetravalent actinide complexes ( $M(\text{IV})$  = thorium, protactinium, neptunium and plutonium), are now being investigated. These studies will be extended to include other sulphur, nitrogen and oxygen chelates.

*Note added in proof.* The preparation and infra-red spectra of the uranium (IV) and some allied actinide (IV) dithiocarbamates were reported at the 153rd ACS meeting, Miami, Florida, in April 1967 by J. P. Bibler and D. G. Karracker (Abstract L32).

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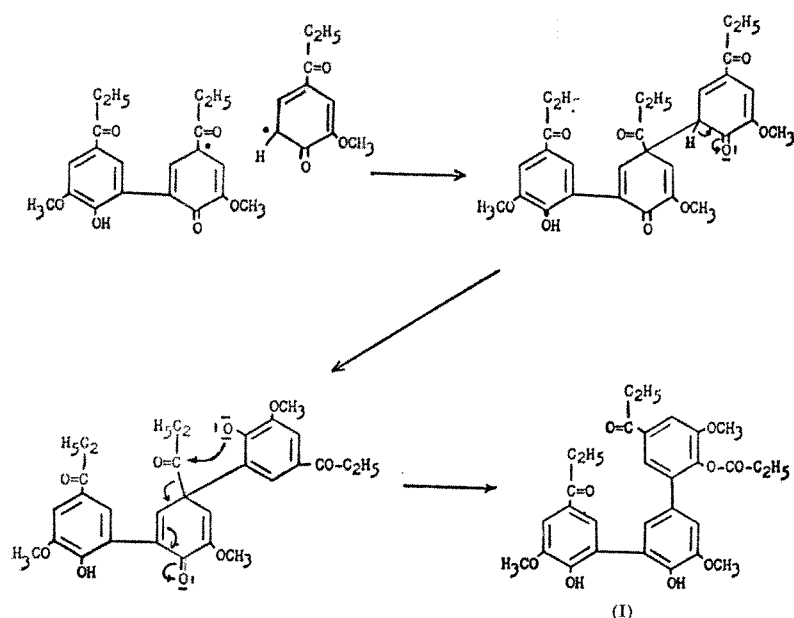
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### New Structures from the Dehydrogenation of Model Compounds related to Lignin

MANY natural products are now known to originate through coupling of free radicals formed by the oxidative dehydrogenation of phenols<sup>1,2</sup>. That this applies to plant lignins is now widely accepted. Much of our present knowledge of the chemical structure of lignin was developed over the past 20 years by the outstanding work of Freudenberg<sup>3</sup> and his associates, who showed that coniferyl alcohol and other *p*-hydroxycinnamyl alcohols are the principal precursors. They have identified more than forty compounds produced initially in the reaction, coupling having taken place through the phenolic oxygen, the position *ortho* to it in the aromatic ring or C-β of the side chain.





We have dehydrogenated simpler compounds, principally without C=C conjugation in the side chain, because many of the dehydrogenation products formed from coniferyl alcohol are of this type.

Previous experiments<sup>4-6</sup> on the limited dehydrogenation of lignin-related model compounds with hydrogen peroxide plus peroxidase showed that guaiacols, substituted in the 4-position with an alkyl side chain, gave rise by free-radical phenol coupling to dehydrodimers, principally the *o,o'*-dihydroxybiphenyl compound and a diphenyl ether. More pertinent models with an  $\alpha$ -carbonyl or hydroxyl group gave the related biphenyl compounds and other products not then identified. These have now been further investigated with surprising results.

Propioguaiacone was dehydrogenated in aqueous solution with hydrogen peroxide in an amount calculated to remove one atom of hydrogen per molecule; the reaction products comprised 60 per cent of the biphenyl, 16 per cent of the starting compound, and 24 per cent of a "resin". This resin showed a pronounced band in the infra-red spectra corresponding to the ester of an aliphatic acid. It consists principally of an amorphous trimer I.

Presumably one of the phenolic groups in the *o,o'*-dihydroxybiphenyl dehydrodimer is attacked and the mesomeric *cyclohexadienone*-type radical formed pairs with a monomeric *ortho* radical. This is followed by re-aromatization of the dienone ring by nucleophilic attack on its side chain carbonyl group by the phenoxyl ion formed by deprotonation of the upper unit. The product (I) contains two structural features that are novel in phenol coupling reactions: the aryl ester group and the biphenyl bond between the two right-hand aromatic residues, which is formed in an orientation that is *ortho*, *para'* to the original phenolic hydroxyls.

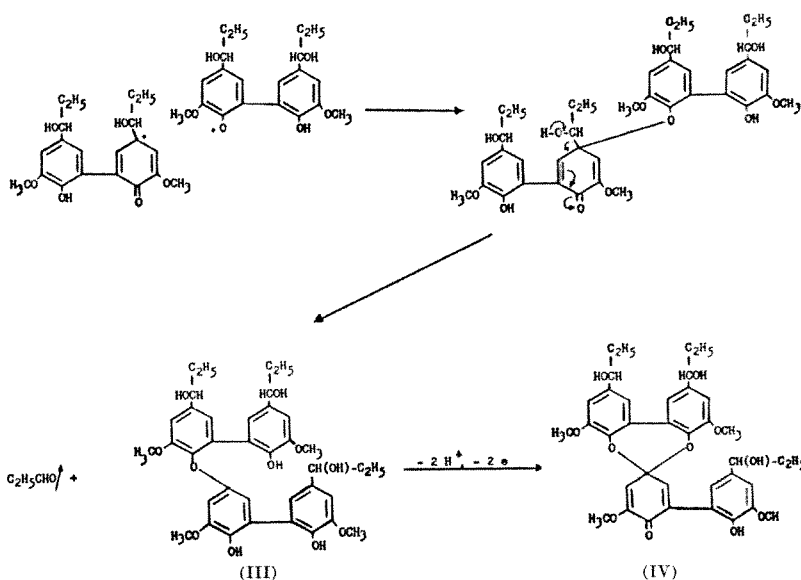
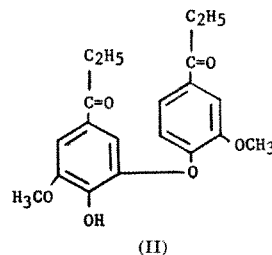
The extent of this unusual reaction is probably limited by the separation of much of the *o,o'*-dihydroxybiphenyl compound in its comparatively unreactive crystalline state. If the reaction is carried out in aqueous alcohol or acetone, more than twice as much ester is formed. A small amount

of the dimer represented by the two right-hand rings of (I) was found as well as the dimer (II). The latter is formed by a straightforward coupling mechanism, but the formation of the former must also involve this novel side chain transfer.

The new reaction appears to be quite general, vanillin and acetovanillone giving esters which can be detected by infra-red spectroscopy or by determination of the volatile acid produced by hydrolysis of the acyl group. Ethyl vanillate gives a product showing a strong aliphatic ester band in the infra-red, presumably the ethoxycarbonyl derivative of the phenol, that is, the product is an aryl ethyl carbonate.

The presence of ester groups in lignin has been claimed from time to time<sup>7</sup> but has usually been thought to be part of the extractive component of the plant or, if in the lignin, to have been the result of secondary esterification of the lignin with *p*-hydroxycinnamic acids<sup>8</sup>. These experiments suggest that ester groups may also be an intrinsic part of the polymeric system of the lignin.

If  $\alpha$ -ethylvanillyl alcohol is dehydrogenated enzymatically in aqueous solution, it is remarkable that when only 2-3 per cent of the calculated equivalent of peroxide is added, an odour of propionaldehyde is plainly evident. This propionaldehyde is also formed by release of the side chain from some molecules after coupling of *cyclohexadienone* radical forms. One oxidation product, the crystalline compound (IV) with a melting point 198°-200° C, can be isolated from the reaction mixture in high yield. The mode of its formation from biphenyl-linked dehydro-



dimers shown here illustrates the mechanism involved in the release of the propionaldehyde.

If 2:1 equivalents of peroxide are added and the propionaldehyde is collected and estimated, it corresponds to 83 per cent of the product referred to here. The reaction must proceed through the coupling of a dienone radical of one biphenyl group to the phenoxy radical of a second. This is then re-aromatized by expulsion of the side chain as aldehyde to give compound (III) which is further dehydrogenated to (IV).

The dibenzo [*d,f*] [1,3] dioxepin (IV) readily breaks up into coloured products when it is treated with dilute mineral acid or alkali at room temperature; these colours are reminiscent of those that develop more slowly in extracted wood meal on similar treatment. An analogous dioxepin structure has been noted by Hewgill<sup>9,10</sup> in products of the dehydrogenation of sterically hindered phenols used as food antioxidants.

As with the carbonyl compounds, this reaction also appears to be rather general, formaldehyde being detected in the case of vanillyl alcohol and acetaldehyde with apocynol. Once isolated and crystallized, the biphenyl compound from  $\alpha$ -ethylvanillyl alcohol proved immune to further dehydrogenation with peroxide-peroxidase in aqueous alcohol or acetone, possibly because of strong hydrogen bonding, but responded readily in dilute aqueous solution.

The dehydrogenation with peroxide-peroxidase of the frequently used and important lignin model guaiacyl-glycerol- $\beta$ -guaiacyl ether is now under investigation.

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## CHEMISTRY

### Mössbauer Spectra of Some Iron 1,2-Dithiolenes

IRON forms a variety of complexes with 1,2-dithiolene ligands, for example,  $[\text{FeS}_4\text{C}_4\text{R}_4]_n^{2-}$  ( $z=0, -1, -2$ ;  $n=1$  or 2),  $[\text{Fe}(\text{NO})\text{S}_2\text{C}_4\text{R}_4]_n^{2-}$  ( $z=+1, 0, -1, -2$  or  $-3$ ) and  $[\text{FeS}_6\text{C}_6(\text{CN})_6]^{2-}$  (refs. 1-3), the structure and electronic configuration of which is uncertain. Mössbauer spectroscopy provides a means of obtaining such information<sup>4</sup>, and we report here the results of a preliminary investigation of some of these iron 1,2-dithiolenes.

The chemical shifts, shown in Table 1, are in the region expected for low-spin iron compounds and the large quadrupole splittings indicate considerable electric field gradients at the nucleus. The complex  $[(\text{C}_6\text{H}_5)_4\text{P}]_2[\text{FeS}_4\text{C}_4(\text{CN})_4]$  (two unpaired electrons) which contains the iron atom in the formal oxidation state +4, may be reduced to  $[(\text{C}_6\text{H}_5)_4\text{P}]_2[\text{FeS}_6\text{C}_6(\text{CN})_6]$  (one unpaired electron), in which the oxidation state may be regarded

Table 1. MÖSSBAUER DATA FOR SOME IRON 1,2-DITHIOLLENES

	Temp (° K)	Chemical shift (mm/sec)	Quadrupole splitting (mm/sec)
$[(\text{C}_6\text{H}_5)_4\text{P}]_2[\text{FeS}_4\text{C}_4(\text{CN})_4]$	77	0.50	1.57
	295	0.42	1.59
$[(\text{C}_6\text{H}_5)_4\text{P}]_2[\text{FeS}_4\text{C}_4(\text{CN})_4]$	77	0.65	1.69
	295*	—	—
$[(\text{C}_6\text{H}_5)_4\text{N}][\text{FeS}_4\text{C}_4(\text{C}_6\text{H}_5)_4]$	77	0.61	2.37
	295	0.53	2.45
$[(\text{C}_6\text{H}_5)_4\text{N}][\text{FeS}_4\text{C}_4(\text{CN})_4]$	77	0.59	2.76
	295	0.50	2.81
$[(\text{C}_6\text{H}_5)_4\text{P}][\text{Fe}(\text{pyridine})\text{S}_4\text{C}_4(\text{CN})_4]$	77	0.59	2.41
	295	0.53	2.51
$[\text{C}_6\text{H}_5)_4\text{P}][\text{Fe}(\text{NO})\text{S}_4\text{C}_4(\text{CN})_4]$	77	0.31	1.68
	295	0.20	1.69
$[(\text{C}_6\text{H}_5)_4\text{P}]_2[\text{Fe}(\text{NO})\text{S}_4\text{C}_4(\text{CN})_4]$	77	0.47	0.97
	295*	—	—
$[\text{Fe}(\text{NO})\text{S}_4\text{C}_4(\text{C}_6\text{H}_5)_4]$	77	0.32	1.65
	295	0.24	1.65
$[(\text{C}_6\text{H}_5)_4\text{N}][\text{Fe}(\text{NO})\text{S}_4\text{C}_4(\text{C}_6\text{H}_5)_4]$	77	0.27	1.98
	295	0.20	1.99
$[\text{Fe}_2(\text{NO})_2\text{S}_6\text{C}_6(\text{C}_6\text{H}_5)_6]$	77	0.25	1.57
		0.55	1.26
	295	0.18	1.58
		0.47	1.27

\* The percentage of absorption was too low to yield reliable data.

formally as +3. The increase in chemical shift on this reduction confirms the decrease in metal oxidation state.

The large quadrupole splitting is the result of distortion from a regular octahedron to either  $D_3$  or  $D_{3h}$  symmetry which lifts the degeneracy of the  $t_{2g}$  orbitals, thus causing an electron imbalance in these orbitals. Relatively little change in the quadrupole splitting occurs on changing the overall charge of the complex from  $-2$  to  $-3$ . Perhaps this is to be expected because one electron outside the symmetric half-filled  $t_{2g}$  levels (that is,  $t_{2g}^4$ ) will have much the same field gradient as a single vacancy in the complete subshell (that is,  $t_{2g}^5$ ). There should, however, be a change in the sign of the field gradient and we hope to investigate this point further.

An X-ray crystal structural investigation of  $[(n\text{-C}_4\text{H}_9)_4\text{N}][\text{FeS}_4\text{C}_4(\text{CN})_4]$  (ref. 5 and I. Bernal, personal communication) has revealed that the anion is dimeric, each iron being surrounded by five sulphur atoms in a distorted square pyramidal arrangement with edge sharing. There is one unpaired electron per iron atom and these electrons are coupled antiferromagnetically. The spectrum of  $[(\text{C}_6\text{H}_5)_4\text{N}][\text{FeS}_4\text{C}_4(\text{CN})_4]$  is similar to that of the  $[(n\text{-C}_4\text{H}_9)_4\text{N}]^+$  salt and the Mössbauer parameters for this compound and  $[(\text{C}_6\text{H}_5)_4\text{N}][\text{FeS}_4\text{C}_4(\text{C}_6\text{H}_5)_4]$  indicate that both these compounds are dimeric. The Mössbauer spectrum of  $[(\text{C}_6\text{H}_5)_4\text{P}][\text{Fe}(\text{pyridine})\text{S}_4\text{C}_4(\text{CN})_4]$  is also similar to the simple dithiolenes, thus indicating that the complex is five co-ordinate but monomeric; J. A. McCleverty, J. Locke and N. Connelly have obtained magnetic measurements which indicate that this complex has three unpaired electrons (unpublished).

The decrease in chemical shift in the nitrosyl complex series, in comparison with the other five co-ordinated species, is to be expected because back-donation to the empty antibonding orbitals on the NO reduces the shielding of the  $4s$  electrons, increases the  $s$  electron density at the iron nucleus, and decreases the isomer shift. A reduction in the quadrupole splitting is also observed which indicates a more symmetric environment around the iron atom than in the other species. Dimerization of the nitrosyls by way of  $\text{Fe} \cdots \text{S}$  interactions to give six co-ordinated iron complexes is possible but, in view of the extensive polarographic and spectral evidence (the latter both in the solid and in solution) obtained by N. M. Atherton and his colleagues (unpublished), this is unlikely.

On the basis of recent analytical evidence, the compound originally formulated as  $[\text{Fe}(\text{NO})\text{S}_2\text{C}_2(\text{C}_6\text{H}_5)_2]_2$  is now formulated as  $[\text{Fe}_2(\text{NO})_2\text{S}_6\text{C}_6(\text{C}_6\text{H}_5)_6]$ . The Mössbauer spectrum consists of two overlapping, quadrupole split, absorptions indicating that there are two distinct iron sites, one of which has very similar parameters to those of the neutral five co-ordinate nitrosyl complex  $[\text{Fe}(\text{NO})\text{S}_4\text{C}_4(\text{C}_6\text{H}_5)_4]$ . The intensities of the two Mössbauer absorptions are approximately equal, but until definitive X-ray

structural data are available we can make no predictions about the structure of this molecule.

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## HAEMATOLOGY

### Haemoglobin Sydney: $\beta 67$ (EII) Valine $\rightarrow$ Alanine: an Emerging Pattern of Unstable Haemoglobins

SEVERAL abnormal haemoglobins have recently been found in patients with inclusion body haemolytic anaemias<sup>1</sup>, and have been called unstable because they form precipitates more readily than haemoglobin A when warmed to 50° C. We now report the identification of the structural abnormality in one of these haemoglobins—haemoglobin Sydney—the haematology and the family study of which have already been described<sup>2</sup>.

Blood was obtained from a mother and her son of German (Hamburg) origin who had migrated to Australia. They had a family history of haemolytic anaemia and it was possible to produce red cell inclusion bodies after 48 h incubation. A haemoglobin fraction precipitable by heat was also present. Haemoglobin electrophoresis on paper using *tris* buffer<sup>3</sup> and in starch gel with discon-

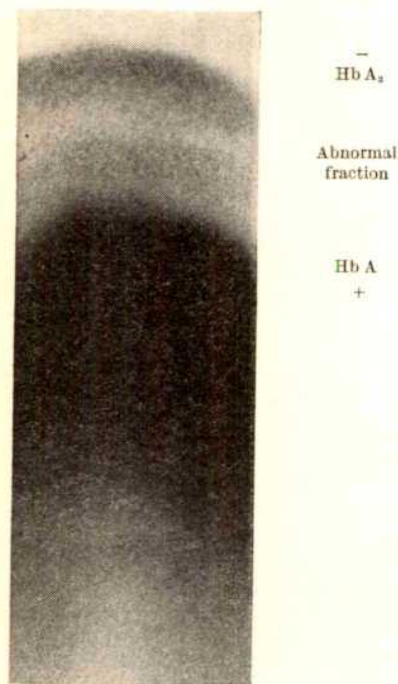


Fig. 1. Paper electrophoresis of the haemoglobin of the propositus. The fraction separating between haemoglobin A and haemoglobin A<sub>2</sub> has been used for preparative work. Ultracentrifugation of the whole haemolysate showed that all fractions had the same sedimentation rate as haemoglobin A.

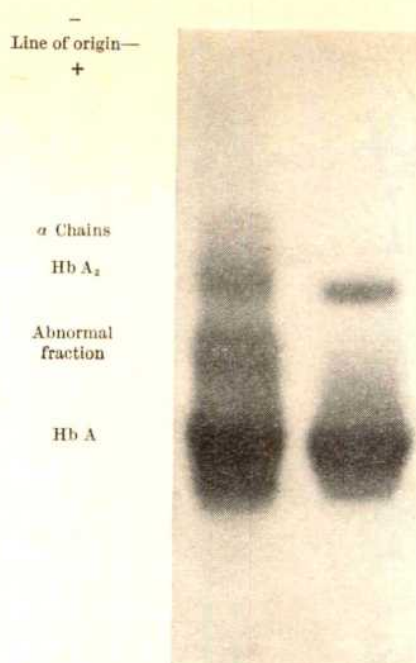


Fig. 2. Starch gel electrophoresis of the haemoglobin of the propositus (left) and of a control (right).

tinuous *tris*-citrate borate buffer at pH 8.6 (ref. 4) demonstrated the presence of a slow moving fraction and another fraction in the position of free  $\alpha$  chains (Figs. 1 and 2). The slow moving fraction was assumed to represent the abnormal haemoglobin, and purification of it was attempted by repeated paper electrophoresis at pH 8.9, as was used for haemoglobin Köln<sup>5</sup>. After five repeated separations, however, haemoglobin A was still present and it was decided to use the fraction as it was for chemical analysis. The finding of free  $\alpha$  chains suggested that, as in some other unstable  $\beta$  chain abnormal haemoglobins<sup>5-7</sup>, the present abnormal haemoglobin was again an unstable haemoglobin with its abnormality in the  $\beta$  chain. This could not be confirmed by hybridization of the haemoglobin with canine haemoglobin<sup>8,9</sup> because precipitation occurred when the pH was lowered to 4.7. Bands with the mobility of normal  $\alpha$  and  $\beta$  chains were found when the globin chains were examined by electrophoresis in starch gel with 6.5 molar urea at pH 8.6 (ref. 10). Similar results had been obtained with haemoglobin Köln and with haemoglobin Genova<sup>7</sup> and had indicated that any change in amino-acid composition of the globin did not in itself involve a change in charge. The likely abnormality was therefore an amino-acid substitution in the  $\beta$  chain involving uncharged amino-acids.

Examination of the globin, and separation and examination of the isolated chains of the globin, were carried out as summarized in refs. 5 and 7. The isolated  $\alpha$  and  $\beta$  chains were hydrolysed in 6 normal hydrochloric acid for 36 and 72 h and the results of amino-acid analysis compared with those of haemoglobin A. The composition of the  $\alpha$  chains of haemoglobin Sydney was normal, but the  $\beta$  chain showed a consistent increase in its alanine content with a corresponding decrease in valine.

Fingerprints of the tryptic digest of whole globin and of the isolated  $\alpha$  and  $\beta$  chains showed a normal pattern with the exception of two extra peptides which were consistently present—one just beneath  $\beta$ Tp IX and the other just beneath  $\beta$ Tp VIII-IX. Both these extra peptides gave a positive reaction for histidine and, as is shown in Figs. 3 and 4, they both had the same electrophoretic mobility as  $\beta$ Tp IX and  $\beta$ Tp VIII-IX at pH 3.5 as well as at pH 6.5. Because it was known that the haemoglobin under examination contained some normal



haemoglobin, it seemed likely that in haemoglobin Sydney a hydrophobic residue had been substituted by a less hydrophobic one in peptide  $\beta$ Tp IX. This would explain why the peptide pairs moved with the same electrophoretic mobility but with different chromatographic mobilities—the upper peptides being derived from haemoglobin A, the lower from haemoglobin Sydney. A curious feature of the fingerprint was that the upper peptide stained more deeply with ninhydrin than the lower in the case of the  $\beta$ Tp VIII-IX pair but that the reverse was true for the  $\beta$ Tp IX peptides. The intensity of the colour with ninhydrin is largely dependent on the N-terminal residues which in both the VIII-IX peptides must be lysine. The reversal of intensities observed in the pair of  $\beta$ Tp IX peptides indicates a difference in the N-terminal residues, particularly as valine, the N-terminal of  $\beta$ Tp IX, is known to stain poorly with ninhydrin.

Table 1. ANALYSIS OF PEPTIDES  $\beta$ Tp IX AND VIII-IX OF Hb SYDNEY AND Hb A

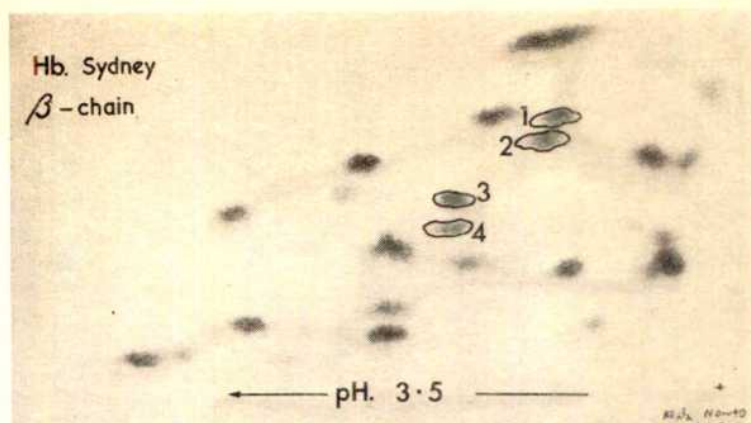
	$\beta$ Tp IX			$\beta$ Tp VIII-IX		
	Sydney	Hb A	Expected	Sydney	Hb A	Expected
Asp	2.6	2.5	3	2.6	2.3	3
Ser	1.0	0.9	1	1.2	1.3	1
Gly	1.9	1.9	2	2.0	2.2	2
Ala	3.2	2.2	2	3.0	1.9	2
Val	0.3	1.1	1	Nil	1.2	1
Leu	4.0	3.8	4	3.9	3.7	4
Phe	1.1	0.8	1	0.7	1.0	1
His	1*	1*	1	1.1	1.0	1
Lys	1*	1*	1	2.1	2.2	2
$\mu$ mole yield per residue	0.08	0.04		0.006	0.014	

\* A positive colour test was considered to represent one residue.

Table 2

Peptide	VIII				IX			
Hb A	Lys	Val	Leu	Gly	Ala	Phe	Ser---	Leu Lys
Hb Sydney	Lys	Ala	Leu	Gly	Ala	Phe	Ser---	Leu Lys
$\beta$ -chain residue	66	67	68	69	70	71	72	81 82
Helical notation	E10	E11	E12	E13	E14	E15	E16	EF5 EF6

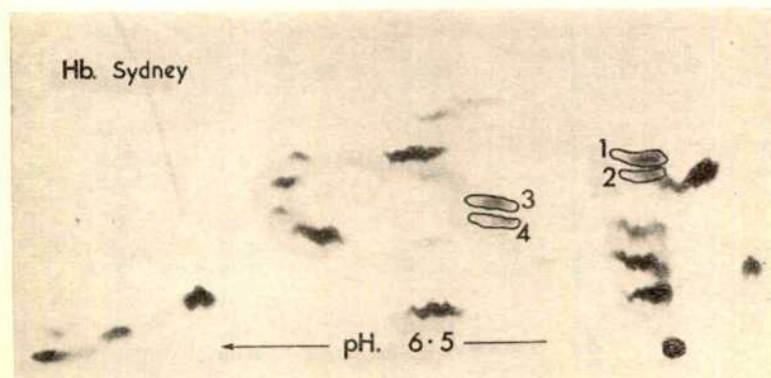
This was confirmed on amino-acid analysis of the peptides (Table 1) which indicated a replacement of the sole valine, the N-terminal residue of  $\beta$ Tp IX, by alanine as shown in Table 2. Additional proof was obtained by determination of the N-terminal of the  $\beta$ Tp IX peptide using the micro dansyl chloride method of Gray and Hartley<sup>11</sup>. This showed that the upper normal peptide has valine as its N-terminal residue and the lower has alanine. Analysis of all the other peptides of the  $\beta$  chain indicated that they have the expected amino-acid composition. It was therefore concluded that the structural

Fig. 4. Fingerprint of the amino-ethylated  $\beta$  chain of haemoglobin Sydney with some  $\beta$  chain of haemoglobin A present. (For details see text.)

abnormality in haemoglobin Sydney was the replacement of valine at position 67 (E11) of the  $\beta$  chain by alanine. E11 is occupied by valine in all known globins with the exception of the lamprey when isoleucine, which is of similar dimension, is found.

A feature of the unstable haemoglobins as a group is their failure to show the same electrophoretic differences from haemoglobin A as the other abnormal haemoglobins. The explanation of this seems to be that they mostly have, like haemoglobin Sydney, amino-acid abnormalities which do not in themselves alter the charge of the haemoglobin from that of haemoglobin A. This is true of haemoglobin Köln<sup>5</sup> and haemoglobin Genova<sup>7</sup> and is also true of the six other unstable haemoglobins that are at present under investigation in Cambridge. Electrophoresis of the globin from these in 6.5 molar urea shows no change in charge from that of globin A. From the genetic code<sup>12</sup> it is to be expected that there will be about twice as many mutations which do not involve a charge change as those that do. Even so, it is surprising to see the preponderance of non-charge changes in the unstable haemoglobins. Examination of the globin molecule as proposed by Perutz, Kendrew and Watson<sup>13</sup> provides an explanation. They point out that the principal forces responsible for the configuration and stability of the globin molecule are the hydrophobic bonds formed by internally sited residues that are invariably non-polar. The replacement of one of these non-polar residues by a charged residue would almost certainly result in a totally non-viable molecule<sup>13</sup>. The replacement of internal hydrophobic residues by other non-polar residues of different dimensions could result, however, in a degree of instability not totally incompatible with survival of the molecule. This is the defect demonstrated in haemoglobin Köln where a methionine replaces the smaller valine at FG5 and again in haemoglobin Sydney when the valine at E11 which bonds with the porphyrin ring is replaced by the smaller alanine.

There will, of course, be exceptions to this rule; the replacement of the distal histidine E7 by arginine to give the unstable haemoglobin Zürich is one. Another exception is haemoglobin M Milwaukee in which the same residue as in haemoglobin Sydney, valine E11, is replaced by glutamic acid. In this case the acidic group can bond to the haem iron, and the  $\gamma$  carbon of the glutamic acid side chain can form the same hydrophobic bond with the porphyrin ring which is formed by valine, thus giving stability to the molecule. From our experience and from theoretical considerations, however, it seems likely that the majority of point mutations giving rise to instability of the haemoglobin molecule will be replace-

Fig. 3. Fingerprint of haemoglobin Sydney containing some haemoglobin A. (1)  $\beta$ Tp IX of haemoglobin A; (2)  $\beta$ Tp IX of haemoglobin Sydney; (3)  $\beta$ Tp VIII-IX of haemoglobin A; (4)  $\beta$ Tp VIII-IX of haemoglobin Sydney. (For details see text.)



ments at internal sites involving non-charged residues.

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## Fibrinolytic Response to Moderate, Exhaustive and Prolonged Exercise in Normal Subjects

PREVIOUS studies of the fibrinolytic response to a short period of moderate exercise and intravenous adrenaline in a group of healthy subjects have revealed a small subgroup whose response to these procedures appears to be impaired<sup>1,2</sup>. It was suggested that these poor responders could be at risk to conditions such as atherosclerosis and thrombosis and irreversible shock in which defective fibrinolysis has been suggested to be an important aetiological factor<sup>3,4</sup>. It seemed possible, however, that our definition of a poor responder was too restrictive, for the fibrinolytic stimulants were submaximal and of relatively short duration. The following communication describes a series of experiments in which healthy subjects aged between 22 and 50 yr (mean, 36 yr) were subjected to moderate, exhaustive and prolonged exercise procedures at intervals of more than a week.

The fibrinolytic assays for this study were identical to those described previously<sup>1</sup> with the exception that the end points for the time of euglobulin clot lysis were recorded on an automatic clot lysis recorder<sup>5</sup>. The

moderate exercise procedure consisted of an 8 min walk on a treadmill moving at 3.4 m.p.h. with 5° elevation. The exhaustive exercise procedure involved walking on a treadmill at 10° elevation for 3 min at 3.4 m.p.h., followed by 3 min at 3.7 m.p.h., then 1 min at 4.0 m.p.h. and if possible a further minute at 4.2 m.p.h. The prolonged exercise procedure was a 3 h treadmill walk on the flat at 4.0 m.p.h. The exercises were performed in a room at 19°–20° C where all subjects were required to rest, lying down, for 30 min before exercising. Fluid in the form of orange juice was provided freely throughout the prolonged exercise procedure.

Samples of blood were obtained from the cubital vein by separate venipuncture with the minimum of venous occlusion. The percentage fibrinolytic response was calculated as  $\frac{A-B}{A} \times 100$  where *A* and *B* represented the

pre- and post-exercise euglobulin lysis times, respectively.

The results of the moderate and exhaustive exercise studies in six subjects are summarized in Table 1. All subjects spontaneously terminated the exhaustive exercise during the last 10 sec of the final minute when the mean pulse rate had risen to 198/min. The findings would indicate that poor fibrinolytic responders to a moderate exercise procedure could not correct this defect when submitted to a short exhaustive exercise procedure and that submaximal exercise procedures could be used for future epidemiological studies.

Table 1. PERCENTAGE FIBRINOLYTIC RESPONSE TO A MODERATE AND EXHAUSTIVE EXERCISE PROCEDURE IN SIX HEALTHY SUBJECTS

Subject	Percentage response (ELT) to moderate exercise	Percentage response (ELT) to exhaustive exercise
G.W.	42	82
J. C.	45	82
G. McK.	50	89
W. G.	12	46
M. A.	10	43
B. G.	10	53

ELT, euglobulin lysis time.

The prolonged exercise was performed by eleven volunteers and the results, along with their moderate exercise grading, are shown in Table 2. Fig. 1 illustrates the times for euglobulin lysis throughout the exercise period. The results would indicate that the fibrinolytic activity increased during the exercise but plateaued at about 2 h in all but one of the subjects. (Subject I. F. exercised for a further 2 h during which time there was no further change in his times of euglobulin lysis.) There were no significant differences in the contents of euglobulin plasminogen, fibrinogen and plasma urokinase inhibitor before and after the exercise in the subjects examined (Table 3).

The demonstration of a plateaued fibrinolysis at the 2 h interval confirms the observations of Ogston and Fullerton<sup>6</sup>, but this finding was not applicable to one of our subjects (M. A.) and could not be explained by the very low level of pre-exercise fibrinolysis, because comparison with another subject (G. McK.), whose pre-exercise time for euglobulin lysis was similar, showed the marked difference between these two subjects (Fig. 2). It is of interest to

Table 2. PLASMINOGEN ACTIVATOR RESPONSE TO A PROLONGED TREADMILL EXERCISE PROCEDURE

Subject	Mean percentage response to moderate exercise	Before		1 h		2 h		3 h	
		Mean ELT (min)	Area on fibrin plates (mm <sup>2</sup> )	Mean ELT (min)	Area on fibrin plates (mm <sup>2</sup> )	Mean ELT (min)	Area on fibrin plates (mm <sup>2</sup> )	Mean ELT (min)	Area on fibrin plates (mm <sup>2</sup> )
G. McK.	60	780	81	81	342	81	40	506	100
A. McG.	58	397	225	123	400	81	65	484	144
P. B.	54	123	340	52	472	—	42	524	—
D. F.	52	115	462	62	525	100	41	629	225
M. D.	50	520	196	120	450	—	48	750	278
J. D.	50	77	—	50	—	—	45	—	—
I. F.	40	132	400	67	515	196	50	552	200
D. J.	39	340	324	75	462	49	42	612	100
I. W.	38	222	340	99	550	121	79	529	156
B. G.	10	234	324	85	484	110	56	589	121
M. A.	10	808	81	476	225	256	300	64	120

ELT, euglobulin lysis time.

Table 3. CHANGES IN EUGLOBULIN FIBRINOGEN AND PLASMINOGEN AND PLASMA UROKINASE INHIBITOR BEFORE AND AFTER A PROLONGED TREADMILL EXERCISE PROCEDURE

Subject	Euglobulin fibrinogen (mg per cent)		Euglobulin plasminogen (c.u./ml.)		Fibrin plate inhibitor assay (area in mm <sup>2</sup> )			
	Before	3 h	Before	3 h	Before 3 h			
					1	1	1	1
D. M.	250	252	4.55	4.51	100	132	81	110
G. McK.	194	187	3.01	3.04	144	225	144	225
I. W.	320	301	3.25	3.31	100	175	100	195
I. F.	182	191	2.10	2.40	—	—	—	—
D. F.	272	282	3.27	3.10	—	—	—	—

note that subject M. A. was a poor responder to both the moderate and exhaustive exercise procedures. Subject B. G., however, was also a poor responder to the moderate and exhaustive exercises but responded to the prolonged exercise in a manner which was similar to the normal group. This would suggest that there are two types of poor fibrinolytic responders: those whose mechanisms are impaired to short duration stimulation only and those who are resistant to both long and short term stimulation. This differentiation may prove to be relevant when further thought is given to the physio-pathological significance of the poor fibrinolytic responder.

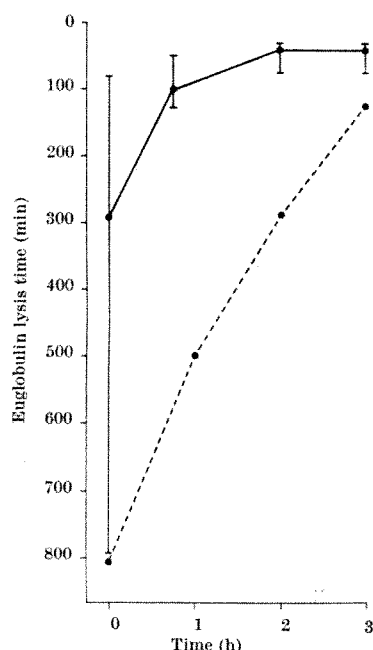


Fig. 1. The time of euglobulin lysis before and during a period of prolonged exercise. The continuous line represents the mean, with the range, of ten subjects. The interrupted line represents the response of subject M.A. (see text).

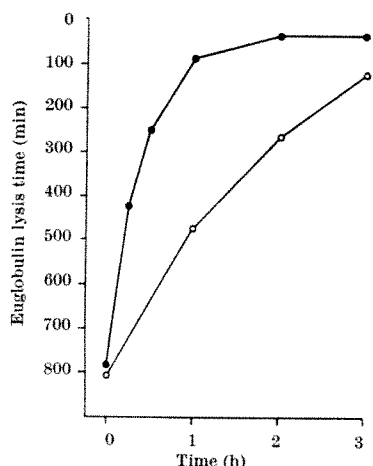


Fig. 2. A comparison of the fibrinolytic response to the prolonged exercise procedure between two subjects, G. McK. (●) and M.A. (○), whose pre-exercise times of euglobulin lysis were similar.

The finding that, despite a wide range in the times of resting euglobulin lysis (77–780 min) in the remaining ten subjects, the final time of euglobulin lysis plateaued at a mean of 49 min with a standard deviation of 12 min was unexpected and of considerable interest. This observation suggests that comparison of the times of euglobulin lysis between normal individuals is valid and that the differences in the resting concentrations of plasminogen activator between individuals represent normal variations in the homeostatic mechanisms controlling the complex system of *in vivo* thrombolysis. It further suggests that the concentration of circulating plasminogen activator, at least in the unstimulated state, may not represent the concentration of active fibrinolysis, because other influential factors, including the mechanisms of fibrinolytic inhibition, may prove to be of great importance<sup>7</sup>. In situations of stress, however, when rapid dynamic changes of fibrinolysis are required, the ability of the individual to augment the concentration of plasminogen activator could be the single most important factor in maintaining homeostasis.

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### Combined Action of Phytohaemagglutinin and RNA on Lymphocytes from Patients with Hodgkin's Disease

It is well known that patients with Hodgkin's disease may exhibit a gradually diminishing delayed hypersensitivity<sup>1</sup>. *In vitro* experiments have also revealed a diminished response by lymphocytes from such patients to the blastogenic stimulus of phytohaemagglutinin (PHA)<sup>2-5</sup>, tuberculin and other antigens<sup>6</sup>. We have found that these phenomena are closely related. Diminished lymphocyte response to such a stimulus can be shown *in vitro* only with lymphocytes from skin-negative subjects, and when skin hypersensitivity remains unchanged the lymphocyte culture produces a normal percentage of blast cells<sup>7</sup>.

The mechanism responsible for this phenomenon is still not clear. Because an increased synthesis of RNA has been shown to be one of the first metabolic changes in the production of lymphocyte blast cells in the case of normal subjects<sup>8-14</sup>, we decided to investigate the effect of the combined action of PHA and of RNA from normal human lymph tissue on cultures of lymphocytes from patients with Hodgkin's disease.

Blood from seven skin-negative and two skin-positive patients suffering from Hodgkin's disease was used to prepare lymphocyte cultures<sup>6</sup>. RNA was extracted from biopsy specimens of normal human lymph tissue according to the technique developed by Kirby<sup>15</sup> and modified by Ralph and Bellamy<sup>16</sup>. The extraction was carried out in phenol at both 4° C and 60° C (ref. 17). The RNA and PHA were added to the cultures at concentrations of 0.8 mg/c.c. and 4 µg/c.c., respectively.

Table 1. PERCENTAGE OF BLAST CELL PRODUCTION IN LYMPHOCYTE CULTURES FROM SKIN-POSITIVE SUBJECTS WITH HODGKIN'S DISEASE AFTER STIMULATION WITH PHA AND WITH PHA + RNA

Case	PHA	PHA and RNA immediately	PHA followed by RNA after 24 h
1	55	38	43
2	75	67	68

Table 2. PERCENTAGE OF BLAST CELL PRODUCTION IN LYMPHOCYTE CULTURES FROM SKIN-NEGATIVE SUBJECTS WITH HODGKIN'S DISEASE AFTER STIMULATION WITH PHA AND WITH PHA + RNA

Case	PHA	PHA and RNA immediately	PHA followed by RNA after 24 h
1	16	33	46
2	7	6	56
3	20	—	38
4	12	8	46
5	9	18	61
6	3	14	53
7	14	15	57

The method used in the experiments was as follows. The first culture was retained as a control; with the second culture PHA was added immediately; with the third culture both PHA and RNA were immediately added; with the fourth PHA was immediately added and RNA after 24 h. All cultures were discontinued at the 72nd hour.

Our results were as follows. With the skin-positive subjects (Table 1) normal blast production was already under way and the addition of RNA either simultaneously with the PHA or after 24 h effected no changes. The simultaneous addition of PHA and RNA to the lymphocyte cultures from skin-negative patients (Table 2) had no significant effect on the percentage of blast cell production. When RNA was added to the cultures 24 h after PHA had been added, the percentage of blast cell production was decidedly higher than that when only PHA was added and in some cases approached the percentages found in cultures derived from normal subjects (Table 2).

Our previous investigations showed that PHA protects lymphocyte cultures from both normal and skin-negative patients with Hodgkin's disease from irradiation in a similar way<sup>18</sup>. We suggested that with the skin-negative group the early stages of culture after stimulation with PHA are biologically the same as those of the normal group but that in a second stage there is a lack of a factor necessary for the further development of blast cells. Our present experiments suggest it is in fact a quantitative and qualitative lack of a particular type of RNA which displays its activity at 24 h and thus carries on and ensures the completion of the normally initiated biological cycle. Our experience suggests that the addition of lymphoreticular RNA to cultures obtained from skin-negative patients with Hodgkin's disease serves to stimulate the transformation of blast cells.

We believe it to be unlikely that after stimulation with RNA and PHA the production of blast cells resulted from the presence of RNA antigen complexes in our RNA preparation. In fact, in our control cultures there was no significant increase in the production of blast cells in either normal or Hodgkin subjects<sup>19,20</sup>. A low level of production of blast cells has frequently been found in lymphocyte cultures from subjects with chronic lymphocytic leukaemia<sup>21-23</sup>. During the course of experiments which are still in progress we have found a considerable increase in the percentage of blast cell production in such cases after the same type of RNA has been added; however, the techniques used were somewhat different to those reported here<sup>20</sup>.

Further research is now being undertaken to determine which fraction of the RNA we used is responsible for the activation of blastogenesis in the lymphocyte cultures from patients with Hodgkin's disease.

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### Immunological Similarity of Horse, Donkey and Mule Haemoglobins

HORSE haemoglobin has been shown to consist of two components with different electrophoretic mobilities at pH 8.6 (refs. 1 and 2). In the donkey a single haemoglobin is present, corresponding in mobility to the "slow" horse component. In the mule and hinny, two fractions can be distinguished by electrophoresis, with mobilities corresponding to those of horse haemoglobin. Mixtures of horse and donkey haemoglobins behave electrophoretically like mule haemoglobin<sup>2,3</sup>.

An attempt is reported here to distinguish between these haemoglobins by immunological methods.

Whole haemoglobin solutions were prepared by the method of Jonxis and Huisman<sup>4</sup>, with final clarification by centrifugation at 15,000g for 1 h. They were converted to cyanmethaemoglobin, adjusted to 10 g/100 ml., and stored frozen at -25° C. Preparations of the "fast" and "slow" horse and mule components were obtained by electrophoresis of haemoglobin in *tris* buffer<sup>5</sup> at pH 8.6 in agar gel, followed by elution of the separate bands in phosphate-buffered saline at pH 7.0. The eluates were concentrated to about 1.5 g/100 ml. by dialysis against 'Carbowax'<sup>6</sup>, and subsequently diluted as required. Immune precipitating sera against whole horse haemoglobin were prepared in two rabbits by intramuscular injection of haemoglobin solutions mixed with Freund's adjuvant. A primary course of four injections, each of 50 mg of haemoglobin, was given at weekly intervals. After a rest period of 4 months a second similar course was given.

The sera were tested against haemoglobin solutions by double diffusion in agar, by immunoelectrophoresis, and in precipitation reactions by the method of Dean and Webb<sup>7</sup>.

In gel diffusion experiments, both sera, when tested undiluted against horse, donkey and mule haemoglobins, showed precipitation which was optimum at an antigen concentration of 80 µg/ml. No precipitation occurred with sera obtained before immunization. When donkey haemoglobin and the "fast" and "slow" components of horse and mule haemoglobins were compared on one plate at the optimum concentration, a single precipitin line was obtained, showing the immunological similarity of all the antigens. Fig. 1 shows the result with serum 1.



A similar result was obtained with serum 2. No precipitation was obtained with human haemoglobin A. Slight diffuse precipitation occurred when anti-horse haemoglobin serum was tested against horse serum, but disappeared when the latter was diluted beyond 1 in 4.

Immunoelectrophoresis confirmed that the sera were reacting with haemoglobin. Horse haemoglobin components separated by electrophoresis in agar were allowed to diffuse towards two anti-horse haemoglobin sera (Fig. 2). A continuous bow-shaped precipitin line developed, corresponding to the "fast" and "slow" haemoglobin components. After washing, this band could be stained with lissamine green, and also with the *o*-dianisidine reagent of Owen, Silberman and Got<sup>3</sup>, because of the peroxidase activity of the haemoglobin in the precipitate. Another weaker precipitin line occurred nearer the origin, presumably from a reaction with non-haemoglobin protein, because it could be stained with lissamine green but not with dianisidine.

Table 1. OPTIMUM HAEMOGLOBIN CONCENTRATIONS FLOCCULATING WITH A 1/10 DILUTION OF ANTI-HORSE HAEMOGLOBIN SERUM

Antigen	Concentration of antigen ( $\mu\text{g}/\text{ml.}$ )	Flocculation time (min)
Whole horse Hb	56.6	10.5
"Fast" horse Hb	50.5-56.6	12.5
"Slow" horse Hb	50.5-56.6	12.5
Donkey Hb	56.6	13.5
Whole mule Hb	50.5-56.6	10.5
"Fast" mule Hb	50.5-56.6	12
"Slow" mule Hb	50.5-56.6	10.5

Precipitation reactions in tubes were carried out with serum 1, using the constant antibody procedure of Dean and Webb<sup>7</sup>. A preliminary rough titration with doubling dilutions of antigen from 5,120 to 5  $\mu\text{g}/\text{ml.}$  was followed by an accurate titration using antigen diluted in 0.05 log steps from 80 to 35.7  $\mu\text{g}/\text{ml.}$ , standardized in a spectrophotometer. Anti-horse haemoglobin serum at one in ten dilution reacted optimally with an equal volume of horse haemoglobin at 56.6  $\mu\text{g}/\text{ml.}$ , with a flocculation time of 10.5 min. The optimum concentrations of donkey haemoglobin and the "fast" and "slow" components of horse and mule haemoglobins are shown in Table 1. Where the optimum lay between two tubes, the antigen concentrations of both tubes are given. Further evidence of the immunological similarity of the haemoglobins is shown by the fact that the optimum ratio was the same for them all. After precipitation had proceeded to completion (2 h at 37° C, 24 h at 4° C), the supernatants in the zone of optimum proportions were colourless and the precipitates faintly pink. No antigen could be detected in these

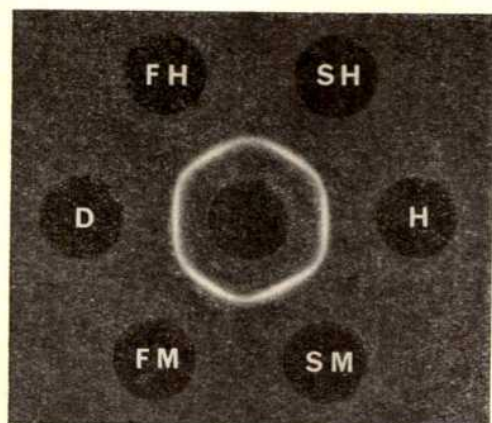


Fig. 1. Double diffusion in agar. Centre well: anti-horse haemoglobin serum, undiluted. Outer wells: FH, "fast" horse haemoglobin component; SH, "slow" horse haemoglobin component; H, whole horse haemoglobin; SM, "slow" mule haemoglobin component; FM, "fast" mule haemoglobin component; D, donkey haemoglobin. The concentration of all haemoglobins was 80  $\mu\text{g}/\text{ml.}$

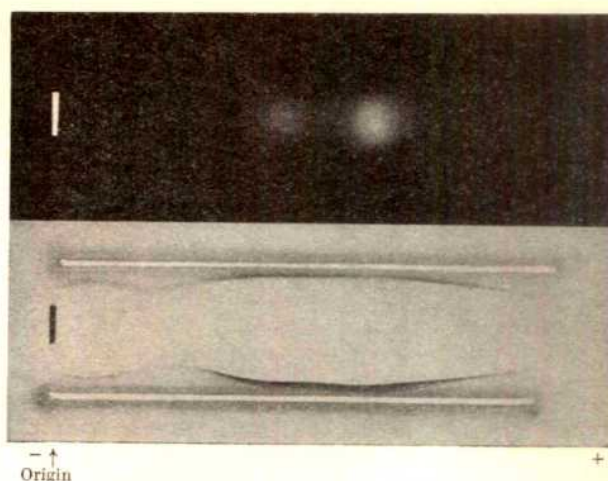


Fig. 2. Immunoelectrophoresis of horse haemoglobin. Above: horse haemoglobin components separated by electrophoresis in Tris buffer at pH 8.6 in agar (contact print on sensitive paper). Below: after diffusion towards two different anti-horse haemoglobin sera. Washed, then stained with lissamine green.

supernatants by precipitin ring tests with immune serum, although the original haemoglobin solutions reacted well.

Spectrophotometric examination showed that at the optimum ratio no haemoglobin pigment was detectable in the supernatant but that all the pigment was released when the washed precipitate was redissolved in one-tenth normal sodium hydroxide.

Kilmartin and Clegg<sup>9</sup> have recently shown that the electrophoretically "fast" component of horse haemoglobin differs from the "slow" component in possessing glutamine instead of lysine at position 60 in the  $\alpha$ -polypeptide chain. In addition each  $\alpha$ -chain may have either tyrosine or phenylalanine at position 24, thus allowing four possible types of  $\alpha$ -chain. The composition of the  $\beta$ -chain is the same in both components. In the single haemoglobin of the donkey, the  $\alpha$ -chain has lysine at position 60, phenylalanine at 24, and differs from horse  $\alpha$ -chains in having asparagine instead of histidine at position 20. The mechanism of the heterogeneity of horse  $\alpha$ -chains is unknown, but if the mule inherits one  $\alpha$ -chain gene from each parent then five types of  $\alpha$ -chain might be possible.

Our experiments show that horse, donkey and mule haemoglobins are immunologically similar, and that the differences between them, now known to involve substitutions of only one or two amino-acids, cannot be detected by the serological methods described in this communication.

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## IMMUNOLOGY

## Large Amounts of Non-agglutinating Anti-erythrocyte IgG in Primary Antisera

It is generally accepted that IgM are synthesized before IgG antibodies. This sequence could depend on the characteristics of the antigen, its route of administration, the dosage used, and the animal species studied<sup>1</sup>. There is some doubt as to whether IgM antibodies are predominant in some early primary antibody responses<sup>2-4</sup>. This question arises in part from the fact that haemagglutination is more sensitive for detecting IgM than IgG antibodies<sup>2,5,6</sup>; and in part from the observation that haemolytically inefficient IgG antibodies are undetected in the Jerne plaque technique<sup>7</sup> unless indirect procedures are used<sup>8,9</sup>. Indeed, sera obtained soon after injection of proteins may have more IgG than IgM precipitins<sup>10-12</sup>. Thus there is a need for re-evaluation of the magnitude of the IgG response in the early stages of antibody production. Evidence is presented that sera from chickens immunized with sheep red blood cells (SRBC) contain more IgG than IgM antibody 6 days after injection.

Primary immune responses were induced in male and female adult White Leghorn chickens by intravenous injection of suspensions of washed sheep red blood cells. Individual sera and fractions were heated at 56° C for 30 min, and haemagglutination titres were determined by two-fold dilutions and addition of 0.1 ml. of 2 per cent red blood cells. Titres are expressed as the reciprocals of the dilutions.

Antibodies were present within 3 days of immunization in all birds which had received either 1 per cent, 5 per cent or 10 per cent red blood cells. Maximum titres were attained 6 days after primary injection. Fractionation by gel filtration in 'Sephadex G-200' (ref. 13) and by diethylaminoethyl-cellulose chromatography<sup>14</sup> indicated that individual primary sera contained mostly IgM agglutinins. This was confirmed by sucrose-gradient ultracentrifugation<sup>14</sup> and by treatment of sera with 0.1 molar 2-mercaptoethanol. Thus, based on haemagglutination, and on fractionation procedures commonly used for delineation of IgG and IgM antibodies, these responses resembled in some respects the anti-bovine serum albumin responses of chickens<sup>14</sup>, that is, little or no haemagglutinating IgG antibody is demonstrated in the early primary response. The following experiments showed that large amounts of IgG non-agglutinating antibody were present.

To elute antibodies from agglutinated cells, 4 ml. of a 6 day primary antiserum was reacted with 4 drops of packed formalized cells<sup>15</sup>/ml. of serum for 40 min at 37° C. The agglutinated cells were washed three times with cold borate buffer (pH 8.2  $\Gamma/2=0.16$ ) by centrifugation, and finally suspended in 1.5 ml. of buffer. Elution was carried out twice at 56° C for 5 min each. Analysis of the eluates by immunoelectrophoresis showed only IgG and albumin arcs. Albumin also may be a co-complexing contaminant in the precipitin reaction, as shown by Orlans *et al.*<sup>16</sup>. The agglutinins (titre, 320) in the eluates were completely inactivated by 0.1 molar mercaptoethanol in conditions known to completely inactivate IgM but not IgG antibodies<sup>17</sup>.

For anti-globulin tests (Coombs's reaction), IgG was isolated by gel filtration from a 6 day primary antiserum (titre, 10,240). A 1.0 per cent solution contained no IgM shown by gel diffusion analysis. After titration of the solution by haemagglutination, the cells were washed three times with borate buffer by centrifugation and resuspended in 0.5 ml. of buffer. To duplicate sets was added either 0.04 ml. or 0.08 ml. of heat-inactivated and absorbed rabbit anti-chicken globulin antiserum. Identical tests were performed with a 1.0 per cent solution of normal IgG. The results, summarized in Table 1, show that before and after addition of rabbit antiserum the titres of the IgG fraction were less than 5 and 640, respectively. Cells

treated with normal IgG failed to absorb detectable IgG. For additional tests to show the presence of non-agglutinating antibodies in this preparation, sheep red blood cells were treated with bromelain<sup>18</sup> and with ficin<sup>19</sup>, procedures used to demonstrate non-agglutinating antibodies for human erythrocytes. Both enzymes increased the titres (Table 1).

To determine the approximate number of IgG and IgM antibody molecules absorbed to the red blood cells, IgG and IgM globulins were isolated from a 6 day primary antiserum by repeated precipitation with sodium sulphate (ref. 13), followed by repeated cycles of gel filtration in 'Sephadex G-200'. The preparations were iodinated<sup>20</sup> with iodine-125. The specific activity of the IgM preparation was 1.38 relative to the IgG preparation, a value in close agreement with that reported by Greenbury *et al.*<sup>5</sup> for iodination of rabbit IgG and IgM. One millilitre each of the IgG (0.280 mg) and the IgM (0.190) preparations were reacted with  $1.25 \times 10^9$  sheep red blood cells at room temperature for 30 min. The cells were recovered by centrifugation, and to the supernates were added the same quantities of red blood cells: a total of  $2.50 \times 10^9$  cells were used to absorb the fractions. The cell suspensions were washed three times with 0.5 per cent bovine serum albumin in borate; the radioactivities of the cells and supernatant fluids were counted after each washing. The final radioactivity count of the cells of the original reaction tube was added to the count of the cells from the second absorption step. The sum of these values was taken as the total absorbable antibody. The experiment was performed in duplicate, using the same globulin preparations but at different times. To control for non-specific absorption, purified normal chicken IgG was labelled with iodine-125 and reacted with sheep red cells as previously described. The molecular weight of chicken IgG was taken as 206,000 (ref. 21), and the molecular weight of IgM was assumed to be 900,000 (refs. 22 and 23). Approximately 1 per cent of the labelled normal IgG was absorbed to cells. As shown in Table 2, when  $8.16 \times 10^{14}$  molecules of IgG were reacted with  $2.50 \times 10^9$  cells, approximately  $2.5 \times 10^{14}$  molecules of IgG antibody were bound, or about  $1 \times 10^5$  molecules in each cell. About 31 per cent of the molecules in this preparation were therefore non-agglutinating anti-sheep red blood cell antibodies. This represents about  $9.02 \times 10^{14}$  anti-sheep red blood cell molecules absorbed/mg of IgG. Of the  $1.29 \times 10^{14}$  molecules of IgM, approximately  $4.23 \times 10^{13}$ , or 32 per cent, were absorbed; this represents about  $1.70 \times 10^{14}$  molecules of IgM antibodies absorbed/mg of protein. Based on these preparations, there were about four molecules of IgG antibody to one molecule of IgM antibody. Greenbury *et al.*<sup>5</sup> reported that at saturation each human group A<sub>1</sub> red blood cell was capable of absorbing  $8.3 \times 10^5$  molecules of rabbit IgG antibody, or  $1.7 \times 10^5$  molecules of IgM antibody. The A<sub>1</sub> cells take up only about one fifth the number of IgM molecules compared with IgG. Although we did not determine whether the

Table 1. HAEMAGGLUTINATION REACTIONS OF 6 DAY PRIMARY CHICKEN ANTI-SHEEP ERYTHROCYTE IgG ANTIBODY AS OBTAINED BY THE ANTI-GLOBULIN TEST AND WITH CELLS TREATED WITH 'BROMELAIN' AND 'FICIN'

Preparation	No treatment	Test		
		Anti-globulin	Bromelain-treated cells	Ficin-treated cells
IgG-normal	< 5*	< 5	< 5	< 5
IgG-antibody†	10	640	160	160

\* Reciprocal of titre.

† Obtained by gel filtration of a 6 day primary antiserum.

Table 2. MOLECULES OF RADIOIODINATED CHICKEN IgG AND IgM ANTIBODIES ABSORBED TO  $2.50 \times 10^9$  SHEEP RED BLOOD CELLS

Antibody preparation*	Titre	Added to SRBC		Absorbed by SRBC	
		(mg)	No. of molecules (cpm)	(cpm)	Molecules/mg of protein
IgG	< 5	0.280	$8.16 \times 10^{14}$	10,777	Mean: 3,305
IgM	160	0.190	$1.29 \times 10^{14}$	9,915	Mean: 3,250

SRBC, sheep red blood cells.

\* Fractions obtained by gel filtration of a 6-day primary anti-SRBC serum. Approximately 1 per cent of radioiodinated normal chicken IgG was absorbed.

sheep red blood cells were saturated with antibody, the values of  $1 \times 10^5$  molecules of IgG and  $1.7 \times 10^4$  molecules of IgM/cell were of the same orders of magnitude as those reported by Greenbury *et al.*<sup>5</sup>.

We have found that 3–4 days after immunization with either bovine serum albumin or haemocyanin many chicken antisera contain only IgM antibodies as detected by radioimmuno-electrophoresis, and that by day 4 or 5 the IgG antibodies predominate—indicating that the period of time in which only IgM antibodies were demonstrable was short—one day (unpublished work). Whether sheep red blood cell IgM antibodies appear in the serum before IgG still remains to be determined. Nevertheless, it is obvious from the present investigation that studies on the temporal synthesis of anti-erythrocyte antibodies in chickens, and most likely in other animal species, should include consideration of the serum non-agglutinating IgG antibodies, which represent as many molecules as do IgM antibody molecules 6 days after immunization.

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### Early Splenectomy and Survival of Inbred Mice

THE spleen is known to function in lymphopoiesis<sup>1–3</sup>, phagocytosis<sup>4,5</sup>, and immunogenesis<sup>6–8</sup>, but these functions are not essential to normal survival or health when the spleen is removed during adult life<sup>9–11</sup>. The spleen appeared in the primitive fishes and has persisted in much the same morphological state in all vertebrates which have evolved since<sup>12–14</sup>. Such phylogenetic constancy implies survival advantage, but the basis of this advantage remains enigmatic.

The syndrome of congenital cardiac disease associated with failure of the spleen to develop is rare, but has been repeatedly associated with overwhelming infection in early life<sup>15,16</sup>. King and Schumacher<sup>17</sup> reported that children with haemolytic anaemia subjected early to splenectomy are prone to develop overwhelming infection. It has been established that splenectomy early in life in children with a variety of haematological diseases<sup>18–22</sup>,

but not necessarily in normal children, causes a great risk of infection with extracellular pyogenic bacterial pathogens, particularly pneumococci<sup>23–31</sup>. These observations suggest that the most important part played by the spleen is in ontogenetic development. Neonatal splenectomy enhances susceptibility to pneumococcal infection in mice<sup>32</sup>. These relationships suggested to Ellis and Smith<sup>33</sup> that the dual phagocytic and immunogenetic role of the spleen is especially important in the early period of exposure to extrauterine life.

Globerson<sup>34</sup> presented evidence from *in vitro* studies that interaction of thymus, spleen, and bone marrow fragments may be essential to the development of certain forms of immunological capacity. Removal of the thymus in the immediate neonatal period in rabbits<sup>35,36</sup> and rodents<sup>37–41</sup> has provided clues to the importance of this organ in developmental biology, and so we decided to look again at the possible significance of the spleen from the same ontogenetic point of view. It is the purpose of this report to describe the effects of splenic extirpation at different ages in the life of inbred mice on survival of the animals.

It was found that neonatal splenectomy, like neonatal thymectomy, produces in mice a high mortality rate during the first 6 weeks of life. Like thymectomy, splenectomy at birth produces runting and wasting disease and interferes with the normal development of the lymphoid system.

C3H/BI mice were subjected to splenectomy in the immediate neonatal period (less than 15 h old) or when 2 or 4 weeks of age. For comparison, mice were thymectomized during the first 15 h of life or 2 and 4 weeks after birth. All mice were kept in air conditioned quarters and nursed by their own mothers until they were 4 weeks old. After weaning the animals were fed 'Purina' fox chow and water *ad libitum*.

The mice were checked every day during the first 3 weeks and at least three or four times a week thereafter. Deaths were recorded and the size and health of the animals noted and recorded.

In one experiment 227 mice were splenectomized during the first 15 h of life, eighty at 2 weeks old, and seventy-five at 4 weeks old. A hundred and nine mice were subjected to neonatal thymectomy, seventy-five to thymectomy at 2 weeks and seventy-two to thymectomy when 4 weeks old.

The survival of the mice of the several groups up to 6 weeks of age is recorded in Table 1. In mice of this strain raised in the conditions existing in these laboratories both thymus and spleen are organs essential to survival. Removal of either organ in the immediate neonatal period had a much more profound effect on survival than did removal at either 2 or 4 weeks. 153 of the 227 mice subjected to splenectomy in the neonatal period had already died by 6 weeks. Most of these deaths occurred between the third and fourth weeks of life. By contrast only twelve out of eighty, or 15 per cent of mice splenectomized at 2 weeks, had died by the sixth week and only five out of seventy-five mice splenectomized at 4 weeks were dead at this age. Further, we observed that mice splenectomized at either 2 or 4 weeks, and kept much longer in our laboratory, very rarely died between the sixth and twelfth week of life.

Mice splenectomized at birth often showed a runting and wasting syndrome similar to that associated with neonatal thymectomy. This process seemed to begin between the twelfth and sixteenth day of life and regularly to have its onset earlier than did the runting and wasting syndrome

Table 1. EFFECT OF SPLENECTOMY AND THYMECTOMY ON SURVIVAL OF C3H MICE

	Splenectomy			Thymectomy		
	New-born	2 weeks	4 weeks	New-born	2 weeks	4 weeks
No. mice operated	227	80	75	109	75	72
Survival to 6 weeks	74	68	70	54	61	64
Dead by 6 weeks	153	12	5	45	14	3
Mortality (per cent)	67.4	15	6.7	41.3	18.7	4.2

observed in neonatally thymectomized mice of the same genetic composition raised in this laboratory. The runted animals seemed always to have loose stools associated with failure of normal growth rate and then later with actual weight loss.

The table shows by comparison that neonatally thymectomized *C3H/Bi* mice had experienced 41 per cent mortality rate at 6 weeks and 16 per cent and 5 per cent mortality rate by 6 weeks when thymectomized at 2 and 4 weeks of age respectively. The latter observations agree with extensive previous experience in this laboratory with neonatally thymectomized *C3H/Bi* mice and seem representative of the effects of early and later thymectomy in the existing conditions.

Haematological studies<sup>41</sup> showed that neonatal splenectomy had effects very different from those observed when splenectomy was carried out at either 2 or 4 weeks of age. We found that neonatal splenectomy greatly inhibited the development of a full complement of circulating lymphocytes during the first weeks of life. This was as pronounced as the effect of neonatal thymectomy on the development of this population of cells. Splenectomy at 2 and 4 weeks, like thymectomy at these ages, had a much less profound effect on the development of the population of lymphoid cells in blood and tissues than did either thymectomy or splenectomy carried out in later life.

These observations, which are being extensively developed, have suggested that, as with the thymus, it may be most incisive to investigate the advantage to survival of the spleen in mammalian biology by considering the function of this organ in the ontogenetic development of the haematopoietic tissues.

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## Suppression of Graft versus Host Reaction by Mitomycin C

At present there is no adequate therapy for human diseases of immunological deficiency. Immunological reconstitution of children suffering from congenital thymic dysplasia has been attempted using allogeneic lymphoid tissues from pre- or postnatal donors, but these efforts remained unsuccessful<sup>1,2</sup>, or were followed by a fatal reaction of the graft against the host<sup>3</sup>.

In animals with experimental diseases of immunological deficiency, reconstitution has been achieved by transplantation of syngeneic thymus, spleen or lymph node cells<sup>4,5</sup>; injection of allogeneic lymphocytes, however, is usually followed by reaction of the graft against the host<sup>6</sup>.

The graft versus host disease following injection of parental strain spleen cells into *F1* hybrid recipient mice can be modified by immunosuppressive therapy of the host<sup>7-9</sup>, but in immunologically deficient states treatment of the host with these drugs would depress resistance to infection even further.

As a first step in an attempt to restore immunologically deficient animals, and even humans with allogeneic cells, we decided to assess the effect of drugs on donor cells in parent versus *F1* hybrid homologous disease in mice. We chose mitomycin *C* because this drug inhibits cell division, but leaves some immunological functions of the cell intact<sup>10-12</sup>. Our results show that mitomycin *C* in appropriate concentrations prevents graft versus host disease.

A strain and (*A* × *C57BL/1*) *F1* hybrid mice were used. Spleen lymphocytes from adult *A* donors were injected into 7-9 day old (*A* × *C57BL/1*) *F1* hybrids, and the graft versus host assay of Simonsen<sup>13</sup> used. Each litter of six or more *F1* hybrid animals was divided into groups, and members of each group injected with either syngeneic *F1* hybrid spleen cells, untreated *A* strain spleen cells, or *A* strain spleen cells treated with various doses of mitomycin-*C*. Spleens from donor animals were made into cell suspensions in Eagle's minimum essential medium by a method described earlier<sup>6</sup>. About 10<sup>7</sup> lymphocytes were injected intraperitoneally into each 7-9 day old recipient in a volume of approximately 0.1-0.2 ml. Injected animals were killed 8 days later and spleen and body weights determined. The "spleen index" was calculated by dividing the spleen weight/10 g of body weight of each of the experimental animals by the mean spleen weight obtained in the group injected with *F1* hybrid spleen cells. Spleen indices greater than 1.30 are considered to indicate significant graft versus host reactivity on the part of the injected cells.

Treatment with mitomycin *C* was performed by incubation with the drug for 20 min at 37° C at a concentration of 100, 10 or 5 µg of mitomycin *C*/ml. of cell suspension. After incubation the cells were washed twice to remove

the drug. Control *A* strain spleen cells and *F1* hybrid cells were simultaneously treated in an identical manner but not exposed to mitomycin *C*. Cell viability before injection (as determined by trypan blue exclusion) was greater than 90 per cent and was not altered by treatment with mitomycin *C*.

From Fig. 1 it is evident that *A* strain spleen lymphocytes consistently caused significant splenomegaly in (*A* × *C57/BL/1*) *F1* hybrids, and that treatment of these cells with mitomycin *C* impaired this ability. One hundred micrograms of mitomycin *C*/ml. of cell suspension abolished the graft versus host reaction completely. In the group of animals injected with *A* strain spleen cells incubated with 10 µg of mitomycin *C*, one animal out of twelve showed significant splenomegaly. In the group injected with spleen cells treated with 5 µg of the drug two animals out of seven showed significant splenomegaly. Thus while mitomycin *C* appears to inhibit graft versus host reactions, this effect is greater when higher doses are used.

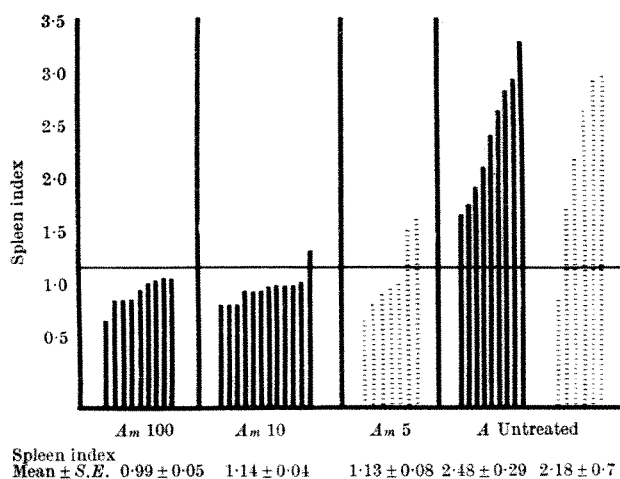


Fig. 1. Graft versus host assays of untreated and mitomycin *C* treated *A* strain spleen cells. Each bar represents the spleen index obtained by injection of the *A* strain spleen cells into one (*A* × *C57/BL/1*) *F1* hybrid. Solid and cross-hatched bars represent separate experiments. *Am* 100, *Am* 10, *Am* 5 are cells treated respectively with 100, 10, and 5 µg mitomycin *C*/ml.

From recent studies it appears that mitomycin *C* has an inhibitory effect on DNA replication, probably because of cross-linkage of the DNA strands<sup>10</sup>. There are indications, however, that protein synthesis continues at near normal rates after cells have been incubated with mitomycin *C* for prolonged periods<sup>11</sup>. In addition, Bloom<sup>12</sup> showed that guinea-pig lymphoid cells incubated with 10 µg of mitomycin *C* for 75 min were able to transfer delayed hypersensitivity, and manifested a vigorous uptake of <sup>3</sup>H-uridine and <sup>3</sup>H-valine into RNA and protein respectively.

From the data presented here it is apparent that mitomycin *C* in appropriate amounts prevents graft versus host reactions in mice. Even when minute amounts of the drug are used, this effect is apparent, although less uniform. Incubation with mitomycin *C* does not impair cell viability as judged by trypan blue exclusion.

A crucial question is whether the treated cells remain metabolically active when injected into syngeneic or allogeneic animals and may confer some degree of immunocompetence on the host. Studies now in progress in our laboratory suggest that allogeneic lymphocytes treated with mitomycin *C* do have beneficial effects in neonatally thymectomized, immunologically deficient mice.

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## BIOCHEMISTRY

### Effect of Tropomyosin on the Calcium-activated Adenosine Triphosphatase of Actomyosin

It is well known that the Mg<sup>++</sup>-activated ATPase of actomyosin extracted as the complex from muscle, "natural" actomyosin, is inhibited by low concentrations of ethylene dioxybis(ethyleneamino) tetra-acetic acid (EGTA), a property not possessed by actomyosin prepared from separately purified actin and myosin<sup>1</sup>. During a study of the factors responsible for the differences in enzyme behaviour of these two preparations a simple procedure has been developed which enables "natural" actomyosin to be readily converted to so-called "desensitized" actomyosin, the behaviour of which resembles actomyosin prepared from the independently purified actin and myosin in that its Mg<sup>++</sup>-activated ATPase is no longer sensitive to EGTA<sup>2,3</sup>. It was noted that in addition to the difference in sensitivity to EGTA of the two preparations the specific ATPase activity of "natural" actomyosin in the presence of Ca<sup>++</sup> was 30–50 per cent of that of the desensitized actomyosin.

During the process of conversion of the "natural" to the desensitized form of actomyosin the Ca<sup>++</sup>-activated ATPase rose roughly in parallel with the loss of sensitivity to EGTA and was accompanied by the removal of about 10 per cent of the total protein. When this protein fraction was added to desensitized actomyosin its enzymatic properties were in part restored to those of "natural" actomyosin. An extract with similar properties to that extracted during desensitization of "natural" actomyosin can be obtained from whole myofibrils by prolonged extraction with 5 mmolar *tris*-hydrochloric acid (pH 8.6), the so-called "soluble fraction"<sup>2,4</sup>. The study of this myofibrillar fraction and the extract removed during desensitization of "natural" actomyosin has indicated that the factors responsible for the sensitivity of the Mg<sup>++</sup>-activated ATPase of "natural" actomyosin to EGTA (EGTA sensitizing factor)<sup>5</sup> and for inhibiting the Ca<sup>++</sup>-activated ATPase of desensitized actomyosin are not identical.

Although the factor inhibiting the Ca<sup>++</sup>-activated ATPase had the properties of a protein, it was much more resistant to treatment with heat and trypsin and the effect could be demonstrated at much lower concentrations of the "soluble fraction" than were required to restore



EGTA sensitivity to a desensitized actomyosin preparation (Fig. 1). In view of the stability and solubility properties of the factor modifying the  $\text{Ca}^{++}$ -activated ATPase and the fact that one of the main components of the soluble fraction was known from earlier studies<sup>1</sup> to be tropomyosin, it seemed possible that this protein was responsible for the effect.

All the evidence indicated that this is the case, because three times precipitated preparations of tropomyosin<sup>6</sup>, which gave a single peak in the ultracentrifuge in 1 molar potassium chloride at pH 7.6 and 13.0, were very active in reducing the  $\text{Ca}^{++}$ -activated ATPase of desensitized actomyosin. The maximum effect was obtained when myosin and tropomyosin were present in the systems in approximately molar ratios, under which conditions the ATPase was reduced by about 50 per cent (Fig. 2). Purified tropomyosin preparations of the Bailey-type prepared in the presence<sup>7</sup> or absence of thiol reagents were equally active with respect to their effect on the  $\text{Ca}^{++}$ -activated ATPase, although only the latter preparations had strong EGTA sensitizing activity. Further purification of tropomyosin by ethanol-ether treatment<sup>6</sup> did not impair its inhibitory effect on the  $\text{Ca}^{++}$ -activated ATPase. Tropomyosin precipitated by 5 per cent trichloroacetic acid and redissolved at neutral pH retained unchanged the inhibitory action on the actomyosin ATPase. Likewise tropomyosin denatured by 4.2 molar guanidine-hydrogen chloride and then exhaustively dialysed showed no appreciable loss of its inhibitory effect. Under these conditions tropomyosin has been shown to regain up to 80 per cent of its original helicity<sup>8</sup>.

Tropomyosin had no inhibitory effect on the  $\text{Ca}^{++}$ -activated ATPase of myosin, implying a requirement of actin for the effect. Although the inhibition was pronounced at the conditions of assay used it was much reduced at higher ionic strength, disappearing completely at  $\mu = 0.15$  with desensitized actomyosin. The inhibitory effect of tropomyosin was not obtained with the  $\text{Ca}^{++}$ -activated ATPase of natural actomyosin nor with the

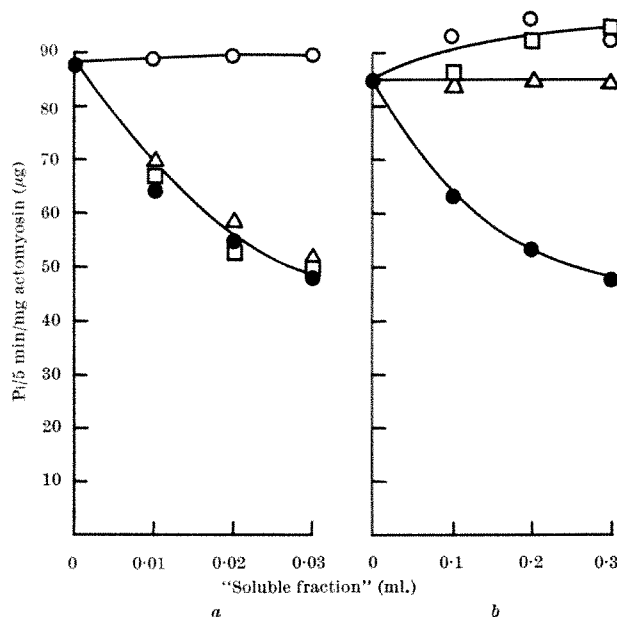


Fig. 1. Effect of heat and tryptic digestion on the ability of the "soluble fraction" of myofibrils to inhibit the  $\text{Ca}^{++}$ -activated ATPase and to confer sensitivity to EGTA on the  $\text{Mg}^{++}$ -activated ATPase of desensitized actomyosin. "Soluble fraction" ( $E_{280}^{1\text{cm}} = 3$ ) treated as indicated before addition. 1/50 part by weight of trypsin was used and the digestion stopped by adding twice the amount of soy bean trypsin inhibitor. Digestion carried out in 5 mmolar *tris*-hydrochloric acid, pH 8.6. ●, Untreated; △, 10 min at 100° C; □, trypsin digestion for 30 min at 0° C; ○, trypsin digestion for 30 min at 25° C. Assay conditions: 0.57 mg actomyosin in 2 ml. containing 25 mmolar *tris*-hydrochloric acid, pH 7.6, 2.5 mmolar *tris*-ATP incubated for 5 min at 25° C with (a) 2.5 mmolar calcium chloride, or (b) 2.5 mmolar magnesium chloride plus 1 mmolar EGTA.

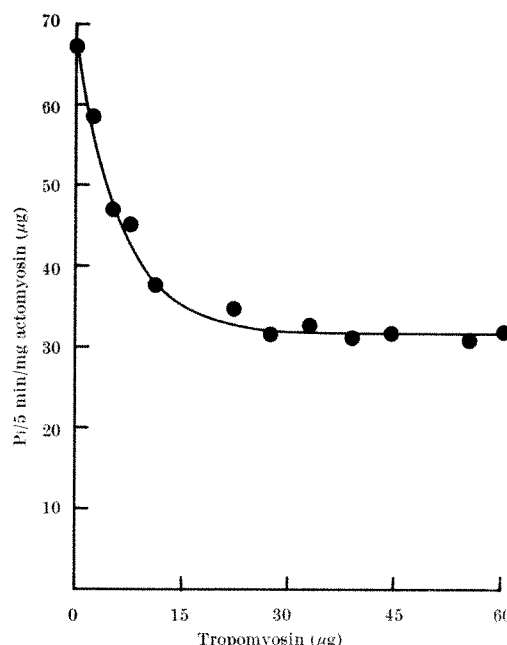


Fig. 2. Effect of tropomyosin on the  $\text{Ca}^{++}$ -activated ATPase of desensitized actomyosin. Bailey-type tropomyosin after two isoelectric precipitation cycles added as indicated to 0.37 mg of actomyosin. Assay conditions as for Fig. 1 in presence of 2.5 mmolar calcium chloride.

$\text{Mg}^{++}$ -activated ATPase of desensitized actomyosin. It is therefore different from that obtained with the inhibitory factor on the  $\text{Mg}^{++}$ -activated ATPase of desensitized actomyosin reported earlier<sup>9</sup>.

According to Ebashi and Kodama<sup>10</sup>, the EGTA sensitizing factor complex consists of tropomyosin associated with another protein, troponin. If this is so, and in view of the role of  $\text{Ca}^{++}$  in controlling the  $\text{Mg}^{++}$ -activated ATPase of the myofibril, the effect of tropomyosin described in this communication might represent one aspect of the mechanism of action of the EGTA sensitizing factor complex. Although the EGTA sensitizing and the  $\text{Ca}^{++}$  ATPase inhibitory effects may be different properties of the complex system regulating the actomyosin ATPase, the effects can be independently demonstrated, and whereas the former acts on the  $\text{Mg}^{++}$ -activated ATPase the latter is only apparent in the  $\text{Ca}^{++}$ -activated system. Further, the properties of the factor responsible for the inhibitory effect on the  $\text{Ca}^{++}$ -activated ATPase parallel those of tropomyosin much more closely than do the properties of the EGTA sensitizing factor. Activity resulting from the latter can be easily lost from tropomyosin preparations whereas the effects on the  $\text{Ca}^{++}$ -activated ATPase persist so long as the tropomyosin structure remains intact. Indeed, this latter property, which is the first clearly defined enzyme effect of tropomyosin on actomyosin, appears to be specific enough to be used as a sensitive test for the presence of this protein.

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# Potential of Cortisol Induction of Hepatic Tyrosine Transaminase by $\beta$ -Diethylaminoethyl Diphenylpropylacetate

$\beta$ -DIETHYLAMINOETHYL diphenylpropylacetate (*SKF 525A*) has been demonstrated in the rat to inhibit the metabolism of drugs both *in vitro* and *in vivo*<sup>1,2</sup>. We have demonstrated that the conversion of cortisol to a polar metabolite or metabolites by the 9,000*g* supernatant of the male rat liver is inhibited by the addition of *SKF 525A* to the incubation mixture<sup>3</sup>. The similarity between liver enzymes which metabolize drugs and those which hydroxylate steroids<sup>4</sup> suggested the possibility that *SKF 525A* would inhibit the hydroxylation of cortisol *in vivo*. In turn, it was thought that this inhibition could manifest itself in an increase in the biological activity of administered cortisol. The administration of cortisol is known to increase the activity of rat liver tyrosine transaminase<sup>5,6</sup>. The present investigation demonstrates in the male rat a further increase in cortisol induction of tyrosine transaminase by *SKF 525A*.

Table 1. EFFECT OF *SKF 525A* ON THE INDUCTION OF TYROSINE TRANSAMINASE BY CORTISOL IN ADRENALECTOMIZED MALE RATS

Experiment	Treatment	p-Hydroxyphenylpyruvate ( $\mu$ g)
1	Control (4)	55 $\pm$ 7
	<i>SKF 525A</i> (5)	35 $\pm$ 3
	Cortisol (3)	212 $\pm$ 13*
	Cortisol + <i>SKF 525A</i> (3)	276 $\pm$ 9*
2	Control (3)	38 $\pm$ 7
	<i>SKF 525A</i> (2)	45 $\pm$ 9
	Cortisol (4)	158 $\pm$ 30†
	Cortisol + <i>SKF 525A</i> (5)	244 $\pm$ 17†
3	Control (2)	25 $\pm$ 4
	<i>SKF 525A</i> (2)	34 $\pm$ 8
	Cortisol (5)	222 $\pm$ 18†
	Cortisol + <i>SKF 525A</i> (5)	286 $\pm$ 14†

The average and range of weight (g) of animals in the three experiments were for experiment (1) 153 (136–164); for experiment (2) 185 (165–206), and for experiment (3) 140 (123–158). Animals were dosed subcutaneously with cortisol (500  $\mu$ g) in 0.2 ml. of 0.5 per cent carboxymethylcellulose (CMC) or with 0.2 ml. of CMC. One half hour (experiment (1)) or 45 min (experiments (2) and (3)) before and 2.5 h after the injections of cortisol or CMC, the specified animals were intraperitoneally injected with *SKF 525A* (each injection 25 mg/kg body weight). Assays for p-hydroxyphenylpyruvate were conducted according to a procedure<sup>8</sup> described before in which the concentration of the incubation constituents was modified; an incubation volume of 5 ml. of deionized water contained 3.3  $\mu$ moles of L-tyrosine; 50  $\mu$ moles of sodium phosphate buffer, pH 7.5; 0.113  $\mu$ moles of pyridoxal phosphate; 5  $\mu$ moles of sodium diethyldithiocarbamate, and 0.2 ml. of liver 9,000*g* supernatant. Reaction was initiated by the addition of 0.2 ml. of a solution of 12  $\mu$ moles of  $\alpha$ -ketoglutaric acid and was terminated with 0.5 ml. of 50 per cent trichloroacetic acid solution. After centrifugation, 3 ml. samples were assayed for p-hydroxyphenylpyruvate by a modified Briggs method<sup>12</sup>. Values refer to the mean  $\pm$  standard error of the mean of the product (p-hydroxyphenylpyruvate) formed by the supernatant from 67 mg of liver in 10 min of incubation. Numbers in parentheses refer to number of animals.

\*  $P \leq 0.025$  when the treatment with cortisol and *SKF 525A* was compared with cortisol alone.

†  $P \leq 0.05$  comparisons as in previous footnote.

Male albino Wistar strain rats were obtained from Royal Hart Farms and were fed freely on 'Purina' chow and water. Experiments were conducted on intact and adrenalectomized rats (fourth day after adrenalectomy); after adrenalectomy animals were given 1 per cent saline instead of water. On the day of the experiment, cortisol was suspended (2.5 mg/ml.) in 0.5 per cent carboxymethylcellulose aqueous solution with the help of a Potter-Elvehjem glass homogenizer; *SKF 525A* was dissolved in deionized water so that about 0.4 ml. contained the required dose. Animals were injected with the drugs or with an equivalent volume of the vehicle as described in the tables. Six hours after the injection of cortisol animals were killed by decapitation, blood was drained and livers quickly removed and placed on ice. Supernatant from liver centrifuged at 9,000*g* was prepared as previously described<sup>3</sup>. Tyrosine transaminase assays were carried out on 0.2 ml. samples of the 9,000*g* supernatant by a modification of the method of Sereni *et al.*<sup>6</sup> (Table 1).

Subcutaneous administration of cortisol to mature adrenalectomized male rats caused an increase in the activity of hepatic tyrosine transaminase (Table 1).

When both *SKF 525A* and cortisol were injected a greater increase in enzyme activity was observed. The administration of *SKF 525A* alone did not alter enzyme activity.

Table 2 shows that there was an increase in tyrosine transaminase activity after the administration of *SKF 525A* to intact rats. There was a larger increase in activity of the enzyme after the administration of *SKF 525A* than after the administration of cortisol. When both cortisol and *SKF 525A* were given to the same animal, enzyme activity was similar to that observed after the administration of *SKF 525A* alone.

Our previous observations suggest that cortisol metabolism *in vitro* proceeds in a sequence of two steps: reduction of ring A followed by transformation of the reduced metabolite into a more polar (possibly hydroxylated) derivative<sup>3</sup>. (Hydroxylation of C19 steroids reduced at ring A by rat liver microsomes has been proposed by Chamberlain *et al.*<sup>7</sup>.) Only the latter transformation seems to be inhibited by *SKF 525A*. These findings and the demonstration that the hydroxylation of barbiturates is inhibited by *SKF 525A* both *in vivo* and *in vitro*<sup>1,2</sup> suggest that the larger increase in the activity of tyrosine transaminase caused by the administration of both cortisol and *SKF 525A* as compared with the increase in enzyme activity after the administration of cortisol alone may result from interference with the polar pathway of cortisol metabolism.

Further support for this conclusion comes from the observation that an increase in cortisol metabolism in male rats caused by thyroxine is accompanied by a decrease in the induction of tyrosine transaminase by cortisol<sup>8</sup>. There are other possible interpretations for the potentiation of cortisol activity by *SKF 525A*, such as an increase in the sensitivity of a "receptor site" to cortisol action and/or facilitation of the accumulation of cortisol at its site of action. The latter interpretation may derive support from the observation that *SKF 525A* potentiates the action of curarizing agents presumably by increasing their concentration at certain specific receptors<sup>9,10</sup>.

Table 2. EFFECT OF *SKF 525A* AND CORTISOL ON THE INDUCTION OF LIVER TYROSINE TRANSAMINASE IN INTACT MALE RATS

Experiment	Treatment	p-Hydroxyphenylpyruvate ( $\mu$ g)
1	Control (5)	72 $\pm$ 11
	<i>SKF 525A</i> (5)	227 $\pm$ 10
	Cortisol (5)	156 $\pm$ 5
	Cortisol + <i>SKF 525A</i> (5)	233 $\pm$ 10
2	Control (3)	40 $\pm$ 6
	<i>SKF 525A</i> (3)	173 $\pm$ 18

In experiment (1) the average animal weight was 180 g and in experiment (2) it was 160 g. Animals were dosed subcutaneously with 1 mg of cortisol in 0.2 ml. of 0.5 per cent carboxymethylcellulose (CMC) or with 0.2 ml. of CMC. Injections of *SKF 525A* and other details are described in Table 1; time sequence of injections as in Table 1, experiment (1). Assays were conducted as described in Table 1. Values refer to mean  $\pm$  standard error of the mean of the product formed by the supernatant from 67 mg of liver in 10 min of incubation. Numbers in parentheses refer to number of animals.

The marked increase in tyrosine transaminase after the administration of *SKF 525A* to intact rats (Table 2) and the lack of effect by this compound in adrenalectomized animals (Table 1) suggests that in the intact animals the effect of *SKF 525A* is mediated through stress. The observation that subjecting mature rats to stress (shaking) does not increase the activity of tyrosine transaminase<sup>11</sup>, however, casts doubts on this interpretation. Obviously, it is possible that in intact animals *SKF 525A* merely potentiates the activity of endogenously produced corticoids in a manner proposed for the potentiation of cortisol action by *SKF 525A* in adrenalectomized animals.

The observation by Magus and Fouts<sup>12</sup> that *SKF 525A* causes an increase in the hepatic tryptophan pyrrolase in both the intact and the adrenalectomized rat points to the differences in the inducibility of this enzyme and tyrosine transaminase.

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### Sensitive Location Reagent for the Simultaneous Detection of Sugars, Amino Sugars and Sialic Acids

Most methods for the detection of sugars on paper and thin-layer chromatograms are tedious and frequently involve the use of toxic reagents. No single location reagent so far described is capable of detecting reducing sugars, non-reducing sugars, amino sugars, and sialic acids simultaneously. In the case of thin-layer chromatography there is an additional disadvantage in that, unless a cellulose layer is used, the sensitivity of many of the reagents is reduced, probably because of the nature of the adsorbants used. Schwartz has described a very sensitive method for the detection of serine on paper chromatograms<sup>1</sup>. The chemical basis for the method is the liberation of formaldehyde from serine after periodate oxidation. The liberated formaldehyde, after condensation with acetylacetone in the presence of ammonium salt yields 3,5-diacetyl-1,4-dihydrolutidine, which is a yellow coloured, highly fluorescent compound. In his original paper, Schwartz pointed out that the reaction was not necessarily limited to serine or hydroxyaminoacids and suggested that a variety of compounds would be expected to give a positive reaction on chromatograms containing biological materials other than protein hydrolysates.

We have slightly modified the original method<sup>2</sup> and have now applied it to the location of sugars, sugar amines and sialic acid, obtained after mild acid hydrolysis of glycoproteins, or liberated from such materials after degradation by enzymes. The method offers, for the location of carbohydrates, a range of usefulness corresponding to that of ninhydrin as an amino-acid location reagent.

For paper chromatography, the dry chromatogram is dipped through a reagent containing 2 ml. 40 per cent periodic acid, 2 ml. pyridine and 100 ml. acetone. When the paper is seen to be dry, it is further dipped through a solution containing 15 g ammonium acetate, 0.3 ml. glacial acetic acid and 1 ml. acetylacetone in 100 ml. methanol. A visible yellow colour appears in 30–60 min, but yellowish green fluorescent spots can be detected within 10–15 min. For thin-layer chromatography, the method is essentially the same as that described for paper, but the reagents are used as spray rather than dip. The plates are left, after application of the first reagent, until no smell of acetone can be detected.

Hexoses and pentoses are detectable in ultra-violet light at concentrations of less than 1  $\mu\text{g}/\text{cm}^2$  and in daylight at concentrations of between 1 and 2  $\mu\text{g}/\text{cm}^2$ . Glucosamine, *n*-acetyl glucosamine, galactosamine, *n*-acetyl galactosamine and *n*-acetyl neuraminic acid are all detectable at the same level of sensitivity as the monosaccharides. When phenol was used as a solvent for chromatographic separation, the sensitivity was, in all cases, very much reduced. All other commonly used solvent systems gave perfectly satisfactory results.

Any substance capable of yielding formaldehyde after periodate oxidation will give a positive result and it is therefore obvious that the method is not completely specific for sugars and hydroxyamino acids. Indeed, the reagent might possess advantages over the other currently used reagents for the detection of formaldehydogenic ketosteroids and for some of the organic acids which are presently detectable in minimum quantities of 10  $\mu\text{g}/\text{cm}^2$ . The preliminary extraction procedures used for steroids and the ion exchange techniques for organic acids would ensure a prior knowledge of the type of compound being examined chromatographically.

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### Teratogenic Response by Hamsters, Rats and Mice to Aflatoxin B<sub>1</sub>

PEANUTS and cereals may become contaminated with *Aspergillus flavus*, a mould of which the metabolites (aflatoxins) are potent carcinogens for rat liver<sup>1,2</sup>. One of these metabolites, aflatoxin B<sub>1</sub>, is a strong teratogen for the hamster<sup>3</sup>, but its effect in rats is equivocal<sup>4,5</sup>. It has been demonstrated that it binds to DNA *in vitro* and alters RNA metabolism *in vivo*<sup>6</sup>.

Although comparative teratogenesis has not been extensively investigated, results of various investigations suggest that the teratogenic behaviour of a drug in one species cannot be predicted on the basis of its effect on another. The results of the investigations reported here show that the teratogenic activity of aflatoxin B<sub>1</sub> may vary according to the species, dose and the developmental stage at which it is applied.

Virgin 8–10 week old female golden hamsters, *Cricetus auratus*, and virgin 3.5 month old C3H/He mice were used. Hamsters were mated in the evening, one couple to a cage, and observed for oestrus and copulation. Those animals which were observed to copulate were separated the next morning and the time of separation was designated as day 1 of pregnancy. To mate the mice, three females were placed with two males in a cage and observed twice daily. Zero time of pregnancy was considered to be the time when a vaginal plug appeared and the pregnant females were then placed in individual cages.

Treated animals were injected with aflatoxin dissolved in purified triethyleneglycol (1 mg/ml.); control animals were injected with equal amounts of solvent. Hamsters were intraperitoneally injected once on day 8 (closed neural tube stage)—the time when the embryos were most susceptible to the drug and likely to develop abnormalities—and on day 13 of pregnancy. Mice received intraperitoneal injections for several days during the period of rapid organogenesis which started on day 6 or 7. Animals were killed and the uterus was removed and transferred to a Petri dish containing warm saline; implantations

Table 1. INFLUENCE OF DIFFERENT DOSES OF AFLATOXIN  $B_1$  ON DEVELOPMENT OF MALFORMATIONS IN HAMSTER EMBRYOS

Day of gestation	Treatment		Day of exam.	Normal No.	Foetuses		Living foetuses	Weight (mg)
	Dose of aflatoxin (mg/kg/day)	Pregnant animals			No.	Per cent	Size (mm)	
8	0	6	9	76	0	0	10	11.5
8	0	4	11	44	0	0	7	13.7
8	0	8	12	94	0	0	12	11.3
8	2	1	9	9	0	0	2	18.1
8	2	3	11	33	0	0	7	17.5
8	4	4	9	27	15	29.4	9	17.6
8	4	5	12	20	14	23.4	18	30.0
13	0	2	15	18	0	0	1	5.2
13	4	3	15	35	0	0	3	7.8
								24.1 $\pm$ 0.72
								1,695 $\pm$ 153
								1,632 $\pm$ 205

were counted, and each sac was opened and the contents examined under a dissecting microscope for viability, state of degeneration and gross abnormalities.

Previous tests had indicated that 4 mg/kg given on day 8 produced the greatest number of externally malformed foetuses<sup>1</sup>. When hamsters were killed on day 9, 29.4 per cent were malformed and 17.6 per cent were dead or resorbed; if killed on day 12, 23.4 per cent were malformed and 30 per cent were dead or resorbed (Table 1). Malformed live foetuses were found in all litters of these mothers. If hamsters were given 2 mg/kg and killed 1 or 3 days later, or if they were given the teratogenic dose (4 mg/kg) subsequent to the time of completion of most of the organogenesis, no malformed foetuses were found. There was a very slight increase in the incidence of dead and resorbed foetuses, but there was no difference in the size or weight of the living foetuses between controls and foetuses exposed to a dose of 2 mg/kg early in gestation or of 4 mg/kg late in gestation.

The response of C3H mice was extremely limited. Preliminary experiments with a single treatment of 8 or 12 mg/kg on day 8 of gestation resulted in the death and resorption of 90 per cent of the foetuses. Fractionated (daily) doses which produced rates of re-absorptions and deaths comparable with those obtained with hamsters were therefore used in an attempt to obtain foetuses with abnormalities. No malformations and no statistically significant differences in weight (0.05 level) were found between foetuses from control and treated mothers, but varying incidences of dead or resorbed foetuses were noted (Table 2).

Histological examination of the placenta of pregnant mice and hamsters which had been treated with solvent did not reveal any obvious differences. Moreover, the placentae of treated mothers did not show any macroscopic or histological signs of abnormality. Although there were signs of toxic injury to the liver of foetal hamsters with abnormalities<sup>2</sup>, there were no histological signs of liver injury in foetal mice which had been subjected to repeated injections of aflatoxin  $B_1$ .

It is possible that between the mouse and hamster there are differences in the metabolism of aflatoxin  $B_1$  and in the rates at which it is discharged in the faeces and urine. The amount of teratogen reaching the foetus would therefore differ in the two animals. The absence of gross malformations, growth retardation and of liver damage in foetal mice subjected to aflatoxin  $B_1$  may indicate, moreover, that differentiating tissues of the mouse and hamster differ in their susceptibility to aflatoxin  $B_1$ .

Table 2. INFLUENCE OF DIFFERENT DOSES OF AFLATOXIN  $B_1$  ON DEVELOPMENT OF MALFORMATIONS IN C3H/He MICE

Day of gestation	Treatment		Normal No.	Foetuses		Living foetuses	Weight (mg)
	Dose of aflatoxin (mg/kg/day)	Pregnant animals		No.	Per cent	Size (mm)	
6-11	4	8	37	30	44.7	21.58 $\pm$ 0.42	1,070 $\pm$ 200
6-11	4	4	26	7	21.2		
6-11	2	6	38	21	35.5	21.92 $\pm$ 0.48	1,190 $\pm$ 148
6-11	1	12	73	33	31.1		
7-12	1	3	17	8	32.0		
7-11	1	4	26	9	25.7		
7-10	1	3	15	6	19.3		
7, 8, 11, 12	1	3	17	4	12.9		
Control	0	8	68*	6	8.0	22.36 $\pm$ 0.42	1,160 $\pm$ 155

\* One malformed foetus was also found.

Numbers in italics refer to animal killed on day 14; all others were killed on day 18.

Butler and Wigglesworth have reported that in rats a dose of less than 200  $\mu$ g of aflatoxin  $B_1$  caused retardation of growth and a dose of 300  $\mu$ g caused foetal death with haemorrhages at the uteroplacental junction<sup>3</sup>. In another study with Wistar rats aflatoxin  $B_1$  at a dose equivalent to a quarter of the  $LD_{50}$  dose for non-pregnant female rats, a slight reduction of placental weight was found at term. On that occasion the drug was given orally rather than intraperitoneally, as with the experiments with the rat, mouse and hamster reported here. Treatment on day 16 caused a retardation in the growth of the foetuses and this was considered to be a secondary effect resulting from toxicity for the mothers or because of damage to the liver of the mothers<sup>4</sup>.

These investigations demonstrate that the teratogenic response to aflatoxin  $B_1$  is species specific. There is evidence that other teratogens such as cortisone<sup>5</sup> are species specific and even that the incidence of cleft palate induced by cortisone in mice is strain specific<sup>6</sup>. The incidence of malformations produced by trypan blue in rats varies from strain to strain<sup>7</sup> and thalidomide produces malformations in A strain mice<sup>10</sup> but not in C57BL/6 mice<sup>11</sup>.

Aflatoxin  $B_1$  was kindly presented by Richard W. Jackson, Chief Fermentation Laboratory, US Department of Agriculture, Peoria, Illinois, and by Gerald N. Wogan, assistant professor of food toxicology, Department of Nutrition and Food Science, MIT.

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## Removal of Macroglobulins from Serum by Ultrafiltration through Agarose Membranes

ULTRAFILTRATION as a method for separating proteins of different sizes has been used in the past<sup>1-3</sup>, particularly in the separation of viruses from other smaller proteins<sup>4</sup>, as well as in the separation of viruses from one another<sup>5-9</sup>. The scant use currently made of this method is astonishing, for it seems much more attractive to push a protein solution through a membrane-shaped gel (ultrafiltration) than to let it seep through a column filled with grains of a



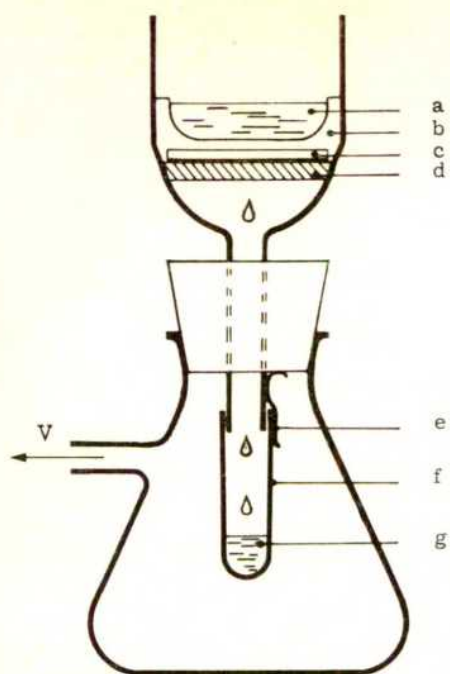


Fig. 1. Cross section of ultrafilter. *a*, Serum to be ultrafiltered; *b*, upper agarose layer, the top surface of which is provided with a skin; *c*, agarose slice, lying on the fritted glass filter *d*; *e*, adhesive tape attaching plastic collector tube *f* to stem of filter and cork; *g*, ultrafiltrate; *V*, vacuum connexion.

gel and to depend on pure diffusion for the separation of proteins of different sizes (gel filtration chromatography). These considerations induced us to attempt the separation of 19S macroglobulins (molecular weight of about 1,000,000) from the other serum proteins (molecular weight of about 160,000 and 70,000) by ultrafiltration. Such a separation, when successful, is very useful in immunohaematology, immunochemistry and immunology in general, especially where the differentiation between primary and secondary antibody forming responses is of interest.

Analytical ultracentrifugation was used to monitor the molecular weight distribution of all the serum ultrafiltrates obtained in various conditions. The membranes were made of agarose<sup>10</sup> (Seakem), a neutral derivative of agar-agar which is often used in gel filtration chromatography. Preliminary experiments had shown that the retention of macroglobulin is better the drier the atmospheric conditions during the setting of the gel. A certain degree of drying evidently conditioned the outer layer of the gel by forming a skin which determined the semi-permeability of the membrane. The exact conditions of "skinning" were determined for the purpose of preparing membranes which consistently retain all 19S macroglobulins, while allowing maximum amounts of 7S globulins, as well as albumin, to pass through them.

The best method of forming these membranes is to lay a disk of solid agarose in a Buchner funnel, and seal it with hot agarose solution, thus forming a cup-shaped membrane, in the following way. Agarose (5 per cent) (w/v) is dissolved in physiological saline by heating and stirring in a 100° C water bath. A vacuum is applied two or three times while the solution is still hot in order to remove dissolved air which might give rise to bubbles causing leaks in the membrane. To make the disk, part of the hot solution is poured into a glass tube (inside diameter of approximately 27 mm), in the bottom of which a small hole is bored after the gel has set. By blowing through the hole the sausage-shaped gel can be removed from the tube and 0.5–1 mm thick slices are easily cut off it with a wood planer. A slice is laid on the fritted glass of a Buchner funnel (inside diameter 30 mm, grade "fine" and fitted with a long stem,

which facilitates attaching the collecting tube), to prevent the pores in the fritted glass from becoming clogged up by molten agarose. (The sausage shaped gel can be used again, for cutting slices, and is best stored refrigerated.) Hot agarose solution (15 ml.) is then pipetted onto and around the slice with the aid of a Pasteur pipette with a tip which has an opening of 3 mm diameter. When the slice on the bottom of the funnel is completely covered with hot agarose solution the rest is deposited along the sides of the funnel, while it is being constantly turned around its axis, mouth downward at an angle of about 30°. When all the necessary agarose has been deposited rotation of the funnel is continued at an angle of 60°, and just before the gel sets a vacuum is applied at the stem of the funnel to draw the membrane tightly to the walls of the funnel.

When the gel has completely set, any portion protruding more than a few millimetres above the expected level of the liquid is trimmed away, to avoid undue osmotic attraction for the smaller proteins by the unwetted part of the gel. Immediately after trimming the surface skin is formed by directing the hot air stream of a hair drier for 10 sec from a distance of 25 mm, warming the rim of the filter to approximately 62° C and the inside of the funnel to 58° C. As soon as possible the serum is deposited on the membrane. The liquid should touch nothing but the membrane surface to avoid leakage (Fig. 1). A collecting tube is attached to the stem of the funnel with adhesive tape and the whole arrangement is attached to a vacuum flask and vacuum is applied (see Fig. 1).

With a vacuum of approximately 36–45 cm of mercury, 2 ml. of serum filters completely in about 4 h. When 2 ml. of serum is applied, approximately 2.2 ml. of a  $2.0 \pm 0.2$  per cent protein solution is collected as ultrafiltrate, containing the normal proportion of albumin and 7S globulin and no trace of 19S macroglobulin, as shown by analytical ultracentrifugation (Fig. 2) within the limits of resolution of this method, which can show up the presence of less than 0.01 per cent of a given protein. The absence of IgM in the ultrafiltrate was further demonstrated by immunodiffusion tests. Immuno-electrophoresis has confirmed that the principal medium molecular weight immune globulins have ultrafiltered and zone electrophoresis has shown a decrease in the  $\beta_2$  and  $\alpha_2$  bands, corresponding to the absence of  $\beta_2$ M (IgM) and  $\alpha_2$ M macroglobulins.

With anti-D (albumin-agglutinating antibody) all the activity (+ + + at 1/16) was found in the ultrafiltrate, whereas with anti-D (saline-agglutinating antibody) none of the activity (+ at 1/64) was found in the ultrafiltrate, and with anti-A<sub>1</sub> (saline-agglutinating antibody) none of the activity (+ at 1/8) was found in the ultrafiltrate. These

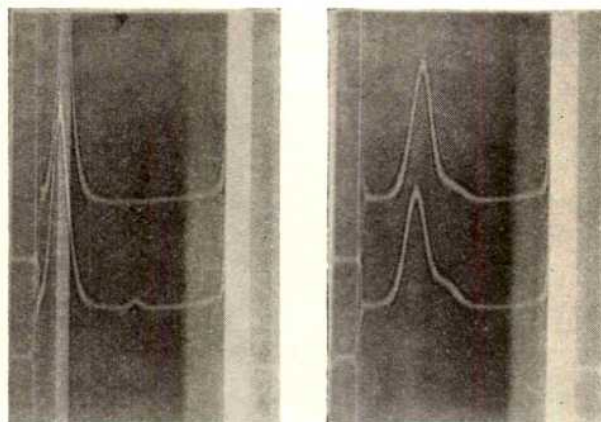


Fig. 2. Ultracentrifuge schlieren (60° phase plate angle) patterns of serum (below) and its ultrafiltrate (above) taken at 20 min (left) and 52 min (right) after attaining the speed of 59,780 r.p.m. The concentration of protein was 1.0 per cent in each case. The absence of 19S macroglobulin in the upper left curve is quite evident.

results were as expected; only the first mentioned antibody, a 7S immune globulin (IgG), passed the membrane while the other two, being 19S macroglobulins (IgM), were retained on the membrane. With anti-*Lub* (indirect Coombs antibody) all the activity (+ + at 1/2) was found in the ultrafiltrate. This indicates that this antibody must be a 7S immune globulin; further tests showed it to be of the IgA type<sup>11</sup>.

Preliminary tests indicate that a 10 per cent agarose membrane can be used for ultrafiltering albumin (molecular weight about 70,000), while retaining 7S globulins (molecular weight about 160,000). This result is sufficiently encouraging to attempt the separation of the *L* and *H* chains of immune globulins with agarose of an even greater concentration, a possibility which is now being investigated.

Because of the thickness of these hand made membranes, some of the serum proteins remain in the filter. They can be washed out of it at the cost of further dilution. The thickness of the agarose membrane is also the principal reason for its very slow flux. When much thinner synthetic membranes of the same pore size are made it will be reasonable to expect an increase in flux of up to two orders of magnitude, as well as a very noticeable decrease in loss of protein caused by retention inside the membrane. All known viruses are larger than 19S macroglobulins, and so the manufacture of such membranes may well be most useful for making virus free solutions in general, and in particular for the purpose of obtaining hepatitis free plasma.

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### Increased Permeability to Albumin induced with Protamine in Modified Gelatine Membranes

THE passage of macromolecules through living membranes is facilitated by cationic forms of proteins<sup>1</sup> and by histones, protamine and alkaline polyaminoacids as demonstrated by Ryser and Hancock<sup>2</sup>. Generally, the cell membrane surface is considered to carry a net negative charge, a property which should be especially pronounced in the case of many tumour cell surfaces<sup>3</sup>. Alterations reported in the permeability of cell membranes may be dependent on changes in membrane surface charges, caused by the adsorption of large cations. I have demonstrated that similar effects are produced by polycations on negatively charged artificial protein membranes.

Membranes were prepared from thiolated gelatine—gelatine reacted with a thiolactone according to Benesch and Benesch<sup>4</sup>. Peptide bonds are formed through the free amino groups of the protein chain and the thiolactone, resulting in addition of free reactive sulphhydryl groups to

the protein. The thiolated gelatine used ('Thiogel B' from Schwarz Bio Research, Inc.) has a molecular weight of about 100,000 and has attached twelve equivalents of sulphhydryl/100,000 g. On oxidation intermolecular cross-linking is established through disulphide bridges. The resultant increase in molecular weight gives a gel which is insoluble in water.

An 8 per cent solution of thiolated gelatine in hot M/20 triethanolamine buffer, pH 7.4, was prepared and poured into a Petri dish. The solution was ready for use after 5–10 min, and glass tubes, of inside diameter 11 mm with stoppers in one end, were dipped into the gelatinous solution. In this way a diaphragm was formed over the open end of the tubes, which were placed in a moist chamber at room temperature for 15–20 h. Melted paraffin was painted on the outer side of the tubes up to the cross-linked thiolated gelatine membranes, and they were submerged into the triethanolamine buffer. Into the tubes was poured 1 ml. of 1 per cent 'Blue Dextran' in buffer, which should not pass through the membranes.

Iodinated human serum albumin, labelled with iodine-131, was added to human serum albumin purified from commercial albumin ('Kabi') by a precipitation procedure<sup>5</sup> with 1 per cent trichloroacetic acid in 96 per cent ethanol and freed from label low molecular weight material by shaking with 'Sephadex G-25'. Protamine sulphate prepared from salmon sperm with a molecular weight of about 5,000 and 60 per cent arginine was supplied by the late Dr Paul Stroier Rasmussen. Polyornithine-HBr was purchased from Yeda Research and Development Co., Ltd., The Weissman Institute, Israel.

Continuous recording of diffusion rate was accomplished by pumping the outer solution through a coil inserted into the well-type scintillation crystal of a one channel analyser connected with a ratemeter and a d.c. recorder. The outer solution, 14 ml. of triethanolamine buffer and 5 mmolar calcium chloride, was placed in a plastic vial which was covered on the inside with thiolated gelatine in order to avoid adsorption onto the plastic. The membrane mounted on the glass tube was submerged 3–5 mm below the surface of the outer solution.

Human serum albumin labelled with iodine-131 (1 ml. of 0.2 per cent or 1.0 per cent) in the triethanolamine buffer was used as the inner solution, and when the rate of diffusion was constant, polyornithine-HBr or protamine sulphate was added to the outer solution.

The membranes probably carry a negative surface charge. This may explain the results of dialysis obtained with such membranes by Johnson<sup>6</sup>, who finds decreased diffusion of nicotinic acid and increased diffusion of an alkaloid. In order to exaggerate the negative charge, the membranes were allowed to adsorb the negatively charged azo-dye Congo red by exposing the membranes to  $2 \times 10^{-4}$  molar Congo red in buffer.

Fig. 1 shows the rate of diffusion of albumin through two comparable membranes, one of which has been submerged into Congo red. To both membranes 100  $\mu$ g of protamine sulphate was added and there was a considerable rise in the rate of dialysis through the membrane treated with Congo red. The untreated membrane was unaltered after addition of protamine sulphate. Fig. 1 also shows that treatment with Congo red decreases the diffusion rate for albumin. Table 1 shows the rates of diffusion through different sets of membranes.

The results indicate that the diffusion of human serum albumin labelled with iodine-131 is accelerated when protamine sulphate and polyornithine-HBr are added to the outer solution in experiments with membranes loaded with Congo red. This effect of polycations is not observed on untreated membranes with a relatively fast rate of diffusion for albumin. As indicated in the last line of Table 1, membranes which exhibit a slow rate of diffusion will respond in the same way as membranes loaded with Congo red, which suggests that they have a smaller negative surface charge.



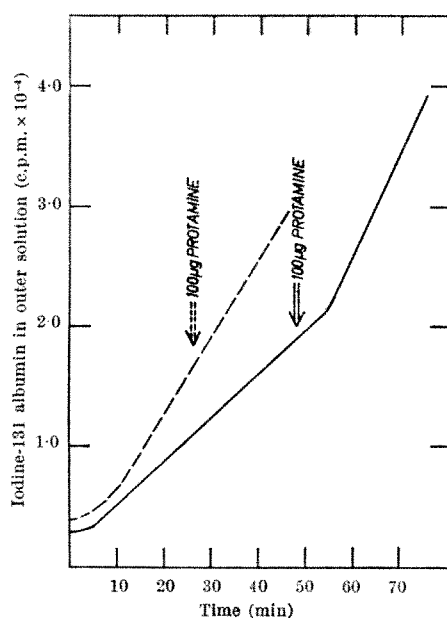


Fig. 1. Continuous recordings of albumin labelled with iodine-131 in the outer solution after addition of 1 ml. of 1 per cent albumin labelled with iodine-131, total c.p.m.,  $2.55 \times 10^5$ , to tubes with cross-linked thiolated gelatine membranes submerged in 14 ml. of the outer solution at pH 7.4 at room temperature. —, Permeability of an untreated membrane; ---, permeability of a membrane treated with Congo red. The arrows indicate addition of 100  $\mu$ g of protamine (1 mg/ml.) to the outer solution.

Addition of surface active polycations like protamine sulphate to such membranes is commonly assumed to result in the adsorption of monomolecular layers of polycations to the surfaces. Thus more cationic groups are bound to the membranes and any functional behaviour caused by surface charge may be altered.

Table 1. PERMEABILITY OF THIOLATED GELATINE MEMBRANES TO LABELLED HUMAN SERUM ALBUMIN BEFORE AND AFTER ADDITION OF PROTAMINE SULPHATE OR POLYORNITHINE-HBr

Pre-treatment of membrane	Rate of diffusion of albumin before addition of polycation (mg/h/membrane)	$\mu$ g of polycation added (1 mg/ml.)	Rate of diffusion of albumin after addition of polycation (mg/h/membrane)
4.5 h in $2 \times 10^{-4}$ molar CR* outside	0.25	100 $\mu$ g protamine sulphate	0.50
7 h in $2 \times 10^{-4}$ molar CR outside	0.18	200 .. ..	0.33
None	0.45	100 .. ..	0.45
1.5 h in $2 \times 10^{-4}$ molar CR outside	0.056	50 .. polyornithine-HBr	0.125
3 h with $10^{-3}$ molar CR inside	0.060	50 .. ..	0.132
4 h in $2 \times 10^{-4}$ molar CR outside	0.145	50 .. ..	0.240
None	0.190	50 .. ..	0.190
None	0.017	100 .. ..	0.045

\* Congo red in triethanolamine buffer M/20 pH 7.4.

Finally, my data provide good evidence for the supposition that surface charge of membranes plays a part in the permeability of charged macromolecules. The present observation may prove to be of biological importance in an understanding of the process of pinocytosis in cells.

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## Electron Tunnelling in Cytochromes

OXIDATION of a c-type cytochrome by light absorbed by the photosynthetic system of the purple sulphur bacterium, *Chromatium*, at the temperature of liquid nitrogen was discovered by Chance and Nishimura<sup>1</sup>. Vredenberg and Duysens<sup>2</sup> made further measurements confirming that quantum efficiencies did not change by more than a factor of two, if that much, at the lowest temperatures. The light and instrument limitations on these measurements make any further conclusions about rates difficult.

Rates not limited by light intensity have been measured with a fast spectrophotometer using a pulsed ruby laser for excitation<sup>3,4</sup>. The chief advantage of the laser compared with a flash lamp is the absence in the former of after-glow which, in the latter, interferes with spectrophotometric measurements on turbid samples for milliseconds after the flash. Our previous results, which went down to 32° K, have now been extended to 4.4° K, using a recently acquired liquid helium cryostat. Fig. 1 shows oscilloscope traces obtained at 4.4°, 4.5°, 15° and 88° K, respectively, on (separate) samples from the same batch of *Chromatium* (strain D).

The techniques used were essentially the same as those described before<sup>4</sup>, with some modifications as follows. A concentrated suspension of *Chromatium* in fresh growth medium was kept at least 5 min in the dark at room temperature in order to establish anaerobiosis and then frozen in the dark in liquid nitrogen and inserted into the measuring chamber. The cytochrome oxidation is irreversible below about 200° K. Each measurement involved the freezing of a separate sample. The measuring chamber was surrounded by helium (gas and sometimes liquid) at 1 atm., issuing through a throttle and diffusing ring from a liquid helium storage tank. The temperature of a copper block above the sample was measured with a carbon resistance thermometer whose calibration depends on the measurement at the boiling point of helium. Uncertainties in the interpolation formula used for higher temperatures set limits of about -3° to +5° K for the 15° K measurement and -0.2° to +0.5° K for the 4.4° and 4.5° K measurements. Measurement at 88° K ( $\pm 0.5^\circ$  K) was made with a calibrated platinum resistance thermometer in the copper block. In all cases the difference between the sample temperature and that of the copper block was measured with a gold-cobalt to copper thermocouple<sup>5</sup> provided by Dr Cesare Bucci.

The suspension used assayed 162  $\mu$ molar in bacteriochlorophyll. The light path through the sample was 1.6 mm. The laser was used in "normal" mode (0.5 msec burst) giving a total pulse of about 0.6 mJoules  $\text{cm}^{-2}$  at the sample at wavelength 6943 Å.

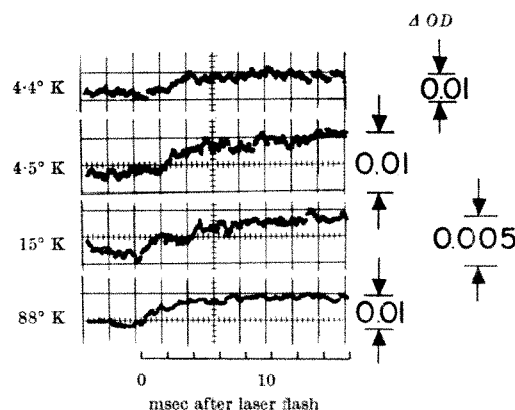


Fig. 1. Oscilloscope traces of optical density changes occurring at 422.5  $\text{m}\mu$  as a result of a pulse of laser light at 694.3  $\text{m}\mu$  in *Chromatium*. Ordinates vary from curve to curve as shown. Optical path length, 1.6 mm; bacterial chlorophyll, 162  $\mu$ molar. Upward deflexions represent decreasing optical density.

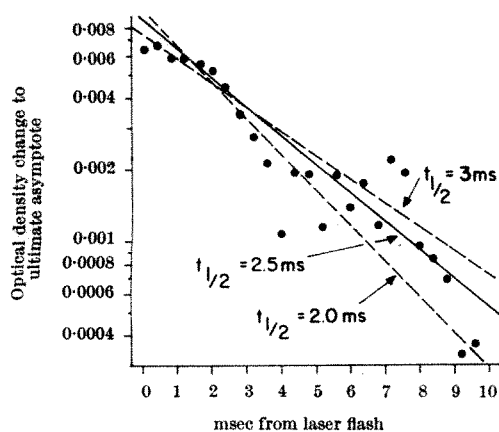


Fig. 2. Determination of half-time from the two measurements at  $4.4^\circ\text{K}$  and  $4.5^\circ\text{K}$ .

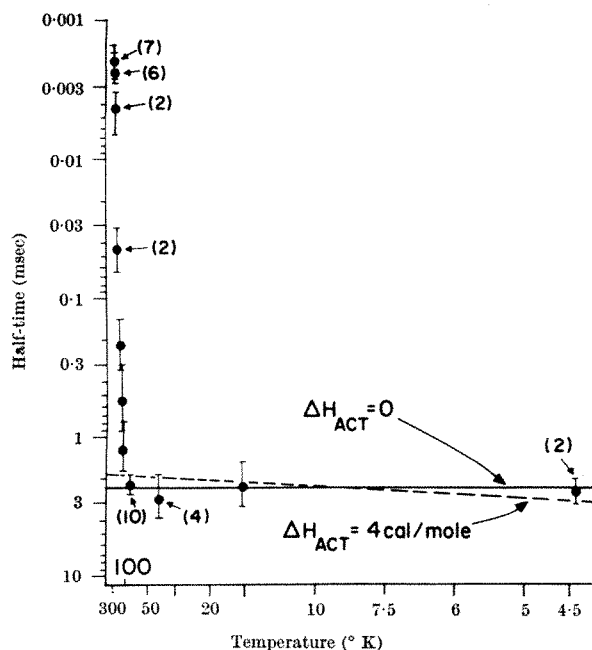


Fig. 3. Arrhenius plot of rate of laser-induced cytochrome oxidation in *Chromatium*. Numbers beside some points indicate the number of observations averaged to the single point.

The measurements of the two responses at  $4.4^\circ$  and  $4.5^\circ\text{K}$  subtracted from the asymptotes reached by them at long times are averaged and plotted in Fig. 2 on a logarithmic scale against the time after the laser flash. From it the half-time was estimated to be  $2.5 \pm 0.5$  msec.

The new data, together with those obtained before, are presented in an Arrhenius plot in Fig. 3. From this plot it is evident that there is a temperature dependent region above  $120^\circ\text{K}$  and a temperature independent region below  $100^\circ\text{K}$ . This may be interpreted as either two different cytochromes or two cytochrome pools which are oxidized in parallel or as two parallel mechanisms oxidizing the same cytochrome. The temperature dependent part falls off from a half-time of  $2\ \mu\text{sec}$  at room temperature to about  $2\ \text{msec}$  at  $130^\circ\text{K}$ , corresponding to an activation energy of  $3.3\ \text{kcal/mole}$  (ref. 4). The reaction at temperatures less than  $100^\circ\text{K}$  is almost constant at a half-time of  $2.3\ \text{msec}$  for a twenty-fold range of absolute temperature. The data would not allow an activation energy larger than  $4\ \text{cal/mole}$ .

We have interpreted the constancy of the rate of the low temperature reaction, together with its slowness, as evidence of a quantum-mechanical tunnelling of the

electron from cytochrome, presumably to bacteriochlorophyll<sup>6</sup>. We consider that the slowness indicates a barrier to electron transfer and the lack of activation energy indicates penetration through the barrier. There seems to be no alternative explanation in terms of semiconductor mechanisms the temperature coefficients of which fortuitously cancel<sup>7</sup> because of the wide range of constancy now established. It also seems unlikely that any movement of atoms is a necessary concomitant of a cytochrome oxidation which can occur at  $4.5^\circ\text{K}$ . Even in the case of proton transfers activation energies of  $2\text{--}4\ \text{kcal}$  are reported<sup>8</sup>.

The whole curve of Fig. 3 can be analysed as a single process involving tunnelling from several levels which are in thermal equilibrium. The method would be similar to that used by Fowler and Nordheim in explaining thermionic emission of electron from metals<sup>9</sup> or could be much simplified by assuming only one excited tunnelling level,  $3.3\ \text{kcal}$  above the ground state. The time course of the reaction recorded for the point at  $141^\circ\text{K}$  in Fig. 3 looks, however, bi-phasic, for the half-time plotted in Fig. 3 is an average value. If this is confirmed by further observation it will argue for separate pools not in rapid equilibrium with each other.

On purely quantum-mechanical grounds we would expect tunnelling to be important in chemical and biological transfer of electrons. The mechanism is well established in physical processes<sup>10,11</sup> and was proposed by Libby<sup>12</sup> and elaborated by others<sup>13-15</sup> for chemical electron transfers. Bell<sup>16</sup> had concluded that tunnelling should be important in hydrogen atom transfers. The penetrability decreases exponentially with the square root of the mass of the particle, and so similar barriers will be enormously more transparent to electrons. The requirements for electron transfer by tunnelling are (a) sufficiently close approach for there to be a measurable overlap of the wave function of the electron in the donor with that of the prospective wave function in the acceptor; (b) orientation suitable for overlap, if the wave-functions are not isotropic; and (c) suitably matched energy levels in donor and acceptor, considering the requirements of the Franck-Condon principle<sup>12</sup>, and phonon emission which may stabilize the process<sup>17</sup>.

The dependence on distance follows an exponential law in which the exponent to the base  $e$  is nearly equal to  $1\ \text{\AA}^{-1}$  for a barrier height of  $1\ \text{electron volt}$  (excess over the energy of the electron) and which is proportional to the square-root of the excess barrier height. (Compare Bohm's derivation of the formulae for rectangular and other barrier shapes<sup>18</sup>, and Eckart's exact solution for a special class of curved barriers<sup>19</sup>.) Thus if we assume an electron frequency (of approach to the barrier) of  $10^{15}\ \text{sec}^{-1}$  and a barrier height of  $1\ \text{eV}$ , we obtain half-times for penetration of about  $10^{-3}\ \text{sec}$  for a width of  $28\ \text{\AA}$  and  $10^{-6}\ \text{sec}$  for a width of  $21\ \text{\AA}$ . A barrier height of  $0.14\ \text{eV}$ , equal to  $3.3\ \text{kcal/mole}$ , would give a half-time of  $10^{-3}\ \text{sec}$  for a width of  $74\ \text{\AA}$ . These figures show the possibility of tunnelling over distances as large as the diameter of cytochrome  $c$ , now known to be  $36\ \text{\AA} \times 25\ \text{\AA}$  (ref. 20) but, of course, do not predict rates in specific cases where steric requirements, mismatching of energy levels, Franck-Condon limitations, or a smaller frequency of approach could act either to reduce the transmission rates or to give the same rates at smaller distances. Satisfying these requirements usually necessitates some activation energy before tunnelling can occur<sup>13-14</sup>, and this has tended to obscure the fact that a barrier is being penetrated. The occurrence of a clear cut case with zero activation energy is, perhaps, remarkable.

We propose that in general it is unnecessary to postulate any more complicated mechanism than tunnelling for moving electrons over distances of a few tens of Ångströms at the rates of biological processes. The requirement of matched energy levels may be used as a switch to forbid or allow the process and thus contribute to the specificities of donor and acceptor that are observed with cytochromes



and to the mechanisms which control electron transport for biological purposes. (The correlation of carcinogenicity of aromatic hydrocarbons with their fluorescence limits by Birks may also illustrate the necessity for matching energy levels in electron transfer<sup>21</sup>.)

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### Localization of Acid Phosphatase in Oral Epithelium

THE presence of acid phosphatase in epidermis and oral epithelium has been well established by histochemical procedures such as Gomori's acid phosphatase method. Several workers<sup>1,2</sup> have interpreted the particulate distribution of the enzyme reaction as indicating the presence of lysosomes, acid phosphatase being an accepted marker for such organelles<sup>3</sup>. At an ultrastructural level, however, there have been few reports of lysosome-like bodies in these tissues. Rhodin<sup>4</sup> has illustrated lysosomes in epidermis and they have been described in epithelium in tissue culture<sup>5</sup>. Their form in oral epithelium has been discussed<sup>6</sup> but not illustrated and they are frequently seen in the dendritic Langerhans cells of this tissue<sup>7</sup>, and of epidermis<sup>8</sup>. Cytochemical studies of normal skin have shown conflicting results. Eisen, Arndt and Clark<sup>9</sup> concluded that the acid phosphatase in epidermal cells was not contained in organelles and that lysosomes played little part in the metabolic processes of human epidermal cells. Mishima<sup>10</sup>, however, showed acid phosphatase activity in vesicles containing melanin granules in epidermal keratinocytes; he termed these bodies lysosomes. Olson and Nordquist<sup>11</sup> demonstrated acid phosphatase activity in membrane-bound vesicles in human epidermis and recently<sup>12</sup> showed the presence of this enzyme in small vesicles in human epidermal cells which had taken up injected ferritin.

The work described here was carried out on oral epithelium from albino rat cheek. Thick sections, cut on a cryostat, were lightly fixed in glutaraldehyde before rinsing and incubating in a medium for acid phosphatase modified from Gomori<sup>13</sup>. Some of the tissue was treated

with osmium tetroxide before dehydration and embedding. The results from osmicated and non-osmicated sections were similar, and staining the ultra-thin sections did not affect the localization of activity.

Deposits of lead salt were frequently visible in the basal layer and prickly cells of the epithelium as discrete parallel strands (Fig. 1), sometimes with small adjacent vesicles. A few larger vesicles were seen, often close to mitochondria; in Fig. 1 the mitochondrion appears to be contained within a membrane. In the superficial prickly cells and the granular layer small deposits of lead were abundant, invariably associated with mitochondria (Fig. 2), but no activity was associated with keratohyalin granules. Some deposits appeared adjacent to the plasma membrane of cells in the granular layer. The keratinized layer contained very little intracellular activity, but a dense precipitate of lead was seen between most of the cells. Occasional cells (Fig. 3) showed a fine uniform intracellular deposit. Control sections incubated without substrate were free from lead precipitate.

The arrangement of discrete elongate deposits in the basal cells strongly resembles a Golgi system. Acid phosphatase has frequently been reported in this organelle in other tissues<sup>14-17</sup>. Larger deposits may represent pure lysosomes, and the structure in Fig. 1 may be considered a cytolysosome. The frequent association of mitochondria and enzyme deposits in the granular layer suggests that acid phosphatase plays a part in the loss of such organelles, which precedes keratinization. Activity was observed by Eisen *et al.*<sup>9</sup> at the cell boundaries of the superficial cells,

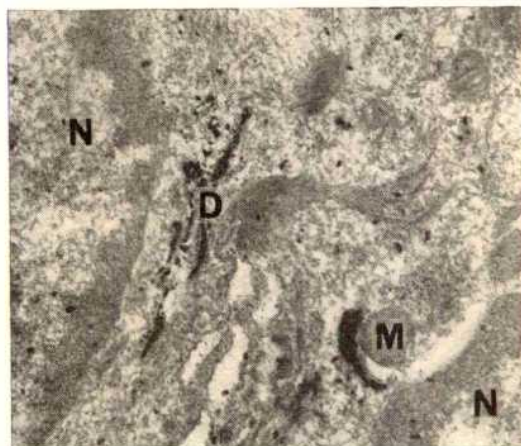


Fig. 1. Elongate lead deposits (D) in a prickly cell. A lead deposit lies next to the mitochondrion (M) enclosed in a membrane. N, Nuclei. Stained with uranyl acetate,  $\times 21,000$ .

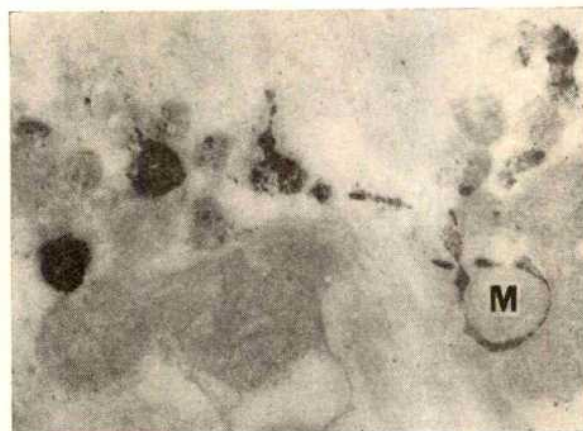


Fig. 2. Part of a cell in the granular layer. Lead deposits are adjacent to a number of mitochondria. One deposit virtually encloses a mitochondrion (M). Unstained,  $\times 30,000$ .



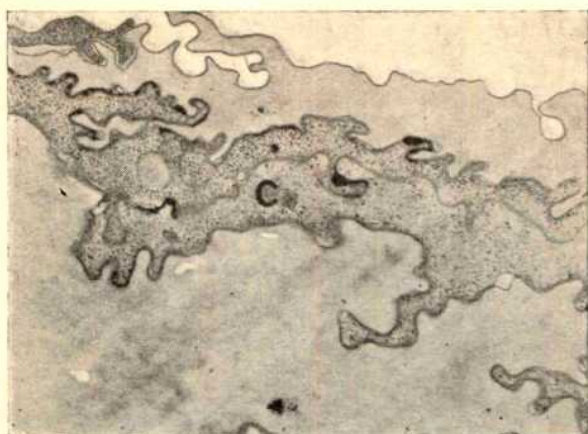


Fig. 3. Section through the stratum corneum. There are intercellular deposits and one cell (C) shows a fine uniform deposit of lead. Unstained,  $\times 7,500$ .

where it may be associated with the loss of desmosome attachments and the formation of a thickened plasma membrane. Such deposits possibly originate from the vesicles observed near cell membranes in the granular layer. Striated bodies have been described in this position by Frithiof and Wersäll<sup>18</sup>. An occasional uniform deposit in a deep cell of the stratum corneum could well be a physiological expression of Brody's transition (T) cell<sup>19</sup>, in which metabolic activity is thought to occur.

The presence of acid phosphatase, presumably aggregated in Golgi vesicles and clearly associated with other cell organelles, suggests an involvement of bodies of a lysosomal nature in the metabolic activities of oral epithelium. Preliminary cytochemical work on the localization of non-specific esterase indicates a distribution in this tissue which supports our suggestions.

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### Function of Copper in the Metabolism of Iron

THE importance of copper in the metabolism of iron has been recognized since Hart *et al.*<sup>1</sup> showed that rats become anaemic when fed a diet composed solely of milk, and respond to the administration of iron only if copper is

provided in addition. The observation made by a number of workers that, following the administration of copper to rats deficient in copper, the concentration of iron in their livers is reduced and that of haemoglobin increased has led to the assumption that copper is required for the utilization of iron<sup>2</sup>. The possibility that copper may be involved specifically in the release of iron from storage sites has not been investigated.

Examination of our data, obtained from experiments in which rats were fed a milk diet deficient in both iron and copper and on which treatments of copper and/or iron were superimposed, shows that rats whose rations were deficient in iron only had less iron stored in their livers and more iron circulating as haemoglobin and in the plasma than rats whose rations were deficient in both copper and iron, and led to formulation of the hypothesis that copper is required for the release of iron from storage sites. Results of subsequent experiments, one of which is described briefly here, support the hypothesis.

Twenty-two weanling rats were fed a diet deficient in both iron and copper. After 4.5 weeks on this diet all of the animals showed obvious signs of the deficiency syndrome and two, Nos. 8 and 12, died. The remaining animals were divided, on the basis of degree of depigmentation and reduction in rate of growth, into two evenly matched groups, groups A and B. Each animal was bled from the tail. Immediately following the collection of blood each animal in group A was given 200  $\mu$ g of copper in 0.25 ml. of solution; each animal in group B was given the same volume of 0.9 per cent sodium chloride; both by intraperitoneal injection. A second sample of tail blood was collected from each surviving animal approximately 24 h later. Nos. 19 and 22 died during the 24 h subsequent to injection with saline and the plasma from No. 16 (group A) was discarded because of haemolysis—no sample in which haemolysis of the red cells (sufficient to be detected by eye) had occurred was accepted.

The concentrations of iron in the plasmas of the deficient animals were so low (about 0.1 of normal) that meticulous care was necessary to prevent contamination; it was virtually impossible to prevent a slight degree of haemolysis and the figures quoted in Table 1 have been corrected for haemoglobin iron as estimated by the benzidine reaction<sup>3</sup> (S.D. of a single observation from the duplicate analyses: 4.3). The method of Giovanniello and Peters<sup>4</sup> was used, with slight modification, to estimate the concentration of iron in plasma. In samples in which sufficient material was available for duplicate analyses the standard deviation of a single observation was 3.0.

The increase in the concentration of iron which occurs in the plasmas of rats deficient in both iron and copper 24 h after the administration of copper (Table 1) is significantly higher than that which occurs subsequent to the administration of physiological saline solution (difference of mean differences 24.9 for which  $P < 0.05$  by the *d*-test) and supports the hypothesis that copper is necessary for the release of iron from storage sites.

In other experiments the time interval between injection and collection of the second sample of blood was

Table 1. EFFECT OF AN INTRAPERITONEAL INJECTION OF COPPER COMPARED WITH THAT OF PHYSIOLOGICAL SALINE SOLUTION ON THE CONCENTRATION OF IRON IN THE PLASMAS OF RATS FED A DIET DEFICIENT IN BOTH IRON AND COPPER

Group A			Group B		
Rat No.	Plasma iron ( $\mu$ g/100 ml.)		Rat No.	Plasma iron ( $\mu$ g/100 ml.)	
	Pre-injection	Post-injection*		Pre-injection	Post-injection†
1	27	43	2	21	20
3	14	43	4	23	16
5	17	26	7	21	28
6	13	34	10	26	25
9	24	31	13	26	35
11	18	32	15	24	41
14	20	88	17	41	59
18	31	76	21	27	30
20	25	93			

\* 24 h subsequent to the injection of 200  $\mu$ g of copper.

† 24 h subsequent to the injection of 2.25 mg of sodium chloride.

varied. Two hours after the injection of copper into deficient animals the concentrations of iron in their plasmas were slightly but significantly higher than those to which saline had been administered (difference of mean differences 12.2 for which  $P < 0.05$  by the  $t$ -test); at 18 h the difference of mean differences was 34.9 for which  $P < 0.01$  by the  $d$ -test. Forty-eight hours after the administration of copper to deficient animals no significant increase in the concentration of iron in the plasma was noted; however, the average concentration of iron in the livers of these animals was 94  $\mu\text{g/g}$  dry wt., approximately half of that of litter mates which had received no copper by injection.

Total iron binding capacity is normal in the plasmas of copper and iron deficient rats.

Statistical analysis of the results was carried out by Dr E. A. Cornish.

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## Synthesis of Proteins by Alveolar Cells

ALVEOLAR cells are important because they are thought to produce surfactant<sup>1-3</sup>, a lipoprotein<sup>4-6</sup> which lines the alveoli and is responsible for much of the stability of the pulmonary airspaces<sup>7</sup>. Previous work has shown that alveolar cells induced with *M. bovis* (B.C.G.) suspended in Hanks medium can incorporate leucine into protein and that such incorporation is inhibited ( $\geq 99$  per cent) by puromycin. Extracellular radioactive protein appears during the incubations, which suggests either a non-specific release due to cell disruption, a more specific release dependent on energy or a combination of both factors<sup>8</sup>. This communication describes the release of radioactive protein into the suspending media by alveolar cells labelled with DL-leucine-1-<sup>14</sup>C and illustrates the depressive effect on this release of metabolic inhibitors.

Alveolar cells induced by B.C.G. were collected from rabbit lungs<sup>9,10</sup> and suspended in 5 volumes (v/wet weight) of Hanks solution. Fifty ml. of cells was incubated at 37° C with 0.125 mc. of DL-leucine-1-<sup>14</sup>C, specific activity 4.77. After 30 min, puromycin dihydrochloride was added to a final concentration of  $1 \times 10^{-3}$  moles/l. and the incubation continued for 10 min. The suspension was then divided into two equal portions. 2,4-Dinitrophenol ( $5.0 \times 10^{-4}$  moles/l. final concentration) in absolute ethyl alcohol was added to one batch of cells and an equal amount of ethyl alcohol alone was added to the other. Incubation was continued at 37° C and samples of 1.5 ml. were removed from each flask at one minute intervals from zero time to 10 min and maintained on ice. The cells were collected by centrifugation, the supernatant fluid was removed and assayed for protein<sup>11</sup>, and the cells were then suspended in water, homogenized and their protein content measured.

The material, assayed for radioactivity in a scintillation counter, was insoluble in cold and hot trichloroacetic acid and in ethyl ether-acetone-chloroform (2:2:1 v/v) at 50° C, but was made soluble by heating in 0.2 molar sodium hydroxide at 80° C for 30 min. All measurements are corrected for efficiency. Sufficient counts were made to have a counting accuracy of 97 per cent.

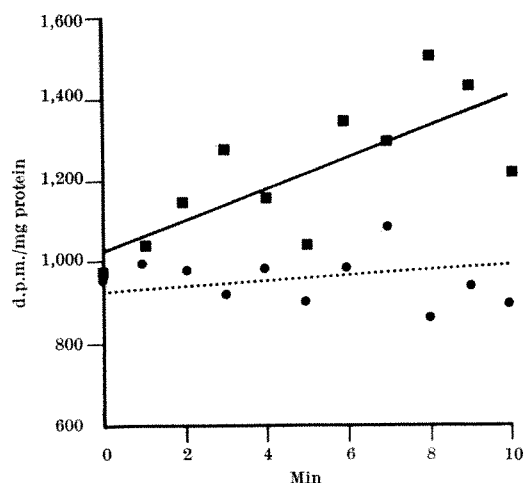


Fig. 1. Appearance of extracellular radioactive protein in media suspending cells (without dinitrophenol ■—■; with dinitrophenol ●---●).

Fig. 1 compares the rise in the specific activity of protein with time between the media of the cells treated with dinitrophenol and those untreated. The slopes of the lines were calculated by the least squares method. The correlation coefficients are 0.72 ( $P = 0.02$ ) for the media without dinitrophenol and 0.3 ( $P = 0.09$ ) for the media with dinitrophenol<sup>12</sup>. The time course of the protein specific activity of the cells was comparable in both groups. There was no rise in specific activity of the cellular protein with time, indicating that an increase in specific activity within the cells did not account for the rise in protein specific activity in the media.

To test the effect of other metabolic inhibitors on the release of radioactive protein into the suspending medium, 0.15 mc. DL-leucine-1-<sup>14</sup>C, specific activity 5.24, was incubated at 37° C with 6 ml. of alveolar cells for 30 min. The cells were collected by centrifugation and washed three times with cold Hanks solution; the supernatant fluids were discarded. The cells were suspended in 5.0 ml. Hanks solution and divided into two groups, to each of which puromycin dihydrochloride was added to a final concentration of  $2.0 \times 10^{-3}$  moles/l. In addition, potassium cyanide, potassium fluoride and sodium arsenite to  $3.0 \times 10^{-3}$ ,  $9.0 \times 10^{-3}$ , and  $4.5 \times 10^{-3}$  moles/l. respectively were added to one group of cells to depress metabolic activity (designated "inhibited" in Table 1). These two batches of cells were again incubated at 37° C for 10 min. The reactions were stopped by chilling and the cells washed as after the first incubation. The cells were again suspended in equal volumes of Hanks solution and each group then divided into three portions for incubation for 0, 10 and 20 min at 37° C. Following this, the procedures were identical to those previously described.

In a final experiment, 6 ml. of alveolar cells was incubated at 37° for 30 min with 0.15 mc. DL-leucine-1-<sup>14</sup>C. After washings and incubation with puromycin exactly as described, the cells were resuspended in Hanks media and

Treatment	Time (min)	Protein (mg/ml.)	RNA (mg/ml.)	Extracellular <sup>14</sup> C (d.p.m./mg protein)	Cells <sup>14</sup> C incorporated (d.p.m./mg protein)
1. Control	0	0.17	0.02	3,280	15,750
	10	0.33	0.04	6,470	13,880
	20	0.43	0.04	9,040	13,600
Inhibited*	0	0.20	0.03	3,260	17,590
	10	0.28	0.04	3,580	16,420
	20	0.44	0.04	4,860	16,690
2. Control	0	0.11	0.02	9,528	17,029
	10	0.21	0.03	11,713	20,700
	20	0.22	0.03	11,322	15,915
0° Incubation	0	0.12	0.02	10,267	15,522
	10	0.22	0.04	4,850	17,638
	20	0.26	0.03	4,258	16,073

\* Potassium cyanide  $3.0 \times 10^{-3}$  moles/l., potassium fluoride  $9.0 \times 10^{-3}$  moles/l. and sodium arsenite  $4.5 \times 10^{-3}$  moles/l.



divided into two groups. One group, designated "control" in Table 1, was divided into three portions and incubated at 37° C for 0, 10 and 20 min. The other batch of cells, designated "0° incubation" (Table 1), was similarly divided and maintained on ice for the indicated times. Cells and media were then handled as previously described.

The rise of protein and RNA<sup>13</sup> is not different in the media supporting cells with energy production altered from that in the cells in which no attempt was made to inhibit energy metabolism (Table 1). The similarity of the amounts of protein appearing in the media of the control group and in the inhibited or 0° incubation groups suggests that interference with energy metabolism has no effect on most of the protein appearing in the media.

The increase of specific activity of the extracellular protein was always at least twice as great for the control group of cells as for its counterpart with the energy metabolism impaired (Table 1). The fall in the specific activity of protein in the media of cells incubated at 0° represents practically complete cessation of release of the radioactive protein into the media although a two-fold rise in the protein in the unlabelled media took place. The lack of further incorporation of leucine-<sup>14</sup>C into the cells with continued incubation is shown in Table 1 and is attributed to the effect of puromycin. This indicates that the differential rise in the specific activity of protein between the control and inhibited or 0° incubation media is not accounted for by a difference in the protein specific activity of the cells.

These experiments provide evidence that alveolar cells induced with B.C.G. can release newly synthesized protein into a suspending medium. This release is depressed by the metabolic inhibitors tested, which suggests that it represents active release rather than release due to cell disruption or death.

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### Reduction in the Concentration of Phosphatidyl Serine in the Plasma of Birds with Avian Erythroblastosis

PLASMA  $\alpha$ -lipoprotein, obtained from birds affected with erythroblastosis, shows a slower than normal mobility in starch gel and paper electrophoresis. The protein moiety had the same electrophoretic mobility when it was derived from lipoproteins isolated by ultracentrifugation from the plasma of either normal or erythroblastotic birds<sup>1-3</sup>. This suggests that the change is in the lipid moiety. We have now observed a difference between lipid

extracts of normal and leukaemic plasma involving a phospholipid with the same chromatographic behaviour on silica gel *H* as phosphatidyl serine.

Lipid was extracted with chloroform-methanol<sup>4</sup> from red cells washed with either plasma or saline. The extracts were applied directly to the plates (thus avoiding the necessity for evaporation and re-solution in other solvents). Samples varying from 10–50  $\mu$ l. were spotted on silica gel *H* plates and the chromatograms were developed with chloroform-methanol-acetic acid-water (50:25:7:3 v/v<sup>5</sup>). All the solvents used contained 100 mg of butylated hydroxy-toluene/l. to prevent auto-oxidation<sup>6</sup>.

Plates were sprayed either with ninhydrin<sup>7</sup> to show the phosphatidyl serine and ethanol amine or with 1 per cent iodine in chloroform<sup>8</sup> or 4 per cent in phosphomolybdic acid ethanol<sup>7</sup> in order to show the lipids. Dragendorff's reagent<sup>8</sup> was used to demonstrate phosphatidyl choline. Commercially available preparations of phosphatidyl choline, phosphatidyl ethanolamine and phosphatidyl serine were used for reference. These preparations showed multiple components, but the specific component was recognized by the appropriate reagent. In the case of commercial phosphatidyl serine the contaminants were removed by column chromatography on silica gel<sup>9</sup>.

Fig. 1 illustrates a thin-layer chromatogram in which the migration of these phosphatides is compared with the migration of lipid extracts from normal and leukaemic plasma. This chromatogram was sprayed with 4 per cent phosphomolybdic acid, heated in an oven for 10 min, and the spots which appeared were outlined with a pencil point. The "phosphatidyl serine" spot from the leukaemic sample did not appear at this time but could be recognized 2 h later when the plates were photographed.

Material responsible for the spot migrating with the same speed as phosphatidyl serine was greatly reduced in erythroblastosis. In addition there seemed to be less phosphatidyl choline and phosphatidyl ethanolamine, but the reduction was not as great as for phosphatidyl serine (Fig. 1). The spot produced by "phosphatidyl serine" was at best only faintly visible when 50  $\mu$ l. of the extract of leukaemic plasma was applied to a chromatogram, but it was very obvious with only 10  $\mu$ l. of the extract of normal

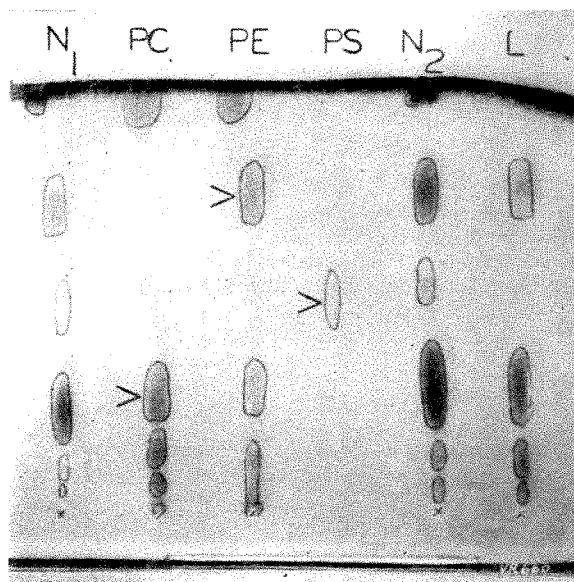


Fig. 1. Thin-layer chromatogram plate of lipid extracts of normal and leukaemic plasma. The normal plasma extract was applied in 10  $\mu$ l. (*N*<sub>1</sub>) and 50  $\mu$ l. (*N*<sub>2</sub>) quantities and compared with the results following a 50  $\mu$ l. application of the leukaemic plasma extract (*L*). The positions reached by the known phosphatides (PC, phosphatidyl choline; PE, phosphatidyl ethanolamine; PS, phosphatidyl serine) used for comparison are indicated by (>).



plasma. This indicates a more than five-fold reduction of this phosphatide in leukaemic plasma.

Lipid extracts from washed red cells showed a pattern of lipid very similar to that of normal plasma extracts, except that the amount of "phosphatidyl serine" was much larger. There was no appreciable difference in the content of "phosphatidyl serine" in extracts from washed cell suspensions from normal and leukaemic bloods.

To summarize, the level of phosphatidyl serine (or a substance closely related to it) in normal plasma is greatly reduced in erythroblastosis. Lipid extracts derived from red cells from infected birds do not show this change. No claim is made that the reduction in phosphatidyl serine is specific for erythroblastosis, because we have not been able to investigate other leukoses or other diseases of the fowl in the same way. It is not yet clear whether this alteration in the concentration of phosphatidyl serine could be involved in the electrophoretic changes in plasma  $\alpha$ -lipoprotein in erythroblastosis, but further work in this direction is being carried out.

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### Immediate Action of Phytochrome in Light-stimulated Lettuce Seeds

SEEDS of *Lactuca sativa* (var. 'Grand Rapids'), which we have used in these studies, require a short exposure to red light in order to germinate at 25° C. The promotive effect of red light can be completely nullified if the latter is followed closely by an exposure to far-red light. This involves the reversion of the active form of phytochrome ( $P_{FR}$ ), produced by red light, to its inactive form ( $P_R$ ). If a dark interval of more than a certain critical duration is intercalated between the red and far-red irradiations, some action of  $P_{FR}$  is permitted which is expressed subsequently as the germination of a number of seeds. After a certain period in darkness (the escape time) after red light, short far-red fails to have any inhibitory effect<sup>1</sup> and all the seeds germinate. The escape time therefore gives a physiological measure of the course of  $P_{FR}$  action in the seed population, as shown in Fig. 1 for our sample.

Little is known of the processes in seeds set in motion by  $P_{FR}$ , but in view of the work of Köhler<sup>2</sup> it seems possible that gibberellin synthesis is initiated. Using extraction methods he found that a significant increase in gibberellin-like substances occurred after only 60 min of  $P_{FR}$  activity. Thus events apparently occur in the first part of the escape time which are not expressed as germination (compare Fig. 1). We can assume that because these events involve the formation of gibberellins the amounts of the latter substances must be suboptimal for germination. If this is the case, the supply of further but still suboptimal levels of exogenous gibberellin might magnify the germination-promoting potential of the endogenous hormone which has been produced even by a short period of  $P_{FR}$  action.

In the present experiments seeds were imbibed on filter papers moistened with water in darkness at 25° C

for 1 h. They were then exposed to red light (1,800 ergs  $\text{cm}^{-2}\text{sec}^{-1}$ ) for 2 min, after which they were returned to darkness for varying lengths of time before being irradiated with far-red (700 ergs  $\text{cm}^{-2}\text{sec}^{-1}$ ) for 5 min. After the far-red treatment the seeds were transferred to other dishes containing either gibberellic acid ( $\text{GA}_3$ ) at 5, 10 or 20  $\mu\text{g}/\text{ml}$ . or water in the controls. These manipulations were carried out under a dim green safe light.

In the first experiment  $P_{FR}$  was allowed to act for 1, 2, 3 and 4 h (Table 1) before  $\text{GA}_3$  was supplied. The results show that gibberellic acid interacts with the product of  $P_{FR}$  action over these times to cause much higher germination percentages than it can when in total darkness throughout. Further, it is clear that the combined effect of  $\text{GA}_3$  and  $P_{FR}$  activity in stimulating germination is much more than additive. Because in this experiment activity of  $P_{FR}$  for 1 h is so evident, a second experiment was performed using shorter times of  $P_{FR}$  action of up to 30 min. The synergism between  $\text{GA}_3$  and the product of phytochrome activity is again apparent (Fig. 2). Surprisingly, as short a time as 5 min of  $P_{FR}$  action can easily be detected. Saturation with added  $\text{GA}_3$  at 10 and 20  $\mu\text{g}/\text{ml}$ . is achieved after phytochrome has been allowed to act for only 10 min.

Table 1. INTERACTION BETWEEN  $P_{FR}$  AND SUB-THRESHOLD CONCENTRATION OF  $\text{GA}_3$ \*

Length of $P_{FR}$ action (h)	Germination percentage	
	Observed	Calculated †
0	14 ± 3	12
1	71 ± 1	19
2	69 ± 0	35
3	91 ± 0	61
4	91 ± 2	90

\* 5  $\mu\text{g}/\text{ml}$ .

† The calculated germination percentage is the sum of the water control (Fig. 1) and the germination in darkness on  $\text{GA}_3$  alone. From this the value for dark germination on water (2 per cent) is subtracted.

One important fact emerges from these experiments; this is that the action of  $P_{FR}$  is almost instantaneous and can be revealed by this technique of supplying sub-threshold concentrations of gibberellic acid. Determinations of the escape time (Fig. 1) would indicate that, at least as far as germination is concerned,  $P_{FR}$  activity for 2-3 h is somewhat ineffectual; in fact, it now becomes clear that the action of phytochrome for only 5 min succeeds in producing highly significant changes in seeds.

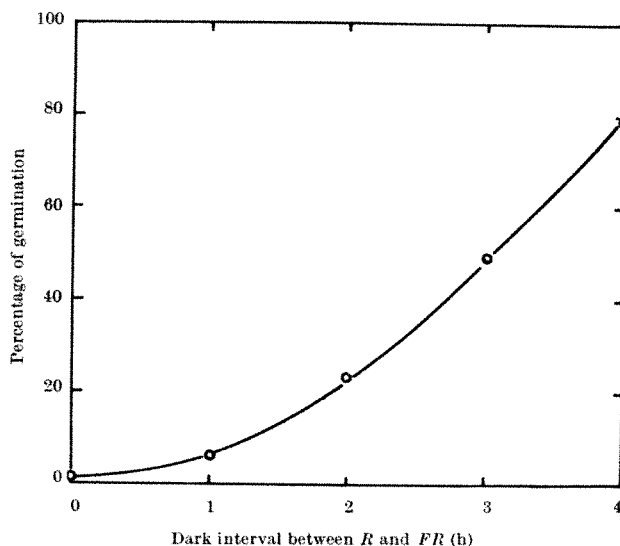


Fig. 1. Time course of escape from the inhibitory effects of far-red ( $FR$ ) irradiation. Seeds irradiated after 1 h in darkness at 25° C with 2 min red light followed by 5 min  $FR$  irradiation, either immediately or after varying intervals of darkness. Controls in darkness and in red light were 2 per cent and 98 per cent respectively. Germination counted 24 h after beginning of imbibition.

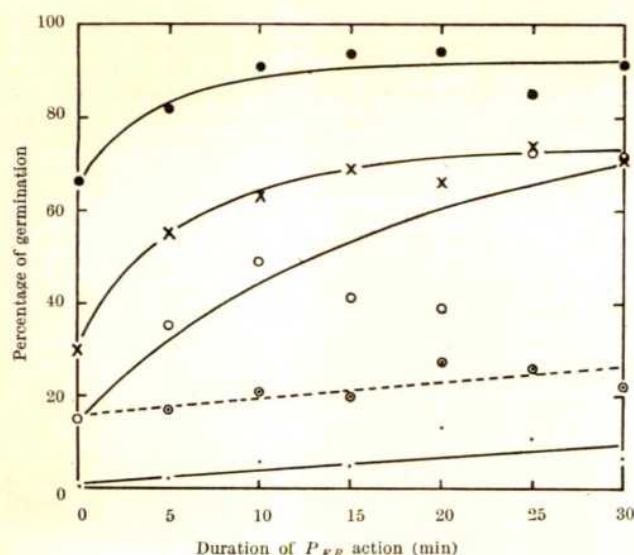


Fig. 2. Interaction between  $P_{FR}$  and sub-threshold concentrations of  $GA_3$ . ●—●, Water; ○—○, 5  $\mu g/ml.$   $GA_3$ ; ×—×, 10  $\mu g/ml.$   $GA_3$ ; ●—●, 20  $\mu g/ml.$   $GA_3$ ; ○—○, calculated curve for additive effect of  $P_{FR}$  and 5  $\mu g/ml.$   $GA_3$ .

The great rapidity of action of phytochrome must be taken into account in any hypothesis of its mechanism of operation. This is borne out by the work of Fondeville *et al.*<sup>3</sup> on the phytochrome control of pulvinar movements in *Mimosa pudica*. These workers consider that the phytochrome control of gene activation as proposed by Mohr<sup>4</sup> is unlikely; our results strengthen this conclusion. If gene activation is involved in the control of germination by phytochrome it seems certain that it is not the primary effect of the pigment.

Köhler<sup>2</sup> has suggested that gibberellin synthesis in lettuce seeds is promoted by  $P_{FR}$ . This might indeed be the case; but one must conclude from our results, which indicate synergism, that  $GA_3$  itself is not produced. It still remains possible, however, that another gibberellin is formed in the seeds immediately after exposure to red light; this gibberellin would have to act synergistically with  $GA_3$ . This hypothesis therefore attributes to phytochrome a role in controlling a metabolic pathway for gibberellin biosynthesis. On the other hand, another substance which is not a gibberellin but which can interact with  $GA_3$  might be produced by  $P_{FR}$ .

It is also possible that  $P_{FR}$  induces changes in cell permeability. This could plausibly be the cause of the effects reported here where the enhanced penetration of exogenous  $GA_3$  might account for the strongly stimulating effect of a short duration of  $P_{FR}$  activity. It is interesting in this context to recall that in a number of seeds gibberellic acid is relatively ineffective unless some exposure to red light has occurred<sup>5</sup>.

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## MICROBIOLOGY

### Virus-like Particles of a Fraction of Statolon, a Mould Product

STATOLON, a fermentation product of a mould, *Penicillium stoloniferum*, is capable of stimulating the production of interferon in animals and in tissue culture<sup>1,2</sup>. It was previously reported that the activity of the partially purified statolon is associated with a sedimentable anionic polysaccharide<sup>3</sup>. Attempts to refine further the statolon preparations were hampered by its low solubility and its tendency to aggregate with itself and other materials causing inactivation. Elucidation of the physical characteristics of statolon has recently been extended by means of centrifugation on sucrose density gradients and electron microscopic studies. It was found that it was possible to avoid aggregation if sodium chloride was added to the gradients and if the pH was kept near 9.

Dried preparations of statolon, dissolved in 1 per cent sodium bicarbonate, or the filtered fermentation broths, were placed on sucrose gradients for centrifugation. Two visible bands formed after centrifugation for 16–24 h. Fractions were collected and assayed for their ability to induce interferon. The assays were conducted with the mouse L-cell-vesicular stomatitis virus (VSV) system<sup>2</sup>, either directly on the collected fractions or by injecting mice with these fractions and then determining the concentration of interferon in their sera.

Electron microscopic examination of the heavy active fraction (45 per cent sucrose) revealed numerous particles of typical viral morphology. Examination of negatively stained preparations under high magnification disclosed hexagonal particles measuring about 30 m $\mu$  across (Fig. 1).

All the evidence suggests that these virus-like particles are associated with interferon-inducing activity. The heavy band contained such particles and was capable of inducing

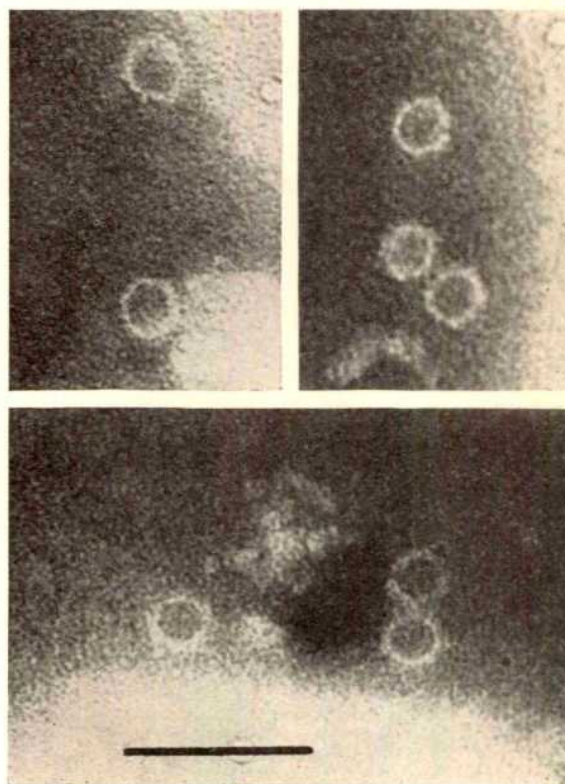


Fig. 1. Particles in an isopycnic band of statolon. Stained with phosphotungstic acid. Scale equals 0.1  $\mu$ .



interferon in both tissue culture and animals. Adjacent fractions contained less interferon inducing activity and correspondingly fewer particles. No particles were found in the inactive fractions. These observations have been corroborated by additional combined rate zonal and isopycnic centrifugation experiments in swinging bucket rotors and with larger volumes in the zonal centrifuge<sup>4</sup>.

It would seem that the inoculated mould, *Penicillium stoloniferum*, is the source of the particles. Uninoculated media incubated for the same period as the normal statolon fermentation (5–7 days) showed neither interferon stimulating activity nor particles when examined in an electron microscope. Both the particles and the interferon stimulating activity were detected only after incubation for 4 days. Electron microscopic examination of thin sections of mycelia of *Penicillium stoloniferum* demonstrated particles possessing the morphology of those obtained by gradient centrifugation (Fig. 2).

Although some reduction of mycelial mass occurs after 6–7 days of fermentation, no evidence of true lytic action has been observed, either in fermentation broths or on agar media (W. M. Stark, W. H. Jackson, C. E. Higgins and M. M. Hoehn, unpublished results). Dr Richard Elander of these laboratories has suggested that failure to detect viral infectivity by cellular lysis could be due to the rigid cell wall of the mycelia of the mould. On the other hand, cell destruction is not a characteristic of all viruses and replication of this particle may also occur without lysis. Particles are not detected in the other band of interferon-inducing activity (30 per cent sucrose). Characterization of this fraction must await further investigation.

To the best of our knowledge this is the first time that virus-like particles have been found in association with moulds belonging to the form class Fungi Imperfecti. It is possible that two disease abnormalities of Fungi Imperfecti are caused by viruses. Lindberg<sup>5</sup> has reported the presence of a transmissible agent in *Helminthosporium victoriae*, and Jinks<sup>6</sup> has suggested that a suppressive cytoplasmic gene, which may be a virus<sup>7</sup>, causes an infectious condition in *Aspergillus glaucus*. In neither of these instances, however, has the pathogenic agent been identified. Lindegren has reported the occurrence of a phage in yeast<sup>8,9</sup>; however, micrographs of particles identified as the phage reveal structures lacking the uniformity normally associated with viruses. Hollings *et al.*<sup>10,11</sup> have clearly identified the virus that causes die-back disease in cultivated mushrooms (*Homobasidiomycetes*).

Tsuda and Tatum observed large hexagonal intracellular inclusions of ergosterol in *Neurospora crassa*<sup>12</sup>. We detected ergosterol in our heavy fraction, but it was possible to remove it by either chloroform or ether without significant loss of activity or particles.

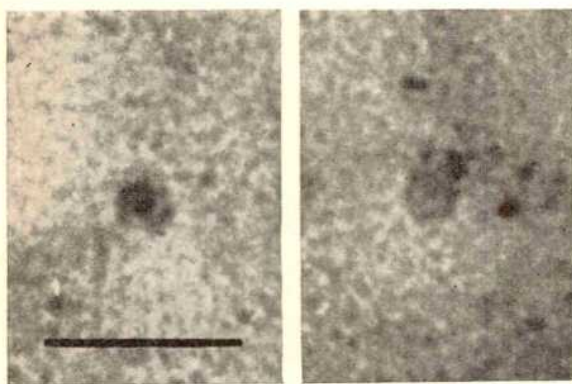


Fig. 2. Two particles from a thin section of *P. stoloniferum* hyphae. Fixed with glutaraldehyde and then with osmium tetroxide. Scale equals 0.1  $\mu$ .

Viruses of fungi may not be so rare as was once thought<sup>13,14</sup> and it is possible that the problem lies in distinguishing them from other cellular components. Interferon assays, in combination with centrifugation on a density gradient and electron microscopic studies, may be helpful in revealing the existence of other viruses of fungi.

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## PHYSIOLOGY

### Need for Calcium in Isoprenaline-induced Relaxation of the Depolarized Rat Uterus

THE contraction of depolarized smooth muscle induced by acetylcholine requires calcium<sup>1-3</sup>, and it has been proposed that calcium is released from a bound site and acts as an intracellular transmitter of the acetylcholine effect<sup>4,5</sup>. More recently, evidence has been presented that calcium may also be required for the action of a relaxant drug, isoprenaline, in depolarized smooth muscle<sup>6</sup>. This

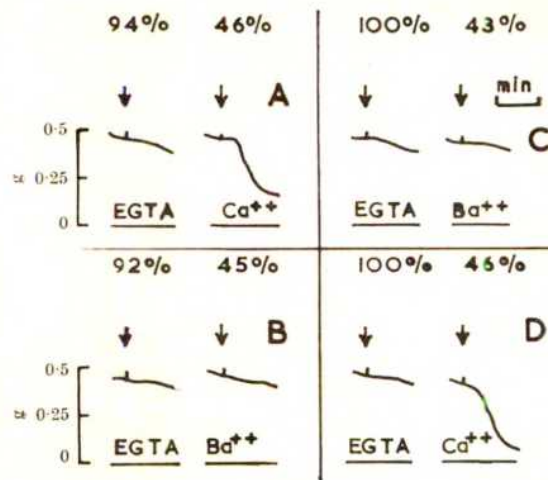


Fig. 1. Isometric tension of isolated rat uterus in potassium chloride-Ringer, 25° C. Relaxation by isoprenaline (iso) given at arrows is inhibited in the presence of EGTA ( $10^{-3}$  molar). Calcium chloride ( $2 \times 10^{-3}$  molar) causes the muscle to shorten isotonically to about half its original length and restores iso responses. Barium chloride ( $2 \times 10^{-3}$  molar) produces equal shortening but fails to restore iso responses. Panels A, C, one horn; B, D, other horn. Between panels A, B and C, D, there was an interval of 80 min during which the preparation was repolarized in Tyrode solution and then again depolarized. Percentages on the top of panels are for the length of muscle in terms of maximum length under EGTA as measured on a Vernier scale. Concentrations of iso  $5 \times 10^{-8}$  in A, B;  $2 \times 10^{-8}$  in C, D.



communication provides further evidence of the essential part played by calcium in isoprenaline-induced relaxation of the depolarized rat uterus.

When an isolated rat uterus which has been depolarized by immersion in sodium-free potassium chloride- or potassium sulphate-Ringer is treated with isoprenaline it relaxes, but if a calcium chelating agent such as EDTA (ethylenediamine tetraacetic acid) or the more specific EGTA (ethyleneglycolbis - aminoethylether - tetraacetic acid) has previously been added to the solution no relaxation occurs with isoprenaline<sup>6</sup>. Chelating agents also abolish papaverine-induced relaxation. These effects of chelation seem to suggest that the removal of calcium from a strategic site has rendered the relaxant drugs ineffective, but because chelating agents themselves relax the depolarized uterus it could be objected that the failure of isoprenaline to relax further is simply due to the contractile element being already fully extended. This objection is disproved by the following experiment (Fig. 1).

The two horns of an isolated rat uterus were suspended in calcium-free potassium chloride-Ringer (145 mmolar potassium chloride, 12 mmolar potassium bicarbonate, 6 mmolar glucose) and treated with  $10^{-3}$  molar EGTA until they ceased to respond to isoprenaline. At this point calcium was added to the bathing solution of one horn and barium to the other, whereon both horns shortened maximally. After readjustment to the original tension isoprenaline was applied which then produced a relaxation in the horn treated with calcium but none in the horn treated with barium. The experiment was then repeated in a crossover arrangement with identical results.

The finding that isoprenaline relaxes the muscle contracted by the calcium but not that contracted by the barium suggests that this relaxation involves a mechanism which is specifically adapted to calcium. (In this connexion, it is of interest that I have found barium also to be a rather inefficient substitute for calcium in supporting the contractile effect of acetylcholine in the depolarized muscle.) It is a possibility that calcium is removed from the contractile element during isoprenaline relaxation and the question arises whether it is then taken up by an intracellular storage site or extruded into the extracellular space. An intracellular process seems functionally more probable in spite of the lack of a prominent endoplasmic reticulum in smooth muscle<sup>7</sup>; thus it has been found that isoprenaline relaxes the depolarized smooth muscle irrespective of the magnitude of the external calcium concentration<sup>6</sup>. An attractive hypothesis is that regional intracellular calcium shifts of opposite sign underlie contraction by acetylcholine and relaxation by isoprenaline of the depolarized uterus.

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### Ionic Requirements of Synaptic Transmitter Release

THE release of a transmitter substance by the nerve impulse depends on the presence of calcium, rather than sodium, on the outside of the axon membrane. There is increasing evidence that inward movement of calcium is one of the first steps in the "electro-secretory" process. This view has been strengthened by recent experiments with tetrodotoxin<sup>1-3</sup>, which eliminates the regenerative

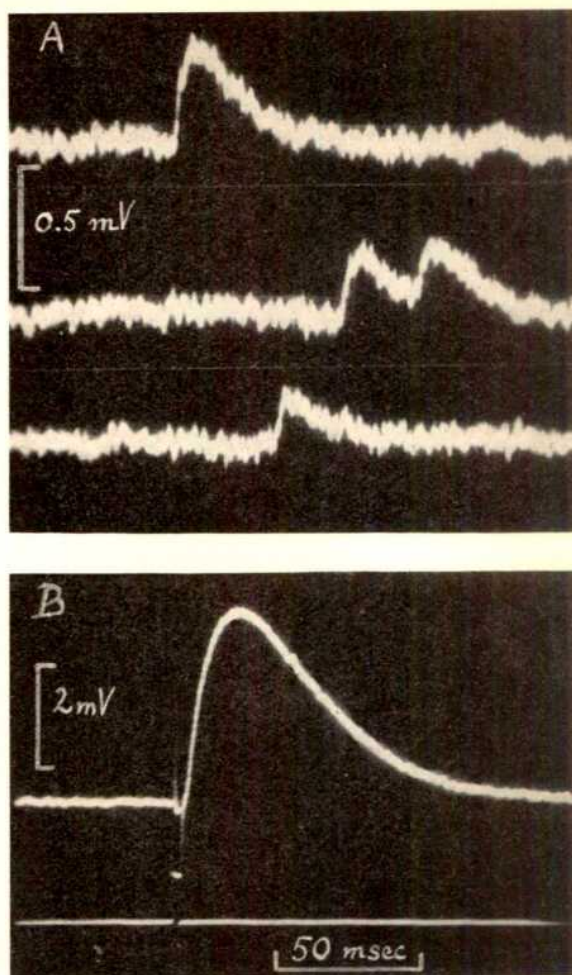


Fig. 1. Intracellular records of spontaneous miniature end-plate potentials (A), and evoked end-plate potential (B), from a frog sartorius kept in "calcium Ringer". Temperature 6.5°C. The lower trace in B shows the depolarizing current pulse (about 2 msec) applied to the nerve ending.

influx of sodium ions but does not interfere with the release of the transmitter by locally imposed depolarization provided calcium ions are present in the external solution.

Further evidence was obtained in an experiment in which a frog sartorius muscle had been equilibrated in a "sodium-free Ringer" containing 83 mmolar calcium chloride, 2 mmolar potassium chloride, and  $2 \times 10^{-6}$  g/ml. of neostigmine methylsulphate. When intracellular records were obtained from junctional regions of muscle fibres, spontaneous miniature end-plate potentials—though much reduced in size—could still be recorded (Fig. 1A). When the nerve ending was depolarized locally, by passing negative going pulses through a closely applied micro-pipette (filled with 1 molar calcium chloride), end-plate potentials were elicited similar to those previously observed in the tetrodotoxin experiments<sup>1</sup> (Fig. 1B).

Thus release of acetylcholine in response to membrane depolarization occurs in the absence of external sodium, when the sodium ions in the bath have been totally replaced by calcium.

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### Induced Maturation of Gonads in *Aplysia punctata* Cuvier

STUDIES of development and reproduction in marine invertebrates are usually limited to the natural breeding seasons of the animals concerned. In order to overcome this time restriction with regard to *Aplysia punctata* (Gastropoda, Opisthobranchia), and in the hope of establishing a method which might be applicable to other animals, we tried to induce early spawning in *Aplysia* collected from a sublittoral population in Treaddur Bay, Anglesey. These collections were made in early February 1965, when the field sea temperature was  $6.7^{\circ}\text{C}$ . The natural breeding period for this species of *Aplysia* lasts from early May, when the sea temperature is about  $9^{\circ}\text{C}$ , until October, when it is about  $14^{\circ}\text{C}$  (ref. 1).

Thirty small specimens, weighing 1–8 g, representing the recruitment stock which settled in the Treaddur Bay area in early October 1964, were placed in an aquarium. The temperature of the inflowing sea water was increased during a 2 h period from the field sea temperature to an experimental temperature of  $15.0 \pm 0.5^{\circ}\text{C}$ . The animals were supplied daily with an excess of food, consisting mainly of the red alga, *Plocamium coccineum* Lamour. (which forms the bulk of their natural diet), together with smaller amounts of the red alga, *Heterosiphonia plumosa* (Ellis) Batt. Constant daytime illumination was provided.

On the first day (February 6), and at weekly intervals thereafter, two individuals were removed from the experimental group. These animals were weighed, and anaesthetized by injecting 5–10 mg of 'Nembutal' (in 1 ml. of sea water) into the regions of the cerebral ganglia and pericardium. The posterior reproductive complexes were dissected out and preserved for sectioning in either Bouin's fluid or Susa's fixative (both made up in sea water). They were then embedded in ester wax<sup>2</sup>, sectioned at  $5.0\text{--}7.5\mu$  and stained in Masson's trichrome. Control animals were collected every 2 weeks from the natural population and were treated as described for the experimental animals. Gametogenesis was then compared in the two groups.

The male and female germ cells of *Aplysia* develop side by side in the acini of the ovotestis. Spermatogenesis proceeded rapidly in the experimental animals and by the fourteenth day mature spermatozoa were present in

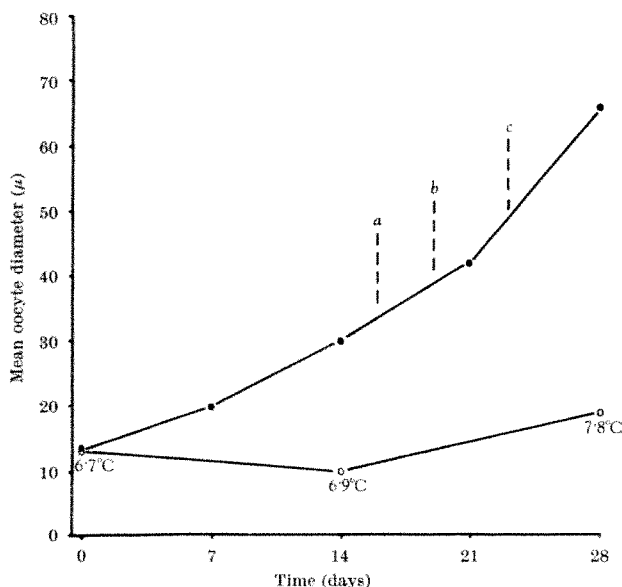


Fig. 1. Change in the mean diameter of oocytes in experimental (held at  $15^{\circ}\text{C}$ ) and field control (field sea temperature shown) groups of *Aplysia punctata* during the 4 weeks from February 6 to March 6, 1965. Where oocytes were asymmetrical, the maximum diameters were recorded. No copulation was seen in the field animals during this time. ●, Experimental; a, first copulation; b, single spawn ribbon; c, continuous spawning to end of experiment. ○, Field control.

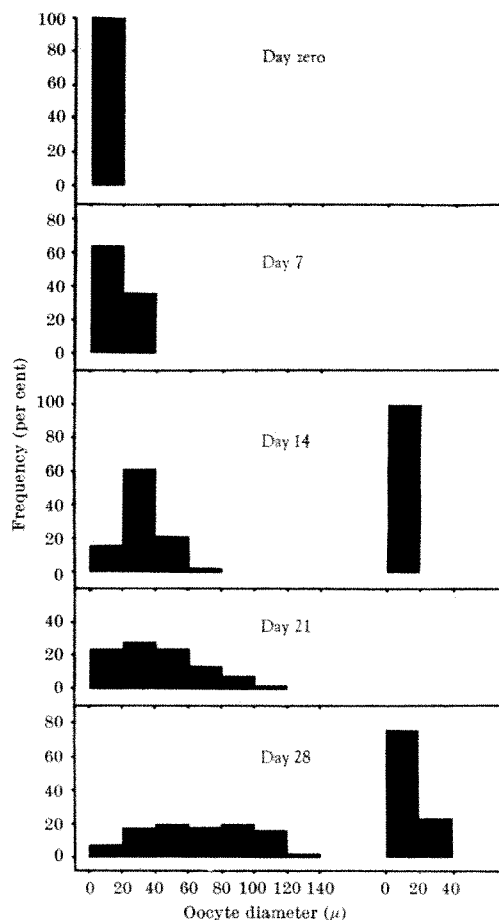


Fig. 2. Size frequency distributions of oocytes in experimental (held at  $15^{\circ}\text{C}$ ) and field control (field sea temperature  $6^{\circ}\text{--}8^{\circ}\text{C}$ ) groups of *Aplysia punctata* during the 4 weeks from February 6 to March 6, 1965.

the small hermaphrodite duct which in *Aplysia* functions as a seminal vesicle. In the control animals, however, male germ cells had not developed beyond the spermatocyte stage even after 28 days. The progress of oogenesis was measured by comparing the maximum diameters of oocytes selected at random from experimental and control animals, using the presence of a germinal vesicle as the criterion for identification of the young oocytes. Fig. 1 shows the comparatively rapid increase in average diameter of the oocytes of experimental animals during 4 weeks, and the change in size distribution of oocytes in each group is illustrated in Fig. 2.

Copulation between animals held at the experimental temperature was first observed on the sixteenth day. At this time the small hermaphrodite ducts of the experimental animals were packed with mature spermatozoa, whereas the oocytes were not fully grown (Fig. 2). This type of protandry, with early copulation, has also been observed in a field population of the cephalaspid, *Retusa obtusa*<sup>3</sup>. Copulation, involving groups of two or three animals, occurred frequently from the sixteenth day until the end of the experiment. After 19 days a single spawn mass was deposited. No further spawning was observed until the twenty-third day, when oviposition occurred in several animals, and numerous spawn masses were deposited each day thereafter (Fig. 1). In comparison, field animals were first seen to copulate in mid-April and spawn was not found in the Treaddur Bay habitat until early May.

Sections of the spermatheca of an animal known to have spawned showed oocytes and spermatozoa in various stages of breakdown. This phenomenon has been described in several other species of opisthobranchiate and pulmonate gastropods<sup>4–6</sup>. All spawn deposited by the experi-

mental animals was viable, and normal veligers hatched after 11–13 days at 17°C.

The experiment was terminated after 4 weeks. During this time, certain of the experimental animals increased in weight from 8 g or less to more than 18 g, while the average weight of the field animals increased from 6 g to only 8 g (ref. 1).

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## Oxygen Transport by Blood in Relation to Body Size

AN understanding of the mechanisms of oxygen transport in mammals requires a knowledge of the rate of oxygen consumption (which represents the quantity of oxygen transported), the arteriovenous oxygen concentration difference (which expresses the amount of oxygen delivered per unit of circulatory load), and the oxygen tension in mixed venous blood (which expresses the "activity" of oxygen in capillary blood). These factors have previously been compared in resting, unanaesthetized sheep and humans and a striking similarity of all three measurements was noted despite great species differences in blood haemoglobin concentration and oxygen affinity<sup>1</sup>.

A wide range of metabolic rates (expressed as oxygen consumption per kilogram body weight) is observed in different mammals and is inversely related to adult body weight. This communication compares the circulatory and haematological characteristics of oxygen supply at rest in species varying greatly in body weight, using previously published results.

In all cases the results were obtained using resting, unanaesthetized animals. Calculation of the oxygen tension in mixed venous blood requires a knowledge of the percentage saturation of mixed venous blood, and the shape of the oxyhaemoglobin dissociation curve at the appropriate body temperature and pH. Because several of the results were incomplete, it has sometimes been necessary to assume one or more of the following: (1) that the pH of mixed venous blood was 7.40 or that the carbon dioxide tension in mixed venous blood was 40 mm of mercury; (2) that the oxygen saturation of arterial blood was 97 per cent; (3) that the body temperature of the species was used by the original workers to construct the blood oxyhaemoglobin dissociation curve. The first of

these assumptions may err in a direction which leads to underestimates of mixed venous oxygen tension.

It can be seen (Table 1) that with the increase of 2,000 times in body weight from ground squirrel to cattle there is a decrease of five times in the rate of oxygen consumption per kilogram body weight. The greater tissue demand for oxygen in the smaller animals is supplied partly by increasing cardiac output per unit of body weight (as much as three times) and partly by increasing the arteriovenous oxygen difference (as much as 1.7 times). Despite the range of blood flow per unit of body weight and arteriovenous oxygen concentration difference, the oxygen tension in mixed venous blood of the seven species listed varies only from 34 to 41 mm of mercury and there is no apparent trend with body size. This relative stability of oxygen tension in mixed venous blood is indeed remarkable when the variations in the blood haemoglobin concentration and the affinity of the blood for oxygen (expressed as  $P_{50}$ , the oxygen tension necessary to half-saturate the blood with oxygen) are taken into account.

The oxygen tension of mixed venous blood reflects the oxygen tension in capillary blood, not of any particular tissue but of a hypothetical integrated mean body tissue. An assumption made in using this as an index for comparing the "mean capillary  $P_{O_2}$ " between species is that there is a relatively constant activity of homologous tissues at rest in the different species. The oxygen tension in capillary blood is directly related to the pressure head for diffusion of oxygen to the sites of its utilization within the cells. Looked at in this way, smaller animals are able to deliver more oxygen to their tissues although the pressure head for oxygen delivery from capillary blood to tissue cells is not demonstrably higher than that seen in larger animals.

On the basis of measurements on three species, Krogh<sup>11</sup> suggested that smaller animals supply the additional oxygen per unit of tissue weight by having smaller intercapillary distances than larger animals. Schmidt-Nielsen and Pennycuik<sup>12</sup>, however, were only able to show an increase in capillarity in very small mammals. Schmidt-Nielsen and Larimer<sup>9</sup> suggested that a mechanism for supplying the higher oxygen need in smaller mammals might be a higher unloading tension for oxygen, because at the same percentage saturation the oxygen tension is usually greater in the smaller animals. We believe, however, that the concept of a constant percentage oxygen saturation in mixed venous (or capillary) blood is an unrealistic criterion for comparing *in vivo* unloading tensions in species of different sizes, because the arteriovenous oxygen difference and oxygen capacity vary among species, and the latter factor is not related to body weight.

We wish to support Krogh's<sup>11</sup> original hypothesis; the relationship between oxygen consumption and body weight is usually presented on logarithmic axes, and then appears as a straight line. In Fig. 1 the relationship is presented on arithmetic axes and an interesting point emerges from the resulting hyperbola: not until body weight decreases below about 1 kg do great increases in oxygen consumption per unit of body weight occur. In this region of animal size (rat, mouse and bat), Schmidt-Nielsen and Pennycuik<sup>12</sup> demonstrated an inverse relationship between muscle capillary density and body weight. They failed to find any distinct relationship in species above this body

Table 1. OXYGEN TRANSPORT BY BLOOD IN VARIOUS MAMMALIAN SPECIES IN THE RESTING STATE

Species	Body weight (kg)	O <sub>2</sub> capacity (vol. %)	$P_{50}$ (mm Hg)	O <sub>2</sub> consumption (ml./kg./min)	Cardiac output (ml./kg./min)	CaO <sub>2</sub> -CvO <sub>2</sub> (vol. %)	$P_{vO_2}$ (mm Hg)	Assumptions*	References
Cattle	414	13.8	30.8	4.2	105	4.0	40	a, c, d	2, 3
Human	75	19.6	28.8	4.1	99	4.1	39.4		4
Sheep	62	11.4	40.6	4.4	96	4.7	40.6		1
Dog	22	19.6	29.1	9.6	154	6.3	36	a, c, d	3, 5
Rabbit	1.9	16.5	34	13.2	218	6.1	38	a, e	3, 6, 7
Rat	0.4	19.6	32	18.3	286	6.4	40	b, c, d	8, 9
Ground squirrel	0.22	19.5	26.8	20.1	313	6.6	34	a, e	10

\* Assumptions made for calculation of mixed venous blood O<sub>2</sub> tension are identified as follows: a, mixed venous blood pH = 7.40; b, mixed venous blood carbon dioxide tension = 40 mm of mercury; c, arterial blood saturation = 97 per cent; d, body temperature = 37°C; e, body temperature = 39°C.

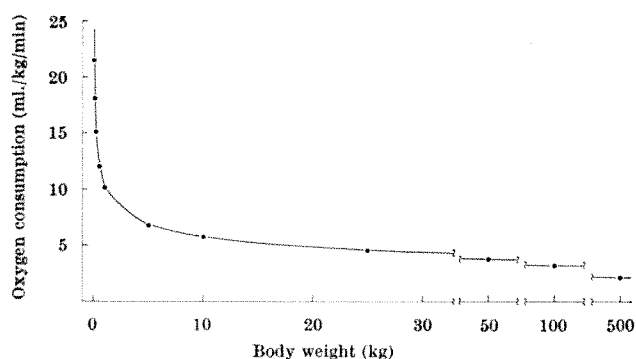


Fig. 1. Relationship between body weight (kg) of mammals and total resting oxygen consumption (ml./kg/min), based on the equation of Kleiber<sup>12</sup>.

weight. One could postulate that only in animals below a certain size (about 1 kg) does the increased oxygen need of smaller species reach a magnitude where a detectably greater capillary density is needed. In animals above 1 kg in weight variations with size in the rate of oxygen delivery per kilogram are small, and similar magnitudes of variation in capillary density may not be detectable by the present methods.

We have made no attempt to extend the generalization of similarity of mean capillary blood oxygen tension to conditions other than those seen in the resting, unanaesthetized animal. Neither have we extended the relationships noted here to specific organs, tissues or cellular components of the body.

From a comparative standpoint, it is not surprising that mixed venous (and capillary) blood  $P_{O_2}$  is maintained at similar levels over the small range of evolution represented by these mammals. It would, rather, be surprising to find, in view of the identity of respiratory enzymes over much wider ranges of evolution, that wide variations in capillary oxygen tension did occur under resting conditions.

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### Stimulus Response Curves of Single Carotid Body Chemoreceptor Afferent Fibres

STUDIES of the characteristics of activity recorded from single carotid body chemoreceptor afferent fibres have so far been concerned with the statistics of the pattern of discharge and the effects of drugs<sup>1-3</sup>. There

has been no adequate description of the activity of single fibres in response to independent changes in blood gas tensions and pH for extended ranges, and it is unclear whether or not the multifibre response curves described by Hornbein and Roos<sup>4</sup>, for example, are composed of a spectrum of differently shaped single fibre response curves showing an array of thresholds. Accordingly, we have investigated the effects of changes in arterial  $O_2$  and  $CO_2$  tensions and pH on the rate of discharge of single chemoreceptor afferent fibres dissected from the sinus nerve of the cat. The animals were anaesthetized with 0.6 ml./kg of 'Dial-Urethane', paralysed with 5 mg/kg of gallamine triethiodide and artificially ventilated. The arterial blood pressure and oxygen tension and end-tidal carbon dioxide tension were continuously recorded on a Grass polygraph. Arterial blood samples were taken for measurement of pH and carbon dioxide tension, which were changed by intravenous administration of 1 molar sodium bicarbonate, 1 molar ammonium chloride or 0.1 normal hydrochloric acid and by appropriate changes in rate and volume of artificial ventilation. The arterial oxygen tension was altered by varying the oxygen content of the inspired gas. Single chemoreceptor afferent nerve impulses were displayed on an oscilloscope and their frequency of discharge was monitored with a ratemeter whose output was recorded on the Grass polygraph. After a change in arterial  $PO_2$ , and, in particular, after a change in either  $PCO_2$  or pH, an average 15–20 min were spent in adjusting the respiratory pump level or infusion until a new steady level of discharge, measured with the ratemeter, was obtained. After a further 5–10 min at this level, the fibre activity was photographed on moving film for 20 sec periods. The numbers of potentials in 10 sec periods were later counted from the filmed records and these counts were used to construct the graphs which show response curves.

The responses of thirteen single chemoreceptor afferent fibres (from five cats) to changes in arterial oxygen tension were recorded at constant arterial blood pH and  $PCO_2$ . Each graph of discharge rate against oxygen tension for a single fibre was similar to a rectangular hyperbola (Fig. 1). In general, the rate of change of discharge with an incremental fall in arterial oxygen tension was almost zero at high oxygen tensions (above 150–250 mm of mercury and up to 650 mm of mercury). Thus response curves of single units formed a spectrum of curves such that below a characteristic  $P_{aO_2}$  in the range 150–250 mm of mercury (mean 180 mm of mercury) the rate of discharge increased progressively from 0.5–5 impulses/sec to 20–25 impulses/sec at arterial oxygen tensions of 25–50 mm of mercury. The rate of discharge at high oxygen

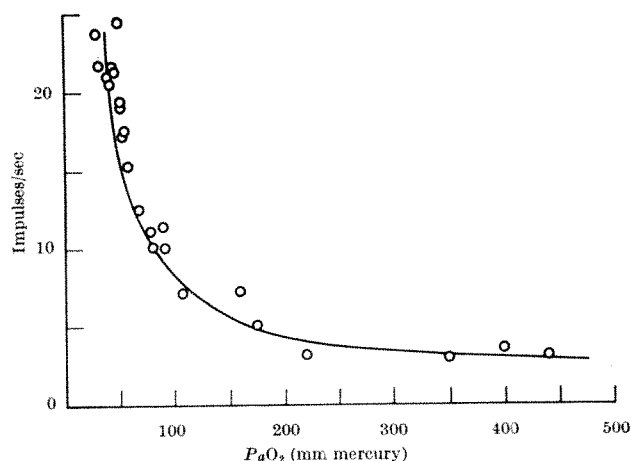


Fig. 1. Graph of activity (i.p.s.) against  $P_{aO_2}$  (mm of mercury) for a single chemoreceptor afferent fibre dissected from the cat sinus nerve. The  $P_{aCO_2}$  was 37 mm of mercury throughout. The line was drawn by eye.

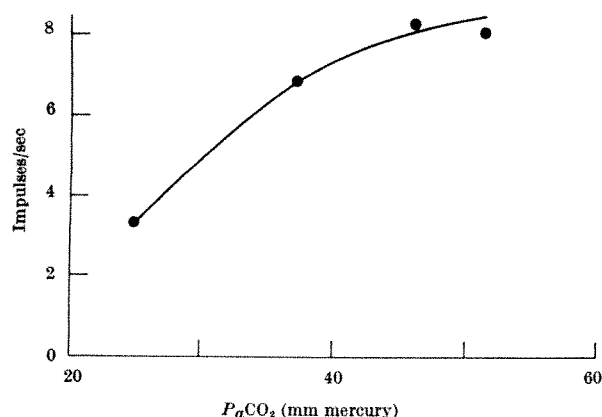


Fig. 2. Graph of activity (impulses/sec) plotted against alveolar carbon dioxide tension (mm of mercury) for a single chemoreceptor afferent fibre of the cat sinus nerve. The  $P_a\text{O}_2$  was 115 mm of mercury, and the arterial pH was 7.30 throughout. The line was drawn by eye.

tensions was in part dependent on the  $P_a\text{CO}_2$  but may also in part be a property of the receptor complex leading to the afferent fibre being investigated. The response curve was shifted to the left by a decrease in  $P_a\text{CO}_2$  or an increase in pH and to the right by opposite changes in these variables. In addition, the discharge persisted at a  $P_a\text{O}_2$  of 650 mm of mercury even when the pH was increased to 7.6. Thus the chemoreceptors could not be shown to have a threshold oxygen tension at which firing commences.

There is a conflict in the literature over the question of the effect of carbon dioxide on the activity of chemoreceptor afferents. Joels and Neil<sup>5</sup> reported that this gas excited these afferents, but Hornbein and Roos<sup>4</sup> did not confirm this. We have tested the effect of changes in carbon dioxide *in vivo* for the range 20–60 mm of mercury while the arterial pH and  $P_a\text{O}_2$  were kept constant. The activity of single chemoreceptor fibres increased with the  $P_a\text{CO}_2$  (Fig. 2) and the slope of this increase depended in part on the pH and was sometimes steeper at a low pH (7.0–7.1) than at a high pH (7.5–7.6). The increase in response to increased carbon dioxide was observed at oxygen tensions up to 250 mm of mercury, the highest  $P_a\text{O}_2$  studied.

The effects of changes in arterial pH on the activity recorded from single fibres at constant  $P_a\text{CO}_2$  and  $P_a\text{O}_2$  were also examined. For the range of pH 6.9–7.6 there was always an increase in the discharge as the pH was decreased. The graph relating the two parameters was usually linear, but in some cases the slope increased as the pH became less than 7.1.

In the whole animal, there are factors that can complicate the relation between rate of chemoreceptor discharge and a given stimulus. Thus at low oxygen tensions it is difficult to obtain a stable arterial blood pressure and, further, there may also be an increase in sympathetic activity, both of which can increase the activity of chemoreceptors<sup>6–8</sup>. The same may also apply to extremes of  $P_a\text{CO}_2$  and arterial pH. Thus the shapes of the response curves *in vivo* are contingent on a number of other variables and do not necessarily represent accurately the response characteristics of the receptor. What is demonstrated is the complex nature of the response of the carotid body for the normal working range of the modalities that excite its activity.

Our experiments show that the response curve relating discharges of the chemoreceptor of the carotid body to changes in oxygen tension is a continuous one. Each individual response curve has a hyperbolic form, and the hyperbola found with multifibre strands does not arise from the superposition of single unit response curves of non-hyperbolic form showing clear cut thresholds. An increase in nerve activity above basal activity com-

mences at a point which varies among fibres and could perhaps be the threshold of the carotid body for the reflexes evoked by changes in oxygen tension, a reflex threshold to be distinguished from a receptor threshold. There seems to be no chemoreceptor threshold for  $\text{PCO}_2$  and pH, for the response is approximately linear in the ranges investigated. Clearly the threshold for any reflex effects produced by excitation of these afferents is also dependent on the behaviour of neurones in the central nervous system.

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## RADIOBIOLOGY

### Age Dependence of Radiation Sensitivity of Haemopoietic Cells in the Mouse

DURING a long-term study of the effect of age at exposure on the incidence of leukaemia after whole-body irradiation, the age dependence of cell killing of the haemopoietic cells of the mouse was investigated. The variation of radiosensitivity with age has been described previously<sup>1</sup>, using as an end-point the  $LD_{50/30}$  days mortality, and might reflect a variation of the radiosensitivity of haemopoietic cells, because death after midlethal doses of radiation is caused mainly by their injury. To test the radiosensitivity of these cells and its dependence on age the following investigation was carried out.

SAS/4 mice, male and female, of two age groups (6 and 30 weeks old) were used. Two methods for assaying haemopoietic stem-cell survival were employed—techniques which involve the endogenous and the exogenous (or transplantation) spleen colonies, first described by McCulloch and Till<sup>2,3</sup>. The technique involving endogenous colonies is based on the fact that mice irradiated with doses of 400–900 rads develop macroscopically visible nodules (colonies) in their spleens approximately 10 days after irradiation. In the technique involving exogenous spleen colonies recipient mice were first exposed to a whole-body dose of radiation which reduced their endogenous spleen colonies to a very small number. Between 2 and 6 h later they received by intravenous injection a known number of nucleated bone marrow cells, obtained from donor mice, which had been exposed to doses of between 0 and 500 rads. Ten days later the mice were killed and the number of colonies in their spleens were counted.

The mice were irradiated with the electron beam of the 15 MeV linear accelerator at Saint Bartholomew's Hospital, at a dose rate of approximately 400 rads/sec. Four mice were irradiated at a time, each in individual 'Perspex' tubes. The remote control mouse machine, previously described, was used for location of each box in the correct position for the irradiation<sup>4</sup>.



The number of mice surviving ten days after irradiation was as follows: 122/179 (69 per cent) for 6 week old mice and 116/171 (67 per cent) for 30 week old mice, when the exogenous technique was used; and 80/108 (74 per cent) for 6 week old mice and 133/155 (86 per cent) for 30 week old mice with the endogenous technique. These values are within the upper limits of what is described as excellent survival in the best circumstances<sup>5</sup>.

For the endogenous technique, the results were expressed as the observed number of endogenous colonies after irradiation as a function of dose. When the exogenous technique was used, the results were expressed as the fractional survival of the transplanted colony-forming cells, exposed to different doses.

The combined results for both techniques are shown in Fig. 1a and b. They have been expressed in the form of cell survival curves because each colony derives from one single cell. Cytological evidence for this is given in the literature<sup>6</sup>.

The results from both techniques were combined, because the slopes of the survival curves were found to be the same within statistical errors. Thus for the 6 week old group, the  $D_0$  values were 93 and 95 rads for the exogenous and endogenous techniques respectively. The corresponding results for the 30 week group were 122 and 129 rads. The weighted average  $D_0$  values obtained from Fig. 1a and b are  $93.9 \pm 4.6$  rads and  $126.2 \pm 6.0$  rads for the 6 week and 30 week groups respectively.

This difference between the  $D_0$  values for the two ages is statistically highly significant, and fits well with the previously reported age dependence of radiation sensitivity at these ages using the  $LD_{50/30}$  days as the end point<sup>1</sup>. The data are also in general agreement with those given in the literature for the same or similar types of cells (compare refs. 7 and 8) although no dependence on age of the radiation sensitivity of these cells has previously been reported, except for colony-forming cells deriving from foetal liver<sup>9,10</sup>. In this system, however, there are many other biological differences in addition to the age. For example, it has been shown that a large proportion (about 40 per cent) of colony-forming cells of foetal liver are in the synthetic phase of the cell cycle, whereas for normal adult bone marrow the proportion of such cells is very small<sup>11</sup>.

The data of Fig. 1a and b give extrapolation numbers of  $2.47 \pm 0.64$  and  $1.42 \pm 0.33$  for the 6 week and 30 week groups respectively. More measurements are required to determine whether this difference is significant. If it were, it might be explained as the result of the accumulation of "faults" with age, thus necessitating fewer events to inactivate the cells in older mice<sup>12</sup>.

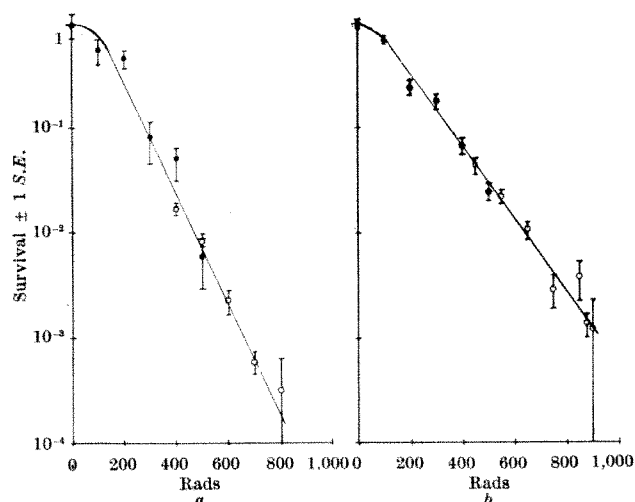


Fig. 1. Survival curve for cells from mice exposed at the age of (a) 6 weeks, and (b) 30 weeks. ●, Exogenous technique; ○, endogenous technique.

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### Prevention of Radioleukaemia by Lymph Node Shielding

IN mice subjected to a schedule of irradiation normally producing a high yield of leukaemia of the thymic lymphoma type, it has been shown that the administration of foetal haemopoietic tissue leads to the presence of dividing cells (which have the chromosome characteristics of the donor almost exclusively) in the target organs of the hosts<sup>1</sup>. A related finding is a significant drop in the mortality from leukaemia. Kaplan has attributed the phenomenon of a lowered incidence of radioleukaemia resulting from the intravenous administration of bone marrow to a bone marrow factor<sup>2</sup>. It has been thought, as a more recent alternative, that inhibition of radioleukaemia follows repopulation of haemopoietic tissues and thymus by unirradiated donor cells<sup>3</sup>. The incidence of the leukaemia induced by radiation which appears to have its origin within the thymus is paradoxically unaltered by the intravenous administration of lymph node material in dissociated form. Other cells intimately associated with the lymph node but difficult to dissociate from its structure may have been excluded in the preparation of such lymph node inocula. Because of this exclusion, and because feedback mechanisms may implicate the endothelial cells of the post-capillary vessels in lymph nodes and perhaps littoral cells<sup>4</sup>, the place of whole lymph node *in situ* in altering the pattern of leukaemia induction was tested.

Exteriorization of lymph nodes<sup>5</sup> as an open wound procedure was not suitable because, in order to induce an incidence of approximately 70 per cent of leukaemia in *C57BL* hybrids, it is necessary to give four divided fractions of 200 rads  $\gamma$ -radiation at intervals of 4 days. Under sodium amytal anaesthesia an incision was made over the lumbar spine and blunt dissection carried down in the subcutaneous tissue until the inguinal node on the right side was exposed. Under direct vision the node was incorporated, by the teeth of a 13-mm Michel clip fastened from outside, in a fold of skin. The dorsal incision was then closed with silk. Again under anaesthesia on each of four occasions, a silk thread was passed through the loop of the Michel clip and traction applied to it so that the fold of skin containing the lymph node was manoeuvred under a 5-cm thick lead block. The lead block was recessed to take the clip and fold of skin. Ties to the extremities and the tail enabled the remainder of the body of the mouse to be pulled clear of the lead shielding during exposure to gamma rays. Seven (*C57BL*  $\times$  *CBA.T6T6*)<sub>F</sub> female hybrids between 30 and 40 days

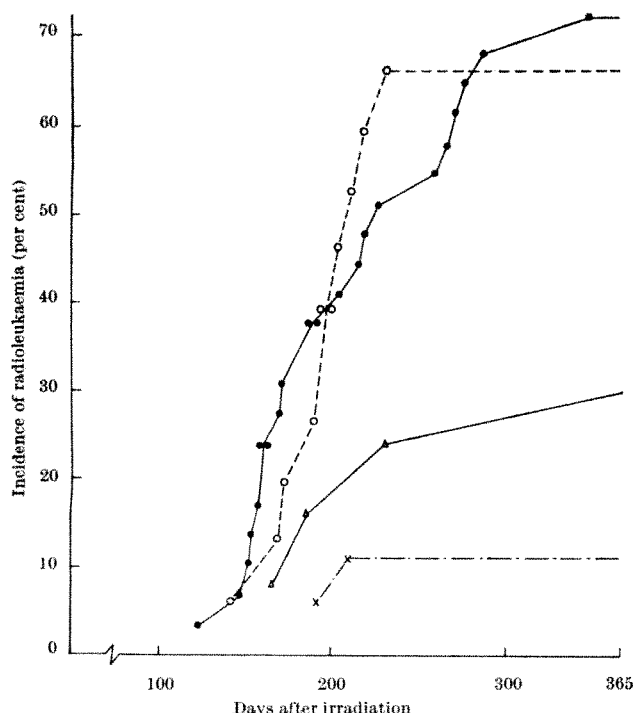


Fig. 1. The incidence of radiouleukaemia in *C57BL* hybrid mice following four fractions of 200 rads  $\gamma$  radiation in relation to time following: no further treatment (●—●); intraperitoneal lymphoid tissue (○—○); intraperitoneal haemopoietic tissue (△—△); lymph node shielding (×—×). (The mice are syngeneic, the *CBA* element having been derived from the backcrossing of *T6T6* mice to *CBA/H* by Dr Mary Lyon.)

of age were subjected to the leukaemogenic schedule of irradiation with a single lymph node excluded from the radiation field by this closed procedure. Death occurred at the following times after irradiation from the following causes: at 231 days, aplastic anaemia; at 576 days, ovarian tumour; at 670 days, reticulosarcoma; at 747 days, mammary tumour; and at 776 days, probably aplastic anaemia. Two mice were still alive at 800 days after irradiation. Approximately 70 per cent of mice given the fractionated radiation without lymph node shielding could be expected to die from thymic lymphoma within 365 days of the last irradiation (Fig. 1). Thirteen and fifteen mice so irradiated received near to  $10^7$  cells of foetal liver and lymph nodes respectively by the intraperitoneal route from (*C57BL*  $\times$  *CBA/H*) $F_1$  female hybrids. As expected, the incidence of radiouleukaemia remained high in the latter. A confirmatory experiment involving thirteen (*C57BL*  $\times$  *CBA.T6T6*) $F_1$  females between 30 and 40 days of age given the leukaemogenic schedule of irradiation and amytal anaesthesia during irradiation, and eleven so treated but with a single lymph node excluded from the irradiation field, gave the following results: 365 days after irradiation six of the former had succumbed to thymic tumours (at 141, 153, 157, 162, 171 and 230 days) and two only of the latter (at 192 and 207 days). Combining the results of the experiments, two of eighteen mice irradiated with a lymph node shielded died from thymic lymphoma (an incidence of some 11 per cent).

It is well known that lymphoid tissue is among the most sensitive to ionizing radiations and it seems clear from these experiments that maintenance of even a small amount by shielding during irradiation is a potent factor in hindering the onset of radiouleukaemia. The place of bone marrow<sup>2</sup> has dominated discussion in this field, although the efficacy of spleen shielding had been demonstrated by Lorenz, Congdon and Uphoff<sup>3</sup>, and Wallis, Davies and Koller<sup>4</sup> have recently reported the effectiveness of spleen cells administered intravenously. Bone marrow

colonization is proceeding within a week of bone marrow grafting, but it is not until three weeks later that an increased mitotic rate in the thymus enables the penetration of donor cells into this organ to be seen<sup>1</sup>. Failure of haemopoietic tissue entirely to prevent lymphoma may result from failure of the colonizing cells completely to outgrow the persisting cells of the host exposed to the leukaemogen, with or without a subcellular mechanism operating. Also during the third week after irradiation the weight of the lymph nodes approaches pre-irradiation levels and may incidentally be a pointer towards direct colonization of lymph nodes from the marrow without a thymic intermediate phase. It seems that early restoration of some immunologically competent cells following haemopoietic grafting is likely to be the important factor and may be the mechanism lowering the incidence of radiouleukaemia by contending with rogue cells or preventing activation of latent virus.

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## CYTOLOGY

### "Phagocytic" Lysosomes in Chromatolytic Neurones

SEVERAL investigators have demonstrated that cells possess a mechanism for the bulk segregation and digestion of portions of their own cytoplasm. Such a mechanism is generally known as "cellular autophagy"<sup>1</sup>, and the digestion of endogenous material is accomplished within vacuoles limited by membranes known as cytolysosomes<sup>2</sup>, cytogrosgosomes<sup>3</sup> or autophagic vacuoles<sup>4</sup>, which show a positive staining reaction for acid phosphatase.

The origin of the membrane surrounding autophagic vacuoles is a matter for speculation. The suggested sources include Golgi cisternae<sup>5</sup> and agranular endoplasmic reticulum<sup>6,7</sup>, or by *de novo* synthesis in the ground cytoplasm<sup>8,9</sup>. The manner in which acid hydrolases enter autophagic vacuoles is also speculative. According to Novikoff *et al.*<sup>6</sup> the enveloping membrane is formed by fusion of vesicles which actually contain the hydrolases although fusion with another lysosome has also been suggested<sup>5</sup>.

Considerable cellular autophagy occurs in neurones after axon section. During an investigation into the fine structural changes associated with axon section in neurones of the superior cervical ganglia in the rabbit, a new mode of formation of autophagic vacuoles was revealed and is reported here. The sectioned ganglia were fixed in acetate-veronal buffered osmium tetroxide, embedded in 'Araldite', and all thin sections were stained with lead citrate.

The neurones responded promptly to axon section by an increased production of small, dense bodies identified as primary lysosomes by the localization of acid phosphatase activity within them, using the Gomori lead phosphate technique<sup>10</sup>.

After 7 days the primary lysosomes had increased in size and were either round, ovoid or disk-shaped with



the periphery thicker than the central region (Fig. 1). Frequently the disks were curved, presenting goblet-shaped profiles (Fig. 2). In many instances a primary lysosome had completely enclosed a small region of cytoplasm and presented a ring-shaped profile the centre of which contained clearly recognizable ribosomes (Fig. 3). Primary lysosomes were also frequently observed partially surrounding mitochondria. Fourteen days after axon section the preponderant type of lysosome was the "cytosome", or irregularly shaped structure bound by a membrane and containing regions of variable electron density, dense membranes and usually a band of extremely dense material around all or part of the periphery (Fig. 4).

These observations suggest that, in chromatolytic neurones, primary lysosomes behave in a "phagocytic" manner, engulfing small regions of cytoplasm, clusters of ribosomes and possibly even mitochondria. By this mechanism structures are formed which are clearly autophagic vacuoles, for they contain recognizable cell organelles, possess acid phosphatase activity and are surrounded by a membrane. The material that is phagocytosed is contained within a vacuole the membrane of which is part of that of a primary lysosome and therefore, of necessity, impermeable to hydrolytic enzymes. This poses the question of how the acid hydrolases reach the engulfed materials in order to digest them. Some change in property of the membrane may occur, allowing diffusion of enzymes into the enclosed region, or the membrane

may dissolve. The next stage in the process of digestion is then represented by cytosomes.

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### Evidence of Age Aneuploidy in the Chromosomes of the Sheep

VARIATIONS in chromosome counts associated with age were first shown in human leucocyte cultures by Jacobs *et al.*<sup>1</sup>. I have examined the chromosomes of twenty-two normal ewes and rams and six freemartin sheep in preparations made by a method similar to that of Basrur and Gilman<sup>2</sup>, and have counted chromosomes in at least fifty metaphases in each case. Several cultures were prepared from each freemartin and were then counted to see if there was a relationship between XY cells and the degree of masculinization as reported before<sup>3,4</sup>; these results are reported elsewhere<sup>5</sup>. In none of the twenty-two normal sheep was any chromosome abnormality observed; in the freemartin sheep, however, there was XX and XY white blood cell chimaerism. The chromosome counts of the six freemartin sheep are recorded in Table 1 and the percentage of hypomodal and hypermodal modal cells in each of these are given in Table 2.

Most of the normal sheep were lambs less than 1 yr old; the oldest case was a Suffolk ram aged 2 yr. Only one freemartin was younger than 2 yr old (Table 2) and the others were aged from 4 to more than 7 yr. In most countries breeding ewes are culled after three or four mating seasons, which means that the usual culling age is 6 yr. While sheep are recorded as living for up to 15 yr<sup>6</sup> it must be assumed that a culling age of 6 yr has evolved with time and that the majority of sheep age rapidly after this. This is usually shown by wear of the teeth. It is reasonable to assume, therefore, that the five freemartin sheep aged 4-7 yr were significantly older than the twenty-two normal sheep which I used.

When the chromosome counts of the five older freemartin sheep were compared with those of the normal

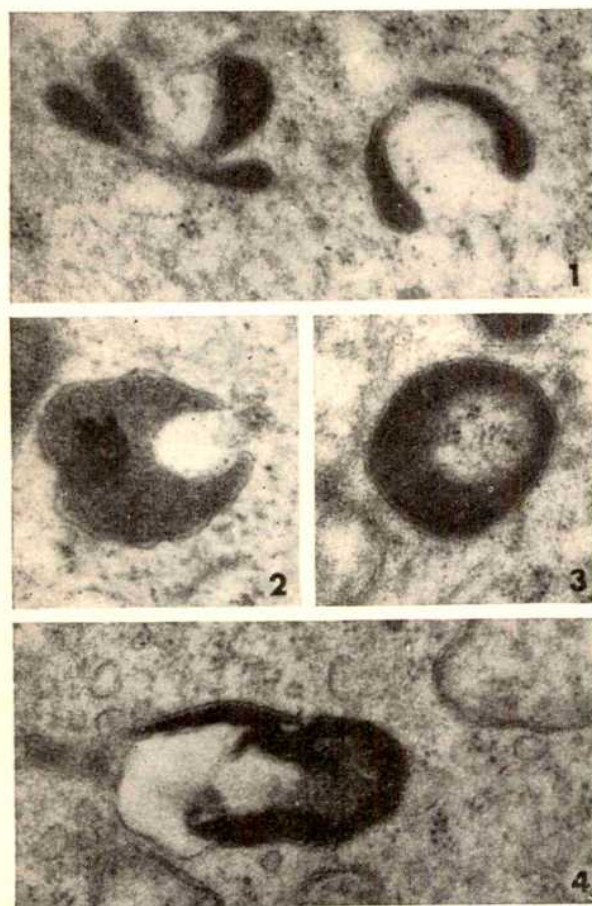


Fig. 1. Curved, disk-shaped primary lysosomes in a neurone 7 days after axon section. ( $\times 48,000$ .)

Fig. 2. A "phagocytic" lysosome containing dense reaction product, indicating the site of acid phosphatase activity. ( $\times 60,000$ .)

Fig. 3. A lysosome with a ring-shaped profile. The enclosed region of cytoplasm contains clusters of ribosomes. ( $\times 48,000$ .)

Fig. 4. A cytosome, resulting from the partial digestion of material engulfed by a primary lysosome. ( $\times 52,000$ .)

Table 1. CHROMOSOME COUNTS FROM LEUCOCYTE CULTURES OF SIX FREEMARTIN SHEEP

Sheep	Chromosome No.							Sex chromosomes	
	50	51	52	53	54	55	Poly	XX	XY
Freemartin No. 1	4	2	2	13	133	3	3	62	75
Freemartin No. 2	7	2	5	18	131	0	4	17	120
Freemartin No. 3	7	3	8	24	151	4	7	43	118
Freemartin No. 4	12	4	21	36	320	8	8	22	314
Freemartin No. 5	2	5	8	20	259	5	4	253	18
Freemartin No. 6	1	1	6	11	186	1	0	80	107
Total cells	33	17	50	122	1,180	21	26	477	752

XX and XY chromosomes were counted on cells with 54 or 55 chromosomes and tetraploid cells only.

Table 2. CHROMOSOME COUNTS FROM SIX FREEMARTIN SHEEP SHOWING PERCENTAGE OF MODAL AND NON-MODAL CELLS

Sheep	Age	No. of cells examined	% Hypo-modal (50-53)	% Modal (54)	% Hyper-modal (55)	% Polyploid (1)
Freemartin 6	18 mths	206	9.22	90.29	0.47	0.0
Freemartin 5	4 yr	303	11.55	85.48	1.65	1.32
Freemartin 4	5 yr	160	13.12	83.13	1.88	1.88
Freemartin 1	5 yr	167	19.16	78.44	0.00	2.39
Freemartin 3	7+ yr	409	17.84	78.24	1.96	1.96
Freemartin 2	7+ yr	204	20.58	74.02	1.97	3.4



Table 3. COMPARISON OF CHROMOSOME COUNTS OF TWENTY-TWO NORMAL SHEEP WITH FIVE FREEMARTIN SHEEP

	Ages	Total cells examined	Hypomodal		Modal (54)		Hypermodal (55)		Polyploid	
			No.	%	No.	%	No.	%	No.	%
Normal sheep	2 years	1,831	198	10.81	1,602	87.49	6	0.33	25	1.37
Freemartin sheep	4-7+ years	1,243	203	16.45	994	79.86	20	1.49	26	2.20
% Difference				+5.64		-7.63		+1.16		+0.83

Comparison of normal and freemartin sheep, regarding this table as a contingency table and using a significance level of 0.05, produced significant values of  $\chi^2$  for the whole table (38.98 with a distribution frequency of 3) and for the proportions of hypomodal, modal and hypermodal cells (19.87, 31.93 and 14.51 respectively with a distribution frequency of 1). The numbers of polyploid cells did not show a significant deviation from their expected values. ( $\chi^2 = 2.40$ , and distribution frequency was 1.)

sheep, a very significant increase in both hypomodal and hypermodal cells was recorded (Table 3). Although small groups of animals are considered it is pertinent that the sixth freemartin sheep, aged 18 months, showed a modal chromosome count which was near to that of the normal younger sheep. In this case only one genuine hypermodal cell was recorded. Furthermore, the two oldest sheep were between 7 and 8 yr old and the number of hypermodal cells (fifty-five chromosomes) was eight and five respectively, which is more than double that recorded from all the normal sheep together. Photographic karyotypes of all the hypermodal cells showed that the extra chromosome was always a member of the acrocentric group (Fig. 1) and both male and female cells were seen.

The biological mechanisms contributing to age aneuploidy have been discussed by Jacobs *et al.*<sup>1</sup> and the same factors could have applied to the sheep. Possibly the increase in aneuploidy was the result of "adaptation" between chimaeric blood cells as considered by Basrur and Stoltz<sup>2</sup> in the bovine freemartin. In the latter case, however, a high percentage of abnormal metacentric cells was seen in all preparations, together with a large number of defective cells showing chromatid breaks, particularly in donor cells. In the freemartin sheep, no observable increase in chromatid breaks was seen in any of the preparations and, out of 1,449 metaphases counted, on only three occasions were abnormal chromosome patterns recorded (Fig. 2). These are apparently seen in human mitotic preparations (personal communication from Ferguson-Smith) and could have arisen in culture in the same way as the translocation reported by Trujillo *et al.*<sup>3</sup>.

It would seem, therefore, that these observations are the same as age aneuploidy reported in man, but they can be

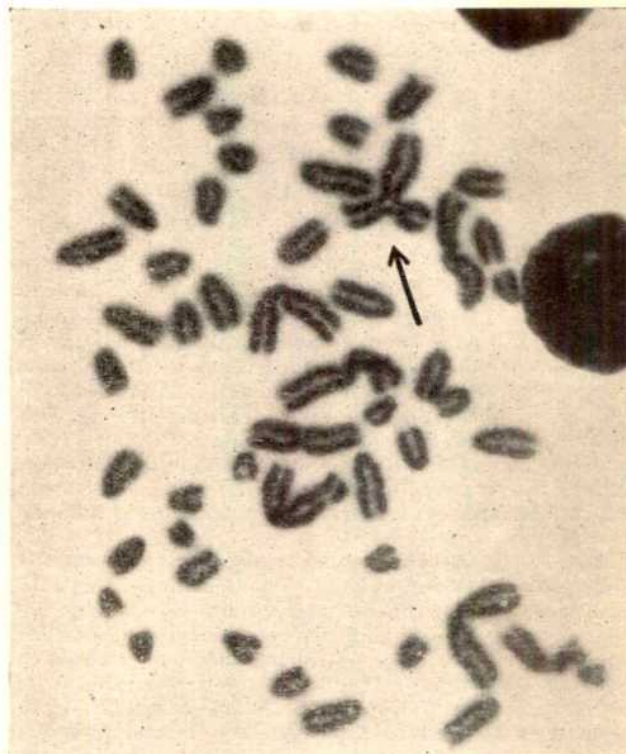


Fig. 2. Triad fusion of three acrocentric chromosomes, presumably caused by breakage and reconstitution of fragments.

confirmed only when larger numbers of normal sheep of differing ages are compared as well. An apparent and interesting difference that has not previously been reported in the sheep or any of the higher domestic animals is, however, suggested.

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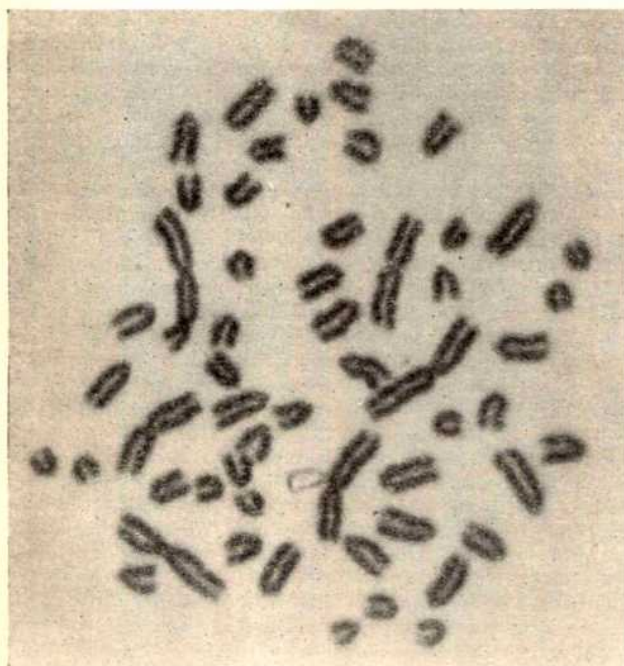


Fig. 1. Hypermodal cell of sheep, showing fifty-five chromosomes. (Normal diploid number, 54.)

### Mitogenic Property of *Wistaria floribunda* Seeds

Two phytomitogens have been described which stimulate blastogenesis and mitosis of human peripheral blood lymphocytes: phytohaemagglutinin (PHA) from *Phaseolus vulgaris*<sup>1</sup> and pokeweed mitogen (PWM) of *Phytolacca americana*<sup>2</sup>. Recently we have discovered mitogenic properties in seed extracts of *Wistaria floribunda* (syn.: *Wisteria floribunda*, Japanese wisteria).



Peripheral blood buffy coat cells of ten normal volunteers were cultured by the method previously described<sup>2</sup> in the presence of saline extracts of *Wistaria floribunda* seeds (final dilution of extract 1:1,500), pokeweed mitogen (Grand Island Biological Company, final dilution 1:1,500), and in the absence of a phyto-mitogen. Cultures were sacrificed at 48 and 72 h and studied for morphology with the Wright-Giemsa stain and for histochemical characteristics.

Haemagglutination was not discernible in cultures prepared with extracts from the seeds of *Wistaria floribunda*, but leukagglutination was apparent. Morphologically and chronologically the blastogenesis occurring in these cultures appeared identical to that induced by pokeweed mitogen. By the time the cells had been cultured for 72 h, mitotic cells were abundant. No plasmacytic differentiation was observed, however, in this culture system.

Metabolically, no distinction could be made between cells undergoing blastogenesis with the extracts from the seeds of *Wistaria* and those responding to pokeweed mitogen or to phytohaemagglutinin. We have recently published a detailed description of the histochemical properties of lymphocytes responding to these mitogens<sup>3</sup>. Acid phosphatase and  $\beta$ -glucuronidase activities were prominent in preblastic lymphocytes; lipid vacuoles were abundant in cells undergoing blastogenesis; glycogen was markedly increased in the responding cells, but rarely present in frank blasts. No  $\alpha$ -amylase stable periodic acid-Schiff positivity was observed during the 72 h culture period. Lymphocytes were never reactive for alkaline phosphatase.

Like *Phaseolus vulgaris*, *Wistaria* is a member of the family Leguminosae, subfamily Papilionoidae. This subfamily is comprised of a very large number of widely distributed genera displaying diverse characteristics. The family Phytolaccaceae, to which pokeweed (*Phytolacca americana*) belongs, is generically widely separated from the Leguminosae. Börjeson and his colleagues have recently isolated and characterized the pokeweed mitogen<sup>4</sup>, but the relationship of this substance to the mitogens of *Phaseolus* and *Wistaria* remains unresolved.

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### Induction of Mitotic Recombination in *Saccharomyces cerevisiae* by Ethyl Methane Sulphonate

MITOTIC recombination can be induced in *Saccharomyces cerevisiae* by exposing them to ultra-violet and X-radiation<sup>1</sup>. The genetic maps so obtained closely parallel those from meiotic recombination<sup>2</sup>. One of the most useful features of mitotic recombination is that it is relatively easy to observe intracistronic recombination. It is possible that this results from a mechanism somewhat different from that leading to meiotic recombination characteristic of higher organisms. For this reason, we decided to study the effects of certain chemical mutagens on the process of mitotic recombination in the hope of being able ultimately to compare these results with those obtained in more usual systems.

For our initial investigations we chose to work with the alkylating agent ethyl methane sulphonate (EMS). This

agent is known to act as a point mutagen, largely through the alkylation of the guanine residue of DNA (ref. 3). As a point mutagen, EMS produces a very low level of chromosome breakage<sup>4</sup>, and thus it was expected that it might be used to provide a test of the breakage and reunion hypothesis of recombination. This idea was reinforced by the report of Morpurgo that monofunctional alkylating agents were not effective in the induction of mitotic recombination in *Aspergillus*<sup>5</sup>. We assumed that a true "point" mutagen would not increase recombination if chromosome breakage were the initiating event and that a deleting agent would. Since we started these experiments, it has been reported that diethyl sulphate will induce mitotic recombination<sup>6</sup>. It is therefore not surprising to find that the results of the experiments reported here indicate that our initial assumption was incorrect, because EMS can dramatically increase recombination.

For these investigations we used mutant strains of the two alternate mating types ( $a$  and  $\alpha$ ) requiring tryptophan ( $tr$ ), uracil ( $ur$ ) or histidine ( $hi$ ). Mutations in the  $hi_5$  and  $hi_4$  cistrons were induced by EMS in  $tr a$  haploid strains, and these were subsequently crossed to either  $ur_1 hi_5 \alpha$  or  $ur_1 hi_4 \alpha$  strains to form the diploids. To facilitate identification the original  $\alpha$  strains were designated  $hi_{5a}$  and  $hi_{4a}$ , and the newly obtained mutants were given other designations ( $hi_{5b}$ ,  $hi_{5c}$ , etc.). We carried out tests of spontaneous recombination with the  $hi_{5a}$  and  $hi_{4a}$  strains, and obtained the relative map distances from the  $hi_a$  mutants<sup>7</sup>. Because EMS induced large increases in recombination frequency, diploids showing relatively low frequencies of spontaneous recombination were selected for use in the experiment.

We treated diploids, freshly generated for each test, with 3 per cent EMS in phosphate buffer, for varying periods of time. After treatment, the cells were spun down and washed twice with 6 per cent sodium thiosulphate and once with distilled water before plating them on minimal medium. We made cell counts by plating aliquots on complete medium. The untreated cells were subjected to all procedures other than the addition of EMS. All treatments and incubations were carried out at 30° C. In all cases, growth was allowed to proceed for 72 h in order to permit the maximum number of recombinants to arise. (Treatment with EMS greatly slows the growth.)

Because only recombinants will grow on the minimal medium, the scoring is relatively simple<sup>1</sup>. On the other hand, only cells wild-type for histidine will grow. Any reciprocal recombination event that produces a doubly mutant individual could not be detected in these tests, and thus there is no indication of the exact nature of the recombination process.

As a test for reversion, homoallelic diploids (having the locus of mutation at the same place on both chromosomes) were used. These were treated in exactly the same manner as were the heteroallelic diploids. Because the homoallelic diploids cannot recombine, it is to be presumed that they offer a satisfactory estimate of the diploid revertants. In addition, reversion of the haploid strains from which one of the diploids was generated was measured.

In each experiment, at least fifteen plates were scored for each treatment, and all experiments were repeated at least three times. The data in Tables 1 and 2 and the points in Fig. 1 are all mean values from these experiments. There is some variability in the data, but it can be accounted for by variations in the initial cell number in each experiment. Doubling the cell number obviously halves the concentration of EMS in the cells, and results in a comparable decrease in recombination frequency. This can be seen by comparing the results obtained with a treatment for 35 min (Table 1) with those presented in Fig. 1. (The data in Fig. 1 were obtained at higher initial cell numbers.)

The data in Table 1 show that EMS causes a drastic rise in the frequency of recombination of relatively closely linked mutational sites in the  $hi_5$  cistron. It can be

Table 1. MEAN NUMBER ( $\times 10^{-3}$ ) OF REVERTANTS AND RECOMBINANTS OF TWO MUTATIONAL SITES WITHIN THE  $hi_3$  CISTRON

Cells treated		Treatment (min)		
		0	20	35
HE	$hi_{3a}/hi_{3b}$	49	177	276
HO	$hi_{3a}/hi_{3a}$	0.1	0.3	0.6
HO	$hi_{3b}/hi_{3a}$	0.1	0.2	0.5
H-a	$hi_{3a}$	1	4	10
H-a	$hi_{3a}$	0.1	1.0	2.0

Symbols are as follows: HE, heteroallelic diploid; HO, homoallelic diploid; H-a, haploid  $a$  strain; H-a, haploid  $a$  strain.

Table 2. MEAN NUMBER ( $\times 10^{-6}$ ) OF REVERTANTS AND RECOMBINANTS OF TWO CLOSELY LINKED MUTATIONAL SITES WITHIN THE  $hi_4$  CISTRON

Cells treated		Treatment (min)			
		0	10	20	30
HE	$hi_{4a}/hi_{4b}$	6	44	81	95
HO	$hi_{4a}/hi_{4a}$	0.1	0.3	0.7	1.0

Symbols are as follows: HE, heteroallelic diploid; HO, homoallelic diploid.

seen that the recombination in the heteroallelic diploid is far in excess of the reversions found in either the haploid or homoallelic diploid strains.

Table 2 suggests that the same effect can be observed in the  $hi_4$  cistron, in which two more closely linked mutational sites were utilized. It should be noted that at the longest treatment time, the  $hi_4$  recombination shows a non-linearity. On the other hand, as indicated in Table 1 and in Fig. 1, for the  $hi_3$  cistron the response to treatment is apparently linear.

The data in Fig. 1 were obtained using a higher initial cell number (and thus giving a lower kill) than used to obtain the data in Table 1. Furthermore, in Fig. 1 the reversion frequency of the homoallelic diploid is subtracted from the total number of recovered clones, and thus the data are frequencies of recombination. It can be seen that, under these conditions, the response is essentially linear over a relatively wide range of treatment times.

It is evident that, although it is a monofunctional alkylating agent, EMS can strongly affect intracistronic recombination in *S. cerevisiae*. Thus it would appear that EMS will not be useful as a "control" for the test of the breakage and reunion hypothesis of genetic exchange. The data presented here are consistent with the model proposed by Holliday<sup>9</sup>, which suggests that any defect arising in the DNA may result in a repair process leading to genetic exchange. Preliminary tests carried out on meiotic recombination in *Drosophila* indicate that EMS is effective in increasing genetic exchange in certain regions. This fact taken with the data presented in this com-

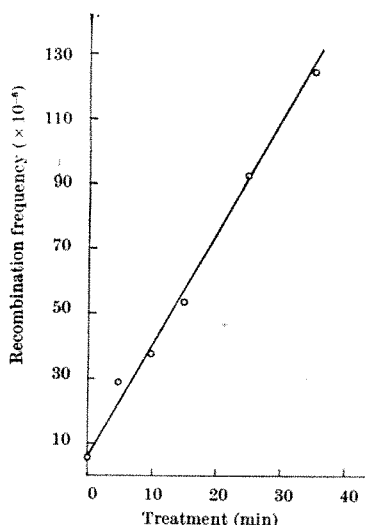


Fig. 1. Variation in recombination of two mutational sites within the  $hi_3$  cistron with time of exposure to EMS. Corrected for reversion. The number killed is half that for the experiments summarized in Table 1.

munication makes it reasonable to suggest that the mechanism of production of genetic recombination is different from that responsible for the production of chromosomal aberration.

Perhaps the most interesting feature of these data is linear response shown to EMS treatment time (and probably to dose, comparing the data in Table 1 and Fig. 1). Thus EMS apparently acts in much the same manner as X-rays but unlike that of ultra-violet light. It will be interesting to continue these investigations to determine whether or not the slope of the curve can be used to map mutants, as can X-rays<sup>1</sup>.

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## GENETICS

### New Genotypes in Cod at Greenland

THE blood sera of cod (*Gadus morrhua* L.) show certain protein types which were believed to be inherited. Møller<sup>1</sup> tested blood samples from cod caught along the Norwegian coast and in the Barents Sea, and described eleven molecular patterns of iron-binding proteins, or transferrins. Ten of the observed patterns represented all the possible genotypes attributable to four co-dominant allelomorphous genes  $TfA$ ,  $TfB$ ,  $TfC$  and  $TfD$ , each controlling one of four proteins. Gel electrophoresis revealed each protein as a discrete band. The alphabetical order of the gene suffixes followed the decreasing order of their electrophoretic mobility. Møller's remaining type of pattern contained a rare protein with an intermediate mobility faster than that of the protein attributed to the commonest allelomorph  $TfC$ , and he proposed a new allelomorph  $TfC^1$ . Møller found six examples of  $TfC^1/TfC$ , and his genetic interpretation anticipated the existence of the genotypes  $TfA/TfC^1$ ,  $TfB/TfC^1$ ,  $TfC^1/TfC^1$  and  $TfC^1/TfD$ . Numerous examples of each of these anticipated genotypes have in fact been discovered in the blood sera of West Greenland cod, and are presented here for the first time.

The testing methods and the notation of the cod transferrins were those of Møller<sup>1</sup>, who kindly provided me with control sera which allowed the identities of the four more familiar proteins to be equated in the tests made in Bergen and Lowestoft.

Fig. 1 shows all five genotypes containing the gene  $TfC^1$  as it appeared in the course of routine tests of unknown sera. Each unknown was tested alongside an established control serum of genotype  $TfB/TfC$ , the most frequently encountered two-band transferrin pattern in cod. Fig. 2 is a diagram based on the photograph and shows the rare genotype patterns as solid lines and the control pattern as broken lines.

The 708 cod sampled at West Greenland showed the following gene frequencies, with their standard errors:



Allelomorph	$Tf^A$	$Tf^B$	$Tf^{C1}$	$Tf^C$	$Tf^D$
Gene frequency	0.129	0.227	0.035	0.524	0.085
Standard error	$\pm 0.009$	$\pm 0.011$	$\pm 0.005$	$\pm 0.013$	$\pm 0.007$

The results of these and previous tests suggest that the order of magnitude of the frequencies of the five cod transferrin genes is consistently  $Tf^C > Tf^B > Tf^A > Tf^D > Tf^{C1}$  throughout the distribution of this species in the North Atlantic Ocean. The West Greenland sample shows the lowest known frequency of  $Tf^C$ , together with the highest known frequencies of  $Tf^A$ ,  $Tf^B$ ,  $Tf^D$  and  $Tf^{C1}$ .

Table 1. THE FIFTEEN TRANSFERRIN GENOTYPES IN 708 COD AT WEST GREENLAND

Genotypes	Observed	Expected	
$Tf^A/Tf^A$	13	12	
$Tf^B/Tf^B$	52	37	
$Tf^{C1}/Tf^{C1}$	4	1	New type
$Tf^C/Tf^C$	227	194	
$Tf^D/Tf^D$	12	5	
All homozygotes	308	249	
$Tf^A/Tf^B$	30	41	
$Tf^A/Tf^{C1}$	7	6	New type
$Tf^A/Tf^C$	100	95	
$Tf^A/Tf^D$	19	15	
$Tf^B/Tf^{C1}$	11	11	New type
$Tf^B/Tf^C$	144	169	
$Tf^B/Tf^D$	33	27	
$Tf^{C1}/Tf^C$	12	23	
$Tf^{C1}/Tf^D$	12	4	New type
$Tf^C/Tf^D$	32	63	
All heterozygotes	400	459	

The observed numbers of transferrin types in cod caught between Disko Bay and Cape Farewell in October 1966 are shown in Table 1. When a Hardy-Weinberg test is applied to this material the very significant departures from numerical expectations indicate that this is a composite sample of two or more genetically isolated stocks. More detailed analyses of the genetic structure of cod stocks at Greenland and elsewhere in the North Atlantic will be published in due course. It is increasingly apparent that this genetic diversity in cod can be used to estimate the degrees of isolation of the various breeding stocks. This knowledge can be applied to stock conservation problems and in the routine classification of fishery statistics.

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## BIOLOGY

### Facultative Autogeny in Arctic Mosquitoes

OVARIAN development in the mosquitoes *Aedes (Ochlerotatus) impiger* (Walk.) and *A. (O.) nigripes* (Zett.) has been studied at Hazen Camp, northern Ellesmere Island (71° 18' W., 81° 49' N.), a site only 150 miles south of the northernmost limit of land. Both species seek blood actively and, if able to engorge, use it to mature eggs in the usual way. A factor thought to limit their reproductive success is the low availability of blood, resulting from the scarcity of vertebrates and the prevalence of weather inimical to host-seeking during the brief flying season of the mosquitoes.

Experiments in 1963 showed that some females of both species show autogeny; that is, they complete ovarian development (the follicles reaching the fifth stage of Christophers<sup>1</sup>) without a blood-meal. During autogenous ovarian development many follicles degenerate and thus fecundity is greatly reduced<sup>2</sup>. Autogeny is found in the first ovarian cycle in several genera of mosquitoes, and in some species both autogenous and anautogenous (blood-feeding) members occur. In these the course of ovarian development is assumed to be predetermined, at the time of emergence, on one course or the other, although so far this has been confirmed only for *Culex pipiens* variety *molestus* Forsk<sup>3</sup>. This type of autogeny may be termed obligate. The 1963 experiments at Hazen Camp, however, suggested that, in some females at least, autogeny during the first cycle is facultative—individuals can mature eggs with or without blood, depending on circumstances. Such a condition had not previously been demonstrated in mosquitoes, but we have confirmed its existence in these two Arctic species. This has posed the further question of when the switch from potentially anautogenous to autogenous development occurs; at this transition a female exchanges the chance of being able to lay many eggs for the near certainty of being able to lay a few.

Experiments at Hazen Camp in 1966 helped to answer this question. Female mosquitoes (a total of about 320) of both species were collected either as nulliparous adults (those that have not yet laid eggs) attempting to bite in the wild when about 3 days old (Table 1, "attracted"), or as pupae that were allowed to emerge in captivity ("reared"). They were confined individually with con-

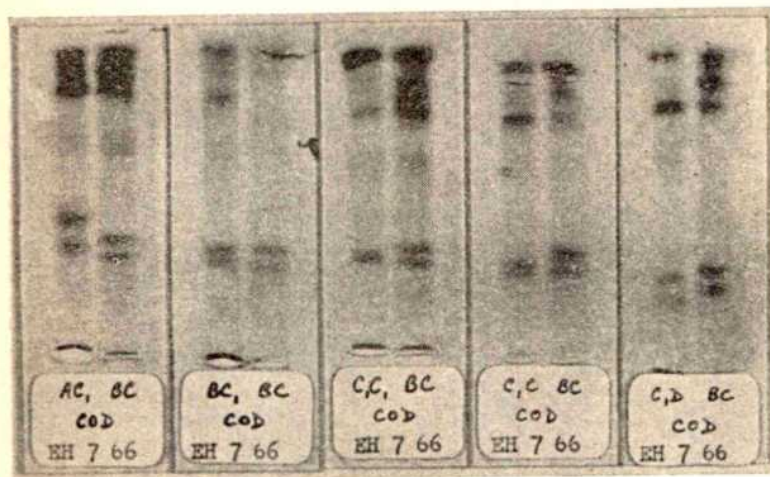


Fig. 1. Selected examples of the results of routine electrophoretic tests which separate the soluble proteins in cod sera. The proteins appear as stained bands on the anodic side of gels supported on five microscope slides measuring 3 in.  $\times$  1 in. Each slide shows the protein components of two sera. Five labelled examples of the uncommon cod transferrin genotypes  $Tf^A/Tf^{C1}$ ,  $Tf^B/Tf^{C1}$ ,  $Tf^{C1}/Tf^{C1}$ ,  $Tf^{C1}/Tf^C$  and  $Tf^{C1}/Tf^D$  were tested in parallel with a control serum of genotype  $Tf^B/Tf^C$ .

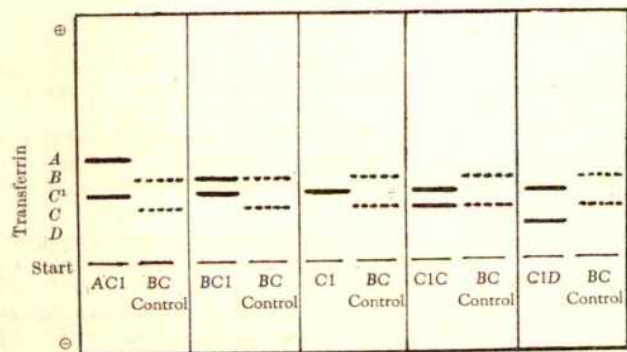


Fig. 2. Diagram outlining the transferrin band patterns seen in the photograph. Certain additional variable proteins seen in the photograph are not relevant. Note the positions of the five cod transferrin bands which are represented in the diagram by solid lines. The control genotype is represented by broken lines.



Table 1. SIZES OF BATCHES OF EGGS IN NULLIPAROUS MOSQUITOES THAT COMPLETED OVARIAN DEVELOPMENT

Category (in order of declining fecundity)	<i>Aedes impiger</i>			<i>Aedes nigripes</i>		
	Median	Range	No. of mosquitoes	Median	Range	No. of mosquitoes
(A) Potential complement (follicles at emergence)	53	(34-71)	16	52	(34-78)	44
Anautogenous (offered blood; engorged)						
(B) Attracted	53	(25-78)	21	39	(22-62)	14
(C) Reared	34	(22-52)	16	31	(24-41)	14
Autogenous (denied blood)						
(D) Reared; obligate	6	(4-8)	2	25	(21-31)	5
(E) Attracted; facultative	6	(2-10)	13	2	(1-5)	3
(F) Reared; facultative	3	(1-7)	6	3	(1-4)	5

Except for (A) all references are to mature eggs.

tinuous access to 25 per cent sucrose solution and two flowers (renewed daily) of *Dryas integrifolia* Vahl., their usual source of nectar. Human blood was offered or withheld according to various regimes; females biting were allowed to engorge and were not offered blood again. Results reported here refer only to the first ovarian cycle and, unless otherwise specified, to both species.

Most females (and probably all females of *A. impiger*) can mature eggs without a blood-meal. In the great majority this autogeny is facultative, but in some it is obligate.

Females exhibiting facultative autogeny, if not allowed to blood-feed, remain for about 10 days with the ovaries in a state of suspended development (the third and fourth stages of Christophers) and then start to mature eggs autogenously: a few follicles enlarge while the rest degenerate until, about 10 days later, a very small number of eggs has matured (Table 1, E, F). By this time a female is about 20 days old. During their first 10 days, however, females readily take blood (maximum on days 2-4), and if they do feed a large batch of eggs matures (Table 1, B, C). The transition from potentially anautogenous to autogenous development probably reflects a female's energy reserves, and so the age at which it occurs can be expected to be somewhat different in the wild, and to vary from year to year according to the extent to which weather permits feeding on nectar.

Females exhibiting obligate autogeny begin to mature eggs promptly after emergence, and are ready to lay them about 10 days later. In *A. impiger* the number of eggs matured is markedly less than in females that obtain blood; in *A. nigripes* it is slightly so (Table 1, D). Females showing obligate autogeny do not normally seek blood (as nullipars) in the wild, an inference based on their absence from baited catches. Nevertheless the behaviour of captive females shows that some at least retain the ability to bite and engorge. So presumably, as in other mosquitoes<sup>5</sup>, and certain blackflies<sup>6</sup>, that exhibit autogeny, females need blood to begin the second ovarian cycle.

These results show that these two Arctic species are more flexible in their ovarian maturation than any other mosquitoes so far examined. This versatility can be seen as appropriate to survival in the exceptionally severe and variable environment in which they live.

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## A *Neopilina* from the Gulf of Aden

On February 22, 1967, R.R.S. Discovery, of the National Institute of Oceanography, dredged a single specimen of the monoplacophoran mollusc *Neopilina* in the Gulf of Aden. This specimen (Figs. 1-3) represents the first record of the genus beyond the depths of the eastern Pacific. First collected in 1952 off Costa Rica, and reported by Lemche<sup>1</sup>, *Neopilina* is representative of a group previously considered extinct since Devonian times. Up to 1960 it had also been found off northern Peru and Baja California, but no further reports have since been noted and the Discovery record is an indication of much wider distribution than could previously be contemplated.

It will be recalled that *Neopilina* displays segmental characters which may be homologized with those present in some annelids and arthropods, previously unknown in molluscs. It is as an indication of a form adopted in the evolution of the ancestors of this very successful marine invertebrate group, the Mollusca, that *Neopilina* is of interest.

The Discovery specimen (described here as a new species) has five pairs of gills (Fig. 3) and in this feature is much closer to the species described as *Neopilina galathea* (ref. 1), and *Neopilina veleronis* (ref. 2), from off Baja California, than to *Neopilina ewingi*, which has six pairs, from off Peru (ref. 3). In *N. galathea* the apex of the shell is almost immediately over the front margin, in *N. veleronis* it is in advance of the front margin, whereas in the present specimen it is set back from this margin (Fig. 1).

*Neopilina adenensis* n.sp. Figs. 1, 2 and 3.

**Type locality:** The Alula-Fartak trench in the Gulf of Aden, 13° 50' N., 51° 47' E., between 3,950 and 3,000 m: R.R.S. Discovery St. 6213.

**Holotype.** B.M.(N.H.) Reg. No. 19672 W.

The holotype measures 10.7 mm in length by 10.1 mm wide; the shell is convex with a maximum depth of about 3.0 mm; the hooked apex of the shell is situated about 0.8 mm from its front margin. The shell is white near its margin, tending to light brown elsewhere; its surface is sculptured with numerous concentric ridges numbering about seventy-five up to the apical region where they run too close together to be counted; in the central region the concentric sculpture becomes irregular, being interspersed with discontinuous grooves of varying depths; faint radiating sculpture occurs in the margin area and towards

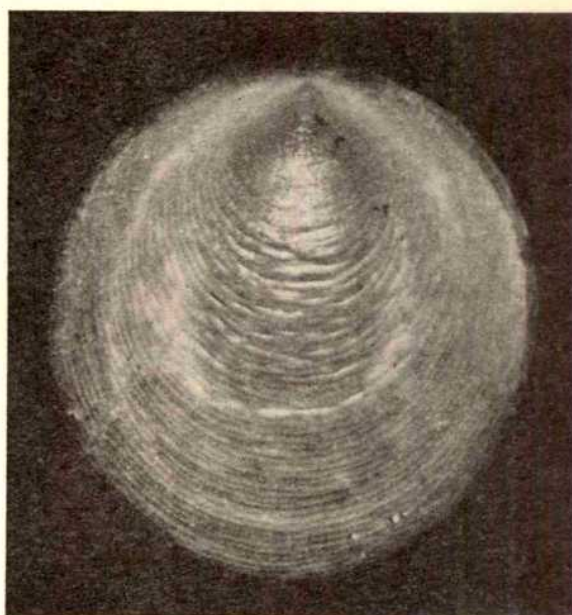


Fig. 1. *Neopilina adenensis* n.sp.; dorsal view of the holotype, which measures 10.7 mm across its longest dimension.



the centre. In ventral view the body of the animal is exposed; it has five pairs of gills, a set of five being disposed in series in the pallial groove on each side of the foot; each gill has six or seven branches running off one side of a main stem. The postoral tentacles are branched.

The sample was collected by Dr J. R. Cann of the Department of Mineralogy, British Museum (Natural

History), during investigations of the structure and origin of the Gulf of Aden by a marine geological group led by Dr A. S. Loughton of the National Institute of Oceanography.

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<sup>1</sup> Lemche, H., *Nature*, **179**, 413 (1957).

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### Standardization of *Bacillus thuringiensis* Products: Homology of the Standard

THE lethality of *Bacillus thuringiensis* to many lepidopterous larvae has led to its development as a "biological insecticide". Products based on this bacterium contain two active ingredients, the spore and the crystal of toxic protein, and sometimes a third, the exotoxin. The water-soluble exotoxin is secreted into the medium during vegetative growth and the crystal is formed alongside the spore at sporulation. Because the crystal is by far the most important ingredient against many Lepidoptera and cannot be estimated chemically, the realization that the insecticidal activities of different preparations cannot be consistently measured by counting the spores was an important advance in the problem of standardization. Bioassay, with species of insect sensitive only to the crystal, is taking the place of the spore count. The recent adoption of one batch of stable bacterial preparation, E61, as the international standard has provided both a recognized material with which other materials can be compared and a basis for establishing toxicity units. Such measurement of the crystal content of materials should work well with products containing the same strain of bacterium as the standard, that is, when the standard is homologous.

The use of a standard, however, has its pitfalls. The standard can be regarded as only partially homologous with products containing other strains of the bacterium. Different species of assay insect may give very different comparative toxicity values between bacterial products if the susceptibilities of the different insect species to the various bacterial strains are unlike. This is illustrated by the example in Table 1. The insecticidal activity of the standard has been designated arbitrarily as 1,000 units/mg. In *Anagasta* units, the product S is 10.6 times as active as 'Bakthane', but in *Galleria* units it is 76.3 times as active. This is explained by the different susceptibilities of the two species of insect to the two strains of bacteria. The  $LD_{50}$  values of the present assays, and assays of other bacterial preparations containing these strains of bacteria, show that *Anagasta* has similar susceptibilities to the two strains but that *Galleria* is much more susceptible to strain *galleriae* than to strain *thuringiensis*. Similar relationships exist between other pairs of bacterial strains and host insects.

Thus, when the standard is incompletely homologous, it is impossible to rely on units based on only one species of assay insect in measuring the insecticidal activity of

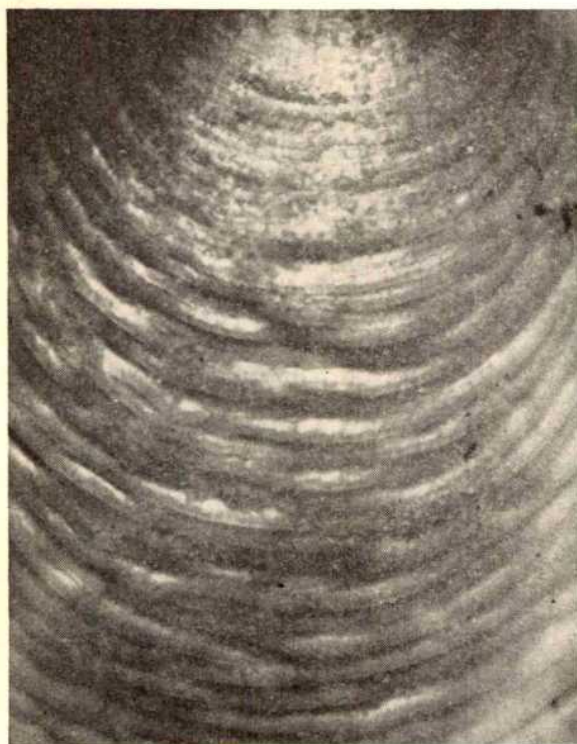


Fig. 2. *Neopilina adenensis* n.sp.; detail of the shell surface.

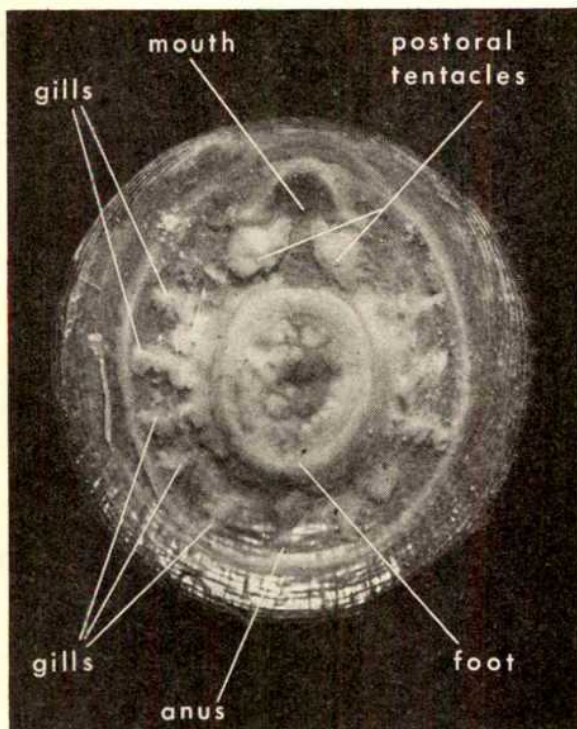


Fig. 3. *Neopilina adenensis* n.sp.; ventral view.

Table 1. INSECTICIDAL ACTIVITY OF *Bacillus thuringiensis* MATERIALS IN TOXIC UNITS

Bacterial strain	<i>Anagasta kuehniella</i> * (units/mg)		<i>Galleria mellonella</i> † (units/mg)	
	Mean	95 per cent fiducial limits	Mean	95 per cent fiducial limits
Standard, E61 <i>thuringiensis</i>	1,000	—	1,000	—
Product S4-275 <i>galleriae</i>	3,662	2,321-5,778	89,373	69,566-114,820
Product 'Bakthane' <i>thuringiensis</i>	345	170-701	1,172	818-1,678

\* Assay technique of Burgerjon and Yamvrais<sup>1</sup>, modified.

† Assay technique similar to that of Hoopingarner and Materu<sup>2</sup>.

crystals in different products. At best, one can group species of pest insects according to their susceptibilities to different bacterial strains and use the most convenient assay insect in each group to provide a unit for its group.

Alternatively, one could erect a separate international standard for each bacterial strain. This is less satisfactory, not only because it would greatly increase the number of available units, leading to severe complication, but also because the characters of the existing strains can be and are being manipulated to increase productivity, etc., and a "manipulated" strain would probably not be homologous with any one of the known natural strains.

These considerations emphasize the need for fundamental study of the toxic action of the crystal of different strains of *B. thuringiensis* in different species of insect. Analogous difficulties are likely to arise in all attempts to standardize pathogens, bacterial or otherwise, active against more than one species.

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### Pentamerism and the Calcite Skeleton in Echinoderms

COCKBAIN<sup>1</sup> has suggested an extension of the suture-line theory<sup>2</sup> of the origin and significance of pentamerism in the phylum Echinodermata, which depends primarily on the fact that all modern echinoderms with extensive skeleton apparently pass through a stage in which the apical system or calyx shows a common arrangement of six plates: a central plate surrounded by a whorl of five. The apical system is of chief importance in establishing the symmetry of the rest of the body. Because the apical system of the young echinoderm occupies a large part, if not all, of one face or pole of the body, the places of greatest weakness in that area of plates (the sutures between them) must be so arranged that: first, they are kept as few and as short as possible; second, that no suture should be directly in line with any other; and, third, that this first whorl of the theca should be as nearly circular as possible. Only with a pentamerous arrangement are these requirements satisfied.

Although Cockbain accepts the main premises of this theory, he seeks to link pentamerism with another unique feature of the phylum—the possession of skeletal elements apparently composed of single crystals—and considers that previous theories have not suggested adequate reasons why five-fold symmetry has an advantage over three-fold for this group of animals. His principal point is that the angle between the faces of the cleavage rhomb of the calcite crystal is about 75°, which is sufficiently close to the angle between five equally spaced radii of a circle (72°) to account for the superiority of pentamerism. A further point is that with this arrangement only two cleavage directions will cut across each plate, reducing the chance of breakage.

If Cockbain's idea is correct, the initial ring of plates should be oriented in such a way that the crystallographic c-axis leaves each plate at the same angle relative to the plane of the plate surface and to the oral-aboral axis of the animal. But this is apparently not so. It has been shown<sup>3</sup> that the c-axis orientation of the genital plates of most echinoids (the only class of echinoderms that has been adequately studied in this respect) departs from perfect radial symmetry: genital plates *G*-1, *G*-2 and *G*-4 (on Lövén's<sup>4</sup> scheme of plate notation, in which *G*-2 is the madreporite) have c-axes perpendicular to the plate

surface, while plates *G*-3 and *G*-5 have axes tangential to it.

It seems therefore that the premise on which Cockbain bases his extension of the suture-line theory of echinoderm pentamerism must in some respects be wrong, if only in the case of echinoids. That there may well be some connexion between the possession by this phylum of a unique type of skeleton<sup>5</sup> and an almost unique type of symmetry has already been suggested<sup>2</sup>, but only in so far as the great strength of the skeletal material emphasizes the relative weakness of the sutures between the skeletal elements.

Fell<sup>6</sup> has pointed out that the larvae of all comatulid crinoids, ophiuroids and asteroids for which the development is known have apical systems consisting of the familiar pattern of five plates surrounding a central plate. This pattern occurs transiently in those forms in which the plating of the disk region subsequently becomes randomized; it occurs in ophiuroids in which the adult disk is completely naked; it even occurs in those forms in which the adult animal secondarily loses pentamerism, as in the Antarctic comatulid *Promachocrinus*<sup>6</sup>. Thus because there appears to be a common pentamerous symmetry in the apical or calycinal plates, albeit briefly, for some time during the immediately post-larval life of so many echinoderms, it seems reasonable to seek the importance of its arrangement at this time in the life of the animal.

The suture-line theory gains cogency from the fact that, when the developing skeletal system of the young echinoderm is relatively weakly interconnected, the apical system may occupy most of its dorsal (aboral) surface (see, for example, ref. 6, Fig. 15, Nos. 6, 9 and 12; ref. 7, Fig. 17 and p. 287; and ref. 8, Plate II, Fig. 4, and Plate IV, Fig. 3).

One possible criticism of the theory lies in its incapacity, at first sight, to explain the advantage of five-fold over three-fold symmetry. In both, a linear projection from the radial suture between two adjacent plates exactly bisects the opposite plate; it is not in line with another suture, as would be the case in tetramerous, hexamerous or any other even number symmetry. Furthermore, the trimerous condition shows two possible advantages: first, there is a sharper angle between the radial sutures on opposite sides of the apical disk than in the pentamerous arrangement (120° as opposed to 144°) and, second, there are fewer suture lines in the complex of plates of the apical disk, which implies fewer lines of weakness. The explanation for the superiority of pentamerism appears to lie in the advantage of having a theca which approaches a circular cross-section without undue deformation or strain to the crystals of the plates; it may well be that an initial whorl of five plates and subsequent whorls of five, or, in some, multiples of five derived from this, is the lowest number that can achieve such a shape while still fulfilling the other requirements of the initial whorl at the critical stage in ontogeny.

The sparse literature on the attributes and significance of pentamerous symmetry has been reviewed elsewhere<sup>9</sup>. Bather's<sup>10</sup> suggestion that the condition arose from an original trimerous symmetry by subdivision of the two lateral radii has been re-stated by Fell<sup>11</sup>. This idea is at first sight attractive, because there is fossil evidence of trimerous symmetry in some edrioasteroids and cystoids; further, edrioasteroids are among the first echinoderms to appear in the fossil record<sup>12</sup>, and some authors<sup>10</sup> regard cystoids as among the most primitive echinoderms, on morphological grounds. Interesting though it is in underlining the theoretical similarity of the trimerous and pentamerous conditions, this idea does little to explain why the pentamerous condition has persisted so tenaciously throughout the history of the phylum, even when the way of life of its members shows such variety. Moreover, if there is advantage to be gained merely from subdividing the two lateral radii of the original three, it is not



easy to see why in the vast majority of echinoderms the process has stopped on attaining five.

In spite of many difficulties in the suture-line theory, particularly the impracticality of demonstrating experimentally its main premise, it remains attractive because it helps to account for so many otherwise strange phenomena of development. But the fact remains, as Cockbain<sup>1</sup> has pointed out, that any theory of echinoderm pentamerism which could be more closely linked with other unique features of the phylum, such as the single-crystalline condition and open-meshwork nature of the skeleton<sup>5</sup>, would be highly attractive; such a theory must, however, be consistent with the facts.

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### Stimulation of Hatching of the Potato-root Eelworm *Heterodera rostochiensis* by Ion Exchange Resins

We have already reported the possibility that de-ionized water might stimulate hatching of the potato-root eelworm<sup>1</sup>. We now present confirmatory evidence: glass distilled water which has been in contact with certain ion exchange resins acquires marked stimulatory properties.

10 g of standard grade 'Amberlite IRA 400' resin was thoroughly stirred up in 100 ml. of glass distilled water and then allowed to stand for 24 h. The decanted supernatant constituted solution A, of unknown composition. In a hatching test of four weeks duration, using dilutions A, A/2, A/4, A/8, A/16, A/50, each tested on twenty-five cysts with a single cyst technique<sup>2</sup>, mean larval emergence per cyst was 263, 111, 209, 250, 262 and 102, respectively. Emergence from 100 cysts in tap-water was 8 per cyst. The "resin water" was therefore very powerful; in an almost concurrent experiment with cysts from the same population, emergence in solutions of a hatching factor concentrate known to be of high activity was about 150 per cyst.

In a repeat experiment which included a series of dilutions of the hatching factor concentrate, emergence was somewhat lower in the resin water. 160 larvae per cyst emerged in the most active of the hatching concentrate solutions, and 97 in the most active resin water. An analysis of variance was carried out with a logarithmic transformation because of the skew nature of the data<sup>3</sup>. Values of  $\log(x+1)$  were used, where  $x$  is the number of larvae to emerge per cyst. Emergence in all dilutions of resin water was significantly greater than in the water controls; the highest values did not differ significantly from the emergence in all but the two most concentrated of the hatching factor solutions.

First experiments show that a number of other resins possess the property. Clearly, de-ionized water must be used with circumspection in hatching tests. Moreover, the use of ion exchange resins, even after exhaustive pretreatment, in procedures directed towards the isolation of

the hatching factor, must be attended by some risk, particularly where recovery rates are on the low side. The nature of the substance, or substances, involved is under investigation.

We are grateful to Dr G. J. Janzen, of Groningen, for the hatching concentrate, and to Mrs A. Stephenson for her assistance.

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### Attempt to find Genetic Recombination in *Anacystis nidulans*

GENETIC relationships in the blue green algae (Cyanophyceae or Myxophyceae) are still obscure. There is some similarity between the nucleoplasmic ultrastructure of blue green algae and that of bacteria<sup>1</sup>, and this similarity might also apply to the genetic processes of these two organisms, but there are no data for or against this idea. Kumar assumed that there was one case of a sexual or parasexual process in *Anacystis nidulans*<sup>2</sup>. We have carried out experiments with this organism similar to those of Kumar, and the results are discussed in this communication.

The Cambridge strain of a strictly autotrophic, mild thermophilic, unicellular blue green alga, *Anacystis nidulans* (from the Collection of Cultures of Autotrophic Organisms, Czechoslovak Academy of Sciences in Prague), was cultured in a slight modification of the liquid basal medium C of Kratz and Myers<sup>3</sup> at 39° C and 1,700 lux on a shaker. This strain was unable to form discrete colonies on agar-agar plates. The standard culture was first tested for sensitivity to ten different chemotherapeutic drugs (see Table 1) and then it was cultured in media supplemented with gradually increasing concentrations of the respective drugs. By this procedure we obtained strains surviving in penicillin, streptomycin and isonicotinhydrazide which were 15, 100 and 200 times more concentrated. The sensitivity to the other chemotherapeutic drugs did not change markedly during ten transfers of the standard strain in the drug supplemented media, as Table 1 shows.

Table 1. SENSITIVITY OF *Anacystis nidulans* TO CHEMOTHERAPEUTIC DRUGS

Drug	Original level of resistance ( $\mu\text{g/ml}$ or IU/ml.)	Final level of resistance	Mode of origin of resistance
<i>p</i> -Aminosalicylate	This drug has no effect even in concentrations of about 1,000 $\mu\text{g/ml}$ .		
Chloramphenicol	1	3	In one step
Cycloserine	10	No increase	—
Isonicotinhydrazide	10	150	In seven steps
Neomycin	0.1	No increase	—
Penicillin	0.1	10	In five steps
Streptomycin	1	200	In seven steps
Tetracycline	1	No increase	—
Vancomycin	50	No increase	—
Viomycin	0.1	No increase	—

The increase of resistance in all three successful cases occurred in several stages; this differs substantially from the analogous processes known in bacteria<sup>4</sup> and could indicate a different kind of genetic control of resistance to the respective drugs in blue green algae.

The strains resistant to penicillin (*PEN<sup>r</sup>*) and to streptomycin (*STR<sup>r</sup>*) were used in an attempt to find genetic recombination. Both strains were tested first for stability of drug resistance by ten successive transfers through basal medium, when no decrease of resistance was observed. Also the cross-resistance to both antibiotic

was tested and found to be negative. Basal medium containing no antibiotics was then inoculated simultaneously into a culture consisting of cells of *PEN<sup>r</sup>* and *STR<sup>r</sup>* strains. Samples were withdrawn from this mixed culture at various times and were transferred into selective medium supplemented with both penicillin (10 IU/ml.) and streptomycin (200 µg/ml.). Growth was observed in seven out of thirty-two culture-flasks in four independent experiments. These "recombinant" cultures, however, did not grow in further transfers in medium containing both drugs or even penicillin only, but it occurred in medium supplemented with streptomycin only or in medium without antibiotics. These observations support the idea that the supposed recombinant cultures are only members of the *STR<sup>r</sup>* strain which have survived contact with penicillin.

This explanation is based on the fact that all seven positive results were obtained only with samples taken from the stationary growth phase of the mixed culture. Considering the similar chemical composition of cell walls of *Anacystis nidulans* and of Gram positive bacteria<sup>1</sup> we can suppose that similar mechanisms of inactivation by penicillin are at work in each case. The cells of inocula withdrawn from the stationary growth phase remain in selective medium for about 6 or 7 days in the metabolic lag phase and that is why they survive in the presence of penicillin. Using the modified Hildick-Smith-Fell micromethod<sup>6</sup> we found that by the sixth day of culture there was no penicillin left in the medium, so that any cells of the strain *STR<sup>r</sup>* which survived the lag could start to grow regularly. In control experiments the medium containing penicillin only was separately inoculated with cells of strain *STR<sup>r</sup>* and also of the standard strain; inocula were withdrawn from the stationary growth phase of the cultures, and in all cases there was growth in medium supplemented with penicillin.

Kumar's idea of the existence of a sexual or parasexual phenomenon in *Anacystis nidulans*<sup>2</sup> was based only on the fact that the growth of the mixed culture of penicillin- and streptomycin-resistant strains (each of them only ten times more resistant than the standard strain) was observed in the medium supplemented with both respective drugs. Our results suggest that the observed growth of the mixed culture in the given selective medium is dependent on the presence of non-metabolizing (and therefore surviving contact with penicillin) cells of strain *STR<sup>r</sup>* in the appropriate inoculum. We assume that the ability of the mixed culture to grow in the given selective medium does not in this case sufficiently demonstrate sexual or parasexual recombination in the blue green alga.

I thank Dr Jan Nečásek for his advice.

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### Accumulation of Copper by Some Zambian Plants

In the areas of Zambia which are rich in minerals there are several species of plants which are both accumulators and geo-botanical indicators of copper. The relation of these plants to soil copper has been surveyed in the initial stage of an investigation of the effects of heavy metals on the metabolism of plant cells. The survey was conducted

mainly in some of the typical "copper clearings", areas in which the soil is rich in copper and where normal bush vegetation is replaced by copper-resistant species.

Soil samples from a number of non-mineralized areas of Zambia contained between 17 and 65 p.p.m. (total) copper. Plants growing on such soils do not accumulate copper to any significant extent. The situation is quite different in mineralized areas.

A wide range of plants, both from mineralized and non-mineralized areas, were collected and analysed for copper. A wet ashing procedure was followed by analysis on a Unicam 'SP900A' atomic absorption spectrophotometer. Several species were found to tolerate a large concentration of soil copper and to accumulate a relatively large concentration of the metal in their leaves. Among these are some "indicator" plants, *Becium homblei*, *Vernonia glaberrima*, *Triumfetta welwitschii* and *Cryptosepalum maraviense*. These are all small herbs or shrubs with woody rootstocks. Some species of Graminae were found to grow on soils rich in copper, but did not accumulate the metal. A specimen of *Trachypogon spicatus*, on soil containing 7,250 p.p.m. of copper, had only 15 p.p.m. of copper in its leaves. Besides these copper accumulators and copper-resistant species were a number of less selective plants, which, while tolerating in certain areas considerably more than a trace of soil copper, usually prefer non-mineralized areas. These include several woody shrubs and small trees such as various species of *Combretum* and *Pterocarpus angolensis*. Many species are cuprifuge and are not found on soils containing more than 20-40 p.p.m. of copper. Two of these, *Becium obovatum* and *B. angustifolium*, are interesting because they are closely related to the copper accumulator, *B. homblei*.

Cell juice was pressed from two species of *Becium* and the *B. homblei* had 0.092 mmolar copper ions, while *B. obovatum* had 0.05 mmolar copper ions. The total copper content for the specimens was for *B. homblei* 65 p.p.m. dry, or 16 p.p.m. fresh weight, and for *B. obovatum* 6 p.p.m. dry, or 1 p.p.m. fresh weight. These results suggest the *B. homblei* can remove copper from free solution in its cell sap, possibly by binding it as a complex with protein.

The results of a study of accumulation of copper by *B. homblei* in different soil conditions are summarized in Table 1. It is seen that, in general, a large concentration of soil copper leads to a large accumulation by the plants. It should be noted that the Allies and Chongwe sites are clearings in which the surface soil has been much disturbed by diggings and is enriched with malachite particles from underlying ore deposits.

A study of plant colonization of some of the old mine dumps at Chongwe is of interest. The soil contains as much as 1 per cent copper and even after more than 20 yr of exposure few plants have managed to establish themselves on the dumps. Only some clumps of *B. homblei* and the grass *Chloridion cameronii* are found. On analysis it was seen that while *B. homblei* accumulated 160 p.p.m. of copper, *C. cameronii*, in the same conditions, contained little more copper than would be found in plants growing on non-mineralized soils.

The toxic effects of copper are not entirely escaped by *B. homblei*. Even in the early growing season (November) chlorosis is frequently seen in the plant. This increases

Table 1. COPPER CONTENT OF *Becium homblei* FROM DIFFERENT SITES

No. of sample	Baluba	Colossus 1	Site Colossus 2	Allies	Chongwe
1	110 (90)	56 (108)	23 (64)	35 (335)	104 (7,509)
2	95 (110)	60 (100)	20 (52)	40 (410)	128 (585)
3	105 (80)	59 (97)	24 (41)	324 (2,300)	117 (760)
4	—	32 (35)	24 (41)	314 (3,400)	112 (3,160)
5	—	40 (35)	30 (50)	197 (71,500)	96 (5,080)
6	—	20 (46)	17 (64)	159 (63,000)	160 (22,000)

Copper content of plant material expressed as p.p.m. dry weight. Dilute acid soluble copper content of soil at roots of plants is given in brackets.



during the rainy season and by March it was observed at one site that 60 per cent of the plants were at least partly chlorotic.

I have concluded from these results that *B. homblei* will serve as a convenient and very suitable experimental plant for further investigations of copper accumulation and of the effects of the metal on metabolism of plant cells.

I thank the University of Zambia, the Ministry of Agriculture and the Ministry of Natural Resources and Tourism, Government of the Republic of Zambia, and RST Technical Services, and Chartered Exploration, Ltd. (Anglo-American Corporation) for their assistance.

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### Phenotypic Restoration of Fertility in a Male-sterile Mutant by Treatment with Gibberellic Acid

THE Tokyo group<sup>1</sup> tested gibberellin on tobacco inflorescences and found them to grow faster and to elongate further; elongation has so far been the most striking plant response to treatment with gibberellins. Floral differentiation, including parthenocarpy, however, has been observed by several workers, among whom are Bonde<sup>2</sup>, Wittwer and Bukovac<sup>3</sup>, Yakar-Olgun<sup>4</sup> and Zatyko<sup>5</sup>. Phatak *et al.*<sup>6</sup> reported that gibberellic acid induced the formation of anthers and the development of pollen in a stamenless tomato mutant. This type of differentiation and development of floral parts is very important in propagating homozygous recessive mutants which involve sterility in one of the sexes in an amphimictic plant species. I describe here my attempts to restore fertility in a spontaneous male-sterile mutant of the barley variety 'Maris Baldric'.

Plants grown in a glasshouse were treated with a single spray of gibberellic acid (GA<sub>3</sub>) at concentrations of 0 (control), 10 p.p.m., 100 p.p.m. or 300 p.p.m. It was applied to leaves of two male-sterile plants when the tip of the bottom awns reached the top of the spike. All ears were bagged just before anthesis. One floret from each of the plants treated at 100 p.p.m. and also one floret from each of the plants treated at 300 p.p.m. had grains formed as a result of the treatment.

Four grains, two from the 100 p.p.m. treatment and two from the 300 p.p.m. treatment, were sown in pots in a glasshouse. One of the grains from the 100 p.p.m. treatment failed to germinate, the other gave a plant which was entirely male-sterile. The two grains from plants treated at the 300 p.p.m. rate germinated and both produced progeny which set from 0 to 12 grains per ear, parts of which were male-sterile.

When the experiment was repeated (results in Table 1) the higher rate showed signs of developing seeds in about 8 days while the lower rate showed these signs in about 14 days. Both the early and the late tillers remained male-sterile.

Spikelets, with restored male-fertility, had anthers which were larger than the normal male-steriles. These enlarged anthers contained pollen with a limited amount of viability—detected by staining with benzedine (J. N. R. Kasembe, unpublished results). Self-fertilization, following the viable pollen formation, resulted in the production of seed which gave homozygous male-sterile plants. These were checked by progeny testing.

Table 1. FERTILITY RESTORATIONS IN SPIKELETS OF MALE STERILE BARLEY FROM 'MARIS BALDRIC' BY A SINGLE SPRAY WITH GIBBERELIC ACID

Concentration	Trial	Total No. of ears	Ears with grains	Total No. of grains
0 p.p.m.	1	16	0	0
100 p.p.m.	1	11	2	10
	2	9	3	21
300 p.p.m.	1	8	5	15
	2	11	8	37

This observation may be important in the production of hybrid barley either as an aid in cereal breeding or in the synthesis of commercial hybrids.

This investigation was carried out under the supervision of Mr R. N. H. Whitehouse, whom I thank for his encouragement and helpful criticism. I also thank the director of the institute, Dr G. D. H. Bell, for his interest in the problem, the Tanzania Government for giving me an extended leave of absence from the Ministry of Agriculture, and the Association of Commonwealth Universities for sponsoring my studies at Cambridge.

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## PSYCHOLOGY

### Genetic Relationships, Choice Models, and Sucrose Preference Behaviour in Mice

BEHAVIOURAL geneticists have criticized mathematical models of learning and choice which do not consider genetic and other individual difference parameters<sup>1,2</sup>. No one, however, has demonstrated empirically that genetically related variables aid in determining how well a given model will fit the data. I have found it necessary to include familial parameters within the framework of probabilistic models.

Luce<sup>3</sup> developed a simple and powerfully flexible model of individual choice behaviour, when he derived equations which utilize data from one experiment to predict the choice of the same subject in an entirely different experiment. Equation (1), adapted from Luce's model, was used in this sucrose preference study for such predictions

$$\hat{P}(i|i,k) = \frac{1}{1 + \left( \frac{P(K|i,j,k)}{P(i|i,j,k)} \right)} \quad (1)$$

where  $\hat{P}(i|i,k)$  is the estimate of the probability of selecting solution  $i$ , when the animal is given a simultaneous choice of concentrations  $i$  and  $k$  (two bottle experiment).  $P(i|i,j,k)$  is the probability of selecting solution  $i$ , when the same subject is given a simultaneous choice of concentrations  $i$ ,  $j$  and  $k$  (three bottle experiment). This equation is an application of Luce's results concerning the independence of irrelevant alternatives and his constant ratio rule. It is relatively common in studies of preferences for liquids to use the proportion of total liquid intake from a solution as an index of preference. To interpret Luce's model in this context, the proportion of liquid intake from a solution will be taken as an operational definition of probability.

The same animals were used in both investigations. In the prediction experiment thirty-seven subjects were taken from a four way cross population ( $AR \times CD$ ) derived from  $A/Crg1$ ,  $R/III/Crg1$ ,  $C57BL/Crg1$  and  $DBA/Crg1$  strains of mice. These animals fell into four full-sib families, with more than one litter in each family, and ranged in age from 99 to 174 days at the start of the experiment.

Each subject was placed in a single cage and simultaneously presented with a choice of 30 per cent, 45 per cent and 60 per cent (w/v) concentrations of sucrose. The

Table 1. COMPARISON OF THE MEAN PREDICTED AND OBSERVED VALUES OF  $P(i|j,k)$ 

	Mean	Standard deviation
Observed value	0.648	0.164
Predicted value	0.620	0.157

$i$  is 30 per cent and  $k$  is 60 per cent sucrose solution. Predictions were generated by employing the data from the first experiment. Standard deviations are also included.

subjects drank these solutions from 25 ml. graduated cylinders fitted with non-reactive stoppers and stainless steel drinking tips. The three concentrations remained in each cage for 9 days; the position of the cylinders was changed after each 3 days. Total daily fluid intake from each solution was recorded to the nearest 0.1 ml. In addition, laboratory chow was available at all times during the experiment.

After about 2 weeks, Luce's model was tested in a different way. In this experiment the same subjects were given a choice between 30 per cent and 60 per cent sucrose for 2 days. Again liquid consumption was measured to the nearest 0.1 ml., and food was available at all times.

The scores for raw intake were converted to proportions of total intake from each solution. In the first experiment preferences change only slightly from day 4 to day 9, and so I decided to use these measures in testing the model. The data from the 2 day exposures to 30 per cent and 60 per cent solutions were also combined for this analysis.

If  $i$  is 30 per cent,  $j$  is 45 per cent and  $k$  is 60 per cent sucrose, then by applying equation (1) each subject's preference in the first study was used to predict its preference in the second experiment. Thirty-seven predictions were made in this way. Table 1 compares the mean and standard deviation of the predictions with the observed mean and standard deviation. At this level of analysis Luce's model fits the data relatively well, but the plot of each subject's prediction on one axis and the observed preference on the other axis shows a large amount of scatter (Fig. 1). If the model accurately predicted each individual's sucrose preference, all points on the graph would tightly cluster around the line  $y=x$ , but this is not so. The correlation,  $r$ , between predictions and observations, is 0.590, and  $r^2$ , the proportion of variability observed in the second experiment which the model accounts for, is 0.35. Thus the predictions only determine 35 per cent of the observed variability in preferences.

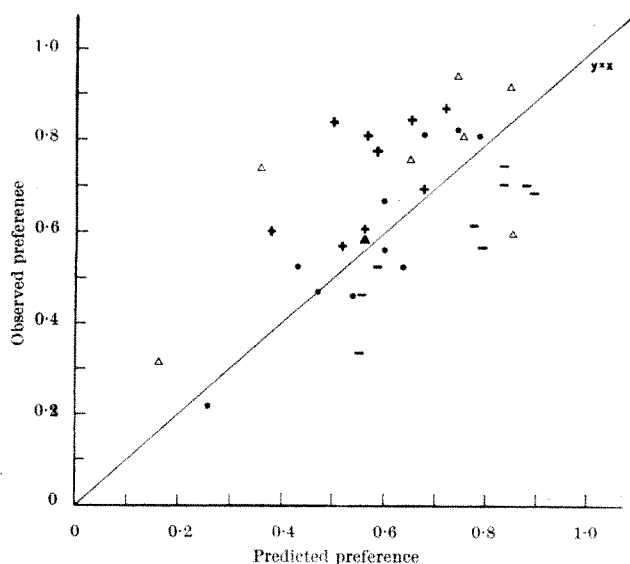


Fig. 1. A scatter diagram showing the relationship between predicted and observed preferences as a function of families. The reference line  $y=x$  has been drawn to demonstrate theoretically perfect predictions, and it does not represent the actual regression line. (●) Sibship No. 1; (+) sibship No. 2; (—) sibship No. 3; (△) sibship No. 4.

Fig. 1 also shows how well each of the four groups fits the model. The first sibship lies in the vicinity of the line  $y=x$ , and therefore the model accurately describes the preference behaviour of this group. With one exception the subjects in sibships 2 and 4 lie above this line, while all the individuals in sibship 3 fall below it. The scatter of points around the line of perfect prediction seems to be intimately connected to membership in family groupings. Thus if one or more of those particular sibships were not included in this study, the close correspondence between the predicted mean and observed mean might not have occurred.

To check the relative importance of this genetically related variable, a deviation score was calculated for each subject. A deviation score is defined as the difference between the observed and predicted proportions,  $P(i|j,k) - \hat{P}(i|j,k)$ , where  $i$  = 30 per cent and  $k$  = 60 per cent sucrose. These scores vary in both magnitude and sign. The data were next linearly transformed to positive numbers and subjected to an analysis of variance ( $F=16.051$ ,  $P<0.001$ , degrees of freedom=3,33). The proportion of total variance determined by sib-relationships, as estimated by the intra-class correlation,  $R$ , equals 0.61. The confidence interval for  $R$ , which can be used as an index of sampling variability, is  $0.21 < R < 0.88 = 0.98$ . Thus it is 98 per cent certain that the true value of  $R$  falls within this interval.

In order to estimate the reliability of these findings in another way, between the first and second experiments the same subjects were given a simultaneous choice of 0 per cent (tap water), 30 per cent and 60 per cent sucrose. The procedures were the same as those used in the first experiment cited. Calculations of  $\hat{P}(i|j,k)$  and deviation scores were again made for all thirty-seven subjects. The correlation between the deviation scores found from both experiments was 0.715, which suggests that these systematic individual differences in predictive accuracy are reliable and consistent differences.

The model was originally intended to describe the choice behaviour of individual subjects. These results show that (a) the close correspondence between the predicted and observed means may be a spurious finding (consider Fig. 1 without including sibship No. 3); (b) the actual correlation between the predictions and observed values accounted for only 35 per cent of the variability in preferences; and (c) sibship groupings determine approximately 61 per cent of the variability in predicted accuracy. Thus because the magnitude and sign of these deviations from perfect predictions can be greatly affected by the sibships used, it seems reasonable to include a genetically related parameter in future models of choice and preference.

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## ANIMAL BEHAVIOUR

### Stimuli provided by Courtship of Male *Drosophila melanogaster*

DURING courtship *Drosophila melanogaster* males perform a movement termed vibration<sup>1</sup>, in which one wing is held out horizontally at right angles to the body and vibrated in the vertical plane. This movement is very important in stimulating sexual receptivity in the female<sup>2</sup> and the

stimuli produced by vibration are perceived through the females' antennae<sup>3</sup>. The exact nature of the stimulation is unknown, although there is evidence that it is not olfactory<sup>4</sup> as was previously thought, nor is it visual, for success of mating is not significantly reduced in the dark<sup>5</sup>. Sound is produced during vibration, and so it is possible that the stimulus is auditory<sup>6</sup>. The sounds consist of pulses of between 180 and 350 cycles/sec with a pulse repetition rate of 29/sec at 25° C. Each sound pulse is possibly produced by one up and down movement of the wing as the sound record shows a single damped oscillation (Fig. 1).

In order to demonstrate experimentally the characteristics of the stimulation we attempted to provide an analogue for vibration and to see whether it was possible to use this to supplement the courtship of males with their wings removed. Such males have been shown to be very unsuccessful in obtaining mates although their courtship remains persistent and they attempt to copulate just as frequently as normal males<sup>2</sup>.

Virgin wild type flies were collected from stock bottles and kept in vials, ten flies in each vial, with standard *Drosophila* medium. Wingless males were obtained by lightly etherizing the flies and amputating the wings with a scalpel as near the body as possible. It is possible by this method to remove between 80 per cent and 90 per cent of the wings. The operations were carried out on the day after ecdysis and all experiments were done when the flies were 3 days old, for females are known to be maximally sexually receptive at this age<sup>7</sup>. A mating chamber 27 mm in diameter and 10 mm deep was used, the roof and floor being of fine nylon mesh and the wall of aluminium. A small aperture in the wall allowed flies to be introduced into the chamber. An electronic circuit was built the electrical output of which faithfully simulated the electrical record of the sounds recorded during vibration. The mating chamber was suspended above a loudspeaker through which the simulated vibration sounds could be reproduced. The initial experiments showed that while the sounds did facilitate the mating of wingless males the number of copulations did not approach the level achieved by the normal winged controls. It seemed possible that net movement of air could occur as a result of vibration. In order to simulate this an extractor fan was arranged to produce a current of air through the cell. The pulse repetition rate of the sounds produced during vibration is temperature dependent, and so all the experiments were carried out at  $25 \pm 1^\circ$  C. The experimental arrangement is illustrated in Fig. 2.

It is difficult either to measure or calculate actual values of sound intensity and velocity of air movement which are produced by the normal vibration of males. The levels were therefore rather arbitrarily set in such a way that the artificial stimuli did not produce any gross changes in the behaviour of the flies other than changes in mating speed. Even slow air movements, however, seemed to reduce ambulatory activity although the flies appeared to habituate to this stimulus in a few minutes.

Ten males and ten females at a time were introduced into the mating chamber and the number of copulations

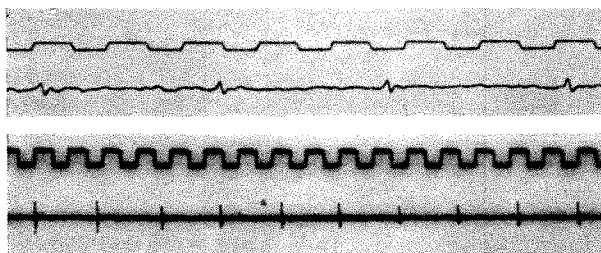


Fig. 1. Oscilloscope records of the sound produced by vibration at slow and fast speeds to show the regularity of the pulse repetition rate and the shape of the sound pulses respectively. Time marker, 50 cycles/sec.

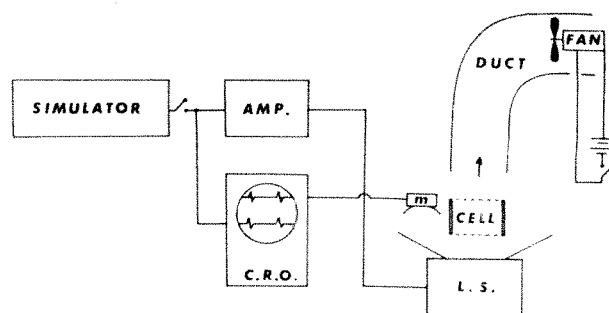


Fig. 2. Experimental set-up for providing simulated courtship vibration. m, Microphone for monitoring audio output from the loudspeaker (L.S.). For further explanation see text.

during the first 15 min was scored. Once copulation had commenced males were not observed to dismount within the test period. Males did not therefore mate more than once. The following experimental groups and controls were tested as follows: normal winged males without sound or air current; wingless males without sound or air current; wingless males with sound but without air current; wingless males without sound but with air current; and wingless males with both sound and air current.

The number of copulations within groups in a series of replicate experiments was not normally distributed in all the groups. This was principally because of the large number of nil copulations scored particularly for wingless males without sound or air current. Also the variances were not independent of the means. For these reasons Wilcoxon's method, which is distribution free, was employed to test for differences between the groups<sup>8</sup>. The percentage copulations in each group are given in Table 1 and the results of the statistical analyses in Table 2.

Table 1. PERCENTAGE COPULATIONS ACHIEVED BY NORMAL WINGED MALES AND WINGLESS MALES SUPPLEMENTED BY ARTIFICIAL STIMULI

Wings	Air current	Sound	No. of experiments	Percentage copulations
+	-	-	14	35.0
-	+	+	14	42.9
-	-	+	14	25.0
-	+	-	14	15.0
-	-	-	13	3.1

Table 2. STATISTICAL COMPARISONS BETWEEN COPULATIONS ACHIEVED BY WINGLESS MALES WITH DIFFERENT ARTIFICIAL STIMULI

	- Wings + Sound - Air	- Wings - Sound + Air	- Wings - Sound + Air
- Wings - Sound - Air	$P < 0.02$	$P < 0.001$	$P < 0.0001$
- Wings + Sound - Air		$P < 0.12$ Not significant	$P < 0.001$
- Wings - Sound + Air			$P < 0.001$

Either the sound or the air current is on its own sufficient to facilitate the mating success of wingless males while the two stimuli provided concurrently have an even more conspicuous effect. It seems from the results that the simulated stimulation is as effective as or even slightly more effective than the natural vibration. It is not justified, however, to make a direct comparison between the number of copulations achieved by winged and wingless males in the mating chamber which we used for these experiments. The wings are used not only to provide sexual stimulation, for competing males flick their wings at one another. This movement has an inhibiting effect on the courtship of males and possibly also has a similar effect on females. In the limited space provided by the mating chamber there is a considerable amount of interaction between winged males, and this is markedly less apparent in experiments with wingless flies. For this reason the results obtained with winged flies have not been treated statistically but are included to give an approximate indication of the natural situation.

The results suggest that the simulated stimulation provided by our apparatus is analogous to that of normal vibration. There therefore seem to be two components of the stimulation, one phasic and one tonic. It is not possible to decide from these results whether the females become receptive because of the heterogeneous summation of the two components or whether the tonic one, that is, net air movement, acts by inhibiting walking in the females, thus making them more accessible to copulatory attempts by males.

It has been shown electrophysiologically in several insects, including *Calliphora*, whose antennae are similar in structure to that of *Drosophila*, that the antennae respond separately to both tonic and phasic stimuli<sup>9</sup>. It is likely therefore that the antennae of *Drosophila* will have similar characteristics. We have observed that the aristae of the antennae resonate in response to a pure tone of between 180 and 220 c.p.s. which is within the range of frequency found during vibration and is also the same as the wing beat frequency during flight<sup>10</sup>. The correspondence between antennal resonance and the wing beat frequency has also been noted in *Anopheles* species where the flight sounds of the females serve to attract males<sup>9</sup>. The arista of *Drosophila* does not appear to resonate in response to either the stimulated or the normal vibration sounds, however, presumably because the energy level is much lower. Further evidence for the involvement of the arista is provided by Manning, who has demonstrated that if the aristae of a female are immobilized she becomes sexually unreceptive (personal communication). It is possible that the intermittent signal of vibration has evolved so that the female can discriminate between the stimuli provided by courtship and flight.

The vibration of different species of *Drosophila* is known to differ with respect to both pulse repetition rate and to intra pulse frequency<sup>11</sup>. These changes could be evolved as an aid to sexual isolation. Our simulator is variable for both these characteristics and this will allow us to analyse whether these signals are indeed species specific in their effects.

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### Divergent Thinking and Science Specialists

EMPIRICAL studies casting doubt on the IQ as an adequate summary of an individual's intellectual capacities<sup>1,2</sup> have lent support to the increasing use of tests of "divergent thinking", and to attempts to show that such tests are usefully related to academic achievement, especially in science<sup>3,4</sup>. These tests are distinguished from more conventional tests of intellectual capacity by the fact that they are designed to measure originality and flexibility. The older tests, by contrast, give greater emphasis to the finding of strictly logical, "correct" solutions. Two chief points about the newer tests can be emphasized: the first concerns their doubtful long-range validity, because it

has not been shown that they correlate with later achievement in the way that ordinary IQ tests do<sup>5</sup>, and the second centres on Hudson's report<sup>6</sup> that sixth form science specialists in British schools are usually convergent in their intellectual bias.

Some data collected at the University of New England are relevant to these two issues. In 1963, out of a total of 336 first year students, the 100 men with the highest marks in the New South Wales matriculation examination were selected. During their first two days in the university this group of men completed five convergent tests (vocabulary, verbal reasoning, verbal comprehension, logical reasoning, and reading speed), and six divergent tests (product improvement, unusual uses, ask and guess, picture construction, figure completion, and parallel lines)<sup>7,8</sup>. The convergent tests were scored in the usual way by tallying correct responses, while divergent tests were scored on the dimensions of fluency, flexibility, and originality in the case of the verbal tests, and fluency, flexibility and elaboration in the case of the non-verbal. Later a single score was obtained for each divergent test by simply summing the three sub-scores for that particular test. After the scores for each individual test had been standardized to avoid difficulties associated with large differences in means and standard deviations, total scores for divergence and convergence were obtained for each man by summing his scores on the divergent and convergent tests. Finally, total scores on divergence and convergence were again standardized and an "intellectual bias" score was obtained for each man by subtracting the convergence score from the divergence score. Positive scores thus indicated a preference for divergent intellectual functioning, negative scores a preference for convergent. No account was taken of level of scores, but, because all the men were very able, level was regarded as relatively unimportant.

Included among these 100 very able entrants to the university were thirty-eight of the sixty-three men who entered science departments in 1963. The performances of these science undergraduates during the 4 yr period, 1963-66, provided the data with which this communication is concerned. Two issues involving these students can be examined. To what extent are they predominantly convergent in their intellectual bias? To what extent are individual differences in bias, as against level, related to undergraduate achievement?

Twenty members of the group displayed the convergent bias and eighteen the divergent. These proportions can be compared with those prevailing in the total group of 100 able men, fifty-five of whom had convergent biases and forty-five divergent. On the basis of the distribution of divergence and convergence in the full group, twenty-one men would be expected to show the convergent bias in any chance sub-group of thirty-eight. Thus the group of able science students did not contain an unusual number of convergent thinkers when compared with all very able men entering the university.

Convergers and divergers did not differ so far as matriculation marks were concerned. The mean matriculation mark of the twenty convergers was 424.7 (standard deviation, 54.9) and the corresponding mark for the eighteen divergers was 411.7 (standard deviation, 41.6). A further comparison of examination performances was made at the end of first year. Each man had been examined in four subjects and allotted a result ranging from FF, indicating a clear-cut fail, to HD, indicating very superior performance. The range of results was converted to a six-point numerical scale ranging from 0 for FF to 5 for HD, and a total achievement score out of a possible 20 calculated for each man. Again, there was no significant difference in achievement between the convergers (mean, 9.30; standard deviation, 4.04) and the divergers (mean, 9.28; standard deviation, 3.97).

When, however, the question of whether or not students proceeded to honours was considered, the position was



quite different. In Australian universities, students enter as candidates for the ordinary degree and are admitted to honours courses on the basis of superior performance in the first 3 yr. Honours requires an additional year. Of the total male science entry for 1963, nineteen completed 4 yr honours degrees in 1966. Fifteen of these nineteen were included in the thirty-eight men with whom this communication is concerned, scarcely surprising in view of the fact that the thirty-eight men were selected on the basis of superior matriculation performance. No fewer than eleven of these fifteen were divergers, only four were convergers. Thus although academic performance at matriculation and first year levels had not differed, markedly dissimilar numbers of the two groups had gone on to honours. The proportions differ significantly from those which would be obtained if there were a chance relationship between convergence/divergence and the taking of honours ( $\chi^2$ , 5.09; degrees of freedom, 1;  $P < 0.025$ ).

The distribution of honours students by departments is also interesting. Convergers receiving honours were confined to mathematics (two), physical chemistry and physics. On the other hand, divergers received honours in mathematics (five), physical chemistry, biochemistry, physics, geology, biology and zoology. Of the total of

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## GENERAL

### Microbarograph Record of Waves from the Chinese Thermonuclear Explosion on June 17, 1967

AN unusual train of waves, characteristic of a distant and violent disturbance of the atmosphere, began to be received on a microbarograph<sup>1</sup> in Aberdeen at about 0545 h G.M.T. on June 17, 1967. The unusual train, which lasted about an hour, was superimposed on a much longer and more usual series of waves of period about 12 min and amplitude 250 microbars; such waves, which are of natural origin, are characteristically received during meteorological inversions.

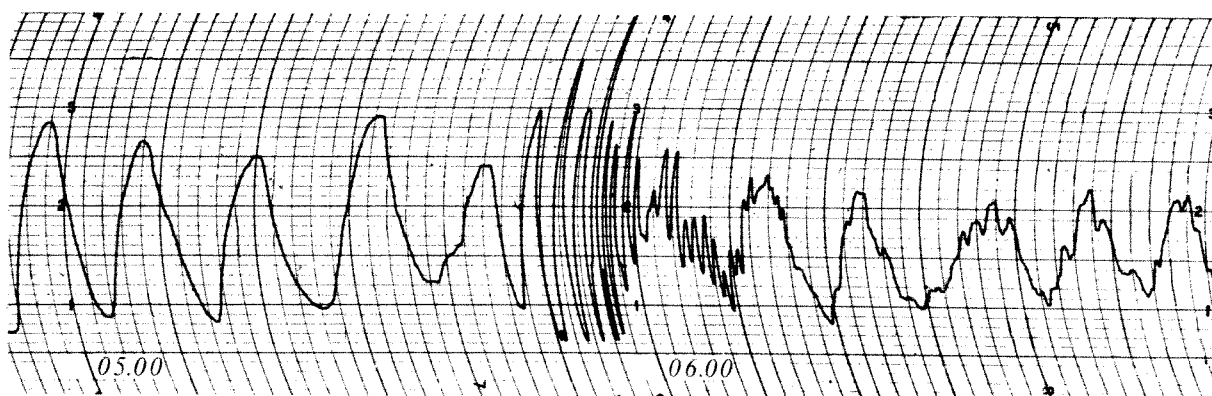


Fig. 1. Microbarograph record of waves from the first Chinese thermonuclear explosion taken at Aberdeen 0500–0700 h G.M.T., June 17, 1967. Full scale deflexion is roughly 270 microbars for waves with a period of 2–4 min.

nineteen honours degrees awarded in 1966, eight were with first-class honours. Seven out of these eight went to the sub-group of thirty-eight, so that only one man who was not in the highest scorers at matriculation level obtained first-class honours in science 4 yr later. Five of the seven firsts went to divergers. In the case of mathematics, three out of four firsts went to divergers.

Although these results are clearly of limited general application because of the small number of students involved, they are interesting in that they do not agree with findings for British sixth form schoolboys<sup>6</sup>. Furthermore, they also suggest that later years of undergraduate study, and especially the honours year, may give students more opportunities to utilize divergent thinking skills, although it is doubtful if any undergraduate course affords many such opportunities.

I thank the Student Research Unit of the University of New England for making available to me data collected by them in 1963.

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<sup>1</sup> Hudson, L., *Contrary Imaginations* (Methuen, London, 1966).

<sup>2</sup> Gibson, J., and Light, P., *Nature*, **213**, 4415 (1967).

The unusual train is likely to have arisen from the first Chinese thermonuclear explosion, announced some hours later. Two small disturbances on a vertical seismograph were also recorded at Aberdeen at 0050 and 0147 h G.M.T. on the same day. If the earlier of these disturbances originated from the explosion, the range was about 4,000 miles, which is consistent with an origin in Western China. The peak-to-peak amplitude at Aberdeen (where I am indebted for the calibration and maintenance of the microbarograph to Mr T. P. Gill) of the largest atmospheric waves from the bomb was about 270 microbars. This may be compared with amplitudes at Aberdeen of 350 microbars and 1,000 microbars for the Russian explosions of 25 and 50+ megatons TNT equivalent over Novaya Zemlya on October 23 and October 30, 1961. Because the Chinese explosion was at roughly twice the range of Novaya Zemlya it thus appears to have been equivalent to that of between 20 and 30 megatons TNT. This figure is consistent with the 1.5 min period of the largest waves in the train<sup>2</sup>.

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<sup>1</sup> Jones, R. V., and Forbes, S. T., *J. Sci. Instr.*, **39**, 420 (1962).

<sup>2</sup> Hunt, J. N., Palmer, R., and Penney, W., *Phil. Trans. Roy. Soc., A*, **252**, 275 (1960).

## BOOK REVIEWS

## HERITAGE OF MENDEL

## The Origin of Genetics

A Mendel Source Book. Edited by Curt Stern and Eva R. Sherwood. Pp. xvi + 179. (London: W. H. Freeman Company, Ltd., 1966.) 36s. cloth; 18s. paper.

## Heritage from Mendel

Edited by R. Alexander Brink with the assistance of E. Derek Styles. (Proceedings of the Mendel Centennial Symposium sponsored by the Genetics Society of America, 1965.) Twenty-seven contributors. Pp. xii + 455. (Madison, Milwaukee, and London: The University of Wisconsin Press, 1967.) 75s. net; \$10.00 clothbound; \$7.5 paperbound.

If we look for the sources of genetics we are bound to look first at Mendel's paper on peas. Eva Sherwood has made a new English translation of this paper and to it she has added a series of other translated and reprinted articles. First there is Mendel's seven-year correspondence with Carl Nägeli, that eminent but opinionated botanist. Secondly, there is Mendel's fruitless paper on the breeding of what we now know to be sub-sexual *Hieracium*. Thirdly, there are the articles and letters of the rediscoverers, both the enquiring and ingenuous Correns and the dogmatic and disingenuous de Vries. And to cap the story there is Fisher's startling re-examination of Mendel's numbers.

What of the new translation? The words used in the first English version of Mendel's paper from "character" to "round and wrinkled" have over a period of sixty-six years passed into the language of genetics. An important step in genetics was taken when Bateson showed that the heterozygote in respect of the character difference round-versus-wrinkled could be recognized and the source of the difference explained. The new translator now speaks not of "characters" but of "traits" and not of "wrinkled" but of "angular". No one probably will follow her unfortunate example. So also with other amendments—whatever they may be, for the translator has given us no help in finding them. Her successes and her failures together are therefore likely to pass unnoticed.

The book as a whole, however, we must treat on a different level. It takes us a step forward in understanding Mendel's work. Fisher has said of this work (on page 171 of the present book) what is true of many smaller revelations, that "each generation perhaps found in Mendel's paper only what it expected to find". The first generation indeed found a jumble of words and numbers concerning hybrids and peas. The second generation found genetics. A later generation (in the coming century) will find that Mendel's ideas in this paper reached out so far as to be almost indispensable for a serious and civilized education.

After Fisher's analysis of Mendel's numbers showing that they agreed too well with Mendelian expectations there follows a neat and just re-assessment by Sewall Wright. Neither is concerned, as Mendel was, with the foundations of biology. On the contrary both offer us an exercise in statistical virtuosity. But what a happy exercise it is! Happy also am I to see so much indignation avoided by a posthumous correction now posthumously corrected.

The fact is that if Mendel had taken and discussed all the statistical precautions which his successors have envisaged he might, even in 1900, have missed having any listeners, let alone readers or successors. And, as we may see here, de Vries was incapable of learning from the sim-

plest practices of Mendel how to set out his own numerical records with veracity: he thought a percentage was a good enough statement of his observations. From de Vries back to Mendel and on to Sewall Wright we are taken in one quick journey through a large part of the evolution of statistical understanding.

In *Heritage from Mendel* twenty-one articles, twelve American, nine foreign, discuss genetics as it seems to their authors today. Nearly all are concerned with looking very closely and deeply at problems covering separately, and with little overlap, a large part of genetics. Of the connexions between these articles the authors and their readers may or may not be aware: we cannot tell.

This book makes clear, if it was not clear already, that the heritage of Mendel has now attached itself to the whole movement of biology. It is not therefore possible to produce a volume with the purpose expressed by the present editors of honouring Mendel without some attempt at co-ordination and elucidation before and after the manuscripts are collected. Of such an attempt this *dysposium* provides evidence in a few articles which indicate the development of their subject. In this way Beermann, Sewall Wright and Crow tell us things which connect one another. And one contributor, Beadle, tells us things which even connect with the origin of genetics. These writers indicate the size of the opportunity that has been missed.

C. D. DARLINGTON

## DEVELOPMENT OF PSYCHIATRY

## Mental Illness

Progress and Prospects. By Robert H. Felix. Pp. 110. (New York and London: Columbia University Press, 1967.) 35s. 6d. net.

THIS book consists of four lectures given by Professor Robert H. Felix, Dean of the Medical School of St. Louis University, at Columbia University. The first lecture describes the history of mental illness from the earliest times until the nineteenth century. The second lecture deals with the realization in America, during the Second World War, that there were thousands of people who were mentally unfit, ranging from the insane to the neurotic. Between 1942 and 1945 out of approximately 15 million examinations for admission to the American armed forces 1,875,000 individuals were found to be suffering from neuropsychiatric disability. Out of every 100 men examined twelve were rejected for this reason. They exceeded the numbers assigned to the Pacific theatre of operations during the war. This was not all. It was found that those in mental hospitals were increasing, as for example, from 183.6 in each 100,000 in 1904 to 412.6 in each 100,000 in 1950.

The third lecture concerns the advances made in dealing with the 47 per cent of the mentally ill patients, out of the 750,000 sick in hospital, on any one day in America. Professor Felix points out that the discovery of tranquillizers which have had such a beneficial effect in reducing the number of patients in mental hospitals was really incidental. Chlorpromazine, for example, was discovered when research was being carried out on hypothermia in France, and reserpine was found during work on *Rauwolfia serpentina* as a hypotensive element.

The last lecture seems to be the most unsatisfactory of the four, although it might have been expected to be the most exciting. The author seems to have become bogged down with administrative matters, instead of scientific possibilities. He does not mention that there are tremendous advances to be made in the causes of mental deficiency, the reduction of organic dementia caused by unsuspected agents, the elimination of psychosis by the discovery and removal of the causes of schizophrenia and manic depression. The tranquillizers, for example, have reduced the resident population of the mental hospitals

of the United States from 558,922 in 1955 to less than 490,754 in 1964 and this in spite of the admission of 302,946, the largest number in history, in 1964. This surely is only the beginning. If research is widened the vaunted mental services will be unnecessary.

In spite of this criticism those interested in American psychiatry, particularly from the administrative point of view, will find this book interesting. It is unfortunately expensive for its size, although well produced.

CLIFFORD ALLEN

## SPACE IN THE BRAIN

### Brain Tissue Electrolytes

By A. Van Harreveld. (Molecular Biology and Medicine Series.) Pp. xii + 171. (London: Butterworth and Co. (Publishers), Ltd., 1966.) 44s.

THE subject matter of this short (171 pages) and rather expensive book is more restricted than the title implies. The author deals with the problem of the size of the extracellular space in the central nervous system, which is usually assumed on the basis of conventional space techniques and electron microscopy to be a mere few per cent of the tissue volume. Van Harreveld assembles convincing evidence that this traditional view is probably incorrect and that the size of the space is not very different from the size in other parts of the body.

The large amounts of extracellular ions, sodium and chloride, found in nervous tissue, the low electrical impedance of the cerebral cortex and the loose packing of neural elements in electron micrographs of tissue frozen within half a minute of circulatory arrest support his contention that the space is about 20 per cent of tissue volume. Earlier measurements of the extracellular space from electron micrographs had not allowed for the very rapid diminution in size of the space that takes place during fixation of the tissue by perfusion. He describes how asphyxia and spreading depression result in a rapid increase in tissue impedance which he attributes to the movement of extracellular ions and water into the depressed cells with consequent reduction in the low impedance extracellular component of the tissue.

Within its compass this is a well written and well argued book on the subject in which the author has done notable experimental work. I warmly recommend it.

J. T. FITZSIMONS

## OUR FAVOURITE REMEDY

### The Salicylates

A Critical Bibliographic Review. Edited by M. J. H. Smith and Paul K. Smith. Pp. xiv + 313. (New York and London: Interscience Publishers, a Division of John Wiley and Sons, 1966.) 80s.

THIS is an exemplary publication—a model of what a monograph can be. It is, above all, about an interesting subject—a family of drugs dating back to the earliest herbal remedies using leaves, bark and fruits of plants containing methyl salicylate ("oil of wintergreen") and glycosides of 2-hydroxybenzyl alcohol (such as salicin), which have withstood all attempts by the pharmaceutical industry to render them obsolete. Aspirin (acetyl salicylic acid), introduced in the 1890s, is still the dominant drug today, not only in tonnage consumed but as first choice whenever an antipyretic, analgesic, mild sedative or anti-rheumatic agent is required. It is also remarkably safe, a property which many less effective drugs conspicuously lack, allowing us easy access to it. To the pharmacologist and physician alike, the question "How does it work?" is

still a challenge. If some answers are found to that question before another 100,000 tons of aspirin are consumed, it will be largely as a result of the work summarized in this monograph.

The book is therefore timely. It is also well written, well documented, well produced, reasonably priced and should be of considerable interest to a wide range of readers. It is a fine tribute to the late Paul K. Smith, whose inspiration is apparent in this volume but who unfortunately died before the manuscript was prepared.

The subtitle and preface indicate that this is not an exhaustive survey of the literature. The aims of this volume, which are to update an earlier monograph of the same title (published in 1948) and to critically evaluate various hypotheses which have been advanced to explain the multiple actions of the salicylates, are certainly achieved. The introduction gives a complete synopsis of the book and is a model of what an "instant survey" should be. Four of the six chapters are written by M. J. H. Smith, who modestly designates himself on the dust jacket merely as a co-editor. There is a brief subject index. Every chapter is a review in itself and, appropriately, each contains a summary—a welcome feature deserving wider emulation.

Considerable attention is given to biochemistry and it is no accident that so much more is known about the salicylates than, for example, colchicine or caffeine, because problems such as the disposition of the salicylates *in vivo* and their effects on enzyme systems have been investigated by essentially biochemical techniques. These particular aspects of a drug's pharmacology are not readily discerned by some of the biophysical techniques and electronic hardware which the average pharmacologist tends to use as his stock in trade.

The description of the absorption and metabolism of salicylate in the first chapter should be required reading for all who hope to investigate the pharmacokinetics of other drugs. Due emphasis is given to the ways in which the pharmaceutical formulation can profoundly influence the rate of gastro-intestinal absorption. There are clear explanations of two paradoxes: the increase in drug half-life *in vivo* with increasing drug dosage, and the fact that bicarbonate can both facilitate and antagonize gastric absorption of aspirin. The second chapter emphasizes the multiple sites of action of the salicylates and indicates how an overall metabolic effect can result from a mixture of superimposed effects of these drugs on several different enzyme systems. The nature and consequences of drug action on the multi-enzyme system of mitochondrial energy conservation ("oxidative phosphorylation"), the aminotransferase enzymes and the nucleotide-linked dehydrogenases, are clearly summarized. Much of the third chapter is taken up with disposing of the old heresy that the antirheumatic properties of the salicylates are mediated only through the adrenal cortex, although toxic (as opposed to therapeutic) doses of these drugs are recognized as stimulators of the pituitary-adrenal axis. The action of the drug on thyroid function is also covered in some detail. The fourth chapter discusses the physiology of pain perception and the problems of measuring pain objectively and assessing the potency of non-narcotic analgesics. Evidence is given that unhydrolysed aspirin may be a more effective analgesic than the salicylate anion and that both these forms are effective only when the pain-evoking stimulus is chemical in nature. The chapter on anti-inflammatory activity opens with a pithy and entertaining survey of that nebulous subject, inflammation. This is followed by a sceptical enquiry into the effectiveness of salicylates in influencing experimental inflammatory states in animals and clinical inflammatory disease. Possible sites of drug action in suppressing inflammation are thoughtfully analysed.

The final chapter, on toxicology, considers the association of gastro-intestinal bleeding with salicylate medication as well as the overt toxicology of these drugs. The

multiple action of toxic amounts of salicylates in deranging the plasma anion-cation balance is detailed. The book ends with a very useful description of the various methods of eliminating excess salicylate from the body and discusses the choice of a suitable procedure for treating a given case of salicylate intoxication.

One of the most impressive features of this book is that the argument is never swamped by a sea of facts, although these are plentifully provided, and the clarity of organization is apparent even on cursory inspection. Ill-warranted assumptions are clearly exposed as impediments to more satisfactory explanations of the duration and mechanism of action of the salicylate drugs *in vivo*. One noticeable omission from this otherwise excellent account of these drugs is any specific discussion of their antipyretic properties.

If there is a serious shortcoming in this book, it is the scant attention paid to the physico-chemical properties of both the postulated drug receptors and the various dosage forms of the salicylates (and their metabolites), which ultimately must determine largely the therapeutic or toxic action of these drugs at the molecular level. Salicylic acid is a remarkable chemical—a relatively strong acid ( $pK_a$  3.0) which, unlike many such acids, can pass from an aqueous phase into or across a lipid phase at physiological  $pH$ . This is possible because the intramolecular hydrogen bond between the ionized carboxyl group and the undissociated phenolic group effectively diminishes the molecular hydration of the drug anion. Some recent studies, reported in *Biochemical Pharmacology*, suggest that the salicylate anion can effectively bind to, and thereby neutralize in more senses than one, essential cationic groups of intracellular enzymes and other key proteins. The physical properties of the drug species *in vivo*, therefore, effectively determine both the translocation of the drug (anion) from the extracellular compartment to these active sites within a cell and the subsequent drug binding at these sites, and thus the overall action of the drug.

This book is warmly recommended to all who are concerned with drugs; all pharmacologists can learn something from it. Future authors of pharmacological treatises would do well to follow Professor M. J. H. Smith's example in combining enthusiasm for their chosen interest with clear-headed objectivity, a lucid style and searching critique.

M. W. WHITEHOUSE

## MEASURING CARDIAC FUNCTION

### Ballistocardiography in Cardiovascular Research

Physical Aspects of the Circulation in Health and Disease. By Isaac Starr and Abraham Noordergraaf. Pp. vi + 438. (Amsterdam: North-Holland Publishing Company, 1967.) 110s.

BALLISTOCARDIOGRAPHY has been around for some thirty years and a recent bibliography on the subject lists 1,500 papers. The subject has been a confusing one, much worked in but with little practical impact on cardiology. This book on the subject is particularly useful for three reasons: it distills the essence of the large bibliography; it is written by someone who has been in the field since the beginning and has acquired a sense of proportion and perspective; and finally because the day of the ballistocardiogram may be approaching.

As Dr Starr repeatedly points out, the ballistocardiogram has two unique advantages as a method of investigating cardiac function. The first is its utter simplicity as compared with the other elaborate and traumatic techniques. The second advantage is that it measures the functional state of the heart which other investigations do not. More and more it is becoming apparent that the acceleration of the blood leaving the heart is a better index of function than either blood pressure or volume output.

Does this book explain why in spite of these advantages the ballistocardiogram has made so little impact on clinical medicine? It does, but only obliquely, for the senior author is himself so convinced of the value of the ballistocardiogram (and with good grounds) that he is eager to put over this idea.

The tone of the book is a very personal one; whenever there is a point of controversy or doubt the senior author firmly states his own point of view and he states it with well-earned authority. Rather than being a fault this personal view is one of the main advantages of the book. The author knows his field thoroughly and can afford to be sane, sound and sensible about a subject which is often brought into disrepute by the over-enthusiasm of those who start using a tool for the first time. This virtue is especially evident in the section on the reading of records. Here the senior author points out that although better instrumentation may make more refined measurements possible, with the older records all that can be done is to classify them into four groups ranging from the normal to the grossly abnormal, on a pattern basis.

The early part of the book contains a clear mathematical exposition of the physical basis of the ballistocardiogram by Dr Noordergraaf. This is probably above the head of the average cardiologist and might on that account have been relegated to an appendix except that the validity of this method does depend so entirely on sound physics and correct instrumentation. Dr Noordergraaf's mathematical model of the circulation gives surprisingly good imitations of the ballistocardiogram, and so do Dr Starr's well-known cadaver experiments, but no description by mathematics or by models can exclude the possibility of alternative descriptions.

The possibility that movement of the heart wall contributes to the ballistocardiogram is rather summarily dismissed by Dr Starr on the dual grounds that his own cadaver experiments with blood movement alone could mimic the ballistocardiogram pattern and also that the experiments purporting to show the contribution of movement of the heart wall were grossly unphysiological. It should, however, be possible to settle this point in a more elegant manner by injecting carbon dioxide into the left atrium of animals while continuously recording the ballistocardiogram.

Most of the book is devoted to the use of the ballistocardiogram in estimating cardiac function in man—and that is as it should be. Much of the early difficulty was caused by inadequate instrumentation, and it is no doubt justifiable to question the adequacy of the instrumentation used to produce records which do not agree with one's own, but it does reinforce the unfortunate impression that the ballistocardiogram record depends far more on the competence of the technique than on the state of the heart—and this is one of the prime reasons it has been distrusted by outsiders. Quite early in the book the shin-bar method of obtaining records is dismissed as being too full of artefacts, yet in the results section a large proportion of the findings offered are stated to have been obtained in this way.

Perhaps the main criticism of the section on the ballistocardiogram findings in various cardiac states is that the authors have tried to be too comprehensive and too fair to other workers. The result is that the good studies are lost among a plethora of rather indifferent studies of which some are no more than anecdotal. Similarly, those conditions in which the ballistocardiogram has shown itself to have a definite use are rather diluted by the inclusion of every possible condition in which the method has been tried.

One of the main troubles with this method points to a dilemma in medicine itself. With increasing age the number of abnormal ballistocardiogram records obtained increases until in the 50–59 age group it may be as great as 34 per cent. Does this mean that some other factor connected with ageing has distorted the record and thus



limited the use of the method (for the number of abnormal records far exceeds the number of patients with cardiac impairment discernible on any other grounds); or should we maintain faith in the method and create a new disease of "presbycardia" which can only be detected by ultra-sensitive ballistocardiography? The data offered on the long-term follow-up of patients with such abnormal records certainly tend to support the latter view. This may not matter too much at the moment because little can be done to improve such hearts, but this may not always be the case. Furthermore, there are times when it is important to exclude the slightest hint of cardiac disease (for example, in testing air-line pilots). It is not made clear in the book whether the ballistocardiogram would be fair for this purpose.

With regard to the follow-up of abnormal records, several examples are shown together with a note of the unfortunate outcome. It may be felt that such anecdotes do not prove anything because normal records might be followed by such an outcome and many abnormal records are not. I feel, however, that there is an advantage in showing such records in order to acquaint the reader with the order of change that might be expected with the method.

One of the most interesting sections of the book deals with the effects of drugs (for example, digitalis and anaesthetics) on cardiac function and this would seem to be a useful area of study. Perhaps too much of the book is devoted to comparing records between different people and establishing norms as opposed to testing cardiac function by comparing the record in one patient before and during some manoeuvre. Such manoeuvres are technically difficult if they involve the patient leaving the ballistocardiography table and then returning. One simple manoeuvre not mentioned is the administration of a drug by aerosol during continuous recording.

A point well made by Dr Starr is the importance of the ballistocardiogram in assessing therapy, as in the excellent work done by Dr Deuchar with reference to aortic valve disease and cardiac surgery. The best way the ballistocardiogram can demonstrate its usefulness to unbelievers is in certain definite conditions rather than as a diffuse multi-purpose test.

In the final chapter Dr Starr effectively indicates the place of the ballistocardiogram in medicine and leaves no doubt that with robust instrumentation this method must become accepted as routine. For this reason, and also on its own account, this book belongs in every cardiology department.

EDWARD F. DE BONO

## FUNGAL COMMUNITIES

### Ecology of Fungi

By R. K. Robinson. (Modern Biology Series.) Pp. 116. (London: The English Universities Press, Ltd., 1967.) 21s. net.

In these days of increasingly precocious specialization in the education of scientists, it is becoming ever more important for young biologists not to miss the right choice through sheer ignorance of all the possible alternatives. Dr Robinson's book is a good example of what can be done in about a hundred pages of text to introduce a beginner to a new field of biology. The fungi, because of their larger cell size and much greater morphological diversity, provide a better introduction to the science of microbial ecology than do either the bacteria or the actinomycetes, so that identification into species is thereby both more rapid and more certain. In this book, Dr Robinson surveys the subject of fungal ecology in four chapters; an introductory one on fungal morphology, organization and classification is followed by others on saprophytic fungi, root-inhabiting fungi, and parasitic fungi, respectively. The book is illustrated by thirty-

eight text-figures, some of them original; among the latter, Fig. 20B is so unlike its subject (conidia of *Helminthosporium sativum*) as to suggest a mistake in labelling. Dr Robinson's treatment of his subject in these four chapters is comprehensive and very readable, as well as being thoroughly up to date. He is likely to be widely successful in explaining to young biologists what the science of microbial ecology in general, and of fungal ecology in particular, is about.

S. D. GARRETT

## ADVANCED LIMNOLOGY

### A Treatise on Limnology

By G. Evelyn Hutchinson. Vol. 2: Introduction to Lake Biology and the Limnoplankton. Pp. xi + 1,115. (London and New York: John Wiley and Sons, Inc., 1967.) 310s.

It is just ten years since the appearance of the first volume of this treatise, treating the geography, physics and chemistry of lake systems which to many limnologists has been a model of concise scientific writing and a mine of information. This second volume continues the fine tradition although some may be disappointed that it does not complete the treatise, but the subject has expanded such that productivity of the plankton, non-planktonic communities, typological, stratigraphic and developmental aspects are left to a third volume. There is no doubt that this was the only solution if the comprehensive coverage of the first volume was to continue through the biological part of the treatise. Let me say at once that this is a most impressive volume, combining as usual in Hutchinson's work an extensive review of the literature with a masterly synthesis of ideas and an original approach to so many aspects. Before the preface is an extract from Hudson and Gosse's classic work on the Rotifera—it is a lyrical passage and typifies Hutchinson's obvious fascination with the natural history of freshwater life. But this book is a natural history carried through to the most rigorous scientific analysis culminating in mathematical models wherever possible.

The opening chapter on the nature and origin of the freshwater biota starts with a synopsis of classification of the plant and animal groups represented in freshwater—it will provoke controversy, as Hutchinson intends. The accent in the discussion of the biota is on the adaptation from the marine to the freshwater environment and on the geological history. The physiological problems of adaptation to freshwater are discussed and the genera illustrated are taken from the world fauna and many obscure forms are illustrated. There is a wealth of fascinating information here, but the reader has to wait until the later chapters for illustrations and discussions of the biology of the commoner plants and animals.

The second chapter is a very brief but valuable account of the terminology of lacustrine communities—the concept of a lake as a single biotope is not strictly adhered to in later chapters and some of the terms are little used. A fairly mathematical treatment of the hydromechanics of the plankton follows in which sinking rates and turbulence are considered. The next three chapters are devoted to the phytoplankton; first its nature and distribution, in which the major algal groups are discussed and related to some physiological parameters; secondly, the associations including multispecific equilibrium, diversity and ecological classification; moving finally to the seasonal succession in the last of these chapters. The examples are selected from a wide series of lake studies and these have been welded into a coherent story in which the author has not merely been content to report the work of others but has analysed it and synthesized it into a valuable discussion. The last three chapters are devoted to the animal components of the plankton. The nature and biology of the zooplankton are largely concerned with

Rotifera and Crustacea, while the non-photosynthetic in contrast to the photosynthetic Protists are shown to be of relatively less importance. The biology of these groups has been considered in its widest sense from life histories, ecology, seasonal succession, to vertical distribution. A mass of diffuse data is summarized, including valuable work on algae as food, a relatively neglected link in the productivity of lakes. This is followed by two rather special topics, vertical and horizontal distribution (especially of Cladocera) and cyclomorphosis. The latter is mainly concerned with rotifers (*Asplanchna*, *Keratella*) and Cladocera (*Daphnia*, *Bosmina*), but also deals with dinoflagellates (*Ceratium*) and, although a little out of place, the size changes in diatoms. As in the first volume, there is finally a list of symbols used in the book, an excellent bibliography and index, index of lakes (including latitude and longitude for most), index of organisms, and finally a general index. Summaries are provided at the end of each chapter and an elementary student may gain a considerable insight into limnology from these alone but would miss a wealth of thought-provoking material.

The production uses the same type and format as the first volume, but there are in this case rather more common mis-spellings, particularly in the captions to the figures, which are themselves excellently produced.

No review can hope to indicate the extensive coverage of literature and ideas, of scholarship and of effort which has gone into this volume. It is an expensive book and it is devoted partly to plants and partly to animals. I trust no serious student of limnology will be put off by either: to search the literature would be even more expensive of time, effort and money, and biological limnology is essentially a synthesis of both or rather all four kingdoms which, under water, assume equal importance; at least awareness of all is essential to good limnology.

F. E. ROUND

## RICE UP TO DATE

### Mechanization and the World's Rice

(Proceedings of a Conference to Support the International Rice Year 1966 of the FAO of the UN held Sept. 26–Oct. 1, 1966.) Pp. 163. (Warwickshire: Massey-Ferguson (Export), Ltd., 1967. Distributed throughout the world by Basil Blackwell, Oxford.) 63s.

RICE is the staple food of more than half the population of the world and most of it is still grown as a subsistence crop on very small holdings. There is urgent need to increase supplies, and in order to focus attention on the problems involved the Food and Agriculture Organization of the United Nations (FAO) made 1966 an International Rice Year within the Freedom from Hunger Campaign.

Available supplies may be increased by extending the area of rice grown, by increasing the yield of each acre, and by decreasing losses during subsequent storage and processing. Mechanization can help to achieve these objectives, and the conference held in 1966 discussed how best to introduce machinery; the types at present available were demonstrated.

The twenty-five papers describe various aspects of production, storage and processing; some speakers deal specifically with mechanization, others do not mention it at all. All contributors treated the subject generally and sometimes superficially. The presentation and technical content are likely to prove more valuable to those engaged in planning and administration than to the specialist.

The book is printed by direct reproduction of typescript in double columns without the type being justified and so presents an uneven appearance. The diagrams are all clear, and the several photographs of rice production and rice machinery improve the presentation. The report

is printed on good quality paper and is therefore pleasant to handle, although the paper-back binding appears somewhat flimsy.

I have no doubts that the conference was very valuable, but I doubt the value for money of the report.

J. K. R. GASSER

## REFERENCE TO LUMINESCENCE

### Luminescence of Inorganic Solids

By Paul Goldberg. Pp. xii + 765. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1966.) 236s.

THIS book represents an attempt to survey inorganic luminescence in terms of a general introduction and eleven chapters on specific themes contributed by separate authors. Experience will have made readers painfully aware of the drawbacks of this treatment, but this volume indicates that a judicious editor can build up, instead of a chaos of fragmentation and repetition, a well-composed picture of the topic by prudent choice of authors and careful correlation of their contributions. In fact, Dr Goldberg has done much to free the field of luminescence from the siege mentality engendered in a topic which, in relation to theory, developed before its time, and which has often been stultified by an intractable technology.

The core of the book consists of articles on luminescence in halide, diamond and ZnS lattices, that is, those areas in which luminescence is in useful contact with other active areas of solid state physics. Oxygen-dominated phosphors are treated separately, and other sections deal with the materials problems of the development of lasers, and the luminescence of glasses and of thin films, providing some good surveys of the newer technical extensions of luminescence. There are two chapters on electroluminescence, one dealing with  $p-n$  junctions and the other with the less certain field of zinc sulphide. The use of electron spin resonance to study luminescence centres is admirably surveyed, and in the final chapter the different aspects of particle-excited luminescence, separated by historical accident, are unified.

The index in a book of this type is of great importance, but this one is, unfortunately, rather inadequate—for example, there is no cross-reference from “Optical Maser” to “Laser”. None the less, the book should serve well as both reference book and textbook for some time to come.

G. F. ALFREY

## THERMODYNAMICS AGAIN

### Thermodynamics

An Advanced Treatment for Chemists and Physicists. By E. A. Guggenheim. Fifth, revised edition. Pp. xxiii + 390. (Amsterdam: North-Holland Publishing Company, 1967.) 86s.

THIS edition, the fifth in eighteen years, has the same virtues of clarity, brevity and authority that marked its predecessors.

There is a new chapter of two pages on systems in motion according to the special theory of relativity. Here the problem of the temperature of a moving body—a problem that has given rise to recent correspondence and an editorial in *Nature*—is characteristically disposed of in one sentence: “There is no need to mention temperature and indeed the property of temperature will depend on its precise definition”.

Much of the rest of the book has been revised in detail. The discussion of critical phenomena and of transitions of higher order, however, is essentially unchanged, although powerful inequalities, discovered in the past four years, have rendered obsolete all discussions before 1965.

One striking innovation is the use of an unnamed symbol for the Helmholtz free-energy. It is formed by running together A and F. J. S. ROWLINSON

## SOLAR SYSTEM ASTRONOMY

### Moon and Planets

Edited by A. Dollfus. (A Session of the Seventh International Space Science Symposium held in Vienna on May 10-18, 1966.) (Sponsored by COSPAR and IAU.) Pp. 321+26 photographs. (Amsterdam: North-Holland Publishing Company, 1967.) 90s.

THE progress which space science has made during its first decade has astonished even some of its more optimistic proponents, and it has become difficult in these days of the information explosion for astronomers pursuing their work in extra-solar-system astronomy to keep abreast of developments in space science (apart from a few well-publicized glamorous discoveries). And in spite of the developments which have taken place, it must be recognized that our new knowledge is fragmentary, and that new questions are being asked faster than they can be answered. Many of us look forward to the day when it will be possible to publish an important textbook, with all its chapters fully integrated one with another, and covering the main aspects of planetary and lunar astronomy, with a full and adequate account of modern developments. Clearly, such a book, if written now, would be premature. We must therefore be grateful for the present volume. Its most valuable feature is undoubtedly a number of survey papers, which will be of interest to a wider audience than some of the individual contributions and abstracts. I single out for mention articles by G. P. Kuiper on the lunar surface and the US Ranger programme, by A. D. Kuzmin on the results of radio observations of the planets, by I. I. Shapiro on planetary radar astronomy, by O. W. Nicks on the Mariner IV results, by R. K. Sloan on the Mariner IV photographs of Mars, and by W. C. DeMarcus on the general theory of (the internal structure of the) planets. The volume is a required purchase for libraries of physics and astronomy because of these surveys, and the latter will be valuable as basic references for astrophysics and space science students until such a time as more integrated treatment of solar system astronomy becomes available.

R. H. GARSTANG

## QUANTUM THEORY OF FIELDS

### Lectures on Quantum Field Theory

By Paul A. M. Dirac. (Belfer Graduate School of Science Monograph Series, No. 3.) Pp. viii+151. (New York: Belfer Graduate School of Science, Yeshiva University, 1966. Distributed by Academic Press, New York and London.) 60s.

PROFESSOR DIRAC'S book, as its title and colloquial style suggest, is based on a lecture course given by him in the session 1963-64 at Yeshiva University, New York. This is not a textbook in the conventional sense: it is, rather, a critical essay in which the essayist describes lucidly his thoughts on the difficulties and illogicalities of the traditional approach to the quantum theory of fields; then in developing the theory of quantum electrodynamics he demonstrates just how he has come to terms with the problems he has raised.

The underlying philosophy of the book has already received considerable publicity<sup>1,2</sup>. Letters to *Nature*<sup>3,4</sup> on the subject of the first article indicate that the ideas put forward are not altogether uncontroversial.

The essential point which Professor Dirac makes is that, in the field theoretic context, "the Heisenberg

picture is a good picture and the Schrödinger picture is a bad picture and that the two pictures are not equivalent". The essence of this point is made explicit when a simple field theoretic model is analysed. Thereafter the author sets about the task of developing a quantum field theory (quantum electrodynamics) working entirely within the framework of the Heisenberg picture and avoiding any direct use of the Schrödinger picture. In any theory which does not admit Schrödinger wave functions the usual probabilistic interpretation of the theory must be abandoned. This problem is tackled by the author and a new basis of interpretation is suggested. Important in these new interpretative proposals is the normal ordering of operators and this, being deemed of physical significance, is taken over from work done in the Schrödinger picture. By developing quantum electrodynamics in the Heisenberg picture many but not all of the "illogical" infinities of the traditional approach are successfully eliminated; it is proposed that other infinities be removed by the introduction of cut-offs, and the arguments presented here seem to me plausible rather than strictly logical. Professor Dirac is careful to point out that each standpoint adopted is merely that which seems to him to be the best at the present stage of knowledge and that we must always be ready to alter our point of view in the light of new facts.

In this formulation there is no place for an *S*-matrix and it would have been interesting to have had Professor Dirac's evaluation of the extensive literature on *S*-matrix theory and other modern outgrowths of traditional field theory.

I am certain that *Lectures on Quantum Field Theory* will provide the serious student with most interesting and stimulating reading: the arguments are presented with great clarity and the layout (despite the absence of an index, which is largely compensated for by the paragraph titles) is good.

JOHN CUNNINGHAM

<sup>1</sup> Dirac, P. A. M., *Nature*, **203**, 115 (1964).

<sup>2</sup> Dirac, P. A. M., *Phys. Rev.*, **139B**, 685 (1965).

<sup>3</sup> Perlman, H. S., *Nature*, **204**, 771 (1964).

<sup>4</sup> Simon, J. M., and Taylor, J. G., *Nature*, **205**, 1305 (1965).

## MAN'S WORLD

### The Science of Society

An Introduction to Sociology. By Stephen Cotgrove. (The Minerva Series of Student's Handbooks, No. 19.) Pp. 310. (London: George Allen and Unwin, Ltd., 1967.) 40s. net.

THIS is an erudite, yet readable and easily assimilated, introductory textbook of sociology. Using the structure-function approach, in particular the "theories" and perspectives developed by Talcott Parsons, the author marshals a judicious mixture of British and American research findings into a text which both serves as an introduction to the elements of sociological analysis and offers the reader a description of contemporary British institutions. This is something of an achievement, for the "theoretical" frame of reference is not a simple one, and its exponents are not noted for clarity and simplicity in their prose style. The book differs from many introductory sociology textbooks by adopting the refreshing approach of assuming that its readers read other books. In particular it was pleasing to find that the author had dispensed with the traditional obeisance to the "Founding Fathers of Sociology" and settled for a discussion of what sociology is about today.

The work of Talcott Parsons has had a great influence on modern sociological teaching and research. Good textbooks using his frame of reference, however, have been rare. Those using it have made the mistake of forcing the material into the conceptual framework in the mistaken belief that it is a verified theory. While Professor Cotgrove closely adheres to the scheme he does not fall into

this trap; consequently he utilizes other theoretical perspectives, particularly "symbolic interactionism" and "social action" theory, when they appear to offer better explanations.

The material is thus organized on the Parsonian assumption that every social system has four functional problems to solve: (a) latent pattern maintenance and tension management dealt with in chapters on the "Family" and the "Educational System"; (b) adaptation to the environment discussed in a chapter on the "Economic System"; (c) goal attainment handled in a chapter on the "Political System"; and (d) integration covered by a discussion of "Belief Systems".

The chapter on education is a particularly good one reflecting Professor Cotgrove's interest in technical education. A passage in the section on politics which states that since the passing of the 1927 Trade Union Act union members "contract-in" to political funds needs amending. The 1946 Act repealed the 1927 Act, thus restoring the 1913 Act, which means that those who do not wish to pay the levy must now "contract-out". The final chapters are given over to a discussion of social processes—"Stratification", "Organizations", and "Deviance, Disorganization and Change".

The undoubted merit of the Parsonian system is its logical coherence and plausibility in explaining certain features of social life. The book is therefore well organized and logically developed. Professor Cotgrove thus capitalizes on the strengths of Parsonian "theory". He also endeavours to avoid its weaknesses. Here he is hampered by the nature of the framework. In particular the notion of society as a homeostatic system with its parts adjusting to each other to achieve equilibrium is implicit in the analysis. From the student's point of view this helps to order and simplify the apparent complexity of social phenomena at the expense of explaining the pervasiveness of conflict in society. Professor Cotgrove acknowledges Dahrendorf's criticism of Parson's work; however, he presents conflict as a second level interaction coming into operation only when homeostasis breaks down. Further, conflict is not adequately defined by contrasting it to other forms of interaction such as competition and co-operation.

An introductory text can serve several functions and must, by necessity, be selective. Criticism of the selection is therefore rather arbitrary. However, I feel that a discussion of the methods of social research and the problems of validity and reliability of techniques would have been useful. It would then have been possible to include more quantitative data in the text, giving the student an opportunity to appraise the research findings more critically.

With its comprehensive bibliographies and discussion topics at the end of each chapter the book would be a good introductory text for sixth forms of grammar schools, for colleges of education, and for first year undergraduate courses.

F. G. COOK

## OBITUARIES

### Professor R. A. L. Black

ROBERT ALASTAIR LUCIEN BLACK, professor of mining and head of the department of mining and mineral technology at the Imperial College of Science and Technology, died suddenly in London on June 22. He was 46.

Black was educated at Stowe and at Imperial College, but his university course was interrupted by the war. From 1940 he served with the air branch of the Royal Navy, first in the Indian Ocean area and then in the Middle East and Malta, where he was awarded the Distinguished Service Cross in 1943. He was demobilized in

1945 as lieutenant (A), RNVR, and immediately returned to the Royal School of Mines to resume his education. A brilliant student, he graduated with first class honours in mining the following year, having carried off three prizes and two medals.

The next seventeen years of his life were spent in South Africa where, in the Witwatersrand gold fields, he first gained practical experience of the engineering and management problems associated with complex mining operations. In very deep mining, ventilation and the structural control of rock movement constitute the major technical difficulties. By the time he was 34, Black had made valuable contributions in both these fields and had gained practical experience up to the level of underground manager. He was therefore a logical choice to be head of the department of mining engineering at the University of the Witwatersrand when the position became vacant in 1955.

He entered academic life at a time when the mineral industry was beginning to face two important and conflicting trends. The first was the application of advanced scientific and engineering developments to the increasingly complex problems of large-scale mining which, together with the introduction of new techniques of management and production engineering, constituted a revolution in an industry which hitherto had been based largely on experience, hard work and common sense. The second was the alarming deterioration of mining schools in the English-speaking world. Black's last twelve years were devoted to advancing the first trend and arresting the second.

At the University of the Witwatersrand he reorganized the undergraduate syllabus on modern, science-based lines and established a new postgraduate school and research unit. The position of consultant to the Transvaal and Orange Free State Chamber of Mines is concomitant with that of professor of mining engineering, and this enabled Black both to enlarge his experience and to put his ideas into practice. In the same period he served as dean of the faculty of engineering, as dean of the university residence, as director of the government miners training schools and as a member of the government commission of enquiry into the methods of training for engineering in South Africa.

A liberal and humane man with a strong sense of justice his distress at political developments conflicted with the loyalty he felt for his adopted country. This dilemma was happily resolved in 1963 when he was asked to return to his old college as professor of mining—an invitation which he could not refuse. He applied himself to his new tasks with characteristic energy and effect; the undergraduate syllabus was revised, postgraduate teaching and research were expanded and, on his initiative, an interdepartmental research group on rock mechanics was established with civil engineering and geology.

He had widespread contacts throughout the mining world and his reputation, both as an engineer and as an educator, was international. His technical writing—mainly on rock mechanics, environmental engineering and mining education—was highly regarded and he had advised on important mining operations and lectured on his speciality in Africa, Australia, Europe and in both North and South America. He was a member of council of the Institution of Mining and Metallurgy and played an active part in the development of the Council of Engineering Institutions.

Black was an unpretentious man with no vanity, and disliked formality. His exceptional intelligence and ability provided an authority that he did not need to stress and his personality was sympathetic and entirely unaggressive. He never pressed a point unless he believed that it was right and fair, but then his argument was convincing and his enthusiasm infectious. His influence, both as a professional engineer and as a teacher, was great and his untimely death is a sad and irreparable loss to the mineral industry and to his many friends.

M. G. FLEMING



## University News :

**Belfast**  
DR P. G. BURKE has been appointed to the chair of mathematical physics.

## Manchester

DR K. W. BAGNALL, at present senior principal scientific officer at the Atomic Energy Research Establishment, Harwell, has been appointed to the chair of inorganic chemistry, in succession to Professor J. Lewis, who has been appointed to the chair of chemistry in University College, London; Professor D. B. G. Edwards, professor of computer engineering in the University, has been appointed to the I.C.T. chair of computer engineering, and Mr A. J. Morton, at present group services engineer of the Southern Projects Group in the Central Electricity Generating Board, has been appointed to an additional chair of engineering. Mr D. L. Burn has been appointed visiting professor of economics for one year from October 1.

## Appointments

DR E. PASSAGLIA, formerly chief of the Polymer Physics Section, has been appointed chief of the Metallurgy Division of the US National Bureau of Standards.

## Meetings

PURE and Applied Chemistry, September 4-10, Prague (The Chairman, Organizing Committee, XXIst International Congress of Pure and Applied Chemistry, POB 139, Praha 6—Dejvice).

INSTRUMENTATION for Engineers, September 4-October 6, University of Sheffield (Professor W. A. Tuplin, Department of Applied Mechanics, University of Sheffield, St. George's Square, Sheffield, 1).

PHYSICS of Quasars, September 5-7, University of Manchester (The Meetings Officer, The Institute of Physics and the Physical Society, 47 Belgrave Square, London, SW1).

SOLID State Devices, September 5-8, Manchester Institute of Science and Technology (The Meetings Officer, The Institute of Physics and the Physical Society, 47 Belgrave Square, London, SW1).

II-VI Semiconducting Compounds, September 6-8, Brown University, Providence, Rhode Island (D. W. Langer, Conference Secretary, Aerospace Research Laboratories, Wright-Patterson AFB, Ohio).

MOLECULAR Spectroscopy, September 10-15, Madrid (IXth European Congress on Molecular Spectroscopy, Serrano, 117, Madrid).

AGRICULTURAL Engineering, September 11-14, National College of Agricultural Engineering, Silsoe, Bedfordshire (Honorary Organizing Secretary, Agricultural Engineering Symposium, The Institute of Agricultural Engineers, Penn Place, Rickmansworth, Herts).

CARBONIFEROUS Stratigraphy and Geology, September 11-16, Sheffield (Dr A. W. Woodland, Secretary-General, Geological Survey Office, Ring Road, Halton, Leeds 15).

8TH ANNUAL Scientific Meeting of the Anglo German Medical Society, September 14-16, Glasgow (Professor W. A. Mackey, Royal Infirmary, Glasgow, C4).

CREATIVE Engineering Design, September 11-16, Queen Mary College (Department of Mechanical Engineering, Queen Mary College, Mile End Road, London, E1).

QUANTITATIVE Methods in Reflected-light Microscopy, September 19-23, Cambridge (Mr G. A. Kingston, Department of Geology, Imperial College, London, SW7).

SURFACE Phenomena of Metals, September 20-22, Brunel University, London (Professor K. S. W. Sing, Department of Chemistry, Brunel University, Woodlands Avenue, Acton, London, W3).

USE of Radioactive Isotopes in Pharmacology, September 20-23, Geneva (Professor B. Glasson, Pavillon des Isotopes, 20 bd d'Yvoy, 1211 Genève).

MASS Spectrometry, September 25-29, Technische Universität, West Berlin (The Institute of Petroleum, 61 New Cavendish Street, London, W1).

DATAFAIR 67, September 25-29, University of Southampton (The British Computer Society, 23 Dorset Square, London, NW1).

MICROBIOLOGICAL Chemistry, September 26-28, Sheffield (Dr E. Haslam, Department of Chemistry, The University, Sheffield, 10).

ERRATUM. In the note "Shape of Universities" (*Nature*, 214, 865; 1967) it should have been stated that Sir Eric Ashby delivered the Queen's Lecture at the Free University of Berlin, not at the University of Belfast.

ERRATUM. In Table 1 of the article "Tale of Two Countries" (*Nature*, 215, 9; 1967) the figures for expenditure as a percentage of GNP have been interchanged. They should read United Kingdom, 2.3; West Germany, 1.3.

CORRIGENDUM. In the communication "Photosensitized Oxidation of Carbon Monoxide on Semi-conductors supported on Silver" (*Nature*, 215, 152; 1967) the values for activation energies should be double the values given in Table 1, with the exception of the activation energies for Ag.

# CORRESPONDENCE

## Equipment Grants

SIR,—Your editorial, "Fighting the Wrong Battle" (*Nature*, 215, 337; 1967)—why such war-like terms?—is misleading in a number of important respects. As Sir Cyril Hinshelwood and his colleagues claimed, it is true that the University Grants Committee dishonoured firm pledges of monies for the equipment of new buildings, and your figure of a deficit of £15.5 million against previous promises confirms this claim. Other requirements were with, or going to, the UGC and these have to be added to this deficit. What you also do not note is that, without this money, science teaching departments will have to open this October without their planned complement of apparatus. Much effort in planning courses, preparing equipment schedules and so forth has been vitiated, and scratch arrangements will have to be made, very late in the day, to the extent that universities are able, and willing, to borrow funds for this purpose. Academic policy is being settled by finance. Further, commitments were made to new staff as to their facilities and equipment, and this, too, is in the melting pot. No new department, in old or new university, can feel confident about the future after this galling experience.

You also say that the UGC's proposed new system "means everyone will be better off". Until we know what this system is, no one can pronounce on it! Two things are clear. A simple, *per capita* scheme will not meet the very different needs of all our universities, since most are at different stages of equipment, and re-equipment, for science teaching and research. Until we know what adjustments are to be made for this, and what the specific allocation of funds will be, all planning for the future involves too many uncertainties to be faced with confidence. The student population, as you note, is growing; the question is: will the equipment and staff be there so that it can be properly taught?

Yours faithfully,

JAMES H. SANG

Biology Building,  
University of Sussex,  
Falmer, Brighton.

## APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

**RESEARCH ASSISTANT IN THE DEPARTMENT OF ZOOLOGY** to work with Dr K. R. Ashby on problems connected with the development of the reproductive system in fish and the effects produced by treatment with sex hormones during early life—The Registrar and Secretary, University of Durham, Old Shire Hall, Durham (August 9).

**LECTURER** (with a first- or second-class honours degree in psychology and research and/or industrial experience) in **PSYCHOLOGY** at Durham University Business School—The Registrar and Secretary, Durham University, Old Shire Hall, Durham (August 11).

**ASSISTANT LECTURER** (preferably with one of the following fields of interest: bacteriology, microbial genetics, whole-plant physiology, physiological ecology, or experimental mycology) in **BOTANY**—The Secretary, Birkbeck College (University of London), Malet Street, London, W.C.1 (August 14).

**TUTOR/SENIOR TUTOR and LECTURER/SENIOR LECTURER in BIOLOGY, GEOGRAPHY, HISTORY OF SCIENCE and TECHNOLOGY, MATHEMATICS** at the University of Papua and New Guinea—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1; or The Registrar, University of Papua and New Guinea, P.O. Box 1144, Boroko, T.F.N.G. (August 15).

**EXPERIMENTAL OFFICER or SENIOR EXPERIMENTAL OFFICER** in the DEPARTMENTS OF BOTANY and ZOOLOGY to assist in the running of an **ELECTRON MICROSCOPE UNIT**—The Registrar, The University, Manchester, 13, quoting Ref. 117/67/Na (August 16).

**ALGOLIST/PLANT PHYSIOLOGIST** (with a first- or good second-class honours degree in botany with a special interest in primary production or plant physiology; but candidates with a degree in biochemistry or an allied subject with an interest in, or knowledge of, fresh water algae will also be considered) to study primary production and factors limiting the production of phytoplankton at Loch Leven as part of an International Biological Programme Study—The Nature Conservancy, 12 Hope Terrace, Edinburgh, 9 (August 18).

**EXPERIMENTAL OFFICER** in the DEPARTMENT OF BOTANY for duties which will include assistance in the teaching of techniques in microscopy, including electron microscopy and in research—The Secretary, Academic Council, The Queen's University, Belfast, Northern Ireland (August 18).

**LECTURER** (with a good honours degree and/or a higher degree in psychology) in **EDUCATIONAL PSYCHOLOGY** in the DEPARTMENT OF EDUCATION—The Registrar, University College of North Wales, Bangor, North Wales (August 18).

**RESEARCH ASSISTANT** (preferably with an interest in physiological ecology) in the DEPARTMENT OF BOTANY, to work with Dr D. J. Boatman on aspects of peat bog development on the Silver Flowe Nature Reserve—The Registrar, The University, Hull (August 19).

**SENIOR RESEARCH OFFICER** (with research experience in some branch of engineering) in the DEPARTMENT OF APPLIED MATHEMATICS for research in Rheology—The Registrar, University College of Wales, Aberystwyth (August 19).

**CHIEF TECHNICIAN** (botanical knowledge helpful but not essential) in the SCHOOL OF PLANT BIOLOGY—The Registrar, University College of North Wales, Bangor, North Wales (August 20).

**BOTANIST** (with a good honours degree and preferably postgraduate research experience) for research on the growth and phenology of arctic-alpine vegetation in the Cairngorms and in Iceland—The Nature Conservancy Unit of Grouse and Moorland Ecology, Blackhall Buildings, Banochry, Kincardineshire (August 21).

**HORTICULTURAL ADVISERS** (with a degree in horticulture or a closely related subject; or National Diploma in Horticulture (sections I to VI); or equivalent or higher qualifications) with the National Agricultural Advisory Service, Ministry of Agriculture, Fisheries and Food (at least 13 posts)—The Civil Service Commission, Savile Row, London, W.1, quoting Ref. 345/67 (August 21).

**SENIOR LECTURER and a LECTURER in MATHEMATICS** at the University College of Rhodesia—The Inter-University Council, 33 Bedford Place, London, W.C.1 (August 21).

**SENIOR LECTURER in VETERINARY MEDICINE** at the University of Queensland, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, August 25).

**ASSISTANT LECTURER or LECTURER in COMPUTATION** in the DEPARTMENT OF MATHEMATICS—The Deputy Secretary, The University, Southampton (August 28).

**SCIENTIFIC OFFICER/SENIOR SCIENTIFIC OFFICER** (with a degree in microbiology or in biochemistry or chemistry with experience in microbiology) in the MICROBIOLOGY DEPARTMENT to be concerned with the extension of work on the nitrogen metabolism of micro-organisms in rumen contents using stable isotopes of N and <sup>14</sup>C and <sup>3</sup>H—The Secretary, Rowett Research Institute, Bucksburn, Aberdeen, Scotland (August 28).

**SCIENTIFIC OFFICER** (with a first- or upper second-class honours degree in mathematics with statistics, or a diploma in statistics) in the STATISTICS SECTION for duties mainly concerned with the design and analysis of field and laboratory experiments, and providing a statistical and data processing service—The Secretary, The Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen (August 28).

**SENIOR LECTURER, LECTURER or JUNIOR LECTURER in GEOGRAPHY, PHILOSOPHY, PSYCHOLOGY/SOCIOLOGY, and PURE MATHEMATICS** at the University of Waikato, Hamilton, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, August 28).

**BIOMETRICIAN** (with an honours or postgraduate degree in, or including, mathematical statistics, and some experience in applied statistics, preferably in biological science) at the Forest Research Institute, Rotorua, New Zealand, to be in charge of the Biometrics Section and act as statistical consultant to officers engaged in the full range of forestry research covering production, protection and utilization, and to carry out statistical analysis and undertake research into statistical methods—The High Commissioner for New Zealand, New Zealand House, Haymarket, London, S.W.1, quoting Ref. B13/8/35/2014 (August 31).

**ELECTRON MICROSCOPIST (Experimental Officer)** (with a university degree or equivalent and experience in the maintenance and use of modern electron microscopes) in the DEPARTMENT OF METALLURGY—The Registrar, University of Sheffield, Sheffield (August 31).

**HEAD** (honours graduate in mathematics, qualifications in statistics and an interest in biological problems) of the STATISTICS SECTION of the DEPARTMENT OF AGRICULTURE and HORTICULTURE, Long Ashton Research Station—The Registrar, Senate House, The University, Bristol 2 (August 31).

**LECTURER in PHYSICS** to pursue research in experimental high energy physics—The Registrar, The University, Manchester, 13, quoting Ref. 119/67 (August 31).

**LECTURER or ASSISTANT LECTURER in APPLIED MATHEMATICS**—The Registrar, The University, Senate House, Bristol, 2 (August 31).

**ASSISTANT/ASSOCIATE PROFESSORS** (with experience in solid mechanics, fluid mechanics and thermodynamics) in **MECHANICAL ENGINEERING**—Dr D. H. Norrie, Head, Department of Mechanical Engineering, University of Calgary, Calgary, Alberta, Canada.

**BIOCHEMIST** (basic grade) for research into lipoprotein metabolism in children—Professor O. H. Wolff, Institute of Child Health, The Hospital for Sick Children, Great Ormond Street, London, W.C.1.

**BIOCHEMIST or MICROBIOLOGIST, SENIOR SCIENTIFIC OFFICER or SCIENTIFIC OFFICER** (preferably with experience of immunology) in the BACTERIOLOGY DEPARTMENT, to join a group working on natural defence mechanisms of the udder against mastitic pathogens, in particular staphylococci—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, Reading, Berkshire, quoting Ref. 67/25.

**BIOCHEMIST** (preferably postdoctoral) to work on physical and chemical properties of the proteins of the lens with reference to formation of lens opacities—Dr A. Pirie, Nuffield Laboratory of Ophthalmology, Walton Street, Oxford.

**ENGINEERS or PHYSICISTS** for work on advanced electro-optical input devices—The Director, Clinical Effects of Radiation Research Unit, M.R.C., c/o 107 Sydney Street, London, S.W.3.

**GRADUATE RESEARCH ASSISTANT** in the Regional Rheumatism Research Centre to work with medical staff—The Administrative Officer, Stoke Mandeville Hospital, Aylesbury, Bucks.

**GRADUATE RESEARCH ASSISTANT** (preferably with experience of, or a keen interest in, modern techniques of analytical biochemistry) in **TOXICOLOGY**, to join in chemical studies of drug action, in association with the Poisoning Treatment Centre, Edinburgh Royal Infirmary—Dr S. S. Brown, University Department of Clinical Chemistry, Royal Infirmary, Edinburgh, 8.

**GRADUATE** (with a good honours degree in chemistry or equivalent qualifications) at the Queen Elizabeth Hospital (Institute of Child Health), Hackney Road, London, E.2, to assist in research on mucopolysaccharides in disease—The Group Secretary, Hackney, and Queen Elizabeth Group Hospital Management Committee, Administrative Offices, Hackney Hospital, London, E.9.

**HIGHER TECHNICAL ASSISTANT** (with sound electronics experience and an interest in research) in the CRYSTALLOGRAPHY GROUP to assist with the maintenance and operation of advanced X-ray diffraction equipment and the work of the group generally—The Superintendent, University Chemical Laboratory, Lensfield Road, Cambridge.

**HOSPITAL BIOCHEMIST** (probationary grade) (recent graduate in chemistry or biochemistry) in the CHEMICAL PATHOLOGY DEPARTMENT, St. Charles Hospital, Ladbroke Grove, London, W.10—The Director of Pathology, c/o Paddington Group Hospital Management Committee, Harrow Road, London, W.9.

**JOHNSON and JOHNSON RESEARCH FELLOW** for work on wound healing or transplantation—Mr H. Cooke, Personnel Officer, Royal College of Surgeons of England, Lincoln's Inn Fields, London, W.C.2.

**LECTURER or ASSISTANT LECTURER** (with an interest in economic or structural geology) in **METAMORPHIC PETROLOGY** in the DEPARTMENT OF GEOLOGY, Fourah Bay College, The University of Sierra Leone—The Inter-University Council, 33 Bedford Place, London, W.C.1.

**LECTURER** (with a special interest in optical instrumentation, including the design of instruments or in modern experimental optics, and preferably experience in the optical industry) to undertake postgraduate teaching and research in the Applied Optics Section of the PHYSICS DEPARTMENT—Professor W. D. Wright, Applied Optics Section, Physics Department, Imperial College, London, S.W.7.

**MICROBIOLOGIST** (graduate) in the DEPARTMENT OF CLINICAL PATHOLOGY—The Assistant Secretary, University College Hospital, Gower Street, London, W.C.1.

**POSTDOCTORAL FELLOW** (preferably with experience in mass spectrometry) in the PROCTOR DEPARTMENT OF FOOD and LEATHER SCIENCE, to undertake research into the chemical basis of the aroma and flavour of foodstuffs, using gas chromatography for the separation of volatile food components and mass and infrared spectrometry for their identification—Dr H. E. Nursten, The University, Leeds, 2.

**RESEARCH ASSISTANT, BIOCHEMIST or VETERINARY PATHOLOGIST** (preferably with postgraduate research experience and/or a higher degree) in the IMMUNOLOGY CANCER RESEARCH UNIT—The Registrar, University of Liverpool, Liverpool, 3, quoting Ref. RV/100/N.

**RESEARCH ASSISTANT** (with a good honours degree, preferably in genetics) for work on the biology of the large bulb fly, *Merodon equestris*, and the genetics of its mimetic polymorphism—W. D. Hamilton, Esq., Imperial College, Silwood Park, Sunninghill, Ascot, Berkshire.

**RESEARCH ASSISTANT** (with an honours degree in botany or agricultural science and a special interest in plant pathology) for work on potato tuber diseases—The Secretary, Edinburgh School of Agriculture, West Mains Road, Edinburgh.

**RESEARCH ASSISTANTS** (preferably with a Ph.D. degree in physics or engineering) at the Central Research Station of the International Flame Research Foundation, Ijmuiden, Holland, to join a small team of postdoctoral investigators to work on problems of combustion physics, in particular aerodynamics and heat transfer aspects of turbulent flames—Professor J. M. Beer, Department of Fuel Technology and Chemical Engineering, University of Sheffield, Mappin Street, Sheffield, 1.

**RESEARCH ASSISTANTS** (with a first- or second-class honours degree in mathematics or statistics) in **MATHEMATICS**—Head of the Department of Mathematics, Northern Polytechnic, Holloway, London, N.7.

**SCIENTIFIC OFFICER** (basic grade) (with an honours degree in science) for the MYCOLOGY RESEARCH LABORATORY—Miss P. Duncan, Secretary, Bernhard Baron Memorial Research Laboratories, Queen Charlotte's Maternity Hospital, Goldhawk Road, London, W.6.

**SENIOR MEDICAL PHOTOGRAPHER and HEAD** (with appropriate professional qualifications and experience of running a small department and the supervision of staff) of the MEDICAL ILLUSTRATION DEPARTMENT—The Secretary, Institute of Ophthalmology (University of London), Judd Street, London, W.C.1.

**SENIOR TECHNICIAN** (Grade II) and a **TECHNICIAN** (Grade III) for an expanding DEPARTMENT OF MICROBIOLOGY concerned with both teaching and research—The Assistant Bursar (Personnel), The University, Reading, quoting Ref. T. 23.

**S.R.C. INDUSTRIAL RESEARCH STUDENT** (graduate in biochemistry, biology, botany or chemistry) to work on the translocation of a number of biologically important compounds in plants—Prof. N. F. Robertson, Department of Botany, The University, Hull.

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deal with people as well as some interest in electronic apparatus)—The Secretary, Goodmayes Hospital, Barley Lane, Goodmayes, Ilford, Essex.  
**VOKES RESEARCH FELLOW** (graduate in engineering, chemistry or physics) in the DEPARTMENT OF MECHANICAL ENGINEERING—The Deputy Secretary, The University, Southampton.

## REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

### Great Britain and Ireland

- Transactions of the Royal Society of Edinburgh. Vol. 67, No. 1: The Proboscis and Oesophagus of some British Turrids. By Edmund H. Smith. Pp. 1-22. 11s.; \$1.80. Vol. 67, No. 2: The Neogastropod Stomach, with Notes on the Digestive Diverticula and Intestine. By Edmund H. Smith. Pp. 23-42. 11s.; \$1.80. Vol. 67, No. 3: Trilobites from the Upper Stinchur Limestone (Ordovician) of the Girvan District, Ayrshire. By Ronald Pearson Tripp. Pp. 43-94 + 6 plates. 25s.; \$4.40. Vol. 67, No. 4: Some Specimens of *Protoclepsyropsis* and *Clepsyropsis* from the Calciferous Sandstone Series of Berwickshire. By Albert G. Long. Pp. 95-107 + 4 plates. 11s.; \$1.80. (Edinburgh: The Royal Society of Edinburgh, 1967.) [126]
- Agricultural Research Council. The Nutrient Requirements of Farm Livestock. No. 3: Pigs—Technical Reviews and Summaries. Pp. xi + 278. 30s. net. No. 3: Pigs—Summaries of Estimated Requirements. Pp. v + 34. 5s. net. (London: Agricultural Research Council, 1967. Obtainable from H.M. Stationery Office.) [136]
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- Bulletin of the British Museum (Natural History). Geology. Vol. 14, No. 6: Lower Carboniferous Trilobites of North Devon and Related Species from Northern England. By J. E. Prentice. Pp. 207-241 + 7 plates. 35s. Vol. 15, No. 2: The Correlation and Trilobite Fauna of the Bedinan Formation (Ordovician) in South-Eastern Turkey. By William Thornton Dean. Pp. 81-123 + 10 plates. 40s. [136]
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- A Survey of the Parasites of Wild Mammals and Birds in Israel. Part 1: Ectoparasites. By O. Theodor and M. Costa. Pp. 117 + 3 maps. (Jerusalem: The Israel Academy of Sciences and Humanities, 1967.) [56]
- Records of the Australian Museum. Vol. 27, No. 8 (26th April, 1967): On *Gangamopteris walkomii* SP. NOV. By J. F. Rigby. Pp. 175-182 + plates 25-27. (Sydney: The Australian Museum, 1967.) 40c. [56]
- Lantbrukshögskolans Annaler/Annals of the Agricultural College of Sweden. Vol. 33, Nr. 1: Studies in the Development of Agricultural Economics in Europe. By J. Nou. Pp. 611. (Uppsala: Agricultural College of Sweden, 1967.) [56]

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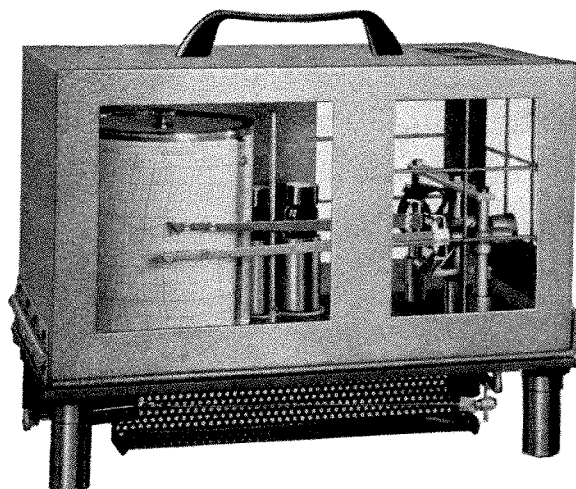
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Mr. R. F. Turnbull, Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, to whom applications (quoting Appointment No. 560/226) should be addressed by the **1st September, 1967.** (500)



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Applications, giving a short curriculum vitae and the names of two referees, should be made in writing to The Secretary, Institute of Neurology, The National Hospital, Queen Square, London W.C.1. (428)

## pharmaceutical biochemist

A vacancy exists for a Biochemist having an interest in enzyme kinetics. The person appointed will join a team studying various aspects of the biochemistry of drugs and will be responsible for the enzymic and certain other specialised studies of the research programme.

Applications are invited from honour graduates in biochemistry who have about three years experience in pharmaceutical biochemistry.

The Company has a non-contributory Pension and Life Assurance Scheme, and a Profit Sharing Plan.

*Please apply, giving full details to:*

D. J. Goddard, Personnel Manager (R/G/7).

**NICHOLAS RESEARCH INSTITUTE LTD.**

225 Bath Road, Slough, Bucks.

A MEMBER OF THE ASPRO-NICHOLAS GROUP

(477)

## SCIENCE RESEARCH COUNCIL

## THE RUTHERFORD LABORATORY

## Scientific Administration

There is a vacancy for a **Principal Scientific Officer** to take charge of the Scientific Administration Group at the Rutherford Laboratory which is the leading research establishment in elementary particle physics in the United Kingdom.

This group, although numerically small, covers a wide range of work, including library and information services, university liaison, public relations, technical writing, documentation and the secretarial servicing of scientific and technical committees and conferences. In addition to supervising the Group, the successful applicant will be expected to make a personal contribution as a writer and interpreter of scientific work for non-specialists.

Applicants should have a good Honours degree, preferably in Physics or in Mathematics or Engineering, and be experienced in the administration of scientific work.

**Salary Scale: £2,410-£3,325 per annum**

There is a contributory superannuation scheme and housing may be available.

*Please send a postcard for application form to Mr. G. N. Pickles, The Rutherford Laboratory, Chilton, Didcot, Berks, quoting reference 409/34.*

Closing Date: 17th August, 1967.

(452)

## IMPERIAL COLLEGE

**ASSISTANT EXPERIMENTAL OFFICER** for field and laboratory work with pesticides and soil organisms at the Field Station. Candidates should possess a degree or equivalent in Horticulture, Agricultural Botany or a biological subject. Salary according to age and experience. Applications to: Dr. N. G. Hague, Imperial College Field Station, Ashurst Lodge, Sunninghill, Ascot, Berks. (480)

**SENIOR MICROBIOLOGIST/BIOLOGIST** required to take charge of Biological Department of Food and Drug Laboratory. Person appointed must be capable of dealing with a wide range of microbiological problems, the identification of insect parts, the microscopic identification of various constituents of foods and drugs (including pollen grains in honey) and the determination of antibiotics. Salary range £1,500 to £2,000 per annum.—Apply Thomas McLachlan, 4 Hanway Place, W.1. (475)

## PLANT BREEDING INSTITUTE CAMBRIDGE

### DEVELOPMENTAL GENETICS

It is proposed to appoint a Scientific Officer in the Cytogenetics Section to work on developmental aspects of the present programme for the cytogenetic analysis of wheat. Applicants should have good degrees and interest in the developmental physiology of plants and in genetics. The salary will be in the range £926 to £1,574 with superannuation under F.S.S.U.

Applications, with full personal particulars and naming three referees, should be sent to the Secretary, Plant Breeding Institute, Trumpington, Cambridge, by September 1. (494)

## NATIONAL INSTITUTE FOR RESEARCH IN DAIRYING (UNIVERSITY OF READING)

Applications are invited from persons with veterinary qualifications or suitably qualified research scientists to join a research team at the Institute where a study is being made of the control of mastitis in the dairy cow and where large-scale field trials are being carried out in co-operation with the Central Veterinary Laboratory of the Ministry of Agriculture. The person appointed will be required to take part in investigations where particular emphasis will be given to the development and testing of skin disinfectants and the cause and prevention of skin blemishes. The appointment will be for a period of three years and it may be possible for the successful applicant to be registered in the University of Reading for a higher degree. The salary will be within the scale for Scientific Officers (£926 to £1,574) according to age and experience.

Apply, naming two referees, to the Secretary, N.I.R.D., Shinfield, Reading, Berks. Please quote ref. 67/26. (440)

## GREAT OUSE RIVER AUTHORITY APPOINTMENT OF ASSISTANT CHEMIST (BIOLOGIST/MICROBIOLOGIST)

Applications are invited for the above appointment in the Pollution Prevention Laboratory of the Authority. Applicants should have an appropriate degree, H.N.C. in Applied Biology or equivalent and preference will be given to those with experience of fresh water ecology and microbiology, and fish diseases. The successful applicant will be expected to help in the chemical laboratory when necessary. Salary will be within the scales AP-2-3 (£1,020 to £1,435), the starting point to be dependent upon qualifications and experience. The National Joint Council conditions of service apply to the post which is superannuable and subject to medical examination. A five-day week is worked. Half cost of reasonable removal expenses will be paid in approved cases.

Applications should be made to The Clerk to the Authority, Great Ouse House, Clarendon Road, Cambridge, by August 21, 1967.

J. S. BISSETT,  
Clerk to the Authority.  
(492)

## THE UNIVERSITY OF MANCHESTER MANCHESTER, 13

### RADIATION OFFICER IN THE

### DEPARTMENT OF PHYSICS

Applications invited from graduates for this post. Knowledge of radiation protection and electronics additional qualifications though not essential. Salary range per annum: either £920 to £1,525; or £1,450 to £2,200, according to qualifications and experience. F.S.S.U.

Particulars and application forms (returnable by August 26), from the Registrar. Quote ref. 120/67/Na. (441)

## THE UNIVERSITY OF MANCHESTER MANCHESTER, 13

### LECTURER IN PHYSICS

Applications invited for above post. The successful candidate will be expected to pursue research in Experimental High Energy Physics. The High Energy group is engaged in experiments using wire spark chambers, counters and on-line computers at the 5 GeV electron synchrotron at Daresbury, Nr. Warrington. Salary range £1,470 to £2,630 per annum. F.S.S.U.

Particulars and application forms (returnable by August 31) from the Registrar. Quote Ref. 119/67/Na. (442)



## ESTABLISHMENT OF PHYSICS GROUP

BCIRA conducts experimental research and development on all aspects of the production, performance in service, metallurgy and inspection of iron castings. The establishment of a **Physics Group** is planned. A physicist is already established as a leader of the group, and BCIRA now has vacancies for physicists to assist in the development of the effort.

The work of the group will cover many topics and will immediately involve responsibility for work with an electron probe micro-analyser, a fundamental project on the electrical and magnetic properties of liquid iron alloys with a view to producing new control techniques, and the development of non-destructive techniques for the determination of structure and properties.

The group will be expected to initiate its own programme of fundamental and applied work which should involve laboratory and industrial experience. The posts provide opportunity for experience in a wide field, and considerable scope, especially for young men with good honours degrees in pure or applied physics, but also for corporate members of the Institute of Physics and for those with HNC.

The laboratories are situated in pleasant rural surroundings within easy reach of Birmingham, Redditch and Coventry. Good opportunities are provided for travel and for publication of results, as well as for maintaining contact with relevant fields of knowledge, both academic and industrial.

Starting salaries will be in the range £1,150–£2,000 per annum, depending on age, experience and qualifications. Staff application forms can be obtained from:

**DIRECTOR,  
British Cast Iron Research Association**  
Alvechurch, Birmingham.

(438)

## microbiologist

A young microbiologist is required in the quality control section of our Microbiological Laboratory. In addition to quality control work, the position will involve working on development projects.

The successful applicant will have a minimum qualification of ONC in biological subjects. Some experience in biological control testing is desirable.

The starting salary will be in the range £750–£950 dependent on age, qualifications and experience.

The Company has a non-contributory Pension and Life Assurance Scheme, and a Profit Sharing Plan.

*Please write, giving full details, to :*

D. J. Goddard, Personnel Manager (R/G/8),

**NICHOLAS RESEARCH INSTITUTE LTD.**

225 Bath Road, Slough, Bucks.

A MEMBER OF THE ASPRO-NICHOLAS GROUP.

(476)

### UNIVERSITY OF EDINBURGH

## Senior Systems Programmer

required to write a LISP 1.5 Interpreter and Compiler for the System 4/75 English Electric multi-access system which is to be installed in the Edinburgh Regional Computer Centre next year. The appointment will be for one year, in the first place, but there should be opportunities for re-appointment in the university or the Regional Centre thereafter. Salary will be from £1,800 upwards, depending on qualifications of candidate. The appointment will be made as soon as there is a suitable candidate.

There is also a possible opening for a junior appointment of a graduate assistant in programming.

Applications, by letter, should be sent to Dr. B. Meltzer, Metamathematics Unit, 5 Buccleuch Place, Edinburgh, 8. (444)

### MINISTRY OF OVERSEAS DEVELOPMENT

The Tropical Pesticides Research Headquarters and Information Unit of the Ministry requires a Scientific Assistant to deal with the maintenance of snail cultures, testing of chemicals as possible molluscicides for the control of Bilharzia and to assist in experiments to investigate the mode of action of these chemicals.

Applicants must have at least four "O" level passes, including English language and a scientific or mathematical subject, together with at least one year's practical experience. Salary according to age in the scale £456 to £1,055 (including Inner London weighting).

Applications to the Director, Tropical Pesticides Research Headquarters and Information Unit, Tropical Products Institute, 56-62 Gray's Inn Road, London, W.C.1, before August 14, 1967. (435)

### UNIVERSITY OF LIVERPOOL

#### PHYSICAL CHEMIST OR BIOPHYSICIST

Applications are invited for the post of Research Fellow or Research Assistant, according to the qualifications of the successful applicant in the Immunology Cancer Research Unit. Postgraduate research experience and/or higher degree desirable, but not essential. Opportunities for both personal research and team-work in excellently equipped new laboratories. Initial salary within the range £1,200 to £2,000 per annum (plus F.S.S.U.), according to qualifications and experience.

Applications, naming three referees, to the Registrar, The University, Liverpool 3. Please quote Ref.: RV/99/N. (404)

### UNIVERSITY OF DUNDEE

#### COX CHAIR OF ANATOMY

Applications are invited for the Cox Chair of Anatomy which falls vacant from January 1, 1968 on the appointment of Professor R. E. Coupland to the Chair of Human Morphology at the University of Nottingham. Salary £4,620. F.S.S.U.; grant towards removal of household effects.

Applications (fifteen copies—overseas applicants one copy), containing the names of three referees to be lodged not later than August 26, 1967, with the Secretary, The University, Dundee, from whom further particulars may be obtained. (439)

# PLANT BREEDING INSTITUTE CAMBRIDGE

**ENTOMOLOGIST** required for a new post to study resistance to aphids in sugar beet and other crop plants. Appointment at Scientific Officer level within the salary scale £926 to £1,574, according to age and experience. First or Upper-Second Class Honours degree essential and post-graduate experience in entomology would be an advantage. Superannuation under F.S.S.U.

Applications, giving full personal particulars and naming three referees, to The Secretary, Plant Breeding Institute, Maris Lane, Trumpington, Cambridge, by September 1. (495)

# COLLEGE OF MEDICINE OF THE UNIVERSITY OF LAGOS

Applications are invited for the following appointments in the newly-created Department of Radiation Biology and Radiotherapy:

(a) Lectureship Grade I in Radiotherapy. Candidates must be medically qualified with D.M.R.T., F.F.R., or equivalent.

(b) Lectureship Grade I or II in Medical Physics or Biophysics. Candidates must possess Ph.D. in Medical Physics.

Appointees will be considered for appointment to honorary consultancies to Lagos University Teaching Hospital. Salary scales: Grade I £2,200 to £2,600 per annum; Grade II, £1,200 to £2,100 per annum. 20 per cent supplementation basic salary (generally free of income tax) payable to U.K. staff. Family passages; children's and car allowances; superannuation scheme; regular U.K. leave.

Detailed applications (six copies), naming three referees, as soon as possible to Inter-University Council, 33 Bedford Place, London, W.C.1, from whom particulars are available. (449)

# UNIVERSITY OF MANCHESTER

Applications are invited for the post of Senior Technician in the Department of Bacteriology and Virology. Applicants should have had previous bacteriological experience and may work either in the preparation of class material or be in charge of the central services unit. Salary in accordance with the University's Scales for Senior Technicians (£942 to £1,180 per annum). Registered technicians may be considered for National Health Service rates of pay (£994 to £1,727).

Applications, with full details of age, experience and names of two referees, should be sent to the Secretary, Dept. of Bacteriology and Virology, Williamson Building, Brunswick Street, Manchester 13. (481)

# UNIVERSITY OF MANCHESTER MANCHESTER, 13

# EXPERIMENTAL OFFICER OR SENIOR EXPERIMENTAL OFFICER IN METALLURGY

Applications invited for this post for work in electronics, on research projects in Department of Metallurgy in Faculty of Science. Expansion requires automation of testing equipment or development and construction of new apparatus for specific measurements. Knowledge and experience of wide range of electronic techniques and devices required. Officer should, under own initiative, be able to consider measurement problems in terms of electronic devices and know how to develop, construct or purchase suitable instruments. Suitable qualifications: Degree, Dip.Tech., or H.N.C. Other qualifications considered if experience relevant. Salary ranges per annum. E.O. £920 to £1,525; S.E.O. £1,450 to £2,200. F.S.S.U.

Particulars and application forms (returnable by August 31), from the Registrar, the University, Manchester 13. Quote Ref. 124/67/Na. (482)

**EAST MALLING RESEARCH STATION.** Vacancies for graduates in Statistics or Mathematics. Research encouraged. Biological or horticultural interests an asset. Access to computers. Appointment in the Scientific Officer grade.—Further details and application forms from the Secretary, East Malling Research Station, Maidstone, Kent. (436)



# Scientific Civil Service

# FORESTRY COMMISSION

**ASSISTANT GENETICIST** (graded S.S.O.) required to fill post at the proposed Forestry Research Station to be built near Edinburgh to carry out research work concerned with problems of flowering and fruiting of forest trees, vegetative propagation problems connected with forest trees, growth analysis and early progeny test procedures. Would take charge of small laboratory, glass houses and growth room facilities. Services of two Scientific Assistants and four foresters available. Candidates should be between the ages of 27 and 35 years and should have a good honours degree in an appropriate subject with post-graduate training in genetics, biochemistry or plant physiology.

**QUALIFICATIONS:** 1st or 2nd class honours degree, or equivalent or higher qualification in appropriate subject and at least 3 years' post-graduate experience in genetics, biochemistry or plant physiology.

**SALARY** (minimum age 26): £1,744 to £2,155.

Non-contributory pension, promotion prospects.

**APPLICATION FORMS** from Civil Service Commission, Savile Row, London, W.1, quoting S/53/FC. Closing date August 29, 1967. (451)

# GOVERNMENT OF NORTHERN IRELAND SENIOR SCIENTIFIC OFFICER/SCIENTIFIC OFFICER

Applications are invited for a pensionable post in the Agricultural Bacteriology Division of the Ministry of Agriculture.

# QUALIFICATIONS:

Honours Degree in Agriculture, Science or Food Science preferably with post-graduate experience in Bacteriology.

# DUTIES:

The successful candidate will be expected to undertake research and investigational work in connection mainly with the bacteriology of meat and meat products and may be required to undertake teaching duties in the Faculty of Agriculture, Queen's University, Belfast. Facilities are available for registration for a higher degree.

# SALARY SCALES:

Senior Scientific Officer £1,744 to £2,155

Scientific Officer £926 to £1,574

Grading and starting salary will depend on qualifications and experience. There are prospects of progression to Principal Scientific Officer.

Application forms, obtainable from the Secretary, Civil Service Commission, Stormont, Belfast, 4, must be returned by August 18, 1967. Please quote SB92/67/64. (454)



# THE ARTHUR D. LITTLE RESEARCH INSTITUTE

wishes to recruit a

# Physical Chemist

to join a group concerned with long-range research into the study of new materials. Research experience in the field of polymer characterization, optical studies or electrical behaviour would be an advantage, but is not essential. The Institute is concerned generally with a variety of long-range projects in the fields of physics, chemistry and biology, and is situated in pleasant rural surroundings within 6 miles from the centre of Edinburgh.

Applications, giving particulars of qualifications and experience should be sent to: The Personnel Officer, Arthur D. Little Research Institute, Inveresk Gate, Musselburgh, MIDLOTHIAN. (469)

## Patent/Licensing Officers

A major Company in the plastics and chemical industries employing 11,000 people, wishes to appoint two Patent/Licensing Officers to work in the Patents Department at Tyseley, near Birmingham. General terms and conditions of employment are good, as are the career prospects for the men appointed.

The successful candidates will undertake general patents work in the fields of plastics manufacture and fabrication with particular emphasis on either:

- a) preparation and filing of British and overseas patent applications
- or b) Licensing of patents and technology.

Applicants should have a good honours degree in chemistry, chemical engineering or engineering and at least 3 years experience in patents and/or licensing work.

**Please write, giving brief details of qualifications and experience to:**

**The Staff Manager, Bakelite Xylonite Ltd.  
Enford House, 139-151 Marylebone Road,  
London, NW1**



(478)

## NEW ZEALAND

University of Canterbury  
Christchurch

### READER IN MATHEMATICS

Applications are invited for the above-mentioned position. Qualifications in either pure or applied mathematics will be acceptable but applicants having interests in the fields of numerical mathematics or statistics would be particularly welcome. The Department is equipped with an I.B.M. 1620 computer, and an I.B.M. 360/44 computer will be installed in August, 1967. The salary will be between \$NZ5,600 and \$NZ6,600 per annum, commencing salary being in accordance with qualifications and experience. (\$NZ2=£1.) Approved fares to Christchurch will be allowed for the appointee and his dependent family, together with actual removal expenses within specified limits.

Further particulars and information as to the method of application may be obtained from the Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1. Applications close on September 30, 1967. (484)

### BUREAU OF SUGAR EXPERIMENT STATIONS

QUEENSLAND, AUSTRALIA

Applications are invited for appointment to the position of Assistant Director. The appointee will be required to take up duty at the Bureau's Head Office in Brisbane on July 1, 1968.

The Bureau of Sugar Experiment Stations is the official research organization of the Queensland sugar industry, and is administered by the Sugar Experiment Stations Board; it is a semi-Government instrumentality.

Applicants should be graduates of an approved university, and should submit with their applications evidence of scientific and administrative ability. The position to be filled is a technical-administrative one and the appointee would be required to assist in administering the activities of the organization in the fields of agronomy, entomology, cane breeding, pathology, extension and sugar-mill technology. His training and experience should be in the agricultural field.

The salary applicable to the position is within the range of \$A8,626 to \$A9,026 per annum (£1 Sterling is equivalent to \$A2.50). The appointee would be required to join the Bureau's Superannuation Scheme after three months' service. Housing or quarters are not supplied.

Previous experience in the cane-sugar industry is not essential, but preference would be given to applicants with a wide experience in a research phase of sugar-cane agriculture.

Applications, supported by complete details of academic training, experience and published work should be addressed to the Director, Bureau of Sugar Experiment Stations, 99 Gregory Terrace, Brisbane, Australia, so as to arrive by November 15, 1967. Applications may be endorsed on the envelope "Confidential—Application for Position." (459)

### UNIVERSITY OF SHEFFIELD DEPARTMENT OF PHYSIOLOGY

Applications are invited for a post of LECTURER or ASSISTANT LECTURER in PHYSIOLOGY duties to begin as soon as possible. The Assistant Lectureship would be suitable for a candidate wishing to study for primary F.R.C.S. Initial salary according to qualifications and experience in the ranges: for candidates holding a registered medical qualification: Lecturer, £2,005 to £2,755; Assistant Lecturer, £1,470 to £1,830; for other candidates: Lecturer, £1,470 to £2,630; Assistant Lecturer, £1,105 to £1,340 with F.S.S.U. provision.

Further particulars from the Registrar, to whom applications (six copies), should be sent by September 2, 1967. (487)

THE LONDON HOSPITAL, WHITE-chapel, E.1. GRADUATE PHYSIOLOGIST required for research project in this large Teaching Hospital. For a suitable candidate, facilities may be available to obtain either a M.Sc. or M.Phil. degree. Salary according to age, experience and degree.—Written applications, naming two referees, to the House Governor by August 19, 1967. (455)

### SIR JOHN CASS COLLEGE

#### Department of Chemistry

Applications are invited for the post of LECTURER or ASSISTANT LECTURER with special interest in OPTICAL SPECTROSCOPY, to commence in either October 1967 or January 1968.

Applicants should be qualified to lecture in Inorganic or Physical Chemistry to honours degree standard, and must have active research interests in Spectroscopy.

Salaries are within the following scales: Assistant Lecturer (Grade B): £1,175 to £1,845; Lecturer: £1,875 to £2,140; plus, in each case, London Allowance of £70.

Application forms and further particulars may be obtained from the Secretary, Sir John Cass College, Jewry Street, London, E.C.3. The closing date for applications is August 21, 1967. (456)

### UNIVERSITY OF LIVERPOOL

#### IMMUNOLOGY CANCER RESEARCH UNIT

SENIOR TECHNICIAN required for the Research Laboratory with either Associate-ship or Fellowship of the I.M.L.T. Experience in the handling of staff is desirable with a practical understanding of the problems of routine supply and administration of a research laboratory. Familiarity with histological, immunofluorescent, or radio-isotope techniques an advantage, but not essential. Excellently equipped new laboratories.

Salary on the scale £994 to £1,303 per annum according to qualifications and experience.

Application forms may be obtained from the Registrar, The University, Liverpool 3. Please quote Ref. RV/96/N. (402)

### PLANT BREEDING INSTITUTE CAMBRIDGE

#### CEREAL CYTOGENETICS

It is proposed to appoint a Scientific Officer to work on applied and fundamental aspects of the chromosomal organisation of barley. This work will be associated with that of the Cytogenetics and the Cereal Breeding Sections of the Institute. Applicants should have good degrees in Genetics or Botany. The salary will be in the range £926 to £1,574 with superannuation under F.S.S.U.

Applications, with full personal particulars and naming three referees, should be sent to the Secretary, Plant Breeding Institute, Cambridge, by September 1. (493)

### BIRMINGHAM (DUDLEY ROAD) GROUP OF HOSPITALS

Biometric or Cardio Respiratory Function Technician required for Respiratory Function Laboratory and Cardiological Department at Dudley Road Hospital. The person appointed should have had previous experience in the field and be suitably qualified. Salary scale £1,333 rising to £1,727 per annum.

Applications in writing naming two referees, to Group Secretary, Dudley Road Hospital, Birmingham 18, by August 19, 1967. (457)

## PROBLEMS OF SCALE

THE publication of the report on the Aberfan disaster, when a coaltip slipped down the side of a Welsh valley last year and killed 145 people, most of them children, has been a dramatic business (HMSO, 15s.). For one thing, the occasion has released a great flood of comment, criticism and emotion which was necessarily pent up while the judicial tribunal was in session. But the report has also been unexpectedly forthright in its criticism of the National Coal Board and surprisingly frank in singling out for blame individual officials of the board. The tribunal's conclusion, from which nobody has yet dissented, is briefly that the disaster could and should have been prevented. It only happened because of "ignorance, ineptitude and a failure of communications" within the board. Lord Robens, the chairman of the coal board, comes in for deliberate criticism, partly because of a hasty but ill-considered attempt to claim that the disaster could not have been foreseen, but more seriously because the coal board did not willingly admit its responsibility to the tribunal.

In reality the circumstances which led to the disaster were neither unusual nor unforeseeable. Indeed the particular coaltip which eventually engulfed the school at Aberfan had slipped some hundreds of feet down the mountainside in 1963. A companion coaltip had slipped almost as far as the village in 1944. For several years the villagers had complained to the coal board of what seemed to them a serious and an imminent danger. In the circumstances it is only natural that Lord Robens should have chosen to offer his resignation. (This remains in abeyance until the end of August, when the coal board is due to make its own proposals for reform to the Minister of Power.) It is also natural that everybody should now be determined that coaltips should never slide again, and this objective will probably be easily attainable. It is, however, much less easy to be sure that other problems made obvious by the Aberfan disaster, and which are in many ways bound up with the growth of industries of all kinds, will be as closely attended to, or even as fully appreciated.

The growing scale on which modern industry—and even the modern coal industry—must function is one aspect of the Aberfan disaster which tends to be forgotten. Last October there were close on three million cubic yards of colliery waste on the hillsides above Aberfan. For more than half a century until the newest of the seven spoil heaps slid down the mountainside,

tipping had continued at a rate which works out at an average of 57,000 cubic yards a year. This rubble was being heaped on to a hillside falling uniformly into the valley beneath at a gradient of one in five. As it happens, the complex of coal tips intersected a geological fault (plainly to be seen on the appropriate maps) and the natural springs associated with it, to say nothing of a number of natural water courses. It seems to be agreed that the disaster happened because water accumulated within the seventh coaltip to such an extent that hydrostatic pressure was able to set in train a bodily movement of one part of the tip. When that had begun, it is no surprise at all that the downward movement of the mass of rubble should have carried for 2,000 feet downhill and that it should in the process have carried away several houses and most of the school. It is now clear that the moving mass scoured the thin layer of boulder clay from the sides of the glacial valley and thus released water stored in the natural aquifers of the underlying sandstone, which then discharged itself catastrophically as a flood on the village underneath.

Plainly the scale of this phenomenon was almost geological and this, no doubt, is part of the reason why the officials of the National Coal Board now held to have been responsible for the neglect of safety should have failed to appreciate the danger of tipping on such a water-sodden hillside. They would have been used, in their daily work, to seeing truckloads of coal, five or ten tons at a time, piled neatly into stable heaps. There would have been little in their everyday experience to suggest that a heap of waste one hundred thousand times as big might behave in quite a different fashion. There is plenty of evidence in the report of the tribunal to show how whimsical were some of the attempts at foreseeing what might happen. One colliery official, for example, who stands out as being alone prepared to consider the possibility that waste might actually reach the village from the coaltips, seems to have thought that even in this unlikely event—to him—there would be plenty of time in which people could be warned to evacuate their houses. The larger the mass, the slower the acceleration—that seems to have been this unfortunate man's intuitive notion of acceleration down an inclined plane. But for most of those concerned with the tips, large scale movement seems to have been quite beyond the bounds of possibility. The tribunal in its report points out that those in charge of coaltips would have appreciated



the danger at Aberfan more readily if they had thought of how sandcastles on the beach can be brought down by a little water, but this point would have occurred only to people anxiously seeking criteria to decide when coaltips would be safe. So indifferent to the possibility of catastrophe were the engineers in charge of these operations that they were not even alerted by the circulation in 1965 of a memorandum spelling out procedures for tipping colliery waste that would have ruled tips at Aberfan completely out of court. (The memorandum was itself, ironically, derived from what the coal industry is inclined to think of as the bad old days before nationalization.) The tribunal is right to describe this state of affairs as akin to blindness. Without excusing in any way the slackness in the coal board, it is entirely fair to say that the failure of the several officials was a failure to appreciate the scale on which they were operating.

From this it follows that in the search now being made for other potential disasters, coaltips are only some of the artefacts which should be thought of. By now there is plenty of experience to show that gigantic catastrophes can follow large scale reservoir construction, for example. Here too, unfortunately, bitter experience has been more formative than foresight. It took the disaster at Fréjus nearly ten years ago to emphasize the importance of rooting concrete dams in sound rock. Other fatalities have been necessary to show that water in a dammed up lake, like water in a bathtub, can slosh about from one end to another with serious consequences. Huge coastal works can create problems as well as solving them. There seems to be a growing suspicion that tall buildings can form patterns of air movement which cannot easily be anticipated from experience with much smaller structures—the failure of the electricity cooling towers of the Central Electricity Generating Board at Ferrybridge two years ago is yet another sign that scaling up is necessarily difficult. And what will happen when cities become even larger than they are now, so that their output of heat and chemicals to the environment is even greater than at present? Fortunately it is usually possible to work out the probable consequences of gigantic scale. In most fields engineers are now ready to acknowledge that there is no substitute for model experiments intended to throw light on unfamiliar circumstances. To say all this is not to imply that scaling up should be avoided—indeed, it is essential that things should get bigger as time goes on. One of the immediate consequences of the Aberfan disaster should be to make all kinds of engineers more sensitive to the need to anticipate what problems there may be. It is the peculiar fault of the coal industry that it was able smugly to ignore all the risks it ran.

And why so blind? One of the most bitter ironies in the report of the tribunal is that most of the men singled out for blame appear to have been not merely conscientious but even vigorous about almost everything but the management of coaltips. Most of the men concerned were what is called self-made men. Some of them, for example, had gone into coalmining

at fifteen and had been able to work their way to positions of responsibility by hard work and devotion. In one sense it is cruel that their reputations should now be ruined on account of a failure which their training had not equipped them to appreciate. But the same people were led by their long experience to believe that they knew everything there is to know about coalmining—which is why they chose to fob off complaining villagers with fairy stories intended to suggest that they had made a sober appraisal of the situation. It is hard to believe that so many people would have been so complacent if they had had wider experience outside coalmining. In the circumstances it is essential that in the programme of reform on which the coal board must now embark, it should take vigorous steps to see that people with positions of responsibility in the industry are given the kind of further education and training necessary to see that they can do their jobs effectively. To say this is not to suggest that people should not be allowed to work their way up from the bottom, but, on the contrary, that job training is as important for individuals moving up the industrial hierarchy as instruction in arithmetic is for children at school. This is as true for other industries in Britain as it is for coalmining. The coal board should be spurred on by the recognition that the disaster at Aberfan may only be the most spectacular of the deficiencies of its organization which can be blamed on insufficient attention to vocational training.

As it happens, the people trained in engineering seem to have been as indifferent to the dangers at Aberfan as were the self-made men, which only goes to show that formal training is not enough to allow people to operate efficiently in modern industry. The professionals, however, were prevented from contributing efficiently to the prevention of disaster by the absurd way in which old-fashioned caste systems have been imported into the mining industry. The distinction between civil and mechanical engineers seems to have been as clear at Aberfan as it was when Stephenson created the Institution of Mechanical Engineers as a slap in the face for the stuffer civil engineers. (One curious anomaly in mining is that mechanical and not civil engineers are formally responsible for such tipping policies as there are.) From the report of the tribunal it emerges clearly that there will now be a movement to make sure that people in pits are given some formal training in soil mechanics, which will do no harm and possibly some good. But in the long run it is more important that professional people employed in mining, and in other industries for that matter, should be trained for flexibility. It is just possible that the real interests of the coal industry will not be served if there is now a concentrated attempt to make the education of mining engineers still more specialized, so that they are able to do everything they do at present and to manage coaltips as well. It could easily be much more valuable if engineers with experience right outside the industry were tempted into positions where their wider experience could serve not merely to prevent accidents but to introduce new ideas as well.

## NEWS AND VIEWS

### Selling Space

THE House of Commons Estimates Committee has just completed the exhausting task of surveying the British space research effort. The results are described in a highly critical report, published this week (HMSO, £1 18s.). "On the whole it has been a story of wasted opportunities brought about by lack of purpose and the lack of any coherent organization. There has been no real space policy and no space programme as such."

The report deals with each of the international organizations in turn. Although ELDO has so far cost Britain £49.8 million, only one buyer (France) is in view for the ELDO PAS launcher which the project is intended to develop. In ESRO, Britain has spent £11 million, and obtained contracts worth £4 million in exchange. France, the committee bitterly points out, has obtained contracts worth more than double what she has so far contributed. On the scientific side, the committee is no happier; ESRO 11 should have been launched by NASA—free of charge—early in 1967, but the Scout launcher failed. It will now be launched in late 1967 or early 1968, about the same time as ESRO 1. As for the large astronomical satellite (LAS) which was to be launched in 1970 as the climax to the ESRO programme, the committee believes that it will be 1972 or 1973 before it is in orbit. CETS, the committee says, is not an organization but a "continuing conference". "Its continued existence in its present form," the committee declares, "would appear unlikely to achieve any useful purpose." In Britain, the decision to proceed with the Black Arrow programme was the right one, but was taken too late: "the fact that in the end the right decision was reached in no way condones the delay in arriving at it".

As the committee discovered, there is no such thing as a British space programme; expenditures and projects are considered on a piecemeal basis. The committee recommends that a space programme with a budget of its own should be drawn up and agreed for the next five years. The Minister of Technology should take charge of the programme, and appoint a minister of state with responsibilities solely for space. The committee believes the present level of expenditure, about £30 million a year, to be about the right figure. As a proportion of GNP it is rather larger than Germany or Japan, rather smaller than that of Italy or France. A much larger part of this money should be spent within Britain, however, and the correct ratio should be something like two to one in favour of national programmes. This, it admits, will be difficult to achieve within the budget suggested if the large contribution to ELDO (£9.7 million this year) is to continue. This is another reason, the committee feels, for questioning the future of ELDO; if it is to continue, the British contribution should not be allowed to rise. Similarly, Britain should oppose any attempt to increase the number of ELDO PAS launches. It should not

take part in the CETS programme for a television distribution satellite, but should attempt to produce a British military communications satellite to replace the existing Skynet satellites in 1971. Expenditure on the Black Arrow programme should be doubled from £3 million to £6 million, with the intention of using electrical propulsion to launch a small communications satellite.

Not all these suggestions will find favour with the Government. They are too closely identified with the space industry lobby and Conservative Party policy for that. In any case, the Government is unwilling to do anything which can be interpreted as anti-European. It is clear, though, that British space expenditures will not be allowed to rumble on untouched for much longer; within the next few months decisions must be taken to bring things under control.

### Non-treaty

HOPES for a nuclear non-proliferation treaty, so high three months ago, now seem to have sunk almost out of sight. Mr Fred Mulley, the British minister at Geneva, had some strong words to say on the subject last week. "I am extremely disappointed," he said, "to find on August 3 that we have no draft treaty before us and, as far as I can discover, have made no further progress towards achieving a non-proliferation treaty." He went on to say that if a draft is not tabled very soon, there may not be time to turn it into a treaty, and he urged the co-chairmen, particularly the USSR delegate, to get negotiations moving again.

The sticking point for the treaty is still the safeguards arrangements. Euratom countries have insisted that they be allowed to preserve the safeguards worked out within Euratom, while other countries have favoured safeguard arrangements in the hands of the IAEA. This impasse led to the suggestion that the treaty be drafted with the section on safeguards left blank, a proposal the USSR has so far refused to accept. It is clear that even if a draft acceptable to all the parties can be put together, there will be plenty of steam left in the arguments, and the transition from draft to treaty could be a long business.

The desire for a treaty has been given urgency by the very rapid advance of the Chinese to thermonuclear status. Neither China nor France, however, is represented at Geneva, which means that whatever treaty is signed France will not be a party to it. It is distasteful to other Euratom countries to reflect that if they sign the treaty they will be subject to inspections from which France is exempt; this is fundamentally opposed to the philosophy of share and share alike on which Euratom was once based. Of the other states near the threshold of nuclear status, Israel and Japan are not represented, but the UAR and India are. France is entitled to a seat at the negotiating table, but has not taken it up, and will presumably not sign the treaty.

The frustration at Geneva has tended to shift the spotlight to another problem under discussion there—the Comprehensive Test Ban Treaty. This too is making very slow progress, both for technical and political reasons. It is at present impossible to detect all underground nuclear explosions unambiguously by

long range seismic recording alone. The present detection threshold is about magnitude four, and 80–85 per cent of the earthquakes above this magnitude are identifiable. A recent Swedish memorandum to the committee has suggested that by processing the data in a certain way, and by the use of decision theory, the data now available are sufficient to provide at least a degree of deterrence to countries contemplating underground nuclear explosions. It is clear, though, that it is still not possible to distinguish with absolute certainty between explosions and earthquakes without on-site inspections. As Mr Mulley put it, "It may be that with further technological progress scientific techniques alone may provide the answer, even without on-site inspections . . . but my government does not believe that this point has yet been reached". Meanwhile the test ban treaty shares with the non-dissemination treaty the problem of inspection—and, of course, the depressing thought that neither France nor China has any intention of signing it.

## Ten Years Old

It is difficult to look back with much enthusiasm on the ten years in which the International Atomic Energy Agency has been in existence. The acquisition of nuclear weapons by China and France, and the adoption of idiosyncratic foreign policies by these two states, represents just the kind of situation that the IAEA was set up to prevent. Indeed, so limited were the powers accorded to the new organization at its inception that its failure was predictable even then. The agency may inspect nuclear installations only at the invitation of governments, and its fiercest sanction is to report misdemeanours to the General Assembly of the United Nations. At present, more than sixty plants in twenty-six countries are under its observation, but none of these is in France, West Germany, the Soviet Union, or, of course, China. Of the technologically advanced countries, only Japan, inspired by memories as well as reason, has co-operated whole-heartedly with the IAEA. Until more of its member states follow this example, the agency can only continue to gain experience of inspecting installations. This month one of its teams is investigating a newly built fuel reprocessing plant for the Yankee reactor at West Valley, New York. This is the first time that a plant other than a reactor has been inspected.

The IAEA has been more successful where its activities have not impinged on politics. More than two thousand five hundred fellowships have been awarded, and a large number of training courses arranged. Research is being undertaken into desalination, pest control by irradiation, the use of isotopes in medicine and hydrology, and other projects. Yet the budget of the agency is minute compared with that of national atomic energy authorities. In 1967, of a total of twelve million dollars, only three-quarters of a million were spent on research projects. The amount will rise by a mere fifty thousand dollars next year, according to the recently published budget for 1968. Even among the United Nations agencies, the IAEA is something of a Cinderella; WHO operates on a budget of nearly sixty million dollars.

Had the research work of the agency been more extensive, its control over the activity of member states

might have been greater, for its statutes stipulate that all of the agency's aid projects shall be open to inspection. As it is, with the bulk of nuclear research being done in regions far from Vienna, international projects have been set up independently of the IAEA, and only occasionally has the agency been invited to join the agreement, as a superfluous matchmaker. With the authority of scientific achievement, the IAEA might speak with a different voice, and be heard.

## Organizing Steel

DETAILS of the organization of the British Steel Corporation were recently completed, and have been published as a White Paper (HMSO, 4s.). The corporation came into existence on July 28, when fourteen British steel companies were nationalized. The BSC will have a total turnover of more than £1,000 million, and will employ 270,000 people; this, the White Paper notes, will put it among the six largest undertakings in the free world—outside the United States.

The organization takes into account two opposing forces, geography and product. Geographical groupings help in the physical problems of management and communications, but destroy valuable product links between companies. Grouping on product lines makes management, sales and research easier to co-ordinate, but reduces freedom of choice for the consumer and makes competition between the groups harder to sustain. In the event a compromise between these forces has been struck, and four regional groups are named in the White Paper. The Midland Group, centred in Sheffield, will do much of the stainless and special steel production; the Northern Group will look after tube, the Scottish and Northern Group strip milling and coated sheets, and the South Wales Group will account for the whole UK production of transformer sheet and tinplate. It is clear, though, that the groups will not be limited to these products, and a generous amount of overlapping on the more common steel products is likely.

The report says little about research and development in the corporation. Dr H. M. Finnieston, as deputy chairman-technical, will have two directors reporting to him, one on engineering, and the other on research and development. The director of engineering will look after the exploitation of research and development work, advise the groups on the engineering aspects of major capital projects, and distribute technical information. The director for research and development will be concerned with all the central research activities, technical processes, product development and measures designed to improve working conditions. The White Paper makes a point of mentioning that he will use techniques such as operational research. The names of the directors have not yet been announced.

The philosophy of the White Paper seems to be to develop a less monolithic structure than is customary in British nationalized industries. "Within the concept of a single, unified corporation, we consider that the Groups should have the greatest degree of operating autonomy and profit accountability. The spur of competition, other than in price, between the groups and the units below them is in our view highly desirable." Price competition, though, says the White Paper, is both unattainable and undesirable within a

single organization. It then goes on to demolish claims which British steel makers have been making in their battle against nationalization: "... true price competition has never operated successfully in this or any other country". It must have come as a great relief to some members of the board to be able, at last, to admit it.

## How to import Chemicals

RESEARCH workers, both in industry and universities, have been complaining about the difficulty of importing small chemical samples into this country. The samples are of compounds which are not manufactured here, and may be available only from a single firm abroad. Although they are given completely free by the manufacturers, and are clearly marked "For Scientific Purposes Only", they have, in certain cases, been subject to import duty. Even more annoying, delays in delivery are frequent; a box of samples that crosses the Atlantic in eight hours may take three weeks to reach the laboratory from London Airport.

The best way to be certain of the swift arrival of a shipment is to employ one of the freight agencies that handle goods at docks and airports. A laboratory will have to pay such an agent three or four pounds to have a case of samples sent through the customs, and put on the train. This sum may well be ten times the value of the chemicals, so that the privilege of receiving free gifts from American and European companies may cost a research team dear. Dr R. Schmutzler, of Loughborough University of Technology, has recently complained about the problem to the Ministers of Technology and Education. He tells of how he was forced to pay agency dues out of his own pocket; there was no provision for them in his research budget.

Those who try to do without the agencies find themselves at a disadvantage. Among other things, they are required to show that the rare chemicals they have ordered will be used only for research. The proof of this, Dr Schmutzler writes, is not easily demonstrated in letters to the customs or by appearance in person at the airport. Yet when the agencies handle the chemicals, no such rigorous investigation of the possibility of their misuse takes place. The customs will accept that drugs imported for laboratory research will be properly looked after only when the actual importing is done by an agency.

The agency will not, of course, question the motives of a university worker who orders a dangerous drug through them. The moral is therefore clear: research workers who are determined to supplement their salaries by selling drugs to swinging Londoners had better order through chemical import agencies. Only the honest man, insensitive to all insinuations of fraudulence, and disposed of a great deal of time, can handle imports of this kind for himself.

Dr Schmutzler goes on to compare the sympathetic attitude of the American authorities to the international traffic in valuable chemical samples, with the suspicion and lack of understanding shown by the customs over here. He and other frustrated research workers feel that it should surely be possible for them to obtain samples quickly, and without too much expense on the one hand, or trouble and even humiliation on the other.

## Loaves and Fishes

FOR an organization with a budget of only four hundred thousand pounds, the White Fish Authority certainly appears exceptionally versatile. In its research report, published this month, the Authority describes a cockle harvester, which will enable fishermen to exploit sublittoral beds where the density of the mollusc population is low, and gives an account of fish rearing experiments in Scottish lochs, and in tanks in which the water is heated by the effluent of nuclear power stations. This latter experiment is of unusual interest. It has been possible to rear a small number of sole to a marketable size in only one year, a process which would take three years in natural conditions. Other projects include the development of fenders, so that fish can be transferred from one vessel to another at sea. The authority also concerns itself with the processing of caught fish from their initial superchilling at 28° F, to their eventual appearance in specially concocted sausages and fish crisps.

As the report points out, most of the innovations in fishing techniques could have been produced fifty years ago. Conservatism, rather than lack of knowledge, prevented their development sooner. On the other hand, the authority is having to pioneer methods of basic research, in order to find out, for instance, how ships behave at sea in extreme conditions. The chairman of the research committee of the authority, Sir Fredrick Brundrett, has called—not for the first time—for a National Council of Marine Technology, saying that conditions in the fishing industry when the authority was founded had differed little from those obtaining in Galilee two thousand years ago. Perhaps a better comparison could have been drawn; even with the million pounds that Sir Fredrick wants for new research, the authority will be hard put to emulate certain achievements of those days. They did more with bread and fish than make sausages out of them.

## More Aid for Visual Aids

HARD on the heels of the newly formed National Council for Educational Technology, sponsored by the Department of Education and Science, comes the Industrial Council for Educational and Training Technology. This has been formed by four trade associations: the British Electrical and Allied Manufacturers' Association, the British Radio Equipment Manufacturers' Association, the Electronic Engineering Association, and the Scientific Instrument Manufacturers' Association. It has originally been the plan that ICETT should co-ordinate the production and use of technical aids in teaching, from machines to textbooks, but the formation of NCET will no doubt cause the Industrial Council to concentrate on the production of teaching aids, rather than their use. There have already been informal talks between the two organizations.

At present, ICETT is only qualified to work on behalf of manufacturers of hardware; discussions have been held with publishing and film associations. (Individual companies may not join the Council.) If ICETT can widen its interests to include both software and hardware, and if it can achieve close relations with NCET, we may hope to see more and better use of educational aids; this may become more and more important if the teacher shortage worsens.



## Improving Liaison

THE Vice-Chancellors Committee of the Confederation of British Industry (not to be confused with the Committee of Vice-Chancellors and Principals) has set up a working party under the chairmanship of Mr P. Docksey, Research Director of B.P. Ltd., to look into relations between universities and industrial research. Its terms of reference are "to study the existing relationships between universities and industry in the field of research, and to make recommendations".

Industrialists—and others—have a sneaking suspicion that much of the universities' research work is wasted. The decision to set up the working party reflects this concern and also the feeling that too few university graduates enter industry. It was prompted by the Sutherland report on relations between universities and Government research establishments. In addition to the chairman the working party consists of Dr A. A. L. Challis, Laboratory Director, I.C.I., Runcorn; Dr T. L. Cottrell, Principal of Stirling University; Dr K. Mather, Vice-Chancellor of Southampton University; Dr J. A. Pope, Director, Mirreles National Research Division; Dr J. S. Tait, Vice-Chancellor of The City University, formerly Northampton College of Advanced Technology; Mr T. A. Uthwatt, Director of Research, Mather and Platt Ltd.; and Dr F. A. Vick, Vice-Chancellor of the Queen's University, Belfast. Mr R. C. Griffith of the UGC, Mr J. Knox of the Ministry of Technology and Mr C. Jolliffe of the SRC will attend as observers. Also in attendance will be Dr A. V. Cohen of the DES, and Mr P. M. Knowlson of the CBI, who will act as secretary to the working party.

Among the areas covered by the working party will be collaboration between universities and industry, the educational need of the industrial research worker and the role of joint appointments. They will also investigate ways of improving universities' understanding of industry's needs and of making industry fully aware of university research. It is hoped that the working party will produce its report by the end of the year.

## ELDO Frustrated

IF the ELDO launchings are any guide, the weather in Australia does not seem to be all that the immigration authorities pretend. The partially successful launching on August 4 was the eleventh attempt to launch F6/1 from Woomera and most of the delays were caused by bad weather.

The purpose of the launching was to test the separation and ignition of the French second stage, Coralie, from the British first stage, Blue Streak. So far as is known, the French second stage separated but did not ignite. The reason given for the failure is that some of the components had waited so long to be launched that they were approaching the end of their design life.

It is not expected that this partial failure will hold up the programme and the F6/2 launching is expected in early November. ELDO hope to gather the additional information on the ignition of the second stage from this firing. At the same time, F6/2 firing will test the separation of the German third stage from the second stage. The F7 launch will be a test firing of the complete Europa 1 vehicle and is intended to inject a test satellite into orbit; it is still scheduled for mid-1968.

## A Rose by Any Other Name

CERA, the Civil Engineering Research Association, announced recently that it has changed its name to CIRIA, Construction Industry Research and Information Association. Its scope now includes research into all aspects of construction, and with the new terms for the grant to the association from the Ministry of Technology, money devoted to research will effectively double and that spent on information will effectively treble. The association's income in 1966 was almost £200,000 and with the promise of extra subscription already made by contractors, the new grant terms are expected to bring the association's income initially to about £300,000.

Under the new terms of the grant, the association receives £1 grant for every £1 raised on most of their industrial income, as long as it reaches £136,000 a year, with a maximum grant of £300,000. During 1966 the association spent £152,175 on direct projects. Among the projects supported was £14,398 for wind effects on buildings and other structures, £10,000 on striking of formwork, and £8,295 on criteria of concrete strength.

## Uncorking an Enzyme

from a Correspondent in Molecular Biology

THE activation of zymogens is known to involve the enzyme cleavage of one or more peptide bonds, and the generation of an active enzyme by this mechanism has sometimes been referred to as "uncorking". The most intensively studied activation of this type has been the chymotrypsinogen-chymotrypsin conversion. Several variants of chymotrypsin can be produced, depending on the conditions. The first product of activation (by trypsin) is  $\alpha$ -chymotrypsin, in which the peptide bond between residues 15 and 16 is cleaved. The further cleavage of the bond between residues 13 and 14 leads to the formation of  $\delta$ -chymotrypsin, with loss of a dipeptide, and the excision of a further dipeptide (147-148) produces  $\gamma$ -chymotrypsin (the most usual accessible form); by changing the conditions this can be converted reversibly into a further form,  $\gamma$ -chymotrypsin. In addition to the fission of peptide bonds, the chymotrypsinogen-chymotrypsin conversion is accompanied by certain physical changes, which have exercised many protein chemists in recent years.

Some direct observations of the differences between the different chymotrypsins and their parent zymogen now come from the crystallographic studies of Kraut *et al.* (*Proc. US Nat. Acad. Sci.*, **58**, 304; 1967). Although the resolution at this stage is only 5 Å, which means that the course of the polypeptide chain cannot be followed with certainty, it is still possible to draw several interesting, if tentative, conclusions, especially if reference is made to the recent 2 Å X-ray structure of  $\alpha$ -chymotrypsin of Matthews *et al.* The results demonstrate how X-ray studies can give relevant information to the protein chemist, without necessarily producing the complete structure of the molecule.

The clearest conclusion to emerge is the extensive similarity between chymotrypsinogen and the chymotrypsins. Thus activation does not involve any major rearrangement of conformation. A comparison between the zymogen and a  $\delta$ -chymotrypsin derivative reveals

the position of the break introduced into the chain, the difference between  $\pi$ - and  $\delta$ -chymotrypsins being also at this site. At the same time the peptide (residues 1-15) appears to coil back on itself. The remaining important difference occurs in another part of the molecule, in which a short segment of the chain is displaced, with consequent unmasking of a cavity. In this are located two out of the three inhibitor molecules which have been introduced into the crystals. This is evidently the basis of the "uncorking" process. It may be recalled that the chymotrypsin structure described by Matthews *et al.* (*Nature*, **214**, 652; 1967) reveals that the new N-terminal group which appears on activation forms an ion-pair with a residue at the active site, and this may be supposed to stabilize the native conformation.

Kraut *et al.* have also calculated difference-Fourier maps between the different chymotrypsin forms, and variously inhibited states. Apart from the removal of the dipeptides, the differences between the successive enzyme forms are very small and localized. Between active and inhibited  $\gamma$ -chymotrypsin one observes, in addition to the differences due to the inhibitor itself, only one local change, which it is suggested would arise from a movement, involving a displacement of some 6 Å, of one or two side chains only. This is evidently the extent of the "induced fit" effect, and it is interesting that it should be so small. It may well prove possible to define these details more precisely in terms of the 2 Å maps of Matthews *et al.* It will also, of course, be of great interest to have a precise description of the differences between enzyme and zymogen, and to reconcile the small structural differences between the four forms of the enzyme with reported variations in activity.

## Teaching Immunology

It is often difficult for people who have finished their formal education to obtain systematic instruction in a new subject. This is particularly true when the subject is changing rapidly, or is of general interest to specialists in other disciplines. Although the lectures given in technical colleges do something to meet the need, few people can find time to attend weekly lectures. In many subjects, indeed, the general point can be made that there is a need for intensive courses in which people could rapidly acquire knowledge of a subject after their periods of formal education.

The British Society of Immunology and the World Health Organization have recently attempted to provide something of the sort. With organization supplied by Professor Leslie Brent, they held a five day summer school in immunology at the University of Southampton. The object was to provide a summary of the present state of immunology for those who have to teach medical students, and for others who wished to know about the clinical and fundamental aspects of immunology. It was not intended primarily for professional immunologists. The course was attended by about fifty people, including some from Europe sponsored by WHO, and seems to have been hard work. Lectures during the day by invited lecturers were followed by demonstrations and discussion, and the broader aspects of the subject were covered by symposia in the evenings.

The intensive nature of the course and the fact that the participants were accommodated together in a Hall of Residence contrived to give participants the impression of going back to university, and it was possible to provide a reasonably balanced picture of the present state of immunology. Particularly valuable was the arrangement for making copies of the slides used by speakers, for distribution to people concerned with the teaching of immunology. Preparing slides on a range of topics is beyond the scope of any one department. The principles behind this symposium could well be applied in other disciplines, in an attempt to come to terms with the problems of further education in adult life.

## Metabolic Roles of Citrate

THE Biochemical Society held a symposium in honour of Sir Hans Krebs, FRS, at Oxford on July 14. Appropriately enough the topic for discussion was the metabolic roles of citrate. The many different disciplines represented illustrated the diverse ramifications that resulted from the original formulation of the tricarboxylic acid cycle some thirty years ago. Not even the imagination needed for such a discovery could have foreseen the widespread biochemical significance of the cycle.

The first two speakers described studies of purified enzymes of citrate metabolism: Dr P. A. Srere (Dallas) discussed whether kinetic studies on isolated enzymes at concentrations of  $10^{-10}$  molar or less gave a true picture of intracellular enzyme behaviour, since the concentrations of some enzymes at their intracellular sites were calculated to be as high as  $10^{-4}$  molar. Dr D. E. Atkinson stressed the importance of intracellular energy conservation for the control of the tricarboxylic acid cycle, and this was also the theme of Dr P. B. Garland's talk. Whereas Dr Atkinson in Los Angeles had studied purified enzymes and then extended the implications to more complex situations, Dr Garland in Bristol had proceeded in the reverse manner—it was gratifying that both approaches led to the same general conclusions. Dr J. Lowenstein (Brandeis) gave an account of the manner in which citrate linked the extramitochondrial synthesis of acetyl-CoA to the cytoplasmic synthesis of fat, a study which emphasized the significance of permeability barriers and membranes in the organization of cell metabolism. The same theme was continued by both Professor P. J. Randle (Bristol) in his description of the manner in which citrate formed in the mitochondria could control glycolysis occurring in the cytoplasm, and by Dr J. B. Chappell (Bristol) in describing the di- and tricarboxylic acid permeases of mitochondrial membranes. Professor M. Klingenberg from Munich gave an account of the adenine nucleotide translocase of the mitochondrial membrane which has extensive implications for the organization of intracellular metabolism.

The occasion marked the retirement of Sir Hans Krebs from the Whitney Chair of Biochemistry at the University of Oxford. At the conclusion of the symposium, Sir Hans modestly thanked the members of the Biochemical Society for the honour they had bestowed on him. It could not have been better deserved.

## Ribosomal 5S RNA

from a Correspondent in Biochemical Genetics

Two years ago Holley and his collaborators determined the base sequence of yeast alanine transfer RNA, the first RNA molecule to be sequenced. Subsequently Zachau and co-workers reported the sequences of two yeast serine transfer RNAs, Madison and co-workers that of yeast tyrosine transfer RNA and Khorana's group that of the yeast phenylalanine transfer RNA. In these cases the major problem lay in the isolation of several hundred milligrams of pure transfer RNA to sequence. The actual sequence determination was facilitated by the presence of unusual bases in these molecules, thereby helping to obtain overlaps of the fragments produced by digestion of the RNA with either pancreatic or T1 ribonuclease.

In this issue of *Nature* (page 735) Brownlee, Sanger and Barrell report the sequence of the *E. coli* 5S ribosomal RNA. This RNA occurs in the 50S ribosomal sub-unit and may be purified much more easily than a transfer RNA molecule. The techniques they used, which they themselves had developed, depend on handling very small amounts (about 100 micrograms) of RNA highly labelled with  $^{32}\text{P}$ . The difficulties in solving this sequence lay in its length of 120 nucleotides and in the absence of any unusual bases. To establish the sequence unambiguously therefore required the fractionation and sequence determination of many partial digestion products. The molecule, when folded up to allow some base pairing, looks quite different from a transfer RNA molecule. Of particular interest is the apparent homology between the two halves of the molecule. The authors suggest that this may be of evolutionary significance, though it is possibly more interesting to speculate that this homology reflects some symmetry in the construction of the 50S ribosomal sub-unit. This work represents a significant advance in the methodology of sequencing large RNA molecules.

## Feline Vision

from a Correspondent in Neurophysiology

THE receptive field of a unit in an animal's visual system has come to be known as that area of retina over which light stimulation causes a change in excitability of the neurone, usually measured as a change in the rate of production of action potentials. Since Hartline's classic work on the frog optic nerve, in which he discovered single fibres carrying responses to the "on" and "off" of a light stimulus, as well as fibres responding to both, a vast amount of information about the response properties of visual units has been amassed. Hubel and Wiesel, in particular (*J. Neurophysiol.*, **28**, 229; 1965), have investigated receptive field organization of units at several levels in the cat visual system, and have apparently demonstrated the existence of a hierarchy in which increasingly complex features of a visual stimulus are abstracted. There is some evidence that such a hierarchical organization also exists in the human visual system, and artificial pattern recognition devices have been designed to perform feature detection on a hierarchical basis.

Rodieck has recently found a new type of receptive field for ganglion cells in the cat's retina (*Science*,

**157**, 90; 1967). In general these have a centre-surround organization, in which the properties of the centre oppose those of the surround: if the centre responds to the on of a stimulus, then the surround responds to its off, and vice-versa. In two cats, however, one decerebrate and the other anaesthetized with nitrous oxide, Rodieck found two ganglion cells which responded to contrast anywhere within their receptive fields. These were between  $1.5^\circ$  and  $2.5^\circ$  in diameter (in terms of retinal angle) and possessed several unusual features. A black or white disk on a grey background, presented to the centre of the receptive field of either unit, caused an inhibition of firing; its removal was followed by reversion to the previous, maintained, firing rate, with no added "off" response. More complex stimuli, such as chessboard patterns, had similar effects: it seemed that contrast, and only contrast, was causing changes in firing rate. Movement of the stimulus at speeds of up to  $10^\circ/\text{sec}$  and rotations of the chessboard pattern had no effect, and it did not matter whether the stimulus was brighter or dimmer than the surround.

This discovery again poses the question of how much peripheral data processing is done in the mammalian visual system. Barlow and Hill have already shown that in the rabbit retina there are units at the ganglion cell level—and possibly as far out as the bipolar cells—which can detect movement of objects in the visual field in particular directions. Maturana, Lettvin, McCullough and Pitts have suggested that the frog's retina extracts four classes of feature from visual input, sending information to the optic tectum about small round objects ("bugs"), moving edges, contrast and "dimming" of stimuli. This peripheral organization of input is in marked contrast to the hierarchical organization described by Hubel and Wiesel for the cat, so the recent findings of Rodieck and also of Stone and Fabian (*Science*, **152**, 1277; 1966) serve to complicate the issue as they are not yet explained by the known details of retinal organization. Dowling and Boycott, working on the Primate retina (*Proc. Roy. Soc., B*, **166**, 80–111; 1966), found quite complicated patterns of synaptic organization which could well underlie similar functions in man and the rhesus monkey. They describe synapses involving, in the inner plexiform layer, bipolar, amacrine and ganglion cell processes. There seems to be ample opportunity for the lateral spread of excitation and inhibition necessary for movement detection, as well as in border, or contrast, enhancement. There is also the possibility of similar synaptic organization in the outer plexiform layer where horizontal cells might also be involved in the lateral spread of information between receptors.

## Nucleoli are Temperature Sensitive

from a Correspondent in Cell Biology

Simard and Bernhard (*J. Cell Biol.*, **34**, 61; 1967) have discovered that exposure of cultured mammalian cells to temperatures above normal selectively and reversibly affects the structure and function of nucleoli. In 1965 Gharpure reasoned that since DNA viruses fail to replicate in cells given a temperature shock (15 min at  $45^\circ\text{C}$ ) before infection, while RNA viruses replicate normally, there is some temperature sensitive step in DNA dependent RNA synthesis. Simard and

Bernhard followed up this suggestion, using electron microscopy combined with cytochemistry and autoradiography to examine the effect of supranormal temperatures on baby hamster kidney and rat embryo cells in culture.

The nucleoli of these cells cultured normally at 37° C have the four usual components, 150–200 Å granules and a loose reticulum of 50–80 Å fibrils, the nucleolonema both containing RNA, a proteinaceous amorphous matrix and associated chromatin with intranucleolar ramifications. Incubation at temperatures between 37° C and 41° C does not affect the cells but at 42° C a critical point is reached and even 15 min exposure causes striking changes in the nucleolus. The granules and intranucleolar chromatin are lost. The nucleolus exclusively consists of closely packed fibrils 60–100 Å wide. Incubation at 43° and 44° C has the same effect but above this the cells begin to die. Apart from these nucleolar lesions the cells are remarkably resistant to the elevated temperatures. The nuclei appear normal and in the cytoplasm the only changes are slight swelling of the endoplasmic reticulum and mitochondria. Moreover, the nucleolar lesions are fully reversible. Cells returned to 37° C, after 1 h at 42° C, grow happily, regaining the lost nucleolar components. In fact there is an excess of granules in the nucleoli of recovering cells.

At 42° C the incorporation of tritiated uridine into nucleolar RNA is reduced by 90 per cent whereas

nuclear uptake is reduced by only 20 per cent. This selective inhibition of nucleolar RNA synthesis strongly suggests that supranormal temperatures do not inhibit RNA polymerase. If this were the case all nuclear RNA synthesis should be equally affected. One possible explanation is that the nucleolar-associated template DNA is particularly heat labile.

When cells were pre-labelled with uridine and then exposed to a temperature of 43° C, the overall pattern of labelling in the nucleus was like that in cells maintained at 37° C. Thus the pre-labelled nucleolar granules cannot simply move into the nucleoplasm in response to the higher temperature. Simard and Bernhard propose that at 42° C and above the granules unfold to form fibrils by a transformation involving only configurational changes in the ribonucleoprotein molecules and when returned to 37° C the reverse occurs. This is in keeping with the precursor product relationship between at least some of the nucleolar fibrils and the granules which has been claimed by several workers. Recently, however, Hay and Gurdon (*J. Cell Sci.*, **2**, 151; 1967), who have studied the fine structure of the abnormal nucleoli in the so-called anucleolate mutant of *Xenopus laevis*, have questioned this relationship.

This point can be resolved by analysing isolated nucleoli, and Simard and Bernhard's temperature shock technique provides an interesting new approach for future studies of the various nucleolar components.

## Internal Structure and Energy Emission of Jupiter

by

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Jupiter emits much more energy than it absorbs. Explanations of the source of this heat depend upon our knowledge of its interior and of the behaviour of condensed matter at very high temperatures and pressures.

JUPITER emits more energy than it receives from the Sun. Gross and Rasool<sup>1</sup> in their theoretical study of the upper atmosphere of that planet concluded that the ratio of the emitted to the absorbed energy is about four, if the albedo is 0.47, while the more recent measurements of Low<sup>2</sup> indicate a ratio of about three. Thus an internal source of energy of nearly  $10^{33}$  ergs/year must exist. A concentration of radioactive elements characteristic of the Solar System would account only for  $5 \times 10^{-6}$  of the required energy. Similarly, the highest temperatures ever suggested for the inside of Jupiter are orders of magnitude too small to sustain exothermic nuclear reactions and so the gravitational field of the planet appears to be the most obvious source of energy<sup>1,2</sup>. The only other suggestion is that made by Dicke (personal communication), who has proposed that the energy release may be a consequence of a gradual decrease of the gravitational constant  $G$  which would lead to an increasing rate of rotation of the inner denser part of the planet with respect to the less dense outside mantle. This effect may result in generation of heat but so far the model has not been evaluated quantitatively.

Without going into the details of the radial density distribution in the planet, it is easily shown that a contraction of about 0.1 cm a year in the radius of the planet, which is  $7 \times 10^9$  cm, would yield the observed flux of energy<sup>2</sup>. In order to approach this problem in a more quantitative manner it appeared necessary to examine in some detail the existing models of Jupiter. What follows is the result of such an inquiry. This leads to a modification of the existing models, and also to an explanation of the observed energy flux; it appears at the same time to have a bearing on the origin of the large magnetic field of Jupiter and on the composition of its atmosphere.

### Models of Jupiter

Recent theories and discussions concerning the interior of Jupiter stem from the original work of De Marcus<sup>3</sup>, who assumed, in a first approximation, the existence of only hydrogen and helium on that planet. Using the best available equations of state, he arrived at a radial distribution of these elements which satisfied the known gravita-



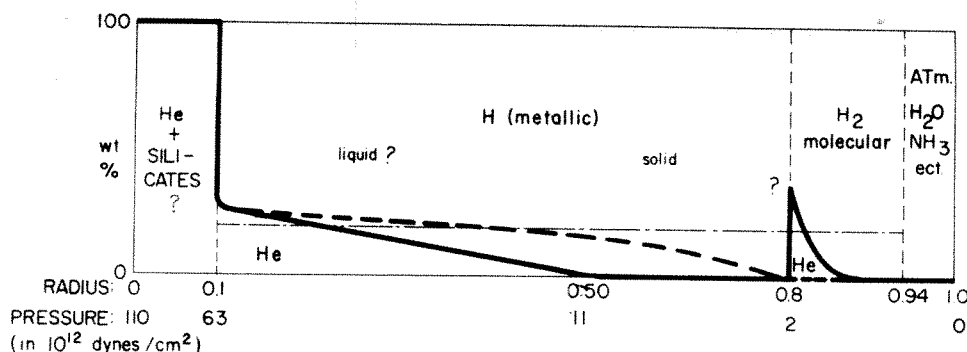


Fig. 1. Schematic radial distribution of elements in Jupiter according to various models. ---, De Marcus; - · -, Peebles; —, modified.

tional multipole components, hydrostatic equilibrium, size and total mass. These results are roughly indicated in Fig. 1, which shows a small helium core surrounded by a thick layer of solid hydrogen in which the helium content decreases to nearly zero at the planet surface. At about 0.8 of the planet radius the solid hydrogen undergoes a transformation from a denser metallic phase to a less dense molecular phase.

Peebles<sup>4</sup> extended the De Marcus model by giving careful consideration to the atmosphere below the visible cloud layer and to the total helium abundance. He assumed that outside a small central core which may contain other elements besides hydrogen and helium the two elements are uniformly mixed—that is, that helium is sufficiently soluble in hydrogen and that convection currents effectively counteract any gravitational segregation. Peebles concluded that the atmosphere is most probably deep and adiabatic and that the mean atomic concentration of helium is 5 per cent, which corresponds to about 20 weight per cent. This model is also represented in Fig. 1. The low value of the critical temperature of hydrogen implies that there is probably a gradual change from a gaseous atmosphere to a liquid layer without there being a well defined surface.

The basic unavoidable weakness of these as well as of other models of planetary interiors has its roots in ignorance of the equation of state of condensed matter at very high pressure and at very high temperature. In particular, the melting points at high pressures are at best obtainable by extrapolation of low pressure experimental data by means of various semi-empirical relations such as, for instance, Simon's equation<sup>5</sup>. This is barely feasible even for the known molecular solid hydrogen; it is very difficult for the metallic form<sup>6</sup>, which has not been observed but the existence of which is almost certain. It is this gap in knowledge of the equations of state which leads to the coexistence of the De Marcus model, in which essentially all hydrogen is solid, and of the Peebles high temperature model, in which everything except perhaps a small inside core is assumed to be to some extent fluid so as to provide uniform mixing.

I now propose to show that a consideration of the hydrogen-helium equilibria leads to a model of Jupiter which is in a sense intermediate between the two described here.

### The Hydrogen-Helium Phase Equilibria

Schematically, the phase diagram of pure hydrogen at high pressures and at high temperatures, omitting the gaseous phase, consists of regions of solid molecular, solid metallic and liquid phases (see Fig. 2). The melting point  $T_M = 14^\circ \text{K}$  at low pressures is well known but the transition pressure  $P_t$  at  $0^\circ \text{K}$  is imperfectly known. Various extrapolations of the semi-empirical equation of state for the molecular solid and of the purely theoretical equation of state for the metallic solid yield values in the range

of  $1-3 \times 10^{12}$  dynes/cm<sup>2</sup> and a change of density of about 50 per cent<sup>4,5</sup>. Alder<sup>7</sup> questioned the validity of the extrapolated equation of state for the molecular form and, by means of entirely different considerations and in analogy to the known similar transition in solid iodine<sup>8,9</sup>, arrived at a transition pressure of  $20 \times 10^{12}$  dynes/cm<sup>2</sup>. Hitherto, all studies of the giant planets have been made using the lower values and I shall also assume a transition pressure of about  $2 \times 10^{12}$  dynes/cm<sup>2</sup>. It should be pointed out, however, that if the higher pressure turns out to be more correct, then drastic changes will have to be made in the variation with radius of both pressure and density for Jupiter and Saturn. Serious consequences for the temperature distribution will also follow because the normal and the radiative heat transfer rates must be expected to be quite different in the two phases. Some of these questions are discussed later on.

The location of the triple point  $S_1$  in Fig. 2 is rather uncertain. Simon's equation<sup>5</sup> can be used to extrapolate experimental points for  $P_t < 3.6 \times 10^9$  dynes/cm<sup>2</sup> or theoretical values for the constants of that equation can be used. It is also possible to calculate the melting temperature directly from the known fact that a solid melts when the root mean square amplitude of the atoms is about 0.2–0.25 of the mean radius of the unit cell<sup>10</sup>. Furthermore, the known Grüneisen constant and an approximate value of the Debye temperature which is obtainable for high pressures by various methods<sup>4,11</sup> can

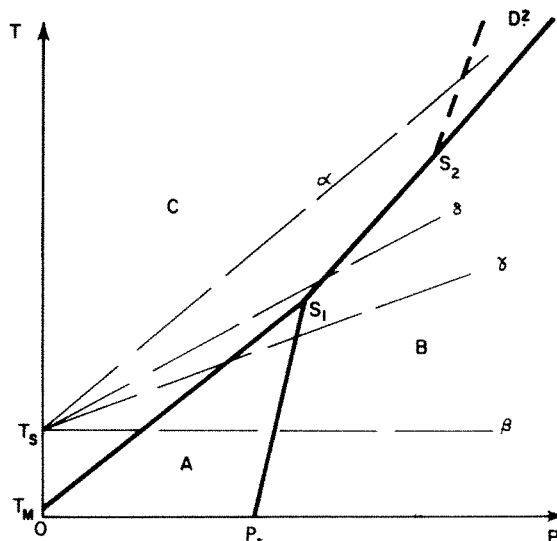


Fig. 2. Schematic  $T$ - $P$  phase diagram of hydrogen: C, liquid molecular; D, liquid metallic; A, solid molecular; B, solid metallic (gaseous phase omitted). Lines  $\alpha$  and  $\beta$  illustrate the Peebles and the De Marcus models, lines  $\gamma$  and  $\delta$  the two modified models.  $P_t$  indicates the polymorphic transition pressure,  $T_M$  the normal melting temperature and  $T_s$  the surface temperature of the planet.

give information about the thermal expansion and vibration frequencies of solid molecular hydrogen near the transition pressure. While none of these methods is very reliable, most of the results lie in the range of 6,000–10,000° K. Because the transition pressure is probably not a steep function of temperature<sup>3</sup>, this temperature can be assumed also to apply to point  $S_1$ .

A much more difficult problem is the question of the existence or absence of a phase change between a molecular and a metallic liquid. So far, no such drastic polymorphic changes have been observed in any liquid. As mentioned here, melting occurs when the amplitude of atomic vibrations reaches a critical value such that the initial density is decreased by more than 30 per cent. This change is comparable with the change of density between metallic and molecular solid hydrogen, so that the chances of the existence of a liquid metal seem remote. On the other hand, a constant density curve starting in the  $P$ – $T$  diagram in the solid metallic phase would probably enter a metallic liquid phase given a sufficiently high  $T$  and  $P$ . Actually, the calculated slope of such constant density curves is not much higher than the slope of the theoretical melting curve of the metallic phase so that the point  $S_2$ , if it exists at all, must be expected to occur at an extremely high temperature.

Next, it is necessary to consider the influence of helium on the phase diagram of hydrogen. It is known that helium is very soluble in liquid molecular hydrogen<sup>12</sup> and this might apply also to the solid form. Theoretical estimates based on the known  $H_2$ – $H_2$ ,  $H_2$ –He, and He–He interactions<sup>13,14</sup> indicate a solubility not less than 50 atomic per cent. One would expect the melting point of this solution to be lower than that of pure molecular hydrogen. The situation with the metallic phase of hydrogen is quite different because noble gases are essentially insoluble in metals<sup>15</sup>. This can also be inferred from the known highly repulsive interaction between H and He (ref. 16). The problem changes considerably when a helium atom is embedded in metallic hydrogen which is being further compressed (J. J. Hopfield, personal communication). Such a compression increases the Fermi energy of the electrons in the metal while at the same time it lowers and narrows the potential barrier between the outside electrons of helium and the metal. Eventually an electron of the helium atom may tunnel into the metal and the positive helium ion will enter the metallic lattice to form an alloy. This is somewhat analogous to the formation of the known stable  $HeH^+$  molecule<sup>17</sup>. An exact solution of the problem is complicated, but an estimate of the required pressure can be made by calculating the density of the metallic hydrogen at which its known Fermi energy  $E_F$  or its crystalline potential  $E_s$  is comparable to the first ionization potential of helium. The value of  $E_s$  can be obtained from its relation to other quantities<sup>18</sup> or from the empirical form  $E_s = 2.5 (E_F)^{3/4}$  (C. Bocciaelli and R. F. Schwartz, personal communication). The result is a minimum pressure in the range of  $7$ – $11 \times 10^{12}$  dynes/cm<sup>2</sup>. It follows that at higher pressures there is a metallic hydrogen–helium alloy which, in accord with the usual behaviour of similar alloy systems, will have a lower melting temperature than pure metallic hydrogen.

### The Modified Model and the Contraction of a Solid Hydrogen Planet

This discussion of the hydrogen–helium system leads to the following conclusions concerning the interior of Jupiter. The transition from molecular to metallic hydrogen occurs at a temperature which lies either below or above  $S_1$  in Fig. 2. In the first case, there is a solid–solid interface. In the second, there is a solid–liquid interface. In either case the molecular hydrogen contains dissolved helium. The metallic layer is essentially pure hydrogen above that depth at which an alloy with helium

can exist. Presumably this helium reached the central regions of the planet at an early stage of the gravitational segregation of the planet when the central pressures were too low to produce metallic hydrogen. The central core is presumably mostly solid helium, but it may contain other heavier elements<sup>3,4</sup>. In the layer of liquid molecular hydrogen there are undoubtedly strong convective currents which lead to mixing and to a rather small temperature gradient. At the interface with the solid metallic form or with the solid molecular form, there has to be the usual boundary layer of essentially motionless liquid which, depending on the value of the assumed non-convective heat transfer rates, can be of the order of 100 m thick.

The melting temperature of a metallic solid solution depends on composition and thus it is not possible to specify the radius at which the He–H alloy becomes liquid. Assuming a required minimum pressure of  $11 \times 10^{12}$  dynes/cm<sup>2</sup>, the maximum radius of this layer is about one-half of the radius of the planet. There will be strong convective currents in this layer.

A schematic comparison of the three models here discussed is indicated in Figs. 1 and 2.

As a first approximation to the problem of the contraction of Jupiter, let us to begin with consider briefly a pure hydrogen planet of a uniform density  $\bar{\rho}$ , radius  $R$  and modulus of compressibility  $K$ . The total free energy of the system, which includes its gravitational energy  $\Omega$ , is lowered if  $\delta E$ , given by

$$\delta E = \delta \Omega - \delta W = -2\pi R \left( \frac{8}{15} \pi G R^3 \bar{\rho}^2 - 3K \Delta R \right) \delta R \quad (1)$$

is positive. Here  $\delta \Omega$  is the change in the total gravitational energy resulting from a decrease  $\delta R$  of the radius and  $\delta W$  is the simultaneous increase in the stored elastic energy produced by a previous strain  $\Delta R/R$ . Thus there is a maximum value  $\Delta R_{\max}$  for which  $\delta E > 0$  and a maximum value of energy which can be released  $(8/45) \pi G^2 R (\bar{\rho}^2) M^2 K^{-1}$ , where  $M$  is the mass of the planet. Because  $\Delta R_{\max}$  is proportional to  $\bar{\rho} M$ , it follows that  $\delta E$  is always positive for  $R$  greater than a certain critical value  $R_c \sim K^{1/2} (\bar{\rho})^{-1/2}$ . For most solids at high pressures  $K \sim \rho^2$  and thus  $R_c$  is a constant characteristic of the solid. For hydrogen, the appropriate values give  $R_c \sim 10^{10}$  cm which is close to Russell's upper limit for cold bodies as obtained by De Marcus from detailed calculations<sup>9</sup>. This assumes, of course, that the pressures are sufficiently low so that the planet can be treated as a solid rather than as a degenerate gas.

Clearly the assumption of a uniform density is not correct for a large body. Fortunately, both for the molecular and for the metallic phase of solid hydrogen, the relationship  $P = A(\rho^2 - \rho_0^2)$  is reasonably well obeyed in the range of the pressures which exist in more than 90 per cent of the volume of the planet. This leads to  $K = 2A\rho^2$  and to the well known Laplace radial distribution  $\rho(r) = \rho_c(L/r) \sin(r/L)$  where  $\rho_c$  is the central density and  $L^2 = A(2\pi G)^{-1}$  with  $L = 3.18$  and  $2.8 \times 10^9$  cm for the molecular and the metallic phase respectively. For  $R < 10^9$  cm, the radial gradient of pressure and of density is negligible for the effects discussed here. The Laplace distribution is not applicable near the surface of the planet, but its use is entirely satisfactory for the purposes of this study which deals primarily with the interior. Expressions for the change in the total gravitational energy  $\delta \Omega$  and for the change in the elastic strain energy  $\delta W$  can be obtained but are quite cumbersome. A good approximate value for  $\delta E$  can be, however, obtained using equation (1) and an average density in a sphere of radius  $R$ :  $\bar{\rho} = 3\rho_R L^2 R^{-2} [1 - RL^{-1} \text{ctg}(RL^{-1})]$  where  $\rho_R$  is density at the surface of the sphere. It is interesting that for a 10 per cent prestrain  $\Delta R/R$  in a hypothetical solid molecular hydrogen planet of the size of Jupiter, nearly 40 per cent of the evolved gravitational energy would be used for compressing the solid and the net rate of energy

emission would be  $\delta E/\delta R = 5 \times 10^{31}$  ergs/cm. The emission rate would be about four times greater for a hypothetical solid metallic planet of the same size.

In reality, however, Jupiter does have a metallic and a molecular phase and thus the gravitational contraction of a two phase model has to be considered<sup>19</sup>. The pertinent equations are

$$\delta\Omega = -\frac{3}{5} \frac{GM_c^2}{R_1^2} \left( R_1 \frac{\delta Q}{\delta R_2} - Q \frac{\delta R_1}{\delta R_2} \right) \delta R_2$$

where

$$Q = 1 - \frac{5}{2\gamma} + \frac{3}{2\gamma^2} + \frac{1}{\gamma^2} \left[ \frac{5(\gamma-1)}{2\beta^2} + \frac{1}{\beta^5} \right]$$

with  $\beta = R_1/R_2$ ,  $\gamma = \bar{\rho}_1/\bar{\rho}_2$ , subscripts 1 and 2 referring to the metallic core and the molecular mantle respectively and  $M_c$  being the mass of the core; further,  $\delta W = \delta W_1 + \delta W_2$  with  $\delta W_1 = 6\pi R_1 K_1 \Delta R_1 \delta R_1$  and  $\delta W_2 = 6\pi K_2 (R_2^3 - R_1^3)^{-1} (R_2^3 \Delta R_2 - R_1^3 \Delta R_1) (R_2^2 \delta R_2 - R_1^2 \delta R_1)$ . The requirement that the pressure should be the same on both sides of the boundary between the two phases leads to  $(\Delta R_1/R_1) [K_1 + \beta^3 K_2 (1 - \beta^3)^{-1}] = (\Delta R_2/R_2) K_2 (1 - \beta^3)^{-1}$ . There is a similar relation for  $R_1$  and  $\delta R_2$ . For  $R_1 = 5.6$  and  $R_2 = 7 \times 10^9$  cm, these equations give  $\delta E/\delta R_2 = 3 \times 10^{33}$  ergs/cm for a 10 per cent prestrain.

If it is now assumed that the radius of the metallic core  $R_1$  increases by  $\delta R_1$  at the expense of the molecular mantle without adjustment of the gravitational shrinkage, then  $\delta R_2 = (1 - \rho_1/\rho_2) R_1^2 R_2^{-2} \delta R_1 = (1 - \gamma) \beta^2 \delta R_1$  and

$$\frac{\delta\Omega}{\delta R_1} = \frac{3}{5} \frac{GM_c^2}{R_1^2} \left( 5Q + R_1 \frac{dQ}{dR_1} \right)$$

For the numerical values appropriate for Jupiter, this gives  $\delta E/\delta R_1 \sim 7.5 \times 10^{33}$  ergs/cm. There is also an additional term  $PdV$  caused by the change of density which is orders of magnitude smaller.

If the enlarged metallic sphere shrinks so as to reach the proper Laplace density distribution, additional gravitational energy will be released and a part will be used for increasing the stored elastic energy. The shrinkage is given by  $-(1-f^{-1})\delta R_1$  where  $f = x^{-2}[1 - 2xtg x + x^2(\sin x)^{-2}]$  with  $x = R_1/L$ . The effect is an increase of  $\delta\Omega$  by about 30 per cent. Thus the net available energy is about  $10^{34}$  ergs/cm with less than 5 per cent of it going into strain assuming, as before, a prestrain of 10 per cent. It should be emphasized that the magnitude of the assumed prestrain is such that its effect on the calculated energy release caused by phase change is less than that due to other approximations. As the phase change progresses, the requirement of hydrostatic equilibrium alters the density and pressure distribution in such a manner that the transition pressure occurs at progressively greater radii.

Thus it appears that a gradual increase of the metallic core at the expense of the molecular mantle of about 1 mm/yr would yield the required amount of energy for the presently observed rate of Jupiter's emission. It remains to explain this rate.

### The Rate of Energy Emission

Because metallic solid hydrogen does not dissolve helium, the phase change discussed here requires that helium dissolved in the molecular phase should diffuse away from the interface towards larger radii. The driving force for this diffusion is, of course, the consequential decrease of the total free energy of the system. The situation is relatively easy to evaluate if the diffusion occurs in solid molecular hydrogen for which the diffusion coefficient and its activation energy are known<sup>20,21</sup>. Unfortunately, there is no experimental information about the corresponding activation volume. In most metals, however, the activation volume is between 0.6 and 0.9 of the atomic volume<sup>22</sup>. For a pressure of  $2 \times 10^{12}$

dynes/cm<sup>2</sup> and a temperature around 3,500° K one obtains  $D = 3 \times 10^{-(10 \pm 1)}$  cm<sup>2</sup> per sec. The corresponding mean diffusion distance of 0.03–0.3 cm/yr is in the right range to account for the observed rate of energy emission. Peebles<sup>4</sup> has also estimated this diffusion coefficient using other approximations.

If at the molecular-metallic interface the molecular phase is liquid the situation is much more difficult to evaluate because experimental and theoretical knowledge of diffusion in liquids is not satisfactory<sup>23</sup>. Although diffusion in liquids near the melting temperature is in general appreciably faster than in solids, it increases more slowly with temperature than in solids because of the much lower activation energy<sup>23,24</sup>. Almost nothing is known about the influence of pressure on this diffusion. Nevertheless, on the basis of extrapolations based on estimated viscosities of liquid hydrogen at high pressures and temperatures, the expected diffusion rate of helium in liquid hydrogen is not in contradiction with the rates calculated here for the solid phase. It should be stressed that the convective currents in the liquid phase have little if any effect on the helium removal process because the critical diffusion occurs over distances several orders of magnitude smaller than the thickness of the stationary boundary layer.

The Elsasser<sup>25</sup> mechanism for generating magnetic fields in planets is based on the principle of a hydromagnetic dynamo, which entails rapid rotation of the planet and strong convective currents in an electrically conducting liquid. Clearly these conditions cannot be satisfied on Jupiter if only the molecular liquid is present because that must be strongly insulating. On the other hand, a liquid metallic hydrogen-helium alloy of the kind discussed here could explain the huge magnetic field, and the proposed size of this liquid layer is just about the same fraction of the total volume of the planet as of the Earth. The central location of the liquid core suggests a centrally located magnetic dipole and contradicts Warwick's earlier interpretation<sup>26</sup> of the decameter radio emission. It is supported, however, by the recent studies of decimeter radio emission<sup>27</sup> and by the studies of decameter radiation, if coexistence of axisymmetric dipole and quadrupole moments is assumed<sup>28</sup>. Such a situation could result from an uneven distribution of the solid phase; this would suitably perturb the roughly centrosymmetric pattern of the convective currents in the liquid. A similar mechanism could account for a local magnetic anomaly suggested by Ellis and McCulloch<sup>29</sup>.

An interesting consequence of the progressive enrichment of helium in the layer of molecular hydrogen is that it implies a higher helium to hydrogen ratio in the atmosphere proper. This enrichment would help to account for the mean molecular weight deduced by Öpik<sup>30</sup> of 4.3, or by Baum and Code<sup>31</sup> of 3.3, without invoking rather complicated mechanisms. It is also suggested by some recent studies of the Jovian atmosphere<sup>32,33</sup>.

Finally, what if the higher rather than the lower transition pressure from the molecular to the metallic form of hydrogen turns out to be more correct? The main consequence would be the presence of a small metallic core made of a presumably helium rich hydrogen-helium alloy. This core would be liquid and would be surrounded by a thick solid molecular hydrogen mantle. It is not certain whether this liquid metallic core would be sufficiently conductive or sufficiently large to account for the huge magnetic field.

### Conclusions

The modified "intermediate" model of the interior of Jupiter described here has certain physico-chemical advantages compared with the earlier models and several interesting consequences as well. Unfortunately, present quantitative uncertainties about the hydrogen-helium

system and the heat conduction and convection in that system make detailed calculations premature. Careful comparison with the known multipole gravitational moments is needed to confirm the presence of higher helium content in the outer layers of the planet. The fair degree of agreement between the calculated and the observed rate of energy emission, although not conclusive, suggests that the proposed mechanism may indeed be operative.

I wish to thank my colleagues Professors Daniels, Deutsch, Dicke, Hopfield, Keil, Peebles and Wigner for many interesting discussions and for advice.

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## Pre-Columbian Maize in Southern Africa

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Maize is indigenous to America and can only be propagated by man. Its presence in Southern Africa in the fourteenth century is therefore particularly intriguing.

MAIZE is an American plant which does not propagate itself, so that its presence is due to human agency. There is, however, evidence for the presence of maize in southern Africa, south of a line from the mouth of the Zambezi in the east to the mouth of the Cunene in the west, which raises several questions about the human agency responsible.

This article comments on observations made in the early sixteenth century which indicate that there was maize in southern Africa when the Portuguese arrived. The same evidence also suggests that people responsible for introducing maize to the coastal regions of eastern Africa were Arabs; that migrating African tribes which had obtained maize from the Arabs introduced it to the interior; and that this introduction of maize occurred before Columbus discovered America. I am not here concerned with the agency initially responsible for bringing maize from America to the Old World.

### European Evidence

The first European to reach Mozambique around the Cape was Vasco da Gama, who recorded *milho* there in March 1498; in an account of the capture of two boats in the Mozambique channel, da Gama wrote: "In the one we took, we found seventeen men, besides gold, silver and an abundance of maize (*milho*) and other provisions". This record, however, was not published until 1512.

The word *milho* is of interest because it is the standard Portuguese name for maize and was used for maize in the early records of Portuguese administration on the east African coast. As early as 1506 at Sofala<sup>2</sup> the following entry occurs in the King's Treasury: "... merchandise and items were spent in buying one hundred and seventy-seven bags of maize with fifteen heads of maize to each bag for the maintenance of the people of this fortress". An entry

on September 1, 1511<sup>3</sup>, which includes reference to sorghum and rice, further clarified the meaning of *milho*: "Bertolemeu Perestrello, factor of this fortress of Sofala ... order you Troylos Bramdam ... to give ... to these forty-nine people ... four hundred *alqueires* of unhusked rice and fifty four *alqueires* of maize (*milho*) and nine *alqueires* of kaffir corn (*mexoeira*) ...". On *mexoeira*, Junod<sup>4</sup> noted: "The word ... nowadays designates the small grey kaffir corn in Lourenço Marques". Kaffir corn is a South African name for *Sorghum vulgare*.

The account of an introduction of maize by the administrator of the Dutch East India Company to the Cape will be found in Van Riebeeck's journal. He expected to find maize in Angola and finally obtained it from West Africa. His Guinea slaves understood its cultivation, which was unknown by his Dutch farmers. To describe this cereal Van Riebeeck used variants of the word *milho*, namely *mily* and *milie*. Such terms for maize are found in use only in South Africa.

In January 1658 Van Riebeeck<sup>5</sup> sent a vessel to Angola to search, among other things, for "maize". Van Riebeeck in his journal<sup>6</sup> had written *milie*, not "maize". In July 1658<sup>5</sup> Van Riebeeck encouraged the planting of maize or "Turkish wheat obtained from Guinea" as the "right time for planting maize is now approaching and the slaves know well how to do it". Van Riebeeck's words were "*mily ofte Turcx taruw*". Burt-Davy<sup>7</sup> held that *mielie* "is derived from the Portuguese word *milho*, from the Latin *milium*, the name for millet".

Van Riebeeck, who joined the Dutch East India Co. in 1639, was brought into contact with Portuguese trading to Africa and the East in the course of his employment. His use of the terms *mily* and *milie* reflect the Portuguese usage of *milho* for maize in the seventeenth century.

The term used today by the Portuguese for maize is still *milho*, and it has been in continuous use. Indeed Santa



Rosa de Viterbo<sup>8</sup> had no hesitation in supposing that the use of the word *milhom* in 1289 referred to maize.

Portuguese documents of the sixteenth century record two Bantu names for a maize-meal loaf. In both Bantu languages there is a separate name for a sorghum-meal loaf. Dos Santos was in Sofala in 1586 and wrote<sup>9</sup>: "The bread commonly eaten in Sofala is made of rice and millet (*milho*) mixed and made into cakes which they call *mocates*". The Manganja language is spoken in the Zambezi valley and the Manganja word for maize-meal loaf is *mkate*. Scott noted<sup>10</sup>: "... the real *mkate* is a roll compounded of maize flour mixed with bananas and honey". The Manganja name of a roll made with sorghum is *nsima*.

When the Santo Alberto foundered in 1593 off the Natal Coast, the pilot's record of the disaster<sup>9</sup> noted: "In exchange for a few tacks, they [the Negroes] gave a quantity of milk and cakes of millet (*milho*) which they call *sincóá*". The Nguni tongue spoken in Natal is Zulu and the Zulu word for a maize-meal loaf is *isinkwa*. The Zulu name for a sorghum-loaf is *isigwamba*. The Nguni example is particularly interesting because the Nguni had come down from central Africa without European contacts. It is impossible to dismiss these two words for a maize-meal loaf, which were recorded phonetically, as aberrations on the part of otherwise highly factual writers.

### The Arab in the Indian Ocean

The question arises of the agency by which maize came to the east African coast. The names used by the coastal Negroes for maize suggest an answer. Many tribal words for maize among the coastal natives consist of a word compounded of their name for sorghum and the name *Manga*. I<sup>11</sup> have shown that *Manga* is a Bantu corruption of Mecca and hence that *Manga* refers to Arabia. Here are examples of such Bantu maize names.

Livingstone, on Nyahasene island in the Zambezi, wrote in his journal of August 1858<sup>12</sup>: "*Mapira* is the name of the large millet or sorghum and *Mapira manga* of Maize (foreign *mapira*)". Posselt<sup>13</sup>, a Rhodesian Native Commissioner, stated that the Chitonga around Sofala and up the Pungwe and Sabi rivers likewise call sorghum *mapira*, and maize *chipira manga* or the sorghum of *Manga*. The *Dicionário Português-Cinyanja*<sup>14</sup> gives *cimanga* as *milho* among the Nyanja of Mozambique. The Gaza people call maize *kimanga*. Doke<sup>15</sup> showed that the Talakundra, Lilima, Kalanga and Numbzya of Rhodesia call maize, *tsimanga*. These *manga* maize names indicate that the Arabs introduced maize<sup>16</sup>. It should also be borne in mind that "1502-1509 Portugal wrests control of Indian Ocean and East African coast from the Arabs"<sup>16</sup>.

These *manga* names are of little help in determining a date for the entrance of maize into Southern Africa, but the names used by Nguni migrants, by tribes in contact with Nguni migrants and by baVenda migrants offer datable information.

### Nguni Migrants and Tribes in Contact with Them

Historical tracing of Bantu word meaning is difficult. Three assumptions, however, may be made. First, where there are two groups of the same stock separated from each other in space and time and each has a similar name for a cereal, it is reasonable to assume that the cereal arrived before the separation took place and that the migrating group departed with both the grain and their common name for it. Secondly, where indigenous people through whom migrants pass give their nickname for the migrants to a cereal, it is feasible to presume that the indigenous group acquired this cereal from the migrating group. Thirdly, where a group which has recently arrived in an area has a cereal name which is not used by any of the group's present neighbours it is probable that their neighbours had the cereal before they arrived.

In comparatively recent times the Nguni migrated into southern Africa. The Zulu, a Nguni group, probably left Nyasaland some 500 years ago, according to Bryant<sup>17</sup> (and by "Nyasaland we mean the Uganda-Kenya region"). Bryant's date places this migration at about 1450 A.D. My own researches put the date at about 1400 A.D.

The Nguni name for maize is *umbimla*. Bryant<sup>17</sup> stated that the word is "obviously of the same derivation as the common East African *mapira* (an *r* elsewhere always becomes an *l* in Zulu and a *p* frequently a *b*) a name applied there, sometimes to maize (for example, in Senga, on the Zambezi), in Tanganyika colony and other tongues..." The conclusion is that the Nguni had maize and their maize name before they migrated. Here one is dealing with a time that is pre-Columbian.

As the Nguni traversed Bantu tribes which were already settled south of them the Nguni would be associated with the arrival of maize among these tribes, as a study of maize names among tribes penetrated by the Nguni suggests.

South of the Zambezi-Cunene line live the Sotho and Sigwanba, who had long preceded the Nguni. These tribes have been in continuous first hand contact with the Nguni through the Transvaal Ndebele and the baKoni, who<sup>17</sup> were an Nguni splinter that became "Sutuized". Among these Sotho and Sigwamba, maize names<sup>18</sup>, are respectively *ma-vele* and *se-fela*. The Mpoche, a branch of the Transvaal Ndebele, call maize *se-peala*. Such names are phonetic dialectical variations of *um-bila*. As maize would be a new crop to the Bantu into whom the Nguni erupted, it is clear that the Bantu, on taking over maize from the Nguni, adapted the Nguni maize name.

### Tribes in Contact with the Nguni

Among other tribes in southern Africa maize names are compounded of *b-n*, *p-n* and *po* stems which can be equated with the Nguni. Thus the Nguni who settled in Rhodesia under Mzilikazi about 1823 are called<sup>19</sup> *ma-Pune* by the Chishona. According to van Warmelo<sup>20</sup>, the Zulu are called *ma-Pone* by the Basuto and *ma-Bunya* by the baVenda. Earlier one finds the name Kabona given<sup>21</sup> to the Nguni by the Hottentots.

I have shown that African tribes are referred to by their salutation term<sup>22</sup> and this is how this basic *b-n*, *p-n* name came to be conferred on the Nguni. The Nguni salutation term is *sa-Kubona*, that is, the Kabonas or Boona or even Poona people, for *b* and *p* are phonetically interchangeable. The Nguni are known to many of the Bantu tribes through whom they migrated north of southern Africa by a name containing the stems *b-n*, *p-n* and *po*, and among these tribes one finds maize names with these *b-n*, *p-n* and *po* stems.

A reasonable explanation for such names is that these tribes obtained maize from the people whom they called by names containing the *b-n*, *p-n* and *po* stems, in which case maize reached these inland Bantu from the north long before da Gama had reached Mozambique in 1498 and before Columbus was born.

In the area between the Sabi and Pungwe rivers in Rhodesia, an area traversed by the migrating Nguni, Johnston<sup>18</sup> gives *ma-bonere* as the maize names among the Cindau, Citeve and Citomboyi. Also<sup>18</sup>, among the Bechuana-land tribes one finds the Sikolo with *m-bonyi* for maize and the ba-Mangwato with two names for maize, *m-bonyi* and *mo-vopu*. Among the ma-Rutze the maize name is *m-boni*<sup>23</sup>. The baSutho, who have long been in contact with the Nguni, call maize<sup>7</sup> *poone* while the Xosa name for maize is *boona*.

The ba-Sotho tribes<sup>24</sup> were in their present habitat before 1300 A.D. Among the baKwena, a Sotho tribe, are settled the baPo. These consist of Nguni groups who<sup>24</sup> migrated thither from Zululand around 1600 A.D. The baKwena word for maize is *m-mopo*, a name derivable from *maPo*, the Sotho name for these Nguni. It is difficult

to explain this *m-mopo* maize name in any other way than in the light of the introduction of maize to the baKwena by these Nguni. The be-Chuana call maize *m-mopo*<sup>25</sup>.

### The baVenda Migrants

The baVenda, like the Nguni, have come from the north. Gottschling<sup>26</sup> concluded that the baVenda "originally came from the great lake regions of eastern central Africa". With this origin for the baVenda their maize name is revealing. Though the present baVenda in the Transvaal are surrounded by people who call maize *um-mbila* and though the baVenda name for the Nguni is maBunya, yet the baVenda maize name is<sup>18</sup> *cikoli*. But maize names around the great lake regions also include the *k-l* or *k-r* stem. Thus it appears that the baVenda when they migrated had, like the Nguni, maize and their own name for it. Because no tribe in southern Africa has to my knowledge a name connected either with the baVenda or with *k-l* or *k-r* stems, I conclude that southern African Bantu encompassing the baVenda had acquired maize and their maize names before the baVenda had arrived. Consequently the baVenda arrived in southern Africa some time after the Nguni.

Von Sicard<sup>27</sup> reckoned that the baVenda reached the Zambezi in the fourteenth century; I think this date is a little too early. Nevertheless the date is clearly pre-Columbian.

From the above evidence it is clear that maize had reached southern Africa before Columbus had discovered the New World.

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## Intensity of the Earth's Magnetic Field in the Geological Past

by

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The intensity of the geomagnetic field in Central Europe during the Permo-Carboniferous, Permian and Plio-Pleistocene did not differ appreciably from the present day intensity.

KNOWLEDGE of the intensity of the geomagnetic field during the geological past is necessary to understand the nature of the geomagnetic field itself, as well as to study the magnitude of remanent magnetization of rocks and minerals. The spatial distribution of the intensity of the geomagnetic field during various geological periods on the individual continents would provide further much needed data for verifying the continental drift theory. For such a study accurate data are needed, which can then be averaged out to exclude such effects as the secular variation of the geomagnetic field. The archaeomagnetic method of Thellier<sup>1</sup> has provided data on the intensity of the geomagnetic field in the historical past. Thellier's method of stepwise heating makes it possible to identify the intensity of the geomagnetic field in the geological past, but the demands placed on the magnetic properties of natural materials by such studies are very high. First the natural remanent magnetization  $J_n$  of samples must be of thermo-remanent origin, the samples must be palaeomagnetically stable (both in direction and modulus of  $J_n$ ) and must not undergo undesirable phase transition during the heating process in the laboratory. I found

three natural materials for determining the intensity of the Earth's magnetic field in the geological past.

Natural cassiterites with iron ions bound to the lattice frequently show outstanding palaeomagnetic stability<sup>2</sup>. The stepwise heating method of two very fine grained cassiterite aggregates was used to derive the intensity of the geomagnetic field in the Permo-Carboniferous. This was found to be  $0.55 \pm 0.06$  oersted at the Horní Slavkov locality (lat.,  $50-12^\circ$  N.; long.,  $12-80^\circ$  E.) in Western Bohemia. As the cassiterite was derived from a greisen type of mineralization this value probably corresponds to the mean intensity of the geomagnetic field with the effects of secular variation averaged out. Detailed tests of palaeomagnetic stability and "mineralogical stability" were carried out on these aggregates<sup>3</sup>. Other samples of cassiterite (from Bolivia, Swaziland and the Far East) were not fully stable and underwent partial phase transitions. They were either not monomineralic aggregates or contained undesirable heterogeneous admixtures of other minerals.

Some ignimbrites are also suitable because they are formed by the settling of clouds of acidic ash at a tempera-

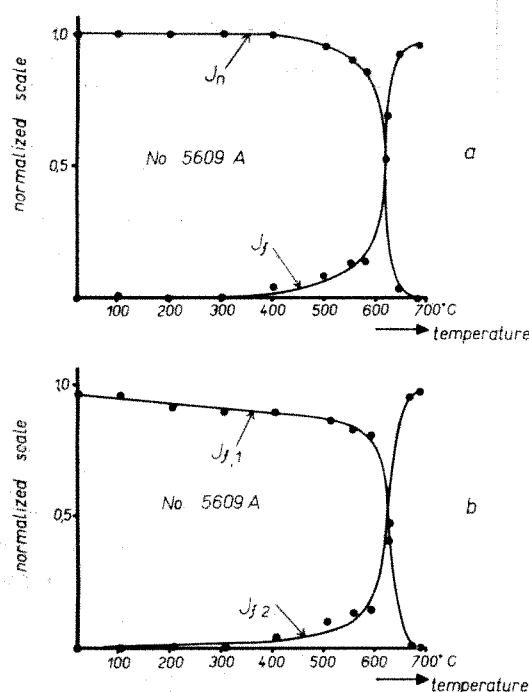


Fig. 1. *a*, Decay of natural remanent magnetization ( $J_n$ ) and acquisition of partial thermo-remanent magnetization ( $J_f$ ) of the ignimbrite sample No. 5609 A, north-east Bohemia, Hynčice, near Broumov. *b*, Decay of thermo-remanent magnetization produced in the laboratory ( $J_{f,1}$ ) and repeated acquisition of partial thermo-remanent magnetization ( $J_{f,2}$ ) of the same sample.

ture above the Curie temperature of the iron oxides contained therein<sup>4</sup>. As the porous ignimbrite cooled down the iron oxides acquired a thermo-remanent magnetization and (provided there was constant access to air) became "mineralogically stabilized". Fig. 1 shows representative graphs of thermal demagnetization ( $J_n$ ) and magnetization ( $J_f$ ) in a laboratory magnetic field of intensity  $F_L$  equal to 0.48 oersted, of an ignimbrite sample from Hynčice, near Broumov, north-east Bohemia. The direction of remanent magnetization of partially demagnetized samples remained identical with the  $J_n$  directions during thermal demagnetization, the declination amounting to  $192^\circ$  and the inclination to  $-3^\circ$ . These values correspond to the Permian palaeomagnetic directions in Central Europe. It thus appears that the  $J_n$  directions of samples examined here do not show any marked evidence of chemical, viscous or other magnetization that might have altered the original magnetization of the ignimbrite. If the thermo-remanent magnetization formed in the samples in a laboratory field  $F_L$  is designated by the symbol  $J_f$ , and the intensity of the geomagnetic field acting at the time of cooling of ignimbrite by  $F_0$ , then  $F_0/F_L = J_n/J_f$ . After laboratory magnetization, the samples were again demagnetized ( $J_{f,1}$ ) and remagnetized in a laboratory field ( $J_{f,2}$ ). The results shown in Fig. 1 suggest that the samples were mineralogically stable; similar data were obtained from seven other samples of ignimbrite from the same locality. The results were used to calculate the intensity of the geomagnetic field during the Lower Permian and it was found that  $F_0$  had the value of  $0.50 \pm 0.03$  oersted for the locality Hynčice, near Broumov (lat.,  $50.63^\circ$  N.; long.,  $16.30^\circ$  E.), in north-east Bohemia. In view of the relatively short period of cooling of the ignimbrite, the value undoubtedly represents the geomagnetic field at one instant and the field has been influenced by such factors as secular variation. Ignimbrite samples from France (Nideck) were investigated, but they either displayed slight phase changes in the course of the laboratory process or their  $J_n$  contained a large proportion of non-thermo-remanent magnetization.

The best archaeomagnetic samples are porcellanites, natural baked earths and palaeoslags. These rocks are formed under natural conditions, frequently in large amounts, through the thermal alteration of loams and loesses by spontaneous combustion of coal seams during the geological past. Spontaneous combustion of coal can occur wherever air enters a coal seam exposed by landslides, erosion or dislocation of overlying strata. In these rocks the  $J_n$  is of thermo-remanent origin and the magnetization of the minerals was stabilized at the time of thermal alteration. The carriers of  $J_n$  of these rocks are composite mixtures of iron oxides which give the rocks their typical red or purple colour. The loam rocks give rise to porcellanites, and the loesses to natural baked brick loam. If the temperature at the time of alteration exceeds  $1,400^\circ$ – $1,500^\circ$  C, palaeoslags are formed; these pass through a liquid stage. Fig. 2 shows an example of a curve of thermal demagnetization ( $J_n$ ) and magnetization ( $J_f$ ) of porcellanite from the locality, Březno, near Postoloprty, north-west Bohemia. In that region, in the so-called Chomutov-Most-Teplice coal basin, the strata overlying seams of soft coal contain dozens of extensive occurrences of porcellanite, natural baked brick loam and palaeoslag which were formed at different periods within the Plio-Pleistocene<sup>5</sup>. Fig. 2 also shows curves of repeated thermal demagnetization ( $J_{f,1}$ ) and magnetization ( $J_{f,2}$ ). The linear relationship follows from plots of  $J_n - J_f$  and  $J_{f,1} - J_{f,2}$ . Fig. 3 shows histograms of  $k$  and mean values of  $k$  for the localities studied,  $k$  being equal to  $J_n/J_f$ . Numerical data are summarized in Table 1. Samples from the locality of Vtelno near Most contained only reverse directions of  $J_n$  both for the porcellanites and for palaeoslags. Samples from Tušimice and Březno, near Postoloprty, contained only normal directions of  $J_n$ . The table shows that the intensity of the geomagnetic field within the Plio-Pleistocene showed variations in intensity, similar to values found archaeomagnetically<sup>6</sup>. These differences are probably caused by secular variation of the geomagnetic field. In all samples (the data for which are shown in Table 1) the relationship of  $J_n$  to  $J_f$  is linear. In typical representatives, the linearity of  $J_{f,1}$  to  $J_{f,2}$  was also confirmed, the  $J_{f,1} - J_{f,2}$  lines subtending  $45^\circ$  angles with the abscissae and the ordinates; this suggested that repeated laboratory procedure did not bring about any phase transitions in the samples<sup>7</sup>.

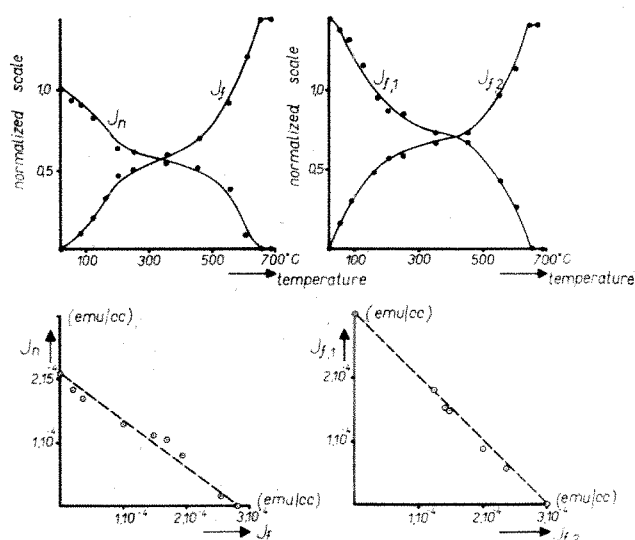
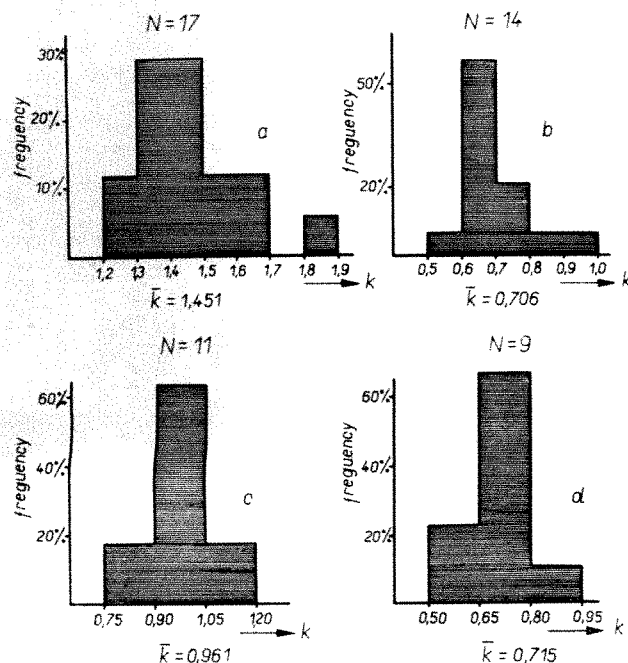


Fig. 2. Decay of remanent magnetization and acquisition of partial thermo-remanent magnetization of the porcellanite sample, No. 5878 A, north-west Bohemia, Březno, near Postoloprty. The symbols  $J_n$ ,  $J_f$ ,  $J_{f,1}$ , and  $J_{f,2}$  the same as in Fig. 1.

Table 1. INTENSITY OF THE GEOMAGNETIC FIELD IN THE PLIO-PLEISTOCENE DERIVED FROM THERMO-REMANENCE OF PORCELLANITES AND PALAEOSLAGS COLLECTED IN THE CHOMUTOV-MOST-TEPLICE COAL BASIN, NORTH-WEST BOHEMIA (MEAN GEOGRAPHICAL CO-ORDINATES: LAT., 50°38' N.; LONG., 13°37' E.)

Locality	$\bar{k}$ (mean ratio of $F_0/F_L$ )	S.D.	No. of samples analysed	Intensity of the geomagnetic field (in oersteds)	Mean direction of $J_n$	
					Declination	Inclination
Tušímece	1.451	$\pm 0.173$	12 porcellanites and 5 palaeoslags	$0.699 \pm 0.083$	$339.5^\circ$	$76.2^\circ$
Březno, near Postoloprty	0.706	$\pm 0.094$	9 porcellanites and 5 palaeoslags	$0.340 \pm 0.045$	$352.6^\circ$	$55.4^\circ$
Vteln, near Most	0.961	$\pm 0.103$	11 porcellanites	$0.463 \pm 0.050$	$209.1^\circ$	$-60.1^\circ$
	0.715	$\pm 0.119$	9 palaeoslags	$0.345 \pm 0.057$		

Fig. 3. Frequency histograms of  $k$  ( $\bar{k}$  the mean value).  $N$ , Number of samples analysed from the localities in north-west Bohemia: a, Tušímece; b, Březno, near Postoloprty; c, Vteln, near Most (porcellanites); d, Vteln, near Most (palaeoslags).

The intensity of the geomagnetic field determined for the Permo-Carboniferous and for the Lower Permian for Central Europe on the basis of an analysis of the thermo-remanence of cassiterite and ignimbrite is similar and

does not appreciably differ from the present day field intensity, which is 0.482 oersted. These data can be compared with some previous calculations<sup>8</sup>. Similarly, the intensity of the geomagnetic field during the Plio-Pleistocene, determined on the basis of an analysis of thermo-remanence of fifty-one samples of porcellanites and palaeoslags, does not differ much from present day intensity which is in agreement with the results reported from New Mexico, Arizona<sup>9</sup> and from Armenia<sup>10</sup>. At the same time, some cassiterites and ignimbrites can be used for such tests. Cassiterites with the necessary properties are relatively rare, but ignimbrites are common in New Zealand, in Western USA, Mexico, Peru, Italy, Indonesia and elsewhere. Without exception, porcellanites and palaeoslags prove ideal for the determination of intensity of the geomagnetic field during the geological past. These materials frequently occur in various coal basins, but the great problem is to determine the age of the thermal alteration.

I thank Professor A. E. M. Nairn and Dr M. Westphal for samples of ignimbrite, and Dr K. Tuček for samples of cassiterite. I also thank Professor M. G. Rutten, the director of the Swaziland Geological Survey and Mines Department, and Dr D. M. Pechersky.

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## Initiation of Vinyl Polymerization by Manganese Carbonyl and Carbon Tetrachloride

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Kinetic investigations of the reaction of manganese decacarbonyl have shown that two rate determining processes operate; one involving the formation of a reactive intermediate, which may be a pair of  $Mn(CO)_5$  radicals trapped in a solvent cage, the other involving the dissociation of a carbon monoxide molecule. Kinetic data are used to reinterpret the results of Bamford *et al.* on the use of mixtures of manganese carbonyl and carbon tetrachloride to initiate vinyl polymerization.

BAMFORD *et al.* have suggested<sup>1,2</sup> that the free radical polymerization of methyl methacrylate, in the presence of mixtures of manganese decacarbonyl and carbon tetrachloride, is initiated by  $CCl_3$  radicals produced by the reaction of carbon tetrachloride with  $Mn_2(CO)_{10}$ , the latter being formed by a slow, rate-determining dissociation of carbon monoxide from manganese carbonyl. An initiation reaction involving manganese-manganese

bond fission and the formation of  $Mn(CO)_5$  radicals has been specifically excluded. The whole question of the formation of pentaco-ordinate radicals of this kind, either as stable products of reaction or as reactive intermediates during reaction, is still subject to considerable confusion.

The existence of  $Mn(CO)_4L$  ( $L = PPh_3, PEt_3, SbPh_3$ ) (ref. 3) has not been confirmed<sup>4-6</sup>, and the only well authenticated pentaco-ordinate radical appears to be  $Mn(CO)_4$ ,



(AsPh<sub>3</sub>) (ref. 6). Basolo and Wawersik concluded that substitution reactions of manganese carbonyl go by means of the rate determining dissociation of carbon monoxide, and that homolytic fission of the manganese-manganese bond does not occur. Quite detailed kinetic investigations, however, have suggested that substitution, oxidation, and decomposition reactions of manganese decacarbonyl, in inert solvents, do involve homolytic fission of the manganese-manganese bond as the rate determining step, the two Mn(CO)<sub>5</sub> radicals formed being trapped together in a solvent cage<sup>7</sup>.

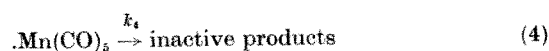
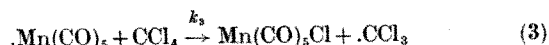
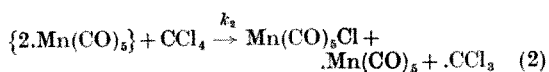
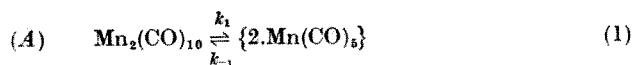
We have now found from further investigations that, for the substitution reactions with triphenylphosphine, the dissociation of carbon monoxide contributes an additional rate determining step (unpublished results). All the reactions are first order in the concentration of manganese carbonyl. The rate of substitution by triphenylphosphine increases with [PPh<sub>3</sub>], but reaches a limiting rate at high concentrations. The rate of decomposition of manganese carbonyl in solvents, saturated with oxygen, is slower than the limiting rate of substitution with triphenylphosphine at the same temperature.

The reaction with iodine produces iodopentacarbonylmanganese in up to 100 per cent yield. The pseudo first order rate constant for this reaction is of the form  $k_{\text{obs}} = k_1 + k_2[\text{I}_2]$ , where  $k_1$  is equal to the rate of decomposition in the presence of oxygen at the same temperature. The second order constant is assumed to be due to direct bimolecular attack on the complex by iodine.

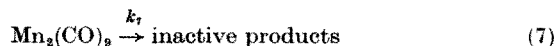
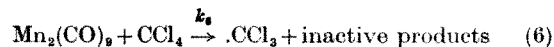
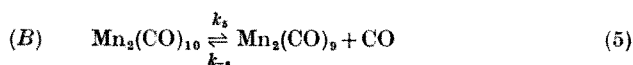
Under one atmosphere of carbon monoxide, the rate constant for the substitution reaction decreases to the same value as for the reaction with oxygen or for the [I<sub>2</sub>]-independent reaction with iodine. One atmosphere of carbon monoxide has no effect on either  $k_1$  or  $k_2$  for the reaction with iodine. This suggests that the reaction with oxygen, and the [I<sub>2</sub>]-independent reaction with iodine, proceed by the same mechanism, namely homolytic fission of the manganese-manganese bond, but that the substitution reaction with triphenylphosphine, in the absence of carbon monoxide, can also proceed by way of a slow dissociation of carbon monoxide from the complex. The ratio of the rate constants for reaction with high concentrations of triphenylphosphine to those for the oxidation reactions is constant at  $1.37 \pm 0.10$  in a temperature range of 80–135°C. This means that activation energies for the manganese-manganese bond fission and for carbon monoxide dissociation are essentially the same (37 kcal/mole<sup>7</sup>). Both mechanisms should lead to the same type of dependence on [PPh<sub>3</sub>].

These results, which will be reported in detail elsewhere, lead us to comment on the mechanism of the initiation of vinyl polymerization by mixtures of manganese carbonyl and carbon tetrachloride. The similarity of the kinetics of the polymerization reactions to those which we have obtained for the reactions of manganese carbonyl suggests that a preferable interpretation should include a substantial contribution from manganese-manganese bond fission.

We propose therefore that the initiation of polymerization could proceed by the reaction schemes A and B.



( $\{2\text{Mn}(\text{CO})_5\}$  represents two Mn(CO)<sub>5</sub> radicals trapped together in a solvent cage<sup>7</sup>.)



If we assume, with Bamford *et al.*<sup>1</sup>, that  $\cdot\text{CCl}_3$  is the only radical that initiates polymerization, and that it is 100 per cent efficient, then the rate,  $\omega$ , of reaction of vinyl monomer,  $M$ , by these two processes is given by

$$\omega = \omega_a + \omega_b = k_p k_t^{-1/2} [M] [\text{Mn}_2(\text{CO})_{10}]^{1/2} (a + b) \quad (i)$$

where  $k_p$  and  $k_t$  are the rate constants for propagation and termination of the chain reaction, respectively, and  $a$  and  $b$  are given by

$$a = \left\{ 1 + \frac{k_3[\text{CCl}_4]}{k_4 + k_3[\text{CCl}_4]} \right\}^{1/2} \left\{ \frac{k_1 k_2 [\text{CCl}_4]}{k_{-1} + k_2 [\text{CCl}_4]} \right\}^{1/2} \quad (ii)$$

$$b = \left\{ \frac{k_5 k_6 [\text{CCl}_4]}{k_{-5} [\text{CO}] + k_7 + k_6 [\text{CCl}_4]} \right\}^{1/2} \quad (iii)$$

This rate expression is in excellent agreement with all the data obtained by Bamford *et al.*<sup>1,2</sup>. Thus the rate of polymerization is first order in concentration of monomer, and half order in concentration of manganese carbonyl. At constant  $[\text{Mn}_2(\text{CO})_{10}]$ , the rate will increase with  $[\text{CCl}_4]$  and reach a limiting rate given by

$$\omega = (1.41 k_1^{1/2} + k_5^{1/2}) k_p k_t^{-1/2} [M] [\text{Mn}_2(\text{CO})_{10}]^{1/2} \quad (iv)$$

when  $k_3[\text{CCl}_4] \gg k_4$ ,  $k_2[\text{CCl}_4] \gg k_{-1}$ , and  $k_6[\text{CCl}_4] \gg k_7 + k_{-5}[\text{CO}]$ . At high values of  $[\text{CCl}_4]$  and constant  $[\text{Mn}_2(\text{CO})_{10}]$  the rate will decrease with increasing  $[\text{CO}]$ , and will reach a limiting rate given by

$$\omega = k_p k_t^{-1/2} [M] [\text{Mn}(\text{CO})_{10}]^{1/2} a \quad (v)$$

where  $a$  is equal to  $1.41 k_1^{1/2}$ .

This interpretation is in at least as good agreement with Bamford and Finch's data<sup>3</sup> as is theirs, which predicts a continuously decreasing rate with increasing  $[\text{CO}]$ . The fact that the variation of rate with  $[\text{CCl}_4]$  is of exactly the same form, whether carbon monoxide is present or not, is readily explained, because both  $a$  and  $b$  rise to limiting rates at high  $[\text{CCl}_4]$ , and the data are not sufficiently accurate at low  $[\text{CCl}_4]$  to distinguish between the more complicated behaviour expected in this region from the expressions for  $a$  and  $b$ , and that predicted by Bamford and Denyer<sup>2</sup>.

A quantitative comparison can also be made. Our values for  $k_1$  and  $k_5$  at 80°C in *n*-octane or decalin are  $1.0 \times 10^{-6} \text{ sec}^{-1}$  and  $0.33 \times 10^{-6} \text{ sec}^{-1}$  respectively. Solvent effects appear to be small, both for the substitution and oxidation reactions of manganese carbonyl<sup>8</sup> and for the polymerizations<sup>1</sup>. We calculate therefore that, at  $[\text{Mn}_2(\text{CO})_{10}] = 1.5 \times 10^{-3} \text{ mole l}^{-1}$ , and at high concentrations of carbon tetrachloride, the rate of polymerization is given by  $\omega = k_p k_t^{-1/2} (1.5 \times 10^{-3})^{1/2} (1.41 \times 10^{-3} + 0.57 \times 10^{-3}) [M]$ . For  $k_p k_t^{-1/2} = 0.14 \text{ mole}^{-1/2} \text{ l}^{1/2} \text{ sec}^{-1/2}$ , the gradient of a graph of rate of polymerization against  $[M]$  should be  $1.07 \times 10^{-5} \text{ mole l}^{-1} \text{ sec}^{-1}$ , as compared with the value of  $1.1 \times 10^{-5} \text{ mole l}^{-1} \text{ sec}^{-1}$  obtained by Bamford and Denyer<sup>2</sup>. The excellence of this agreement may well be fortuitous, however, because it is by no means certain that the "inactive products" in reactions (6) and (7) are indeed inactive.  $\text{Mn}_2(\text{CO})_9$  might well react, either with or without carbon tetrachloride, to give  $\text{Mn}(\text{CO})_5$  radicals which we believe to be, very probably, reactive species. Thus  $\text{Mn}(\text{CO})_5$  radicals are considered to react

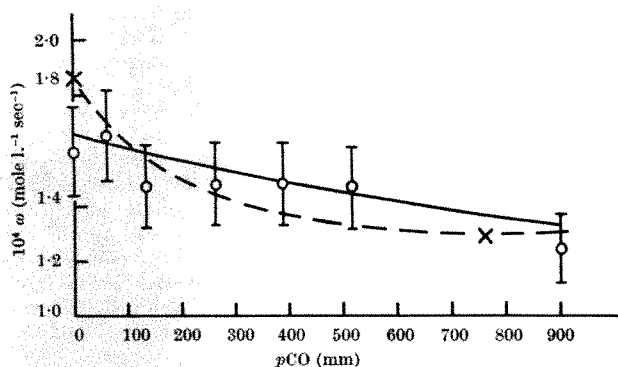


Fig. 1. Variation of rate of polymerization of methyl methacrylate at 80° C with  $p(\text{CO})$ .  $[\text{Mn}_2(\text{CO})_{10}] = 2.37 \times 10^{-3}$  moles  $\text{l}^{-1}$ ,  $[\text{CCl}_4] = 0.191$  moles  $\text{l}^{-1}$ . Values given in ref. 1, Fig. 3;  $\times$ , values calculated according to reaction schemes A and B and our values of  $k_1$  and  $k_3$ . —, Curve calculated according to Bamford and Denyer's first mechanism<sup>1</sup>; ----, curve expected from our mechanism (exact degree of curvature is unknown).

very readily with bromine to form  $\text{Mn}(\text{CO})_5\text{Br}$  and  $\cdot\text{Br}$  in the chain reaction of bromine with manganese carbonyl<sup>8</sup>, and a similar reaction with carbon tetrachloride to form  $\text{Mn}(\text{CO})_5\text{Cl}$  and  $\cdot\text{CCl}_3$  seems highly likely. If either or both of reactions (6) or (7) produce an extra  $\text{CCl}_3$  radical in this way, then the calculated gradient would become  $1.20 \times 10^{-5}$  and  $1.31 \times 10^{-5}$  mole  $\text{l}^{-1}\text{sec}^{-1}$ , respectively.

For  $k_p k_t^{-1} = 0.22$  mole $^{-1}$   $\text{l}^{1/2}\text{sec}^{-1}$ , as used in reference 1, we calculate the rate in the absence of carbon monoxide, with high concentrations of carbon tetrachloride and with  $[\text{Mn}_2(\text{CO})_{10}] = 2.37 \times 10^{-3}$  mole  $\text{l}^{-1}$ , to be  $1.81 \times 10^{-4}$  mole  $\text{l}^{-1}\text{sec}^{-1}$ . The limiting rate in the presence of high  $[\text{CO}]$  is  $1.28 \times 10^{-4}$  mole  $\text{l}^{-1}\text{sec}^{-1}$ . These values are compared with the experimental data<sup>1</sup> in Fig. 1. Uncertainties of  $\pm 10$  per cent have been assigned to each point, as suggested by the mean deviation for  $k_p k_t^{-1}$ , calculated from the data (Table 1, ref. 1).

Bamford and Denyer<sup>2</sup> also measured the rate of evolution of carbon monoxide from solutions of manganese carbonyl, both in the presence and absence of carbon tetrachloride, and in the absence of oxygen. They assumed that, in the absence of carbon tetrachloride, the products were one mole each of  $\text{Mn}_2(\text{CO})_9$  and  $\text{CO}$  from each mole of  $\text{Mn}_2(\text{CO})_{10}$ . We have found no evidence for any such manganese carbonyl species in solution on examining the infra-red spectra of solutions of manganese carbonyl at 80° C, and have concluded that no inter-

mediate manganese carbonyl species is present in appreciable concentrations during the decomposition, that is, 10, not 1, moles of carbon monoxide are evolved for every mole of manganese carbonyl that reacts.

If only one mole is evolved, then the rate constant for decomposition becomes  $4.5 \times 10^{-6}$   $\text{sec}^{-1}$  at 80° C, as compared with our value<sup>7,8</sup> of  $1.33 \times 10^{-6}$   $\text{sec}^{-1}$  for the limiting rates of substitution by triphenylphosphine. On the other hand, if 10 moles are evolved the rate constant for decomposition is  $0.45 \times 10^{-6}$   $\text{sec}^{-1}$ . This is less than the rate of decomposition in the presence of oxygen because the reverse of reaction (1) is significant under these conditions<sup>7</sup>. In the presence of carbon tetrachloride, the rate of evolution of carbon monoxide from  $1.50 \times 10^{-3}$  molar manganese carbonyl is  $1.24 \times 10^{-5}$  mole  $\text{l}^{-1}\text{sec}^{-1}$ , but the nature of the products is uncertain.  $\text{Mn}(\text{CO})_5\text{Cl}$  must be formed<sup>9</sup>, and we find that this decomposes slowly at 80° C ( $t_{1/2} \geq 100$  min). Our value for the limiting rate

constant for the reaction of manganese carbonyl with species such as carbon tetrachloride, which attack both  $\text{Mn}_2(\text{CO})_9$  and  $\{2\text{Mn}(\text{CO})_5\}$ , is  $1.33 \times 10^{-6}$   $\text{sec}^{-1}$ . This means that the number of moles of carbon monoxide evolved from each mole of manganese carbonyl that reacts is  $1.24 \times 10^{-5} \div (1.33 \times 10^{-6} \times 1.50 \times 10^{-3}) = 6.3$ . This is quite consistent with the partial decomposition of relatively stable carbonyl species such as  $\text{Mn}_2(\text{CO})_8\text{Cl}_2$  (ref. 10) and  $\text{Mn}(\text{CO})_5\text{Cl}$  which are formed in the presence of carbon tetrachloride.

Finally it should be pointed out that, although the data for all these reactions of manganese carbonyl are fully consistent with a rate determining formation of a  $\{2\text{Mn}(\text{CO})_5\}$  radical pair, alternative formulations of this reactive intermediate are possible so that the activation energy of 37 kcal/mole may be only a lower limit for the manganese-manganese bond fission.

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## Polymorphism of Lipids

by

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Some divalent cation soaps are known to exhibit a body-centred cubic phase at high temperatures. X-ray crystallography now shows that the polar groups (cations and carbonyl groups) are arranged so as to form interwoven three-dimensional networks embedded in a continuous matrix formed by disordered paraffin chains.

ONE of the characteristics of the class of substances embodying soaps, detergents, phospholipids, sphingolipids—namely the lipids—is that it displays a variety of phases with a long range organization strictly periodic in one, two or three dimensions and yet with a highly disordered short range conformation. Such phases are

usually encountered with lipids and lipid-water systems when temperature and concentration are varied (for a review of the subject see refs. 1 and 2). A large number of phases of this type have been identified by X-ray scattering techniques and the structures of many of them have been determined<sup>1</sup>.

We describe here the body-centred cubic phase, first detected by one of us<sup>3</sup> in the strontium saturated soaps at about 230° C, in barium stearate above 220° C, and in calcium *p*-ethyl- $\omega$ -undecanoate at room temperature. The structure was solved by the application of crystallographic techniques more conventional than those used in previous studies of lipids. The structure displays some novel features: the polar groups are present on rods of finite length which belong to two interwoven infinite three-dimensional networks, and the hydrocarbon chains constitute a continuous paraffin matrix.

The experimental characterization of the body-centred cubic phase has already been described elsewhere<sup>3,4</sup>. Its chief properties can be summarized as: (a) it is optically isotropic; (b) in the "high-angle" region of the X-ray diffraction diagrams (namely for  $s = 2 \sin \theta / \lambda > (10 \text{ \AA})^{-1}$ ) only one diffuse band is observed, near  $s = (4.5 \text{ \AA})^{-1}$ , thus indicating that the conformation of the paraffin chains is highly disordered<sup>1,5</sup>; (c) a fairly large number of sharp reflexions are observed in the "small-angle" region (see Fig. 1 and Table 1), the position and intensity of which are almost independent of temperature; (d) the X-ray diffraction diagrams of the different soaps are remarkably similar—the ratios of the spacings as well as the relative intensity of the reflexions are identical (see Fig. 1).

Experimentally observed spacings and intensities, as obtained on Debye-Scherrer diagrams, are given in Table 1. These data, based on a recent careful inspection of the X-ray diagrams, involve a few minor corrections of the data presented earlier<sup>3,4</sup>. The lattice dimensions observed with various soaps are given in Table 2.

As has already been shown<sup>3,4</sup>, all the reflexions can be indexed on a body-centred cubic lattice (see Table 1). The cubic symmetry is confirmed by the optical properties; the lattice type appears to be defined unambiguously because neither a primitive nor a face-centred cubic lattice is compatible with the spacings of the reflexions.

Further developments in the structural analysis were guided by the following considerations: (a) only the

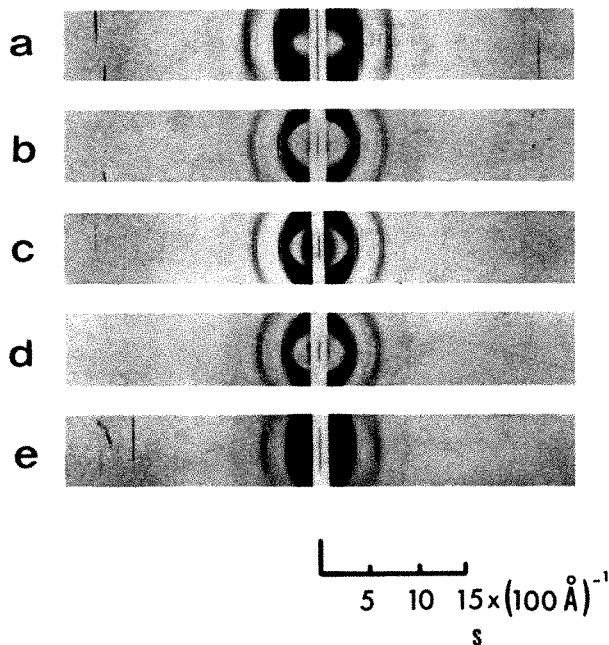


Fig. 1. X-ray diffraction diagrams of some strontium soaps, at 235° C. The camera is of the Guinier type, operating *in vacuo*, in symmetric position, with a sample-film distance of 125 mm. The radiation is the copper  $K\alpha$  line,  $\lambda = 1.54 \text{ \AA}$ . The sharp spots in the outer region are due to the mica windows of the sample holder. Note the similarity of the X-ray diagrams: the ratios of both the spacings and the intensities of the reflexions are the same for all the soaps. a, Myristate; b, palmitate; c, stearate; d, arachidate; e, behenate.

positions of the strontium ions are taken into account—the electron density is assumed to be uniform over the rest of the cell; (b) most of the absent reflexions can be explained by the space group symmetry—indeed the number of reciprocal lattice points with zero intensity is too large (see Table 1) to be ascribed to the form factor of the asymmetric unit; (c) it is probable that the structures of all soaps are related because they show similar X-ray diffraction patterns; (d) the locus of the strontium ions can be presumed to coincide with some symmetry element (that is, the strontium ions occupy special positions)—the shape of the structure elements is, in fact, likely to be defined by the symmetry of the cell, because the paraffinic regions, which constitute the bulk of the structure, lack internal rigidity<sup>1,6</sup>.

The most probable space group with absences to account for most of the observed absences without con-

Table 1. X-RAY DIFFRACTION DATA OF STRONTIUM MYRISTATE AT 223° C

<i>h k l</i>	$s_{\text{obs}}$ (10 <sup>3</sup> Å) <sup>-1</sup>	$I_{\text{obs}}$	$s_{\text{cal}}$ (10 <sup>3</sup> Å) <sup>-1</sup>	$I_{\text{cal}}$	<i>F</i>
0 0 0					+ 460
1 1 0			22.4		abs
2 0 0			31.7		abs
2 1 1	39.0	vvs	38.9	371,000	+ 184.6
2 2 0	45.0	s	44.9	32,900	- 88.8
3 1 0			50.1		abs
2 2 2			54.9		abs
3 2 1	59.3	vw	59.3	1,210	- 12.6
4 0 0	63.4	m	63.4	8,090	- 105.1
4 1 1			67.3		abs
3 3 0					abs
4 2 0	71.1	s	70.9	39,500	- 151.2
3 3 2	74.4	s	74.4	38,200	- 170.0
4 2 2	77.4	m	77.6	8,100	88.8
5 1 0					abs
4 3 1	80.8	w	80.9	1,797	- 33.9
5 2 1			86.9		+ 6.5
4 4 0			89.7	54	+ 17.3
5 3 0			92.5		abs
4 3 3					abs
6 0 0			95.2		abs
4 4 2					abs
6 1 1	97.7	w	97.8	3,450	- 116.8
5 3 2					+ 62.9
6 2 0			100.3	1	- 1.1
5 4 1	102.7	vw	102.8	1,205	- 79.6
6 2 2			105.2		abs
6 3 1	107.4	vw	107.5	1,156	- 101.1
4 4 4	109.4	vvw	109.9	556	- 197.5
7 1 0					abs
5 5 0	112.0	vw	112.1	864	abs
5 4 3					+ 113.9
6 4 0			114.1	25	+ 30.6
7 2 1					+ 2.1
6 3 3			116.5	4	+ 10.0
5 5 2					+ 10.8

$s_{\text{obs}}$  and  $s_{\text{cal}}$  are the reciprocal spacings ( $s = 2 \sin \theta / \lambda$ ) of the observed reflexions, and calculated for the body-centred cubic lattice with  $a = 63 \text{ \AA}$ .  $I_{\text{obs}}$  are the observed intensities, which were visually estimated (vvs, extremely strong; vs, very strong; s, strong; m, medium; w, weak; vw, very weak; vvw, extremely weak; see Fig. 1).  $F$  is the structure factor, calculated with the equation:

$$F_{h,k,l} = K \int_0^{0.1075} A_{h,k,l} \zeta(x, 1/4-x, 1/8) dx$$

where  $A_{h,k,l}$  is given in the *Intern. Tables* (ref. 7, pp. 524-525), and  $K$  is a normalization factor such that  $F_{0,0,0}$  is equal to the total length of the rods contained in one unit cell ( $F_{0,0,0} = 24 \times \sqrt{2} \times 2 \times 0.1075 \times a = 460 \text{ \AA}$ ). The reflexions forbidden by symmetry are indicated by *abs*.  $I_{\text{cal}}$  is the sum of  $(F_{h,k,l})^2$  for all the reciprocal lattice points with the same spacing, multiplied by the convergence factor  $\exp(-524 s^2)$ .

Table 2. SOME DATA RELEVANT TO THE STRONTIUM SOAPS AT 235° C

Soap	<i>a</i> (Å)	$\delta_{235^\circ \text{C}}$ (g cm <sup>-3</sup> )	<i>N</i>	<i>N</i> /24	<i>n</i> (Å <sup>-1</sup> )
Laurate	58.4	0.87 <sub>3</sub>	215	8.9 <sub>6</sub>	0.51 <sub>1</sub>
Myristate	62.6	0.85 <sub>4</sub>	233	9.7 <sub>1</sub>	0.51 <sub>5</sub>
Palmitate	66.2	0.84 <sub>9</sub>	245	10.3 <sub>4</sub>	0.50 <sub>5</sub>
Stearate	69.7	0.83 <sub>8</sub>	261	10.8 <sub>8</sub>	0.50 <sub>7</sub>
Arachidate	73.3	0.82 <sub>2</sub>	278	11.5 <sub>8</sub>	0.50 <sub>4</sub>
Behenate	76.0	0.82 <sub>6</sub>	285	11.8 <sub>8</sub>	0.50 <sub>4</sub>

*a* is the parameter of the body-centred cubic cell,  $\delta$  is the density of the soap, *N* is the number of soap molecules per cell, *N*/24 is the number of molecules per rod, *n* is the number of strontium ions per unit length of the rod.

The densities were first determined for the hexagonal phase, at 265° C, as discussed in the appendix of ref. 4, then corrected by the thermal factor  $\delta_{235} = \delta_{265}(1 + 0.000748 \times \Delta t) = \delta_{265} \times 1.022$ , taken from the data of the hexagonal phase of calcium stearate<sup>8</sup>. *n* is determined with the equation

$$n = (N/24) (0.250 \times \sqrt{2} \times a - 2 \times 1.56)^{-1}$$

assuming that the length of the segment devoid of strontium ions, near the ends of the rods, is 1.56 Å for all the soaps.

flicting with any of the observed reflexions (see Table 1) is *Ia3d* (No. 230 of the *International Tables*<sup>7</sup>). This space group possesses various sets of special positions. Those nominated *a*, *b*, *c*, *d* and *e* in the *International Tables* (ref. 7, pp. 345–346) are discarded, because they involve additional absences at variance with the experimental data. The position *f* (*x*, 0, 1/4, etc.) is also discarded because it involves  $F_{431} \equiv F_{543} \equiv 0$ , in contrast with the observed intensity of those reflexions (see Table 1).

Only one set of special positions is thus possible, nominated *g* in the *International Tables* (1/8, *x*, 1/4-*x*, etc.), which lies along two-fold axes. The structural factors of the various reflexions were calculated (see ref. 7, pp. 524–525) and the results were plotted as a function of *x*. It was noticed then that the agreement with the observed amplitudes is excellent if the structural factors are integrated over the interval  $0 < x < 0.1075$ , or, in other words, if the strontium ions are uniformly distributed over segments of the two-fold axis. The integrated structural factors and the intensity of the reflexions are given in Table 1: the calculations were carried out on a computer ('Univac 1107').

The convergence factor ( $\exp(-524s^2)$ ) (see Table 1) takes account of the actual electron density distribution in the cross-section of the rod, as well as of the disorder, due in particular to the fluctuations in the length of the rods (see below) and the thermal movements.

One projection of the unit cell, with some of the symmetry elements, is shown in Fig. 2*a* (the representation of all the symmetry elements would be confusing for such a high symmetry). The strontium ions are situated along rods of finite length, which lie along two-fold axes. Each end of a rod is grouped with two other rods, from which it is separated by a short gap (see below); the three rods of each group are coplanar, and are related by a three-fold axis perpendicular to the plane. The length of each rod is  $a \times 2 \times \sqrt{2} \times 0.1075$  (*a* is the cell parameter, see Table 2); twenty-four rods are contained in one unit cell. Neglecting the short gap, the rods define two infinite three-dimensional networks, mutually interwoven, and

otherwise unconnected. The structure is depicted in Fig. 2*b*.

The distribution of the strontium ions is uniform along the rods; the length of the gap from the end of the rod to the three-fold axis is (in myristate)  $a \times \sqrt{2} \times 0.0175 = 1.56 \text{ \AA}$ . The number of molecules per rod ( $N/24$ ) and the linear density (*n*) of the strontium ions along the rods are given in Table 2 and the values are based on the assumption that the length of the segments devoid of cations is the same for all soaps (the differences would be very small if the length were proportional to the lattice parameters). The number of cations per rod is not an integer and it may be presumed that it varies from rod to rod and from cell to cell, most likely within very narrow limits, and that  $N/24$  is an average over the whole crystal. The linear density of the cations is the same for all the soaps, at least within the scatter associated with the probable errors of  $\delta$  (see legend of Table 2).

The space between the rods is occupied by the paraffin chains, in a highly disordered conformation; these form a continuous matrix throughout the whole crystal. The distance from any point of the cell to one of the rods never exceeds the fully extended length of the chain; for example, the maximum distance is 11.1 Å in strontium myristate.

The diameter of the high electron density region, around the two-fold axes, is, of course, finite, and is negligible only in the most idealized model; it is clear that, taking into account the resolution of the data and the shape of the convergence function (see Table 1), the strontium ions may well be located at some distance from the two-fold axes, and thus leave room for the intercalation of the carboxyl groups. Furthermore the organization of the strontium ions, and probably of the carboxyl groups as well, appears to be quite ordered. Indeed, it may be noted that at higher temperature, in the phase that follows the cubic, the loci of the cations are rods of infinite length, parallel and organized on a two-dimensional hexagonal lattice<sup>3,4</sup>, the linear density of which is the same for all the strontium soaps ( $n = 0.496 \text{ cations/\AA}$ )

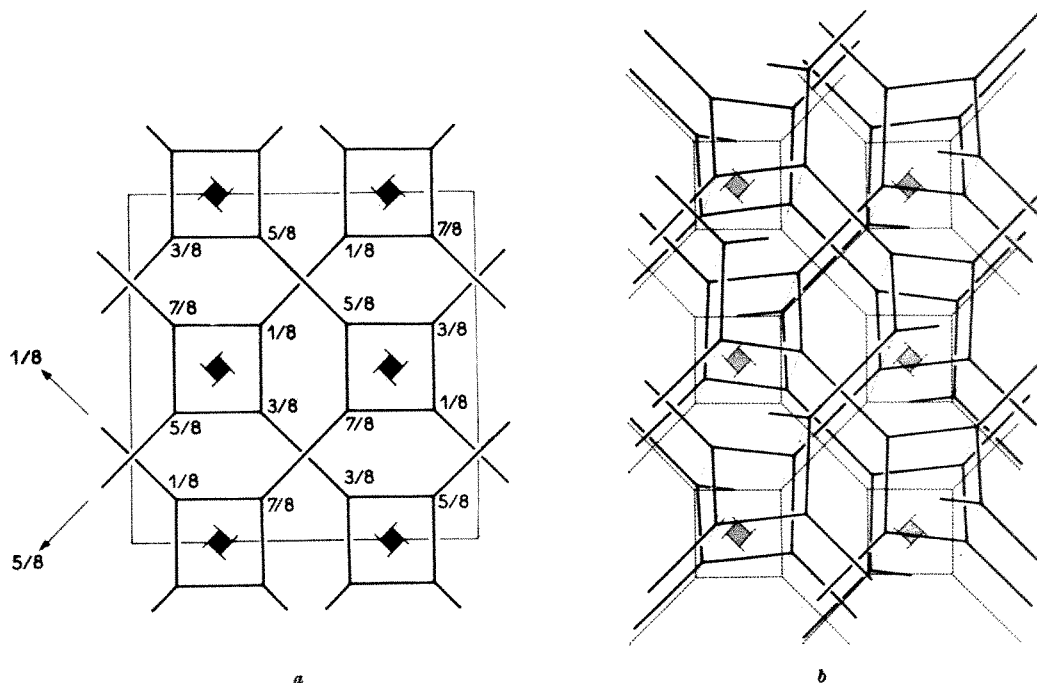


Fig. 2. Representation of the structure. The loci of the strontium ions, represented by heavy lines, are rods of finite length, that lie along two-fold axes. The ends of the rods are joined three-by-three. The gaps at the end of the rods are omitted. (a) Projection on the plane *ab*, with the position of some of the symmetry elements. The thin lines show the limits of one unit cell. The fractional figures are the *z* co-ordinates of the intersections of three rods. (b) Perspective view. The dotted lines are the projection of the heavy lines on the plane *ab*. Note the two three-dimensional networks mutually interwoven, otherwise unconnected.



and is almost identical to that of the cubic phase (Table 2). The organization of the polar regions thus appears to be quite similar in those two phases. This type of hexagonal phase has been observed in other divalent cation soaps, and the organization of the polar rods has been shown to be highly ordered, and this contrasts with the chaotic conformation of the paraffin chains<sup>3,4,8</sup>. A similar conclusion can thus be drawn for the hexagonal and the cubic phases of the strontium soaps.

The finite length of the rods of the cubic phase can be explained by the interplay of the ordered and the disordered regions. Indeed the separation of the paraffin chains along the rods is fixed by the organization of the polar groups. Under these conditions the average orientation of two neighbouring chains may not be quite random, if the flexibility is not sufficiently high. Assuming, more precisely, that the neighbouring chains tend to keep closer than is allowed by the separation of the polar groups, the structure becomes increasingly "strained" as the length of the rods increases. If the length of the rods is limited and the ends joined three by three, this releases the "strain" and preserves efficient space filling. It should be noted that a similar explanation, based on the interplay of the order of the polar groups and of the disorder of the paraffin chains, has been given for some of the phases exhibited by the sodium soaps<sup>9</sup>. It is to be expected that the polar groups at the junction of three rods will be organized in a different way from that of the rest of the rods, and thus the presence of the short segments of low electron density is not surprising.

If the clustering of the polar groups into rods of finite length, and the junction of the ends of the rods three by three, are justified, the precise three-dimensional structure can be visualized as a regular framework constructed with those elements. This class of structures has been discussed by Wells<sup>10</sup>; indeed the structure described here is one of those considered by him.

The similarity of the X-ray diffraction diagrams (see above) requires that the structure of all the soaps be related, that is, that the dimensions of the structure

elements be proportional to the cell parameter. This requirement would be strictly fulfilled if the length of the segments of low electron density, near the ends of the rods, were proportional to the cell parameter. In fact, the intensities calculated assuming that the length is constant are hardly different from those of Table 1, even in the case of behenate.

One final comment on the properties of other phases. All the phases described previously were supposed to be formed by structural elements the symmetry of which is analogous to that of the lattice: planar sheets for the one-dimensional (lamellar) lattice; circular cylinders for the two-dimensional hexagonal lattice; rectangular prisms for the two-dimensional rectangular lattice; spheres for the face-centred cubic lattice, etc. (see discussion in ref. 1). In many cases these hypotheses were confirmed by straightforward crystallographic analyses and by the analysis of some physical and chemical parameters. Nevertheless, for other phases it seems likely—especially as a consequence of the results described here—that some of the structures are in fact more complicated.

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## Electrical Responses in Brain Samples

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Small samples from the mammalian brain remain excitable *in vitro*, and spike discharges similar to many observed *in vivo* are evoked in neocortical samples. The autonomy of such samples will make them very valuable in investigations of the working of the brain.

STUDY of the mammalian brain *in situ* is conditioned and often limited by the complexity and interconnexion with other bodily events, which are characteristic of the brain as an organ. The innumerable interactions possible among the  $10^{10}$  neurones of the human brain have been often noted, and have prompted us to develop suitable means of examining quite small samples excised from the brain when investigating aspects of cerebral functioning. Application of these methods to the neocortex has hitherto given partial success, and it is this major part of the mammalian brain that presents the greatest problem in deciphering cellular interconnexions. We have now demonstrated in neocortical samples, weighing a few milligrams, several of the electrical and metabolic responses to applied stimuli previously recognized in the brain *in vivo*.

Tissue samples, about 0.3–0.4 mm in one dimension and a few mm in the others, were excised<sup>1</sup> and placed in an incubating solution at 38° C in a glass chamber<sup>2</sup>, within 1–2 min of exsanguinating a rat or guinea-pig. Neocortical tissues in these conditions in oxygenated solutions containing glucose have been found to maintain or to recover a variety of their *in vivo* attributes. These include their contents of energy-rich phosphates and sodium and potassium ions<sup>3</sup>, the resting membrane potentials of constituent cells<sup>2</sup> and several metabolic responses to excitation<sup>3</sup>. Thus although some damage is inevitable in excising the tissue sample (it has been quantitatively appraised<sup>4</sup> and concerns an area perhaps 0.1 per cent of the cell surfaces of the sample), the greater part is close to normal in many functional characteristics.

Electrical responses to stimulation were therefore sought. First, there were the results of stimulation for periods of some seconds or 1 min<sup>6</sup>. These were detected by micropipettes with a tip diameter of 0.5–1  $\mu$ , which were used intracellularly. Depolarization on stimulation and a slow subsequent repolarization were found.

Present experiments showed direct responses to individual stimuli, or to limited groups of stimuli, using glass micropipettes with tip diameter of 2–4  $\mu$ , filled with 20 per cent sodium chloride and used extracellularly. Conventional amplification and display techniques were used (see also Fig. 1). Stimuli were applied to the tissue through to two silver-silver chloride ball-tipped electrodes placed 1 mm apart on the upper surface of the tissue sample, while it was supported on a nylon grid at the surface of a glucose saline buffered by bicarbonate<sup>8</sup>. The stimulating electrodes were connected to two coupled 'Grass S4' stimulators through stimulus-isolation units.

Potential changes detected extracellularly (Fig. 1) were elicited in response to individual rectangular pulses of about 5 V and lasting 0.4 msec. Surface responses were positive, negative and negative-positive waves lasting about 10–20 msec, although in a few cases they lasted longer than 100 msec. These responses were detectable only within a 2 mm radius of the stimulating electrodes and the amplitude of the response fell rapidly as the recording electrode was moved away from the stimulating electrode, so that little was found at 2.5 mm. It is interesting that, much earlier, metabolic responses in neocortical samples to pulses similar to those now used were

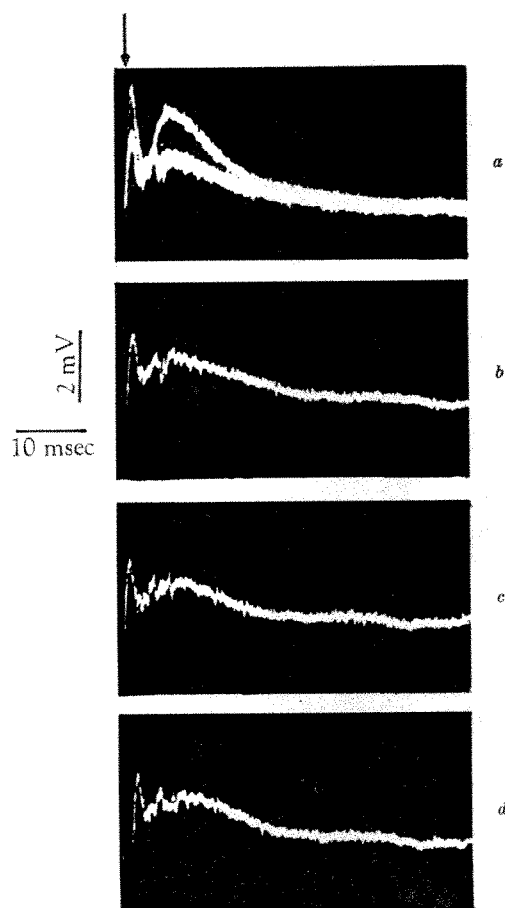


Fig. 1. Potentials evoked in an isolated neocortical specimen in response to surface stimulation. *a*, A surface-positive wave, superimposed on a further response to a subsequent stimulus recorded 130  $\mu$  below the tissue surface. *b–d*, Responses recorded at one location, to identical surface stimuli delivered during 10 sec (in *b–d*, traces retouched). In this and in the other figures, arrows indicate stimuli and upward displacements represent positive potentials.

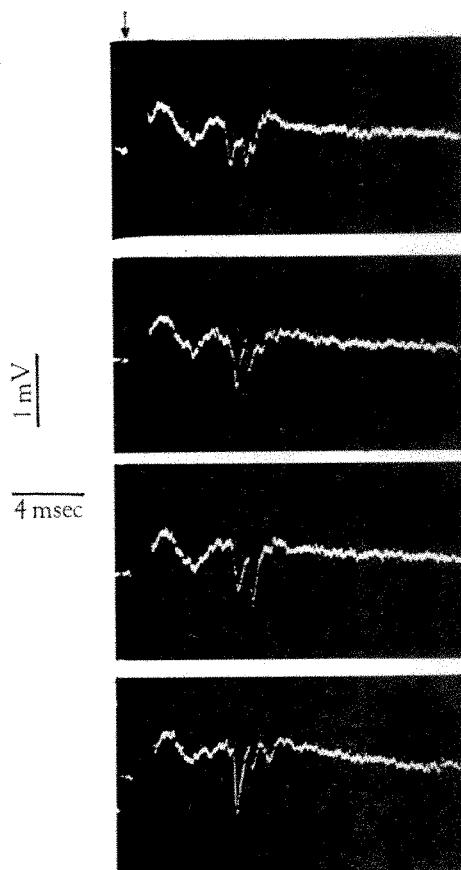


Fig. 2. Variation in evoked responses. The frames were taken at about 2 sec intervals while electrode placement and stimulus characteristics remained constant. The depth of the tip of the recording electrode was 180  $\mu$ .

found to be maximal with electrodes 1–2 mm apart<sup>7</sup>, and that a separation of 2 mm has been adopted in most subsequent metabolic measurements of response<sup>8,9,10</sup>.

Spike potentials have now been recorded at depths of 150–300  $\mu$  in response to surface stimulation (Figs. 1–3). These spikes could be classified into the first two of the three classes listed by Amassian<sup>9</sup>. They were either initially negative, both with and without a smaller positive phase following, or they were large positive-negative spikes. Monophasically positive spikes only occurred rarely as injury discharges. Most of the spikes encountered in penetrations of the cortex were of the initially negative variety. Their amplitude ranged from just above the noise level to about 1 mV and they lasted about 1 msec. These observations are very similar to observations of units recorded in grey matter *in vivo*<sup>9</sup>.

Examples of unit recording from *in vitro* neocortical preparations are shown in Figs. 1 and 2. Fig. 2 shows considerable variation in response to constant stimulus characteristics; possibly some degree of synaptic activation is the cause of this variability. Units in which activity had been evoked by surface stimulation were not susceptible to being driven at frequencies above about 20/sec, although precautions were taken to avoid electrode polarization.

The relationship between changes in the composition of the medium and the excitability of the slices has been examined. When most of the extracellular chloride ions were removed there was substantial increase in the excitability of guinea-pig hippocampus<sup>10</sup>. Similar experiments were attempted with neocortical samples. When sodium ethane sulphonate was substituted for sodium chloride, no seizure discharge was observed; but the surface cortical response increased in amplitude and

duration from 0.5 mV (peak amplitude) and about 20 msec (maximum duration) to 1.2 mV and 40 msec. There was no noticeable increase in the unit activity encountered. When lithium ion replaced sodium in the experiments it did not permit spike generation, but caused an irreversible loss of excitability, which occurred within 5 min of exposure to the lithium-saline.

Maintenance of function in isolation is familiar in excised samples from many parts of the mammalian body including muscle and peripheral nerve, but pessimistic statements have repeatedly appeared regarding tissues from the brain<sup>11,12</sup>. The present series of experiments provide a new outlook; they showed, first, the conduction of impulses along the lateral olfactory tract in small samples from the piriform lobe of the brain<sup>13,14</sup>, maintained *in vitro* with an experimental arrangement similar to that described previously, except that recording was extracellular and from the surface of the tissue sample. Complex responses were also found in the piriform cortex in such samples<sup>13-15</sup>, and in portions from the hippocampus of the brain of the guinea-pig<sup>10</sup>. The micropipette recording now applied to the neocortex also revealed unit activity in the piriform cortex in response to stimulation of the lateral olfactory tract<sup>14</sup>.

Each of these experimental systems offers special advantages and opportunities. Findings now reported with the neocortex have the merit of referring to the part of the brain which is most characteristic of higher animals, which has been most extensively studied by biochemical

techniques and which is available in the greatest quantity. The sample of piriform cortex which carries the lateral olfactory tract gives the best opportunity for examining the spread of the effects of stimulation, by normal routes of conduction and synaptic transmission. Metabolic studies<sup>13,17</sup> have suggested that the proportion of a 35 mg sample which was directly affected by localized stimuli was about 5 per cent, while up to 40 per cent of the tissue responded as a result of conduction and transmission. Thus this preparation showed the augmentation of response during a train of impulses, the alteration in amplitude during repetitive stimulation and the modified responses on high frequency stimulation<sup>13,14</sup>, which are also found *in vivo*<sup>18</sup>.

The piriform preparation has also proved interestingly susceptible *in vitro* to a number of centrally acting drugs<sup>13-15</sup>. Lack of suitable cerebral preparations has in the past partially caused the study of the action of several drugs the actions of which are primarily central (for example, anticonvulsants), to be carried out with preparations of peripheral nerve or ganglia, which are not their points of action as drugs *in vivo*. It is interesting that the hippocampal preparation, *in vitro*, exhibited seizure discharges in response to single stimuli when these were applied in media with a diminished chloride content<sup>10</sup>. The preparation has thus proved valuable in the understanding of central inhibitory phenomena by permitting much more facile control of ionic environment than is possible *in vivo*.

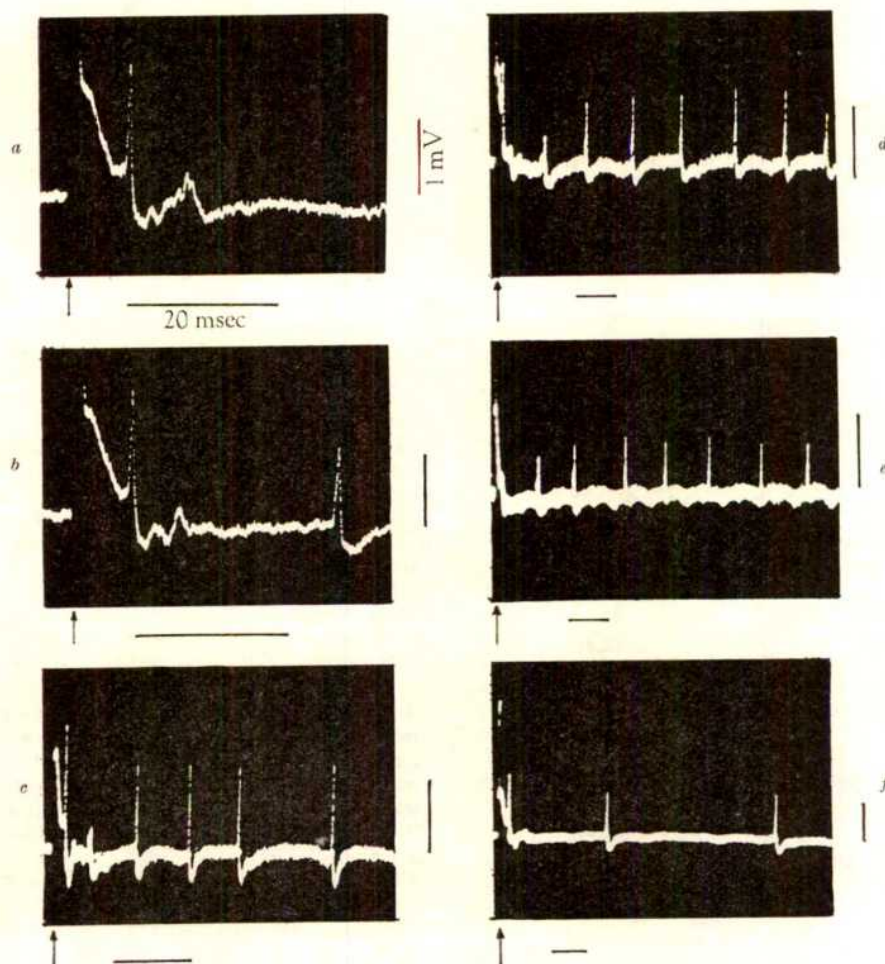


Fig. 3. Repetitive firing induced by surface stimulation in a sodium ethyl sulphonate medium. (a) Initial spike evoked by surface stimulation at the former cortical surface. After about 15-20 sec of continued stimulation at 1 pulse/sec, a second spike was seen (b); then, (c), high frequency discharges began after a further lapse of 20 sec. These discharges lasted about 20 sec (d-f), after which time only a few discharges were seen following stimulation. Calibrations: horizontal bars, 20 msec; vertical bars, 1 mV.



The samples which we have examined are about 1/50,000 of the size of the human brain, and when such samples are derived from the human brain itself, they have shown cellular potentials<sup>5</sup> and responses to stimulation<sup>19,20</sup>. The preparations clearly exhibit a newly recognized autonomy in the action of groups of cerebral cells, very relevant to the dictum "matter works itself" which was intriguingly discussed by Sherrington in relation to cerebral phenomena differently observed<sup>21</sup>.

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## Neuronal Mechanisms of Habituation

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It is possible to account for many aspects of habituation without postulating an active gating process (such as proposed by Sokolov), but by supposing that the gradual waning of the response of a group of neurones to a repeated stimulus is caused by a self-generated depression of sensitivity at one or more places in the group.

An animal suddenly presented with a stimulus may respond by some movement of the body in which the sense organs are directed towards the source of the stimulus<sup>1</sup>. At the same time certain vegetative changes occur, including an alteration in blood pressure and heart rate as well as changes in the electroencephalogram. Repeated presentation of the stimulus at intervals of about a second or more brings about a gradual waning of this orientation response which can, however, be provoked again if the original stimulus is applied after a prolonged interval of time<sup>2-5</sup>. I shall use the term "habituation" to describe these phenomena—the attenuation of response associated with repeated presentation of stimulus and the recovery of response which may afterwards be induced.

Many of the characteristics of habituation can be observed in single neurones. Studies of such cells in invertebrates<sup>6-8</sup> and vertebrates<sup>9-14</sup> have shown that the response to an infrequently presented stimulus decays after a few presentations, although neurones in close synaptic connexion with receptors do not appear to exhibit this behaviour<sup>15</sup>. In vertebrates, the phenomenon does not depend on the integrity of the "higher centres", because it can be observed in cells of the mid-brain after the removal of the neo-cortex<sup>16</sup> and in cells of the spinal cord separated from the brain<sup>13,14,14a</sup>.

In addition to response decrement, these neurones show many of the properties which characterize other aspects of habituation. The recovery of response to the original stimulus usually occurs provided the stimulus is withdrawn for a sufficient length of time before being presented again<sup>8,10,12,17</sup>. In cells which can be driven by different stimuli, for example, acoustic and photic stimuli, Horn and Rowell (in preparation) and others<sup>8,10,12</sup> found that failure of response to one of them may be without influence on the ability of the other to elicit a response.

A view that has gained considerable currency, and which attempts to account for habituation of the orientation response, is that proposed by Sokolov<sup>18</sup>. In this theory an afferent signal is conveyed to the sensory cortex and also, through axon collaterals, to the reticular formation. Access of the signal to the reticular formation is controlled by fibres originating in the cortex. The cortex stores a neuronal representation of stimuli which have been presented in the past. If this representation

matches the incoming signal, impulses descend from the cortex and prevent the signals in the afferent collaterals from activating the reticular formation. If the stimulus is a novel one, it will have no neuronal representation and the descending discharge from the cortex facilitates the passage of signals from the axon collaterals to the reticular formation. Thus response decrement to a repeatedly applied stimulus is the result of an active gating process controlled by the output of a matching system.

As a general theory of habituation, this model is unsatisfactory. For one thing, response decrement can occur in the absence of the cerebral cortex. Then elaborate neuronal circuitry is invoked to account for certain phenomena which could be accounted for more economically. An environment matching system similar to that proposed by Sokolov is probably essential for some but not for all properties of habituation.

The object of this article is to consider what aspects of habituation can be accounted for on the hypothesis that the gradual waning of response of a system of neurones to a stimulus which is slowly and repeatedly applied is a result of a self-generated depression of sensitivity (SGD) at one or more points in the system. This hypothesis differs from that of Sokolov<sup>18</sup> which supposes that response decrement is caused by an active blocking of transmission, the gate being operated by the output from a matching system. The term self-generated depression of sensitivity is used in the present context as a generic term to include such phenomena as after hyperpolarization, conduction block due to accumulation of potassium ions in the extracellular clefts surrounding an active fibre, synaptic depression which probably represents an imbalance between the mobilization and utilization of transmitter and, in certain circumstances, recurrent inhibition and prolonged primary afferent depolarization as found in the spinal cord and thought to be partly responsible for presynaptic inhibition.

### Response Decrement and Recovery

Let us suppose that a neurone ceases to respond to a repeated stimulus but responds vigorously to another stimulus applied to a sense organ of a different modality;



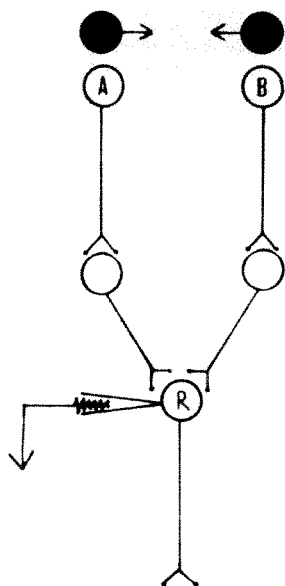


Fig. 1. A spot moved from left to right in the visual field activates a group of cells whose discharge reaches the recorded cell *R* over pathway *AR*. When the spot is moved from right to left a different group of cells is activated and this discharge reaches cell *R* over pathway *BR*.

and that when the original stimulus is withdrawn for some time and then presented again, the cell responds once more. This behaviour would be expected if there were an SGD in the pathway transmitting the signal evoked by the repeated stimulus. Thus if the response of the cell to a tone presented once every 4 sec had waned as a result of such a depression somewhere in the pathway linking the cell to the basilar membrane, a photic stimulus would still be able to drive the recorded cell provided the acoustic and photic stimuli did not evoke activity in anatomically overlapping pathways to the cell. The ability of the original stimulus to elicit a response after a period of withdrawal would depend on the rate at which the SGD recovered.

Thus the whole sequence of events—gradual failure of response, recovery after a lapse of time and the potency of an additional stimulus—could be accounted for by SGD assuming that the activity evoked by the repeated and additional stimuli reached the recorded cell over anatomically independent pathways. It is clear<sup>19-22</sup> that specific features of a stimulus often trigger specific groups of neurones. To take an example, a spot of light moved across the visual field in the horizontal meridian will activate a specific group of cells in the rabbit retina<sup>21</sup>; if the direction of movement of the spot is now reversed, activity will be evoked in another group of cells. If both groups are connected to a cell (for example, cell *R* in Fig. 1) by independent pathways, then a spot moved in either direction will drive it. Should there be an SGD as a result of stimulus repetition in the pathway linking one group of directionally selective cells to the recorded cell (for example, SGD between cells *B* and *R* in Fig. 1), cell *R* will cease to respond to that stimulus. If, however, the spot of light is moved in the reverse direction and so activates the other group of directionally selective cells, *R* will discharge. Thus cell *R* is unresponsive to the repeated stimulus but can be activated if one of the parameters of that stimulus is changed.

A set of experimental results which is difficult to account for in this way is that in which an additional stimulus, which differs from the repeated stimulus only in being less intense, evokes a response<sup>5,12</sup>. Voronin and Sokolov<sup>5</sup> used this difficulty as one of the crucial arguments for their active gating theory. The less intense stimulus might be expected to use a pathway to the

recorded cell coincident with or contained within that used by the original stimulus; if the response of the recorded cell waned because of SGD in that pathway, a less intense stimulus must also fail to activate the cell. Such a conclusion is inevitable if the assumption about common pathways is correct. Is it?

Goldberg and Greenwood<sup>23</sup> recently showed that about a quarter of the neurones in the dorsal cochlear nucleus of the cat which were examined in detail showed a non-linear increase in firing rate as the intensity of a tone stimulus was increased. In fact, as the intensity increased the firing rate of such a unit increased, reached a peak and then decreased. Units which respond in a similar way to photic stimuli have been described in the cat retina<sup>24</sup>. Consider an array of five such neurones, the firing rate of each of which is plotted against stimulus intensity (Fig. 2*A*). At the intensity corresponding to arrow 2, cell *D* fires maximally and the other cells fire relatively slowly. At the intensity corresponding to arrow 1, cell *B* discharges maximally and the others weakly or not at all. The output from such an assembly of neurones will transmit the intensity parameter of the stimulus along specific neurones. This assembly of neurones is shown in Fig. 2*B*. The more intense stimulus evokes activity in the recorded cell *R* over the pathway from cell *D*, and the less intense stimulus over a different pathway from cell *B*. If the response of the recorded cell to the more

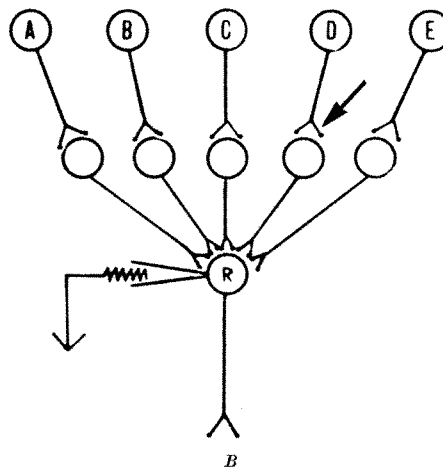
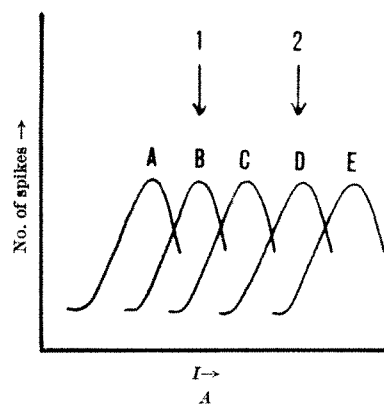


Fig. 2. *A*, Intensity/response curves of five (theoretical) neurones with properties similar to the non-linear units of Goldberg and Greenwood<sup>23</sup>. The number of spikes (ordinate) is plotted against stimulus intensity (abscissa). Arrows below the co-ordinate legends indicate direction of increasing magnitude. *B*, The neurones *A*, *B*, ..., *E* whose intensity/response curves are plotted in 2*A* are connected by separate pathways to recorded cell *R*. The response of cell *R* to an iterated stimulus which evokes activity over pathway *DR* gradually wanes as a result of self-generated depression of sensitivity at the synaptic junction indicated by the arrow.

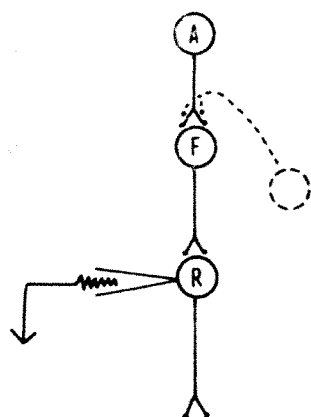


Fig. 3. Synaptic depression at the terminals of cell *A* may temporarily be restored through the activity of the inhibitory neurone (indicated by the broken line) from the reticular activating system.

intense stimulus wanes as a result of an SGD at the junction indicated by the arrow in Fig. 2*B*, the ability of the less intense stimulus to drive cell *R* will remain unaffected.

### Dishabituation

A transitory recovery of the orientation response to the repeated stimulus may occur after the presentation of an additional stimulus<sup>3,18,25-27</sup>. The effect is known as dishabituation. The additional stimulus may be a sudden loud sound, a bright flash of light or a shock applied to the skin. Horn and Rowell (in preparation) observed a similar effect which occasionally occurs in single nerve cells<sup>10</sup>. How this effect is brought about will depend on the mechanism of response waning, that is, on the basis of the SGD. Such depression may be brought about in a number of ways, but for the purposes of the following discussion it is assumed that it represents an imbalance between the processes of transmitter mobilization and utilization similar to that seen at neuromuscular<sup>28-30</sup> and synaptic<sup>31,32</sup> junctions. There is indeed some direct evidence that synaptic depression is the basis of a response failure to slowly repeated stimuli in *Aplysia*<sup>17</sup>. (If the SGD were the result of some other process—for example, accumulation of potassium ions in the spaces surrounding the fine axon terminals—dishabituation might be brought about in ways similar to, but not identical with, those described later in this article.)

We can account for dishabituation in the following way (see Fig. 3—in this and all figures continuous lines indicate excitatory connexions and broken lines indicate inhibitory connexions). A painful or intense stimulus activates in mammals<sup>33</sup> the widely connected system of fibres<sup>34</sup> known as the reticular system. If, for example, neurones of this (non-specific) system hyperpolarize the terminals of cell *A* on cell *F* the effect might be to increase the amount of available transmitter substance<sup>32,35,36</sup>. Thus once the response of cell *F* to the repeated stimulus has failed as a result of synaptic depression an additional, intense stimulus might activate the non-specific system and so lead to hyperpolarization of the terminals of cell *A* on *F*. If the original stimulus is now applied, the discharge it evokes in cell *A* will lead to a larger excursion of the membrane potential at the synaptic terminals of *A* on *F*, more transmitter will be liberated and cell *F* will fire. In this way the additional stimulus has temporarily made the repeated stimulus potent. A sudden increase in the activity of the non-specific system, unprovoked by a sensory stimulus, might have the same effect; so that occasionally there would be a spontaneous recovery to the iterated stimulus, an effect which has been described in single cells and in the intact organism. A similar recovery would be achieved if the non-specific system, activated by the additional stimulus, depolarized and hence facilitated cell *F*. The previously sub-threshold

amount of transmitter substance liberated at the terminals of cell *A* in response to the iterated stimulus would now become threshold to the facilitated cell *F* which would fire. It is thus of considerable interest that Comis and Whitfield<sup>37</sup> have shown that acetylcholine applied locally to neurones in the cochlear nucleus of the cat increases their sensitivity to auditory stimuli; and that the non-specific system connecting with this nucleus is cholinergic<sup>38</sup>.

### Stimulus Generalization

An interesting feature of habituation may be illustrated by the following experiment<sup>12</sup>. While recording from a cell in the optic tectum of rabbit a tone of 1,000 c/s was delivered and evoked a response from the cell; so also did a tone of 950 c/s of similar intensity. The 1,000 c/s tone was repeatedly presented until the cell no longer responded to it. One of the 1,000 c/s tone pips was then omitted from the sequence of presentations and the 950 c/s tone substituted. This stimulus now failed to evoke a discharge. Such a broadening of the response decrement to include stimuli other than the repeated stimulus is known as stimulus generalization, and the two stimuli are then said to be equivalent. To account for stimulus equivalence, consider Fig. 4. The cells marked *R*<sub>1</sub> correspond to cell *R* in previous figures, that is, on repeatedly presenting a stimulus their response gradually wanes. Cells *P* and *Q* are each considered to require activity in three or more *R*<sub>1</sub> cells in order to be discharged. A 1,000 c/s tone is repeatedly presented and cells *R*<sub>1</sub> . . . *R*<sub>4</sub> gradually cease to discharge; so do cells *P* and *R*<sub>1</sub>. The 950 c/s tone is now presented. It may be seen that this tone reaches cell *Q* through cells *R*<sub>3</sub> . . . *R*<sub>6</sub>. Cells *R*<sub>3</sub> and *R*<sub>4</sub> will not discharge, nor will cell *Q*, because it is not excited by the minimum number of inputs. Cell *R*<sub>1</sub> will not therefore respond to the 950 c/s tone and the two tones are said to be equivalent. Stimulus equivalence on this model is a consequence of channel overlap.

### Time Dependent Effects

There is one set of results that certainly cannot be accounted for by the SGD/channel-dependent code hypothesis alone. Sokolov<sup>18</sup> found that after extinction of the orientation response to a regularly repeated stimulus a "response" appeared when one of the stimuli in the sequence was omitted. He also found that if one of the iterated stimuli was lengthened a response appeared at the time the repeated stimulus would have ended; and if the duration of the stimulus was reduced, the response appeared when this briefer stimulus ended. Sokolov<sup>39</sup> has also described single cells in the hippocampus the response of which to a repeated stimulus gradually wanes, but which gives a discharge when one of the stimuli of the sequence is omitted. This effect may be a specialized

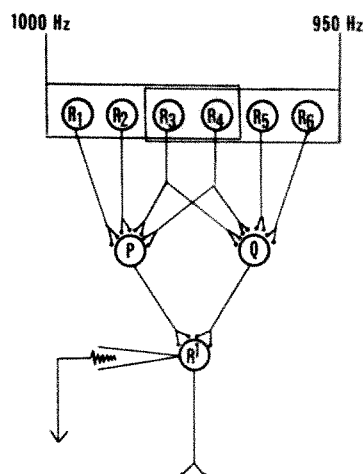


Fig. 4. See text for discussion.

property of certain neuronal assemblies, because it has not yet been reported to occur elsewhere than in the hippocampus.

In order to account for these time dependent effects (of omitting a stimulus from a sequence or of changing the duration of one of the stimuli) it seems essential to introduce a system that detects the difference between past and present activity<sup>18</sup>. The way in which a difference detecting system might operate may be considered with reference to Fig. 5A. Suppose that cells *A* and *K* are simultaneously active and each discharge *N* spikes for *t* sec. Cell *J* will be depolarized by the *N* spikes in *K* and hyperpolarized by *N* spikes from *A*. Thus the excitatory and inhibitory inputs exactly balance (assuming linear transmission) and cell *J* is silent. For similar reasons cell *H* is silent. Because cell *G* is not excited by *J* or *H*, it also is silent. If in another circumstance cell *K* discharges  $N + \Delta N$  spikes and cell *A* discharges *N* spikes, cell *H* will be silent. This is because it is excited by *N* spikes from cell *A* and inhibited by  $N + \Delta N$  spikes from *K*. Cell *J*, however, will fire because it is excited by  $N + \Delta N$  spikes from *K* and inhibited by *N* spikes from *A*. Because *J* discharges  $\Delta N$  spikes, so also will cell *G*. Thus cell *G* discharges the number of spikes equal to the difference between the spike discharges in fibres *A* and *K*.

The difference detecting circuit is incorporated into the neuronal assembly illustrated in Fig. 5B, which contains cell *R*, the response of which gradually wanes if a stimulus

is repeatedly presented. This cell also shows the various time-dependent properties implied by Sokolov's<sup>18</sup> results. Suppose that cell *A* maintains its response to a repeated stimulus and that the waning of response of cell *R* is due to an SGD localized in the terminals of cell *A* on *F*. At no other point in the circuit does this depression occur. Within this circuit, cell *K* is considered to possess certain special properties. These are: (i) if a stimulus is repeatedly presented and then withdrawn cell *K* will discharge at the time the next stimulus would have appeared<sup>40</sup> and the discharge contains the same number of spikes and lasts the same length of time as the discharge evoked by the actual stimulus; (ii) if after this period of "training" by stimulus repetition some parameter of the stimulus is suddenly changed, the discharge elicited in cell *K* corresponds to that evoked by the iterated stimulus<sup>41</sup>. Thus if the iterated stimulus generates *N* spikes for *t* sec in cell *A* it will also evoke the same number of spikes for the same length of time in cell *K*. If the stimulus is now shortened and evokes a spike discharge in *A* for less than *t* sec cell *K* will nevertheless discharge *N* spikes over *t* sec. That is, cell *K* has a memory of certain aspects of the repeated stimulus which is released by activity in fibre *A*. ("Cell *K*" may, of course, be an assembly of cells which collectively possess the properties ascribed to this cell.)

When a novel stimulus is first applied to cell *A*, cell *R* is excited over the pathway *A**F**R*; cell *G* is silent because the discharge in cell *K* is equal to that of fibre *A* (linear transmission at the *A*/*K* synaptic junction). The stimulus is now repeatedly presented and, after the response of *R* has waned, one stimulus of the series is omitted. Cell *K* will discharge at this time and cell *J* will also discharge because it receives no inhibitory input from fibre *A*, which is silent. Cell *G* will discharge *N* spikes for *t* sec, so also will cell *R*, which thus appears to "respond" to the omitted stimulus.

If the duration of one of the iterated stimuli is suddenly changed, cell *G* will fire for the difference between this new duration and the duration, *t* sec, of the iterated stimulus, and so will cells *F* and *R*. Thus the neuronal circuitry illustrated in Fig. 5B will confer on cell *R* all the properties of habituation that have already been described, including the time dependent properties (it is assumed that a stimulus which differs from the iterated stimulus in some way other than duration will reach cell *R* over a different pathway from that illustrated in the circuit). It would, of course, be necessary to introduce time delays between cells *A* and *F*, but this might be done quite easily by having a chain of neurones interposed between the two cells. (It is worth noting that if the duration of the stimulus were transmitted by a channel-dependent code, as proposed for intensity, the only result that could not be accounted for by the SGD/channel-dependent code hypothesis is that in which a response occurs when a stimulus is omitted.)

Most of the properties of short term habituation can be accounted for by the neuronal systems that have already been described. In all these systems it is assumed that recovery of response to the repeated stimulus after a lapse of time is due to restoration of the SGD. Now there is some evidence that the response of a cell to a repeated stimulus may be depressed for several hours<sup>8</sup>. In behaviour, this depression may last for as much as 24 h or even longer<sup>27,42-44</sup>. Whether such long term effects rule out any possibility of accounting for response recovery after a lapse of time in terms of a spontaneous very slow restoration of the SGD can only be decided by a further analysis of habituation and of the properties of synaptic junctions in the central nervous system.

In the meantime, we should bear in mind the possibility that several mechanisms may be used in the central nervous system to bring about the set of phenomena that have been termed habituation.

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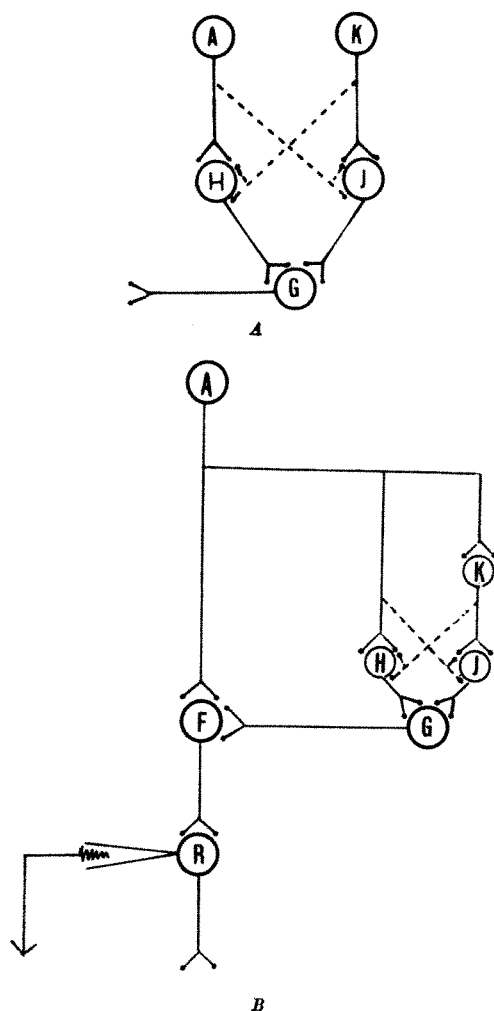


Fig. 5. Excitatory connexions indicated by continuous lines, inhibitory connexions by broken lines (inhibitory interneurons have been omitted for the sake of clarity). A, The difference detecting circuit in which the activity in cell *G* is equal to the difference between the activity in cells *A* and *K*. B, An assembly of neurones incorporating the difference detecting circuit in which the activity of cell *R* shows many of the features of habituation including the time-dependent effects.



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## Histochemistry of the Mucosaccharides in the Epiphyseal Plate of Young Rabbits

by

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Histochemical observations on sections of the epiphyseal cartilage plate of young rabbit bones suggest that free anionic sites are present in the hypertrophic cartilage zone. This finding may help to explain why this cartilage zone takes up certain radionuclides and may throw light on the mechanisms involved in the initiation of calcifications.

IMMEDIATELY after an intravenous injection of americium and plutonium, these radionuclides become concentrated in a narrow band in the zone of hypertrophic cartilage cells at the base of the epiphyseal plate<sup>1</sup> (Fig. 1). To understand why they become concentrated here, it seemed of interest to study the histochemistry of this anatomical site in frozen undecalcified sections and to compare it with the histochemistry of the same site in sections fixed in the usual ways. This comparison appeared essential for at least two reasons. First, it is known that certain mucosubstances capable of binding cations<sup>2</sup> are soluble in the solutions used in ordinary histological procedures<sup>3-6</sup>; and second, the difficulties in identifying histochemically components rich in carbohydrate must be recognized<sup>7</sup>. Tissues of a single age only were investigated because the histochemical properties of young rat epiphysis change markedly with age<sup>8</sup>.

Blocks from the distal end of the femur of rabbits 10 days old were frozen in liquid nitrogen immediately after removal at autopsy. Sections were cut on a Cambridge rocking microtome in a Pearce cryostat at a temperature between  $-18^{\circ}$  and  $-20^{\circ}$  C. Coverslips at room temperature were used to lift the  $5\mu$  sections off the knife. Sections were dried and stored at room temperature. Subsequently they were stained: (i) without further treatment; (ii) after they had been fixed with Lillie's formalin-acetic acid-alcohol (FAA)<sup>9</sup> for 5 min; (iii) after

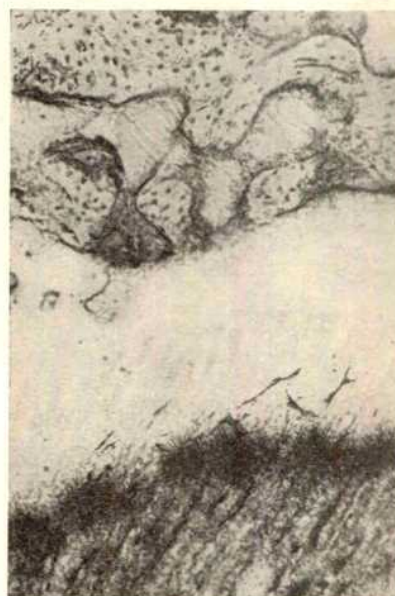


Fig. 1. Autoradiograph left on longitudinal section of epiphyseal plate from the femur of a young dog killed 4 h after an injection of  $5\mu\text{Ci}$  of  $^{241}\text{AmCl}_3$ . Note band of  $\alpha$  tracks over hypertrophic cartilage cells.



decalcification with the disodium salt of ethylenediamine tetraacetic acid (EDTA)<sup>10</sup> at pH 7 for 5 min; or (iv) after fixation followed by decalcification. For comparison, paraffin sections were cut from blocks which have been fixed in either a neutral (Lillie's neutral formalin<sup>9</sup>, NF) or an acid medium (FAA) before decalcification in aqueous EDTA at neutral pH. The blocks were washed in running water for 3 h after both fixation and decalcification.

Two types of histochemical technique were used. First, those dependent on the affinity of tissue anionic groups for cationic dyes, namely, toluidine blue (pH 4) or azure A, aldehyde fuchsin, high iron diamine, and 'Alcian Blue' (at pH 1.0 and 2.5; and at pH 5.6 dissolved in magnesium chloride solutions of various molarities). Details of theory and procedure are summarized elsewhere<sup>11,12</sup>. Second, another dependent on the condensation with salicylhydrazide of the aldehydes engendered by the periodate oxidation of the vicinal glycols of the carbohydrate fraction of mucosubstances. The products thus formed from sialomucins emit a particularly intense blue fluorescence after being treated with a solution of an aluminium salt<sup>12</sup>.

The results of using the cationic dyes are shown in Table 1. Toluidine blue, aldehyde fuchsin and high iron diamine stain the whole plate in all sections, however prepared, but the colour is more intense over the hypertrophic cartilage cells in the frozen undecalcified sections. Toluidine blue does not stain the cartilage remnants before decalcification.

'Alcian Blue' at pH 2.5 imparts to frozen untreated sections a deep stain in the matrix and cells of the lowest hypertrophic layer and in the longitudinal bars which survive as the cores of the metaphyseal trabeculae. The rest of the plate is extremely pale (Fig. 2A). After sections have been fixed with FAA there is a slight extension upwards in the direction of the epiphysis of the deep staining reaction (Fig. 2B). In decalcified frozen sections (Fig. 2C) and in paraffin sections the whole plate is deeply stained.

In frozen fixed sections stained first with high iron diamine and then with 'Alcian Blue' at pH 2.5, only the granules in the hypertrophic cells take up 'Alcian Blue'. This is not seen in decalcified or paraffin sections.

Table 1. STAINING DISTRIBUTION IN FROZEN AND PARAFFIN SECTIONS

	Frozen	Frozen FAA	Frozen EDTA	Paraffin NF
<b>'Alcian Blue' pH 2.5</b>				
Hypertrophic matrix	4	4 } Extending	4	4
Hypertrophic cells	3	3 } slightly	3	3
Rest of plate	1	1	4	4
Calcified remnants	4	4	4	4
<b>Aldehyde fuchsin</b>				
Hypertrophic matrix	4	4	4	4
Hypertrophic cells	3	3	3	3
Rest of plate	3	3	3	4
Calcified remnants	4	4	4	4
<b>High iron diamine</b>				
Hypertrophic matrix	4	4	4	4
Hypertrophic cells	1	1	1	1
Rest of plate	3	4	4	4
Calcified remnants	4	4	4	4
<b>Toluidine blue pH 4</b>				
Hypertrophic matrix	M	M	M	M-B
Hypertrophic cells	M	M	M	M
Rest of plate	M	M	M	M
Calcified remnants	B*	B*	M	M

Intensity of staining estimated on scale 4 (very strong) to 1 (weak).  
M, Metachromasia; B, orthochromasia.

\* Occasional metachromasia which is only pale if present.

Table 2. EFFECT OF pH ON 'ALCIAN BLUE' STAINING OF UNTREATED, CRYOSTAT AND ROUTINE PARAFFIN SECTIONS

	Untreated cryostat	NF/EDTA paraffin	FAA/EDTA paraffin
<b>'Alcian Blue' 1 per cent; 30 min</b>			
<b>pH 1.1</b>			
Hypertrophic matrix	4	4	2
Hypertrophic cells	4-3	4	1
Rest of plate	3-2	4	2
Calcified remnants	4	4	2
<b>pH 2.5</b>			
Hypertrophic matrix	4	4	2
Hypertrophic cells	3	4-3	1
Rest of plate	1	4	1-0
Calcified remnants	4	4	2
<b>pH 5.6</b>			
Hypertrophic matrix	4	3	3
Hypertrophic cells	3-2	2	3
Rest of plate	3	3-2	2
Calcified remnants	4	3	3

Intensity of staining estimated on scale 4 (very strong) to 1 (weak).

The effect of pH on 'Alcian Blue' staining is shown in Table 2. In general, the degree of staining of untreated frozen and NF-fixed paraffin sections is comparable and is always more intense than in paraffin sections fixed in FAA. Likewise, increasing the pH of azure A solutions

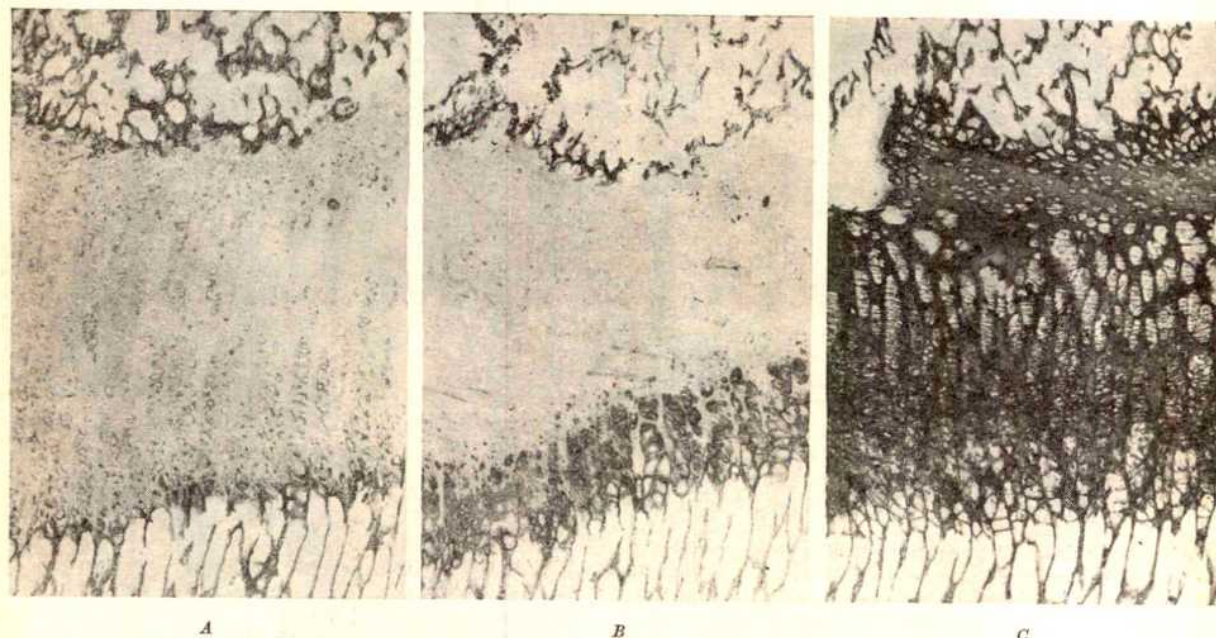


Fig. 2. Longitudinal sections of femoral epiphyseal plate from young rabbit stained with 'Alcian Blue' pH 2.5 ( $\times 60$ ). A, Frozen untreated showing heavy staining of hypertrophic cell zone; B, frozen fixed (FAA) showing extension of heavy staining towards the epiphysis; C, frozen decalcified (EDTA) showing heavy staining of whole plate.

Table 3. ALCIANOPHILIA AT pH 5.6 IN THE PRESENCE OF MAGNESIUM CHLORIDE

	Molar magnesium chloride									
	0	0.05	0.1	0.2	0.3	0.4	0.6	0.8	1.0	
Untreated cryostat										
Hypertrophic matrix	3	3	3	3	3	2-0	2-1	0	0	
Hypertrophic cells	1	1	1	1	±	0	0	0	0	
Rest of plate	2-1	2	2-3	2-3	2-1	2-0	2-0	0	0	
Calcified remnants	3	3	3	3	3	2	1	±	±	
NF/EDTA/paraffin										
Hypertrophic matrix	4	4	4		3	3	2-1	±	0	
Hypertrophic cells	4-3	4-3	3		3-2	2	1	0	0	
Rest of plate	3-2	2	3		1-2	1-2	1	0	0	
Calcified remnants	4	4	4		3	3	2	±	0	
FAA/EDTA/paraffin										
Hypertrophic matrix	3	3	3	2	2	2-1	+	0	0	
Hypertrophic cells	3	3	3-2	2	2-1	1	±	0	0	
Rest of plate	2-1	2	2-1	2-1	2-1	2-1	0	0	0	
Calcified remnants	2	3	3-2	2	2-1	2-1	±	0	0	

Intensity of staining estimated on scale 4 (very strong) to 1 (weak).

favours increased metachromasia of the matrix. Table 3 summarizes the effect of different molarities of magnesium chloride on the uptake of 'Alcian Blue' at pH 5.6. In the absence of magnesium ions the matrix of the entire plate stains heavily; the positive reaction of granules in the cytoplasm of the hypertrophic cells is also marked. The latter is reduced in the presence of 0.2 molar magnesium chloride, whereas the reaction of the matrix is not markedly reduced until 0.6 molar magnesium chloride is reached. It is eliminated in the presence of 0.8 molar magnesium chloride. As is indicated in Table 2, staining decreases more rapidly in the interterritorial matrix than in the matrix immediately around the cell as the molarity of the magnesium chloride increases.

The periodic acid-salicylhydrazide-aluminium procedure induces some fluorescence over the region of the hypertrophic cells. The fluorescence is reduced in sections incubated beforehand in neuraminidase.

The results obtained in this study, though in detail confusing, suggest that there are more free anionic sites in the zone of hypertrophic cartilage cells than elsewhere. The uniform staining throughout the plate in paraffin sections and in frozen decalcified sections indicates that the decalcifying and embedding processes liberate anionic sites which then bind cationic dyes to the same degree and in all sites. Such preparations give no opportunity to discriminate between different mucosubstances. Three of the cationic dyes used, toluidine blue (or azure A), aldehyde fuchsin and high iron diamine, give the same result in both frozen and paraffin sections except that in the frozen sections the zone of hypertrophic cartilage cells is more deeply stained. Their reaction is not affected by pH. 'Alcian Blue' stains the whole plate in frozen sections only at a higher pH and is considered later.

The fact that aldehyde fuchsin stains the whole plate in frozen sections while 'Alcian Blue' does not, except at a high pH, needs an explanation. Perhaps the acid in the aldehyde fuchsin solution strips cations originally present on the anionic groups or mucosubstances. If it does, the anionic sulphate sites thus exposed would take up aldehyde fuchsin in the normal way. Alternatively, aldehyde fuchsin may have a greater inherent affinity for mucin sulphate groups than the metal cations or even hydrogen ions originally bound to them. The cations are possibly peptides or calcium ions. The latter, though they do not show up with the usual histochemical methods for calcified deposits<sup>11</sup>, are present in the rabbit tissues studied here, as shown by micro-incineration (0.4 per cent calcium dry weight). Hjertquist<sup>13</sup> found 1.5 per cent calcium on a dry weight basis in the non-calcified epiphyseal plate in the dog as compared with 10 per cent in the zone of provisional calcification.

Aldehyde fuchsin, high iron diamine and, to a lesser extent, toluidine blue stain sulphated mucopolysaccharides specifically and the results with these dyes substantiate what is already known from chemical analyses<sup>14</sup>, namely, that there are large amounts of sulphated mucosubstances throughout the plate. The fact that in frozen undecalcified sections the cells of the hypertrophic zone are more deeply

stained by these dyes suggests that there is a greater concentration of free ester sulphate groups here than in the rest of the plate. On the other hand, it has been shown chemically that there is no change in sulphur concentration in different regions of the plate<sup>14</sup> and this has been confirmed by autoradiographic studies<sup>15</sup> using <sup>35</sup>SO<sub>4</sub>.

The ester sulphate groups in the hypertrophic zone may have fewer metabolic cations or, as seems more likely, amino-acids or proteins bound to them than those elsewhere in the plate. Thus more of them would be free to take up basic dyes in the histochemical experiments. This is borne out by the experience of others. Amprino<sup>16</sup> shows that the basophilia of undecalcified bone increased following trypsin digestion, and Szirmai, Van Boven de Tyssensk and Gardell<sup>17</sup> have described amino-acids blocking the basophilic groups of nasal septum cartilage. Dziwiakowski and his group<sup>15,18</sup> have also provided immunological and autoradiographic evidence that some protein is removed from the zone of provisional calcification but not from elsewhere.

The results with 'Alcian Blue' are, however, much more informative. In frozen undecalcified sections 'Alcian Blue' at pH 2.5 stains only the region of the hypertrophic cartilage cells at all darkly, thus suggesting that free anionic sites capable of binding this dye are present in this site alone. Frozen sections fixed in FAA are stained in a slightly wider zone (Fig. 2B) presumably because more anionic sites have been liberated, but it was only after decalcification that the whole plate stains at this pH (Fig. 2C). Further, when sections are stained with high iron diamine followed by 'Alcian Blue' at pH 2.5 the cells, as distinct from the matrix, of the hypertrophic zone are stained by 'Alcian Blue'. In this sequence this is considered to indicate the presence of non-sulphated mucosubstances, that is, sialomucins and hyaluronic acid<sup>11,12,19</sup>. This observation alone suggests that in the hypertrophic region the cells themselves contain probably a sialomucin or hyaluronic acid. These mucins may also be present in the matrix of these cells, but their presence here is masked by the far deeper staining of the more highly concentrated chondroitin sulphate. The results obtained with 'Alcian Blue'-magnesium chloride solutions confirm this suggestion<sup>20</sup>. In this laboratory Herring has found that the alcianophilia of an isolated bone sialoprotein and a bone chondroitin sulphate fraction on cellulose acetate strips is inhibited by 0.2 molar magnesium chloride and 0.6 molar magnesium chloride, respectively. In the case of the frozen sections the 'Alcian Blue' staining of the intracellular granules in the hypertrophic cells was greatly reduced at 0.2 molar magnesium chloride, suggesting the presence of a sialoprotein, while at 0.6 molar magnesium chloride the staining of the rest of the plate is less strong and except for the outer zone of the articular cartilage extinguished at 0.8 molar magnesium chloride.

The positive fluorescence obtained with the salicylhydrazide technique substantiates the presence of a sialomucin in the hypertrophic zone. The finding that the fluorescence is reduced by previous treatment with neuraminidase, however, must be treated with caution. The use of enzymes to remove selected compounds should in theory provide good specificity, but in practice there are many difficulties, among them the impurities of many enzyme preparations and the fact that in the process of incubation itself important compounds may be extracted apart from the specific enzyme action<sup>21</sup>.

This histochemical identification of a sialoprotein in the hypertrophic cells is confirmed by some biochemical evidence<sup>22</sup>. Jibril and Lindenbaum<sup>22</sup> have demonstrated, with sodium hydroxide extraction, an appreciable excess of extractable sialic acid in the zone of calcification of the calf scapula compared with the resting zone, 0.7 per cent of dry weight compared with 0.2 per cent. They suggest that this is normally bound to a chondromucoprotein. In 1964 Hjertquist found a slightly higher concentration of



hyaluronic acid in the zone of provisional calcification than elsewhere in the plates. Hirschman and Dziewiatkowski<sup>18</sup> have demonstrated a removal of protein, probably by proteolytic action, from the protein-polysaccharide complexes in this zone which may indeed explain the present observation that there are here free anionic groups capable of binding 'Alcian Blue' and therefore other cations. The finding of a sialoprotein, which in the case of the bone sialoprotein isolated by Herring<sup>4,5</sup> is known to be capable of binding certain cations even more strongly than it does calcium ions, in this zone is of interest<sup>2</sup>. The presence of these free anionic sites in the zone of hypertrophic cartilage cells may explain the initiation of calcification here and the ability to concentrate cations like americium and plutonium.

Thus the appropriate uses of 'Alcian Blue' and the periodic acid-salicylhydrazide-aluminium technique on frozen undecalcified sections of the epiphyseal cartilage plate of young rabbit bones suggest that mucosubstances with anionic sites are present in the hypertrophic cartilage zone and that they are free to bind the dye and therefore possibly other cations. This observation may explain both the initiation of calcification and the uptake of plutonium and americium at this site.

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## Radioimmunoassay of Angiotensin in Human Plasma

by

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Understanding of the renin-angiotensin humoral system has been held up for lack of a really specific and quantitative method of measuring angiotensin in plasma. A very sensitive radioimmunoassay for the hormone, which can be used on small samples of human plasma, has been developed.

We have demonstrated that antibody to angiotensin can be elicited in rabbits by copolymers of the hormone with poly-L-lysine. The octapeptide can be attached by its carboxy-terminal phenylalanine to the  $\epsilon$ -amino groups of the lysine residues by a carbodiimide condensation or coupled by its amino-terminal asparagine either with *m*-xylylene diisocyanate<sup>1</sup>, or by a carbodiimide condensation with succinyl poly-L-lysine<sup>2</sup>. Bovine serum albumin has also been used as a macromolecular carrier for angiotensin<sup>3</sup>. Poly-L-lysine as a carrier offers the advantages of low intrinsic immunogenicity and its  $\epsilon$ -amino group permits specific orientation of the molecule of angiotensin.

Asp(NH<sub>2</sub>)<sup>1</sup>-Val<sup>5</sup>-angiotensin II ('Hypertensin', given by Ciba) is used as the reference standard in the assay, and also as the isotopically labelled tracer after iodination with iodine-125 (ref. 4). After iodination antiserum added in excess is used to select the labelled angiotensin with the greatest affinity for the antibody. It is then liberated from antibody at pH 2 (ref. 1). The labelled material separated by gel filtration has a specific activity of approximately 220,000 c.p.m./ $\mu$ g, and moves as a single peak on high voltage electrophoresis.

The separation of antibody-bound and free angiotensin was achieved by gel filtration<sup>1,5</sup>. Another method of separation has been developed since then, utilizing half-saturated ammonium sulphate to precipitate antibody-bound hormone together with a carrier amount of rabbit gamma-globulin. This method gives results identical to those obtained by gel filtration and permits analysis of a larger number of samples.

Fig. 1 shows a typical standard curve in which displacement by increasing amounts of unlabelled angiotensin II is expressed in the percentage of the initial bound to free ratio ( $B/F$ ). Self displacement achieved by increasing amounts of the labelled hormone follows the same curve indicating that labelled and unlabelled angiotensin have the same reactivity toward the antibody. The association constant ( $K_A$ ) is  $2 \times 10^9$  l./mole. The usual dilution of the antiserum is 1:2,000, giving a sensitivity of 0.05  $\mu$ g for each assay.

The high specificity of this immunoassay is demonstrated by the fact that large excesses of bradykinin and vasopressin, and of peptide mixtures obtained by enzyme hydrolysis of lysozyme, do not displace labelled angioten-

sin from antibody. The binding activities of various analogues and enzyme fragments of angiotensin itself are shown in Table 1. Compound 2 has the sequence of bovine angiotensin II, while compound 3 has been found in horse and hog, and has been reported as the probable sequence in man<sup>6</sup>. These compounds have identical binding properties to compound 1 employed in the synthesis of the immunogen. Angiotensin I (compound 4) and compounds 5-9 obtained by enzyme hydrolysis of compound 1 display diminished affinity for the antibody.

Table 1. RELATIVE BINDING AFFINITY OF VARIOUS ANALOGUES, PRECURSORS AND ENZYME FRAGMENTS OF ANGIOTENSIN II, AS MEASURED BY RADIO-IMMUNOASSAY

Compound No.	Amino-acid sequence	Binding activity
1	H-Asp (NH <sub>2</sub> )-Arg-Val-Tyr-Val-His-Pro-Phe-OH	1
2	Asp-Arg-Val-Tyr-Val-His-Pro-Phe-OH	1
3	Asp-Arg-Val-Tyr-Ileu-His-Pro-Phe-OH	1
4	H-Asp (NH <sub>2</sub> )-Arg-Val-Tyr-Val-His-Pro-Phe-His-Leu-OH	1/20
5	H-Asp (NH <sub>2</sub> )-Arg-Val-Tyr-Val-His-Pro-OH	1/170
6	H-Val-Tyr-Val-His-Pro-Phe-OH	1/2
7	H-Asp (NH <sub>2</sub> )-Arg-OH	1/1,760
8	H-Asp (NH <sub>2</sub> )-Arg-Val-Tyr-OH	1/4,600
9	H-Val-His-Pro-Phe-OH	1/18,700

In our procedure the plasma is adjusted to pH 5.5 and diisopropylfluorophosphate and 'Dowex 50W-X2' in ammonium cycle are added. Duplicate 2 ml. samples of plasma are used for measurement of angiotensin and for renin activity. The first duplicates are kept on ice, while the latter are incubated for 3 h at 37° C with constant agitation. All are then transferred into small columns which are washed with 0.2 molar ammonium acetate, pH 6.0, followed by water. The sample is then eluted with 0.1 molar diethylamine, then 0.2 molar ammonium hydroxide, and the eluate is brought to pH 5.5 with glacial acetic acid. The samples are evaporated to dryness, resuspended and re-evaporated three times from 80 per cent ethanol. Finally, the samples are redissolved in 0.1 molar tris-buffer (pH 7.5) with 1 mg/ml. of lysozyme added to prevent adsorption of angiotensin on glassware, and the labelled hormone and antiserum are added. The mixtures are equilibrated for 18 h at 4° C, after which the bound and free hormone are separated and counted in a well-scintillation detector.

Reproducibility in individual samples is  $\pm 5$  per cent. Recovery of added angiotensin from plasma as compared with recovery from buffer subjected to the procedure described here is 90 per cent to 110 per cent. The concentration of angiotensin in plasma is calculated from

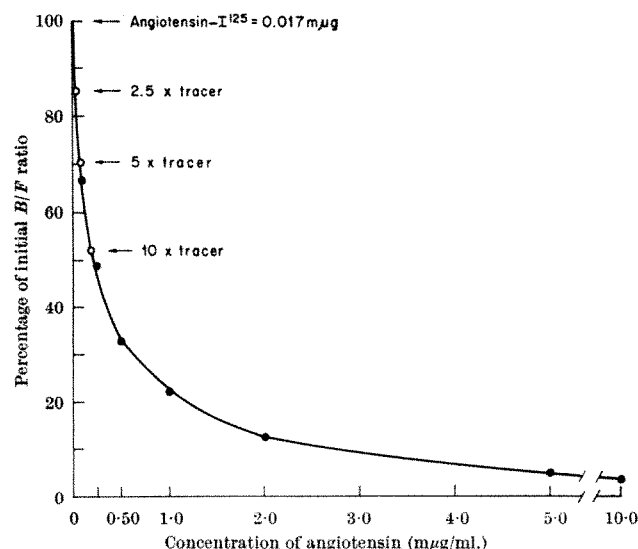


Fig. 1. Radioimmunoassay of angiotensin; displacement obtained with increments of unlabelled angiotensin II (●) and with increments of labelled angiotensin II (○) expressed as a percentage of the initial bound to free ratio (B/F).

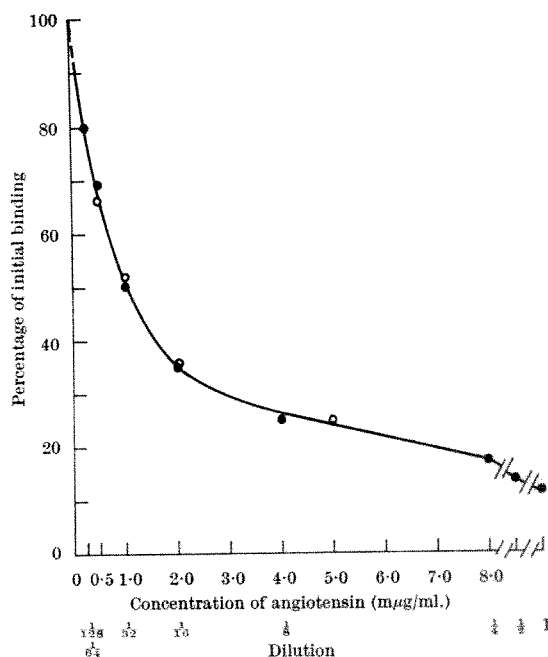


Fig. 2. Radioimmunoassay of angiotensin; comparison of the displacement of labelled angiotensin from antibody achieved by stepwise dilutions of material extracted from human plasma with the displacement achieved by increments of unlabelled synthetic angiotensin II. ○, Synthetic angiotensin II; ●, stepwise dilution of human angiotensin.

the observed ratio of bound to free hormone by comparison with the curve relating ratio to standard solutions of angiotensin. The displacement of labelled hormone by human angiotensin extracted from a plasma with high renin activity is identical with that of synthetic material (Fig. 2). Similar results are obtained when purified human renin (Haas preparation) is added to plasma before incubation.

As in other radioimmunoassays, the values found in human plasma are much lower than the ones obtained by bioassay. Table 2 gives the values found in normals and in certain disease states. These preliminary observations suggest that the method could have wide applicability in studying the role of the renin angiotensin system in man. Its chief advantages are its extreme sensitivity and high specificity, allowing the measurement of circulating concentrations of angiotensin and of renin activity, independently of other vaso-active substances, in small plasma samples.

Table 2. VALUES OF ANGIOTENSIN II FOUND IN HUMAN PLASMA BY RADIO-IMMUNOASSAY IN VARIOUS CLINICAL SITUATIONS (Mμg/100 ML.)

Diagnosis	Not incubated mean (range)	Incubated 3 h, 37° C mean (range)
Control subjects	25 (12-35)	35 (15-82)
Essential hypertension	19 (0-34)	32 (8-80)
Malignant hypertension	44 (0-164)	63 (65-170)
Cirrhosis	66 (20-107)	138 (22-266)
1° Aldosteronism		
Pre-operation	6	6
Post-operation	7	41
Renovascular hypertension		
Pre-operation	34	236
Post-operation	0	34

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# Regulation of Fat Metabolism in the Liver

by

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The fate of labelled free fatty acids in isolated perfused livers shows that on entering the liver they are esterified or oxidized. The more acid which enters the oxidation pathway, the more goes into ketogenesis and the less into the citric acid cycle, so that the total production of energy remains constant.

The free fatty acids of the plasma (or non-esterified long chain fatty acids) arise primarily from the hydrolysis of triglyceride in adipose tissue<sup>1</sup>. Their concentration is increased during starvation and other conditions associated with ketosis. Free fatty acids labelled with carbon-14 are taken up by the liver, where either they are esterified into triglycerides or phospholipids and secreted ultimately by the liver as lipoproteins<sup>2</sup>, or they are catabolized by  $\beta$ -oxidation to carbon dioxide or ketone bodies<sup>3,4</sup>. Fatty acids, however, in the form of chylomicron triglyceride are not metabolized to any appreciable extent by the isolated perfused liver<sup>4</sup>. There is good evidence therefore for free fatty acids being the principal substrate for the production of ketone bodies by the liver. These relationships are summarized in Fig. 1.

It is evident that the pathways in the liver (Fig. 1) leading, first, to the esterification of incoming free fatty acids and, second, to the production of carbon dioxide through the citric acid cycle, could act competitively to the pathway leading to ketogenesis and they may be regarded therefore as potentially antiketogenic. Fritz<sup>5</sup> suggested that an increased capacity of the liver to esterify free fatty acids diverts some of the influx from the oxidative pathway leading to ketogenesis. Glycerol was later shown to be antiketogenic with respect to oleic acid, which is esterified readily, but not with respect to caprylic acid, which is not esterified readily<sup>6</sup>. Exton and Edson<sup>7</sup> have demonstrated that the antiketogenic effect of sorbitol or fructose on the oxidation of <sup>14</sup>C-palmitate by liver slices is accounted for, partly, by incorporation of label into esterified fatty acids. We considered that a study of

the factors affecting the fate of free fatty acids in the isolated intact organ perfused with whole blood would give information reflecting more accurately the mechanism of hepatic ketogenesis and antiketogenesis *in vivo*. An isotopic balance was drawn up between the esterification of labelled free fatty acids on the one hand, and their oxidation on the other, in livers from fed or fasted animals perfused with three different concentrations of free fatty acids.

The perfusion apparatus and method of perfusion of the liver have been described elsewhere<sup>8</sup>. Livers from male rats (weighing 340–360 g) were perfused with whole defibrinated rat blood, care being taken to exclude heparin from both the liver donors and the perfusate to avoid the activation of lipoprotein lipase<sup>4,9</sup>, which catalyses the hydrolysis of lipoprotein triglycerides to free fatty acids. Livers were infused for 90 min with either 150 mg, 50 mg or a trace amount of oleic acid-1-<sup>14</sup>C complexed to bovine serum albumin. The infusion was given for the first 5 min as a priming dose followed by an infusion at one-tenth the rate of the priming dose for the remaining 85 min. The purpose of the priming infusion was to establish as rapidly as possible a steady concentration of labelled free fatty acids in the perfusate. During the course of the perfusion serial analyses were carried out for respiratory <sup>14</sup>CO<sub>2</sub> (ref. 8) and for <sup>14</sup>C-ketone bodies in blood<sup>10</sup>. Serum lipoproteins were separated into  $d < 1.006$  (very low density lipoproteins or VLDLP) and  $d > 1.006$  fractions by ultracentrifugation. <sup>14</sup>C-lipids were separated into classes by silicic acid chromatography and counted as described before<sup>2</sup>.

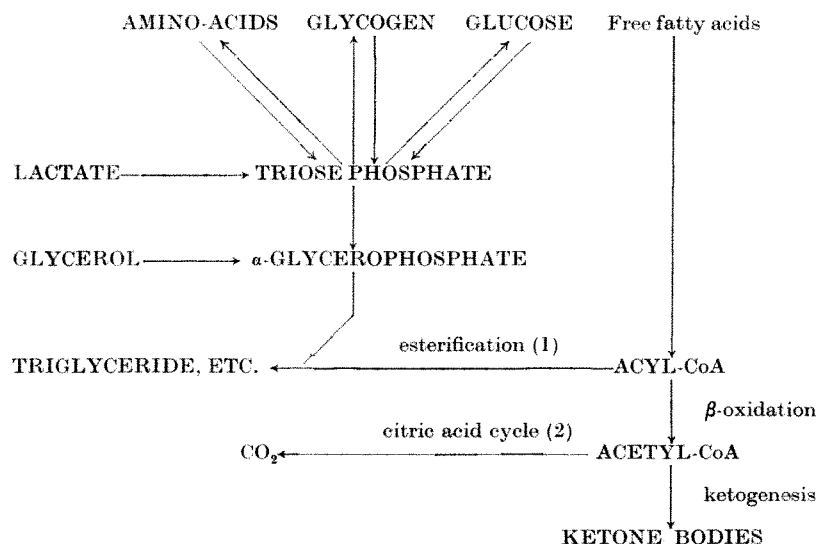


Fig. 1.

Table 1. FATE OF INFUSED FREE FATTY ACIDS (<sup>14</sup>C-OLEATE) IN PERFUSED LIVERS

Nutritional state of liver	Fed			Fasted (24 h)		
	Trace (2)	50 mg (1)	150 mg (2)	Trace (2)	50 mg (1)	150 mg (2)
Weight of free fatty acids infused						
Esterified products						
In liver	51.4 (45.6-57.2)	20.1	28.8 (26.4-31.2)	15.1 (10.0-20.2)	14.4	15.0 (14.2-15.8)
In plasma lipoproteins						
<i>d</i> < 1.006 (VLDLP)	18.2 (12.4-24.1)	22.9	3.3 (3.0-3.6)	0.4 (0.3-0.5)	4.8	1.7 (1.4-1.9)
<i>d</i> > 1.006	1.7 (1.3-2.1)	1.5	1.6 (1.5-1.7)	1.8 (1.7-1.9)	2.7	2.2 (2.0-2.4)
Total	71.3	44.5	33.7	17.3	21.9	19.9
Oxidized products						
In carbon dioxide	2.4 (1.9-2.9)	5.7	6.2 (5.0-7.3)	18.4 (12.4-24.4)	14.5	4.3 (3.3-5.2)
In total ketone bodies	0.8 (0.0-1.6)	4.6	4.2 (3.3-5.0)	5.0 (3.3-6.7)	18.3	19.9 (19.9-19.9)
Total	3.2	10.3	10.4	23.4	32.8	24.2
Total carbon-14 esterified and oxidized	74.5	54.8	44.1	50.7	54.7	44.1
Labelled free fatty acids remaining in perfusate	19.9 (14.5-25.4)	15.7	18.7 (17.5-20.0)	22.1 (22.0-22.2)	27.2	21.0 (20.2-21.8)
Total carbon-14 recovered	94.4	70.5	62.8	72.8	81.9	65.1

Table shows percentages of infused labelled oleate recovered in 90 min.

The number of perfusions and experimental variation between duplicate perfusions is shown in parentheses.

The fate of labelled free fatty acids infused into isolated perfused rat livers is summarized in Table 1. Those labelled acids remaining in the perfusate after 90 min of perfusion were slightly less than 20 per cent of the total dose infused in the fed livers and slightly more than 20 per cent in the fasted livers. In these perfusions, the concentration of serum free fatty acids in the steady state established by the infusion ranged from 0.2 to 1.5  $\mu$ moles/ml. depending on the mass infused. There was a constant rate of blood flow in all perfusions and so these results show that in both fed and fasted livers there is a constant fractional uptake of free fatty acids by the perfused liver for most of the range of concentrations of free fatty acids in the plasma encountered *in vivo*. For any given infusion of labelled free fatty acids the fed livers always esterified more of the influx than the fasted livers and the latter always oxidized more than the former. In the livers infused with 50 mg or 150 mg of <sup>14</sup>C-oleic acid, the total carbon-14 found in esterified plus oxidized products of metabolism was similar in livers from fed and fasted rats for each quantity of free fatty acids infused. The balance of the label not esterified in the fasting livers compared with the fed livers was completely accounted for by the extra label recovered in oxidized products, particularly in ketone bodies. Clearly, there is a reciprocal relationship in the liver between the esterification of incoming free fatty acids and their oxidation.

In livers from fed rats the proportion of the free fatty acids esterified was inversely related to the mass of the acids infused (Table 1). A different pattern of esterification was found in fasted livers. Not only was less of the influx esterified but, in contrast, the fraction of the labelled fatty acid esterified was constant irrespective of the mass of fatty acids infused. It has been generally considered that the availability or concentration of  $\alpha$ -glycerophosphate in a tissue is a primary factor influencing the rate of esterification of fatty acids<sup>5,6,11</sup>. This concept, however, has been challenged in respect of muscle<sup>12</sup> and adipose tissue<sup>13</sup>. Nevertheless, with respect to the liver Bortz and Lynen<sup>14</sup> have produced direct evidence that the concentration of  $\alpha$ -glycerophosphate in livers from fasting rats is depressed compared with the concentration in livers from fed animals, and Wieland and Matschinsky<sup>6</sup> have shown that the addition of carbohydrate (fructose) to the perfusate of isolated livers increases the concentration of  $\alpha$ -glycerophosphate in the tissue.  $\alpha$ -Glycerophosphate is derived in part from intermediates of glycolysis, and so it is probable that in the present experiments the high content of glycogen in the livers of fed rats ensured a high concentration of  $\alpha$ -glycerophosphate, which in turn could account for the greater and characteristic pattern of esterification reported in these livers. While the lower rate of esterification found in the livers from fasted rats can be attributed to a decreased concentration of  $\alpha$ -glycerophosphate, however, the pattern of esterification, showing that a constant fraction (about 20 per cent) of the free fatty acids utilized was esterified, irrespective of its mass, is difficult to explain solely in terms of a low concentration of  $\alpha$ -glycerophosphate. It must be concluded that there are sufficient

precursors of  $\alpha$ -glycerophosphate to maintain this small but constant fractional esterification, even in the face of a large influx of free fatty acids.

A greater proportion of the labelled oleic acid taken up by the liver was returned to the perfusate as lipoproteins in the livers from fed rats than from fasted rats. Most of the label secreted in lipoproteins was found in the very low density fraction (*d* < 1.006) in confirmation of previous results using intact rabbits<sup>2</sup>. As the influx of free fatty acids into the liver increased, so the mass of labelled fatty acid oxidized increased in both fed and fasting livers. The proportion of label entering ketone bodies compared with the proportion entering carbon dioxide also increased, particularly in livers from fasting rats.

For the administration of the radioisotope in the experiments described, the "constant infusion" procedure (as against the "single dose" procedure) was employed to minimize effects on the results caused by variations in the size of intermediary pools and to allow time for equilibration of label in side pools of the main pathways. The substantial total recovery of label (Table 1) indicates that these factors would not significantly affect the general conclusions concerning these data. To eliminate misinterpretation, however, we have examined preparations that were in a completely isotopic steady state. This necessitated taking biopsies from the liver.

In two perfusions, one with a liver from a fed rat and the other from a fasting rat, 150 mg of labelled oleic acid was infused for 90 min as described previously. Within 30 min constant specific activity of the serum free fatty acids was achieved. Samples were taken, including biopsies, after 60 min and after 90 min and analysed for labelled products of the metabolism of labelled free fatty acids. The radioactivity which accumulated in all the measured products in 60-90 min could be equated completely with the radioactivity of the oleic acid removed from the perfusate during this time (Table 2), indicating that all pathways of quantitative importance in the metabolism of free fatty acids by the liver were being measured. While the data in Table 1 indicate the relative importance of the various pathways being exam-

Table 2. FATE OF INFUSED FREE FATTY ACIDS (<sup>14</sup>C-OLEATE) IN PERFUSED LIVERS

Nutritional state of liver	Fed	Fasted
Products of esterification		
(a) In liver		
Triglyceride	56.5	26.0
Phospholipid	6.1	13.9
(b) In perfusate lipoproteins		
VLDLP ( <i>d</i> < 1.006)	12.1	4.8
LDLP + HDLP ( <i>d</i> > 1.006)	3.3	6.9
Total	78.0	51.6
Products of oxidation		
Respiratory carbon dioxide	12.4	6.6
Respiratory carbon dioxide derived from decarboxylation of aceto-acetate	0.4	0.5
Respiratory acetone	0.3	0.5
Blood total ketone bodies	7.7	37.6
Total	20.8	45.2
Total recovery	98.8	96.8

Percentage of <sup>14</sup>C-oleate utilized between 60 and 90 min appearing in metabolic products (150 mg infused in 90 min).

ined, the data in Table 2 indicate the quantitative significance of the pathways, because they are being assayed in a system in complete isotopic equilibrium. These data show that at least 50 per cent of the influx of free fatty acids into the liver, even in the fasting state, is esterified. This fraction approaches 80 per cent in the fed preparation. The reciprocal relationship between esterification on the one hand, and oxidation on the other, confirms the data in Table 1. The partition of the labelled molecules between oxidation to carbon dioxide and oxidation to ketone bodies indicates that oxidation of fatty acid through the citric acid cycle becomes less as ketogenesis increases. These results agree with those of Wieland *et al.*<sup>15</sup>, who observed an inhibition of the oxidation of <sup>14</sup>C-acetate to labelled carbon dioxide in liver slices from ketotic rats, but are at variance with the results of Ontko<sup>16</sup>, who found that oxidation of labelled palmitate to labelled carbon dioxide in liver homogenates was not diminished, but increased asymptotically as the concentration of fatty acid in the medium was increased.

In the six perfusions reported in Table 3, we have calculated and compared the energy yield, as adenosine triphosphate (ATP) resulting from the utilization by the liver of 1 mole of oleic acid. Although the fasting livers oxidized nearly twice as much of the labelled oleic acid as the fed livers, both obtained approximately the same energy from each mole of free fatty acid utilized, because of oxidation of fatty acid through the citric acid cycle which yielded more energy than partial oxidation to ketone bodies. Thus it seems that when the liver is presented with a quantity of fatty acid for oxidation, the molecules are so distributed between the efficient pathway (the citric cycle) and the inefficient pathway of oxidation (ketogenesis), that the total energy yield from the fatty acid remains constant. Apparently the liver is enabled to oxidize an almost unlimited quantity of fatty acid, without increasing its energy production, merely by diverting more of the oxidation from the production of carbon dioxide to ketogenesis. The limit in the quantity of fatty acids that could be oxidized by the liver would be reached theoretically when all molecules were being oxidized to ketone bodies and especially to  $\beta$ -hydroxybutyrate. Further oxidation of fatty acid could only take place by increasing the oxidative requirements of the liver or by means of a mechanism similar to the uncoupling of oxidation and phosphorylation. It has been suggested that a way in which the liver is enabled to change from the oxidation of fatty acids through the citric acid cycle, to ketogenesis, may involve the inhibition of citrate synthase by an increase in the steady state concentration of intramitochondrial ATP<sup>17</sup>.

Studies subsequent to those reported have shown that the oxidation of the infused labelled free fatty acids (Table 2) accounts for approximately two-thirds of the total oxygen consumption of either the fed or starved perfused liver. A respiratory quotient of 0.99 for fed perfused livers (not infused with fatty acids) suggests

that these livers oxidize largely carbohydrate<sup>18</sup>. Present results indicate therefore that when free fatty acids are infused into the fed liver in the conditions described, they displace much of the available carbohydrate as the chief fuel of respiration. This conclusion is similar to that of Randle *et al.*<sup>19</sup>, who observed that free fatty acids depress glucose utilization in rat heart and diaphragm. The oxygen consumption of perfused livers from fed and fasted rats is similar<sup>18</sup> and we have found that it remains essentially the same when a concentration of free fatty acids comparable to that used in the present experiments is infused. It seems probable that in the present conditions, oxidation and phosphorylation in the liver remain tightly coupled when free fatty acids are infused. Because the fasting livers produce substantially more ketone bodies than the fed livers, our results do not support the hypothesis<sup>20</sup> that uncoupling of oxidation and phosphorylation by free fatty acid is a cause of ketogenesis.

Results from this and previous studies<sup>21</sup> suggest that ketogenesis is controlled by metabolism in both adipose tissue and the liver. The primary factor appears to be the magnitude of the flux of free fatty acids entering the liver which is determined by the rate of release of the acids from adipose tissue. The principal hepatic factor responsible for antiketogenesis is the capacity of the liver to esterify incoming fatty acids, which in turn is related to the availability of carbohydrate, the esterifying capacity of livers from fed animals containing appreciable quantities of glycogen, being superior to those from fasted animals containing no glycogen. The degree of ketogenesis is determined subsequently by the magnitude of the flux of fatty acids which escape esterification. The acetyl-CoA formed in  $\beta$ -oxidation of fatty acids can enter either into the high energy yielding, non-ketogenic pathway of oxidation to carbon dioxide, or two molecules can condense to enter the low energy yielding, ketogenic pathway. The factor which determines the relative rates of each pathway appears to be the total energy requirement of the liver. Thus as more fatty acids flood the pathway of  $\beta$ -oxidation, more enter the pathway of ketogenesis and less enter the citric acid cycle, controlled in such a manner that the total yield of energy from oxidation remains constant.

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Table 3. ENERGY DERIVED FROM OXIDATION OF FREE FATTY ACIDS (LABELLED <sup>14</sup>C-OLEATE) IN PERFUSED LIVERS UNDER STEADY STATE CONDITIONS

	Percentage of <sup>14</sup> C utilized			Fasting livers		
	(a)	(b)	(c)	(d)	(e)	(f)
In respiratory carbon dioxide	12.4	12.7	11.8	6.6	6.9	6.2
In total ketone bodies	8.4	6.6	10.7	38.6	30.8	21.7
$\beta$ -Hydroxybutyrate	6.5	4.7	4.2	34.2	7.7	5.0
Acetoacetate	1.9	1.9	6.5	4.4	23.1	16.7
Energy yield (calculated)						
(Moles of ATP/mole of oleate utilized)						
Through CO <sub>2</sub> pathway (citric acid cycle)	17.7	18.1	16.9	9.4	9.9	8.9
Through ketone body pathway	2.1	1.7	3.2	8.9	9.8	7.0
Total	19.8	19.8	20.1	18.3	19.7	15.9

Oleic acid was infused at the rate of 150 mg in 90 min. In experiments a, b, and d, the PO<sub>2</sub> of the blood entering the liver was 70 mm mercury. In experiments c, e, and f, the PO<sub>2</sub> was 95 mm mercury. The calculations are based on the <sup>14</sup>C-oleate utilized over a period of 30 min in the steady state. The percentage of the label appearing in respiratory CO<sub>2</sub> and in total ketone bodies has been corrected for decarboxylation of acetoacetate.

## LETTERS TO THE EDITOR

## ASTRONOMY

## Large Scale Density Inhomogeneities in the Universe

Sachs and Wolfe have recently considered the possibility of large scale density inhomogeneities in the universe with characteristic dimensions of the order of  $10^8$  parsecs.<sup>1</sup> The suggestion<sup>2</sup> that quasi-stellar sources with highly red-shifted spectra are not isotropically distributed has aroused interest in large inhomogeneities. Rees and Sciama<sup>3</sup> have suggested that the tendency to cluster of quasi-stellar sources with large  $z$  implies that the mass of the universe may not be homogeneously distributed on a scale of  $z \sim 1$ . Although the observational status of the anisotropy of the distribution of these sources is in some doubt<sup>4</sup>, we have recently obtained results which appear to set rather stringent limits on the magnitude of possible density inhomogeneities in the universe.

The recently discovered cosmic microwave background<sup>5-8</sup> originates at an epoch corresponding to a very large red-shift, and thus provides a powerful tool for investigating the isotropy and homogeneity of the universe on the largest scale. We have already pointed out<sup>9</sup> that our early results set limits of the order of 10 per cent to the large scale density inhomogeneities of the type considered in ref. 1. Conklin and Bracewell<sup>10</sup> have shown that inhomogeneities greater than 0.2 per cent in the background radiation do not appear to be present on a scale of angular resolution of the order of  $1^\circ$ , at least in the restricted area of the sky they surveyed.

For the past fifteen months, we have measured the isotropy and homogeneity of the cosmic microwave background. A Dicke radiometer operating at 3.2 cm wavelength was specially designed for this experiment<sup>9</sup>. Our measurements were made by comparing the temperature of the background microwave radiation along a full circle running  $8^\circ$  south of the celestial equator with the temperature measured at the north celestial pole, a fixed point in the sky. The temperature difference is displayed as a function of right ascension in Fig. 1, in which each point represents an average of the data obtained from about eighty runs, each of which lasted about 24 h. Before the results were averaged, a constant instrumental offset,  $C_{24}$ , was subtracted from the data of each run (see ref. 9). The temperature difference was averaged over an hour

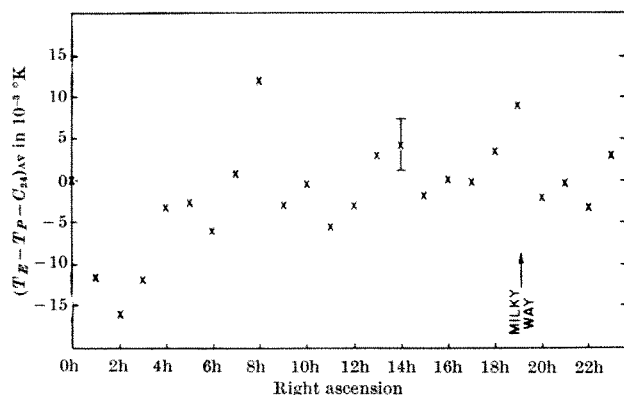


Fig. 1. Changes in the temperature of the background radiation along a circle parallel to the celestial equator at  $-8^\circ$  dec. are displayed. The measurements were made with a 3.2 cm radiometer having an angular resolution of about 1 h in right ascension.

in sidereal time because this is approximately the angular resolution of our horn antenna. It can be seen from Fig. 1 that the largest departure from zero is of the order of  $0.016^\circ$  K, which should be compared with  $3^\circ$  K, the assumed value for the mean temperature of the microwave background<sup>6-8</sup>. Most of the random error in the data arises from fluctuations in the radiation temperature of the atmosphere; we estimate that this random error is about  $\pm 0.003^\circ$  K.

The passage of the central region of the Milky Way through our beam may be indicated by the slight increase in  $T_E$  at 19 h R.A. At  $\sim 2$  h R.A. and near the celestial equator, there appears to be a more extended region of lower temperature. This feature closely corresponds in position and extent to one of the clusters of quasi-stellar sources suggested by Rees and Sciama<sup>3</sup>. Because the depth of this feature is only a few times the estimated error for a single point, however, its existence cannot yet be regarded as well established.

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## PLANETARY SCIENCE

## Luni-Solar Daily Variations of the Geomagnetic Field at Tananarive during the International Geophysical Year

A PROGRAMME for the analysis of IGY data from magnetic observatories for luni-solar variations has recently been extended to the observatory at Tananarive, Madagascar ( $18^\circ 55' \text{ S}$ ,  $47^\circ 33' \text{ E}$ ). This site is of particular interest because it lies at dip latitude  $\varphi = -34.8^\circ$  ( $\tan \varphi = \frac{1}{2} \tan I$ , where  $I$  is the magnetic dip). No data between  $\varphi = -24^\circ$  (St. Helena,  $D$  only) and  $\varphi = -47^\circ$  (Cape Town, Hermanus) have previously been analysed. In addition Tananarive is an inland observatory, so it is unlikely to have an anomalous luni-solar variation in  $Z$ , as is the case for coastal observatories<sup>1</sup>.

The data consisted of instantaneous values of  $D$ ,  $H$  and  $Z$  measured every 2 h at odd hours of Universal Time from July 1, 1957–December 30, 1958. Because of breaks in the records, August 22 and 23, 1958, were omitted for all elements and August 13 and 14, 1958, for  $D$ . Also, for reasons considered earlier<sup>1</sup>, the five International Disturbed Days of each month were omitted.

The method of analysis and derivation of vector probable errors followed that described by Leaton, Malin and Finch<sup>2</sup>, which is closely similar to that of Chapman and Miller<sup>3</sup>. The object of the analysis was the calculation of  $l_n$  and  $\lambda_n$ , the amplitude and phase of  $L_n$ , the luni-solar daily harmonic

$$L_n = l_n \sin(nt - 2v + \lambda_n)$$

where  $n$  is a small integer,  $t$  is the mean solar time measured from local midnight and  $v$  is a measure of the epoch in a lunation which increases from 0 at one new moon to  $2\pi$  at the next.



Each element was analysed as a whole ( $Y$ ) and also as subdivided into Lloyd's seasons: November, December, January and February ( $W$ ); March, April, September and October ( $E$ ); May, June, July and August ( $S$ ). It should be noted that, in the southern hemisphere,  $S$  corresponds to winter and  $W$  to summer months. The results are presented in Table 1.

As usual, the  $L_2$  terms are by far the most important, and most of the determinations of  $l_2$  are significantly different from zero at the 5 per cent level. There are also a number of significant determinations of  $l_1$  and  $l_3$ , including a large value of  $l_3$  for  $D$  ( $W$ ).

Table 1. TANANARIVE LUNAR TERMS

	No. of days	$l_1$ ( $\gamma$ )	$p.e.$ ( $\gamma$ )	$\lambda_1$ ( $^\circ$ )	$l_2$ ( $\gamma$ )	$p.e.$ ( $\gamma$ )	$\lambda_2$ ( $^\circ$ )	$l_3$ ( $\gamma$ )	$p.e.$ ( $\gamma$ )	$\lambda_3$ ( $^\circ$ )	$l_4$ ( $\gamma$ )	$p.e.$ ( $\gamma$ )	$\lambda_4$ ( $^\circ$ )
Declination													
$Y$	457	0.46	0.37	250	1.88	0.29	126	0.81	0.20	282	0.27	0.14	63
$W$	150	1.64	0.56	266	4.61	0.40	108	2.73	0.27	277	0.75	0.16	93
$E$	153	0.87	0.51	257	1.59	0.45	137	0.48	0.32	285	0.47	0.26	50
$S$	154	1.01	0.49	123	0.93	0.35	213	0.51	0.30	76	0.35	0.21	281
Horizontal intensity													
$Y$	455	0.70	0.48	137	0.77	0.22	52	0.25	0.27	265	0.11	0.16	57
$W$	150	1.32	0.76	118	0.60	0.34	50	0.58	0.35	273	0.16	0.20	347
$E$	153	1.25	0.90	145	0.63	0.41	74	0.09	0.48	227	0.13	0.32	0
$S$	152	0.67	0.92	268	1.35	0.40	38	0.18	0.40	241	0.35	0.27	105
Vertical intensity													
$Y$	457	0.47	0.15	23	1.48	0.12	174	0.04	0.08	184	0.02	0.06	300
$W$	150	0.77	0.25	36	1.93	0.20	180	0.24	0.14	357	0.09	0.07	185
$E$	153	0.84	0.28	30	1.59	0.17	174	0.08	0.13	249	0.13	0.11	76
$S$	154	0.32	0.22	249	0.99	0.15	163	0.27	0.11	164	0.19	0.08	296

$p.e.$ , Vector probable error.

Harmonic dials of  $L_2$  are shown in Fig. 1 for Tananarive (filled circles) and for the British Isles (open circles). The British Isles values are means deduced from analyses of IGY data at Eskdalemuir, Hartland, Lerwick and Valentia<sup>1</sup>, and are included for comparison. The dial vectors are indicated only by their end points.

The distribution of the seasonal values of  $L_2$  ( $D$ ) about the mean is closely similar to that obtained in the British Isles, although the range of nearly  $5\gamma$  from summer to winter at Tananarive is much greater.  $L_2$  ( $H$ ) also shows a similar pattern to that obtained for the British Isles,

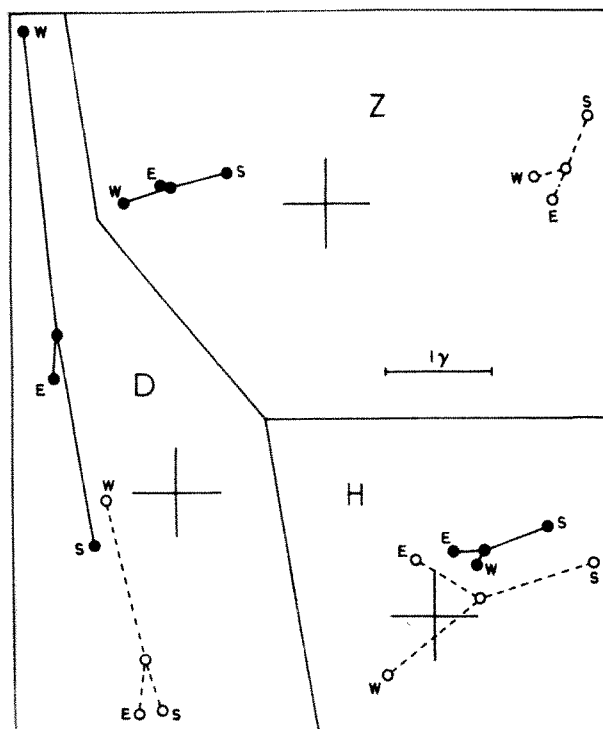


Fig. 1. Harmonic dials of  $L_2$  for Tananarive (●) and the British Isles (○). Seasonal values are marked with a letter. Points with no letter refer to the full year.

with the ends of the  $L_2$  ( $W$ ),  $L_2$  ( $E$ ) and  $L_2$  ( $S$ ) vectors arranged clockwise about the  $L_2$  ( $Y$ ) value. In this case, however, the range of the seasonal variation is less at Tananarive than in Britain.  $L_2$  ( $Z$ ) shows a significant increase in amplitude from  $S$  to  $W$  at Tananarive. As might be expected for a southern observatory, the sign of  $L_2$  ( $Z$ ) is opposite to that for Britain.

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### Daily Variation of Total Magnetic Field over the British Isles

THE use of observatory data as a basis for the correction of diurnal variation in magnetic surveys is common both for secular repeat stations spread over a wide area, and in ground and marine magnetic surveys where limitations of personnel and equipment prohibit the maintenance of a local base. Geographical interpolation of the variations is usually based on local time and latitude, the errors involved in this procedure being assumed to be small. In 1918, Walker<sup>1</sup> stated that such interpolation was incorrect, although he was prevented by the First World War from carrying out investigations to provide a more rigorous alternative. So far as I am aware, the situation today is little changed, at least in the British Isles.

During the reduction of a total field survey in North-West Ireland, comparisons between a local diurnal base station and Valentia Observatory suggested that differences were neither small nor clearly dependent on local time but were nevertheless systematic. It was therefore decided to investigate on a larger scale the inter-relationships of the daily variations over the British Isles with a view to determining whether the common interpolation method did provide agreement with the observations and to assessing the errors involved in using it.

Owing to the time lag in production of year books and to the closing down of Abinger Observatory, it was found most convenient to use published observatory data for the years 1956 and 1957. The figures used were those for the "Mean Diurnal Inequalities", that is the hourly means expressed relative to the mean of the period 0001–2359 G.M.T. Data from the French Observatories differed slightly from this, the tables in the year books giving the actual observed value at the hour rather than a mean, centred at 30 min past. The periods considered were for all days of the year and for the International Quiet Days of the summer (that is May, June, July and August), the latter probably being the period when the signal to noise ratio of the solar daily variation would be at a maximum.

Comparison of the phase and amplitude of the field variations at two stations was made by direct cross-multiplication and the calculation of the correlation coefficient, the process being simplified by the fact that the mean of the twenty-four values was by definition zero. As the use of the hourly values could only give a phase comparison to the nearest half hour, it was found necessary to interpolate values at 6 min intervals from a smoothed curve drawn by eye through the hourly means. Although this could introduce an unwelcome subjective element into the calculations, the pattern and consistency of the results would seem to justify the use of this smoothing in a preliminary analysis. By progres-

sively cross-multiplying the twenty-four values for one station with the series of twenty-four values of another station obtained at the hour, 6 min past, 12 min past, etc., the position of maximum correlation between the two curves could be placed to within  $\pm 2$  min. The mean of the two linear regressions obtained was taken as the measure of the amplitude relationship. The smallest value of the maximum correlation coefficient between two stations for all the comparisons was 0.88, still highly significant for twenty-four values.

Having thus obtained a network of links between stations, values of phase and amplitude at any particular station were expressed relative to Valentia Observatory by calculating a weighted mean for the station of the phases and amplitudes indicated by the link or links between it and Valentia, the weighting being proportional to the correlation coefficients of the links involved. The range of values for any one station, given by these links before taking the mean, was of the order of  $\pm 3$  min for phase and  $\pm 1$  for amplitude where Valentia is taken as 100 units.

The results pertinent to 1956 and 1957 from six observatories are shown in Table 1. As far as their consistency is concerned, there is a marked difference between the values obtained for the International Quiet Days of

the summer and those for All Days of the year. Within these two categories consistency is fair, apart from the phase values at Nantes and the International Quiet Days amplitude values at Chambon. Clearly the variations of the calculated values indicate that the relationships between stations are by no means constant but can change considerably in response to factors which are not at this stage possible to evaluate. Similar calculations on a series of observations made near Dublin suggest that in fact these relationships may even change from day to day. Using these data, Fig. 1, although it contains some values for one year only, shows the resultant contour maps of mean phase and amplitude for All Days of 1956 and 1957 and for the International Quiet Days of the summers of the same years. Also included in brackets are values of phase and amplitude obtained from the data given by Hill and Mason<sup>2</sup> for the one day, 26.6.61, at Ivybridge (near Plymouth) and Buoy 4 ( $47^{\circ} 22' N.$ ,  $07^{\circ} 37' W.$ ). This particular day, while not designated as an International Quiet Day, was not disturbed.

Despite the paucity of stations and the consequent uncertainty of contouring, two chief features seem to be evident in these maps. First, the amplitude of the daily variation of total field decreases eastwards; second, the

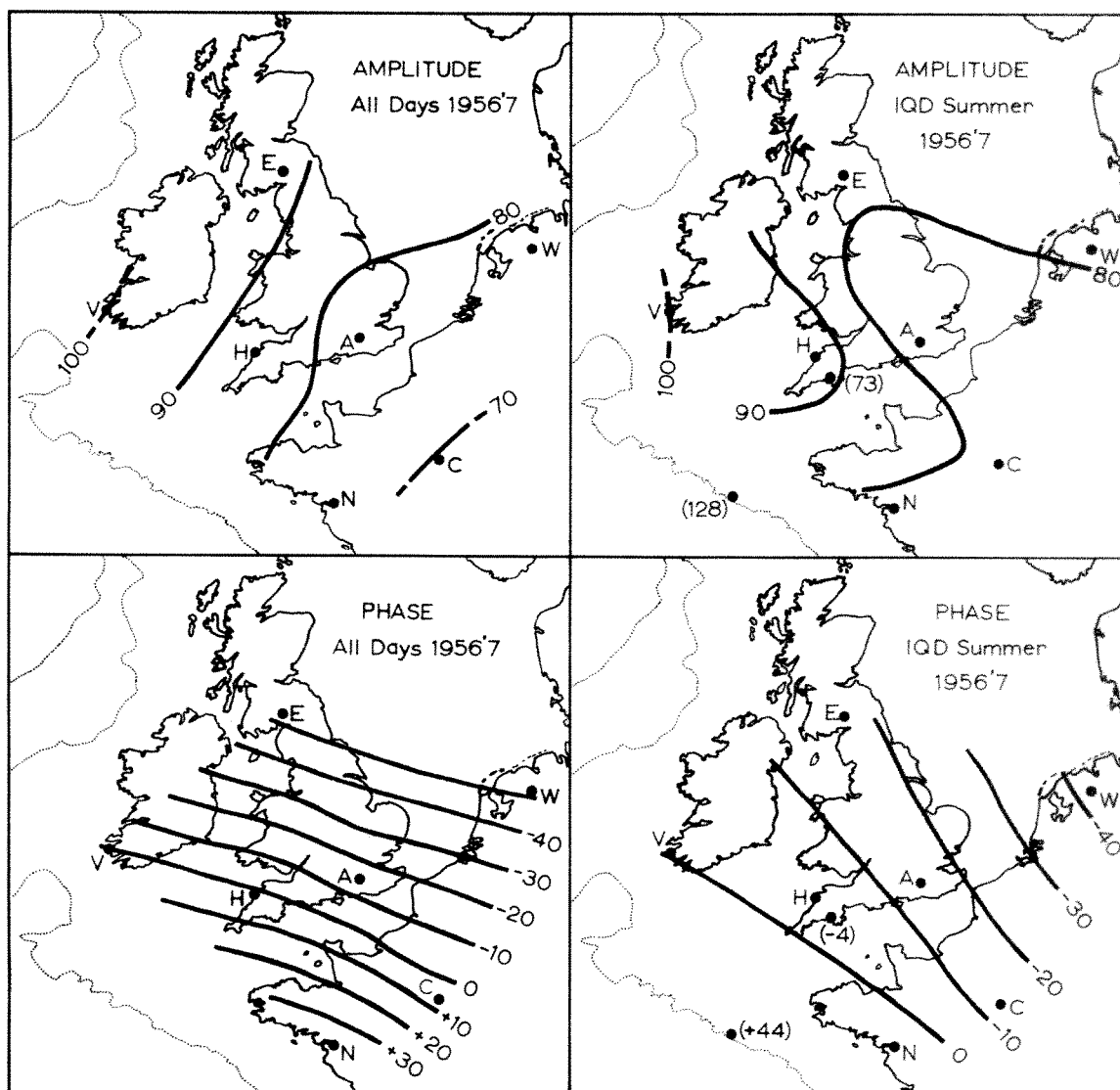


Fig. 1. Contours of phase and amplitude of diurnal variations of total field derived by linear interpolation from the data of Table 1. Amplitude relative to Valentia at 100 units, phase relative to Valentia in minutes; "before" shown negative and "after" positive. 1,000 fathom ocean contour shown dotted, observatories by initial letter.

Table 1. PHASE AND AMPLITUDE OF DIURNAL VARIATIONS OF TOTAL FIELD

Observatory	All days 1956	All days 1957	IQD Summer 1956	IQD Summer 1957
	Amplitude (Valentia 100 units)			
Valentia	100	100	100	100
Eskdalemuir	94.9	92.8	81.8	79.2
Hartland		86.6		93.4
Abinger	72.9		71.8	
Nantes	74.2	68.8	75.2*	80.8*
Chambon Le Foret	70.6	69.1	73.2	80.9
Witteveen		76.4		81.9
Phase relative to Valentia (minutes "before" are negative, "after" are positive)				
Valentia	0	0	0	0
Eskdalemuir	-51	-55	-16	-16
Hartland		+2		-2
Abinger	-15		-15	
Nantes	+29	+47	0*	+14*
Chambon Le Foret	+6	+7	-15	-11
Witteveen		-51		-42

\* Obtained from All Days of the summer because IQD data are not published.

contours of equal phase run approximately north-west-south-east, so that, for instance, the variation at Hartland occurs almost simultaneously with that at Valentia. Taking the contours for All Days as the norm around which other contour patterns will occur, the pattern contrasts considerably with the accepted "local-time" situation and shows some interesting correlations with the type of pattern predicted and discussed by Roden<sup>3</sup> for the edge of an ocean. In particular, the parallelism of the phase contours to the edge of the continent in the south-west as shown by the 1,000 fathom ocean contour, could compare in character with the model variations obtained by Roden for the Western Pacific. The amplitude contours show a decrease away from the edge of the Atlantic Ocean of the same order as predicted by Roden but are not so strikingly parallel to this 1,000 fathom ocean contour line.

A fuller analysis of the geography of the diurnal variations is obviously necessary before the theoretical implications can be properly discussed; however, the data calculated so far do suggest that the "local-time" method of applying diurnal variations over a wide area may be in need of modification, particularly in the light of the increased use of proton magnetometers giving absolute values of the field to  $\pm 1$  gamma. It is hoped

that an estimation of the relevance of shorter period (7-14 days) observations of phase and amplitude relationships to the overall picture may provide a basis for survey work from which a more detailed map of their normal distribution can be built up.

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## Surface Air Radioactivity and Ozone at Amundsen-Scott Station (90° S.), Antarctica

THE Amundsen-Scott station located at the geographical South Pole offers, by virtue of its extreme isolation from man-made pollution, a unique setting for the study of atmospheric tracers. In addition, the permanent snow cover provides for the atmosphere a homogeneous, inert and unchanging lower boundary.

Continuous measurements of surface air radioactivity levels from 1959 to 1963 at the South Pole have previously been reported<sup>1</sup>. Additional data up to 1965, obtained with the same equipment, are presented. Surface ozone records for the period from 1962 to 1965 taken with two types of instruments, a Regener chemiluminescent device<sup>2</sup> and a Brewer-Mast wet chemical detector<sup>3</sup>, are also presented.

The monthly means for the airborne radioactivity (fission products), in c.p.m. (uncorrected) and in pc./100 m<sup>3</sup>, are shown in the lower half of Fig. 1. Above appear the monthly means of the surface ozone concentration, in parts of ozone per hundred million parts of air (p.p.h.m.), for the two types of instruments used. A comparison between the curves discloses a yearly oscillation of the fission products and of levels of ozone concentration in the surface air at Amundsen-Scott; furthermore, the respective oscillations are opposite in phase.

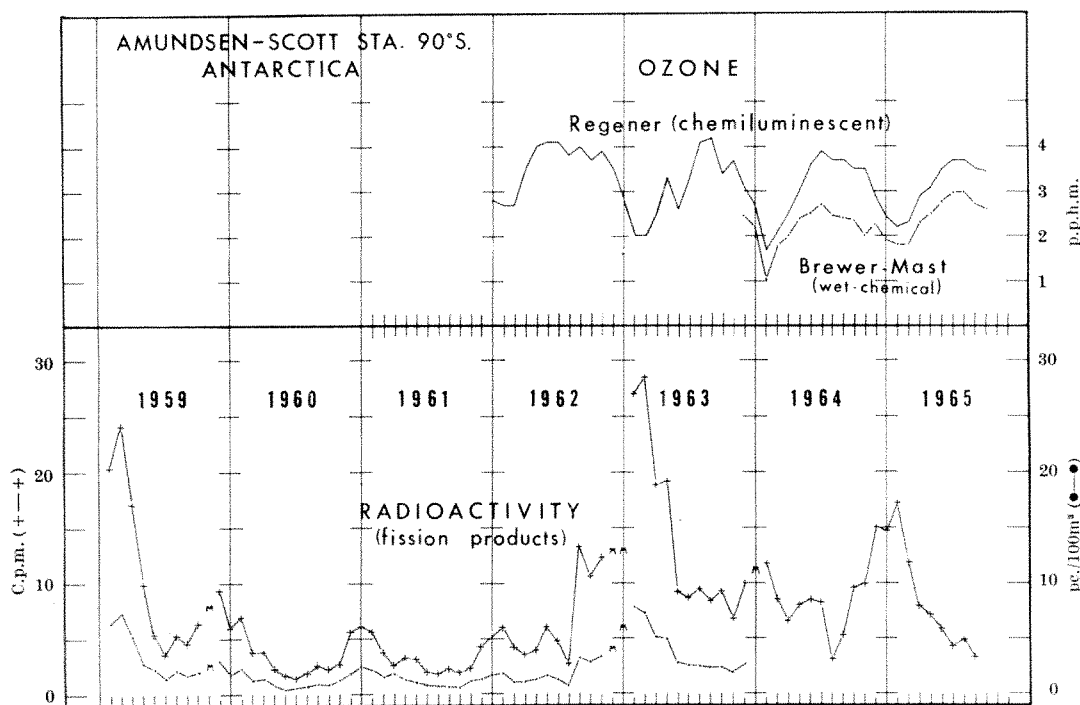


Fig. 1. Monthly means of ozone, in p.p.h.m., and radioactivity, in counts per minute (c.p.m.) or pico-curies per 100 m<sup>3</sup> (pc./100 m<sup>3</sup>) in the surface air at Amundsen-Scott station (90° S.).

In the middle latitudes of the northern hemisphere the time difference at the surface between the seasonal maxima for the same two tracers is approximately one month, with the radioactivity peak lagging<sup>4</sup>.

The six-month phase difference noted at the South Pole merits further investigation. It is possible that seasonal variations in the atmospheric exchange between the middle latitudes and the polar regions are somehow controlling the observed annual oscillation of ozone and radioactivity in the surface air at the South Pole.

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### Implications of the Isotopic Ages of Ignimbrite Flows, Southern Atacama Desert, Chile

In the Andean Cordillera from central Chile to southern Peru, supergene alteration has been of profound economic significance to copper mining, because the unenriched hypogene assemblages rarely attain mineable grades in present conditions. The dimensions, grade, mineralogy and preservation of supergene enrichment zones in this region largely reflect the geomorphological evolution of the mineralized areas in the period since the orebodies were originally exposed.

We have carried out geomorphological studies<sup>1</sup> in the area between the Río Huasco (28° 40' lat. S.) and the Río Salado (26° 25' lat. S.) and have shown that the early stages of topographic development in this region are represented by a succession of extensive pediplains, and, locally, by aggradation deposits, which have accumulated on these erosion surfaces, in tectonic depressions, and in transverse river valleys. Acidic volcanic flows occur on the earliest recognizable surface or topography, on the lowermost and most recent major pediplain, and on and within the aggradation deposits.

Our systematic examination of the workings of the many small copper mines in the Copiapó region (27° 20' lat. S.) has shown the existence of at least two main zones of supergene sulphide enrichment, marked by the development of massive djurleite and subordinate chalcocite, blaubleibender covelline and covelline. In most parts of the area, the lower and more persistent of the enriched zones lies at or near the surface of the lowermost pediplain, and it is clear that the supergene alteration profile was truncated during the formation of this planation surface. We propose that most enrichment took place during the extended period in which the succession of pediplains was developed, but at times when pediplanation was relatively inactive.

In order to date the evolution of the landforms and the periods of supergene mineral alteration we have collected specimens of ignimbrite for potassium argon dating. The ignimbrite carries biotite and has the composition of a sodi-potassic rhyolite. So far two specimens from differing parts of the ignimbrite flow, which lies on the lowermost pediplain in the vicinity of the Quebrada San Andrés (IGS-67: 19, IGS-67: 44) in the Río Copiapó drainage basin, and single specimens from two ignimbrite sheets exposed in the canyon of the Río Salado, have been dated. Of the Río Salado flows, one (IGS-67: 40, from the vicinity of Pueblo Hundido) is interbedded with an alluvial sequence which forms part of the aggradation within the Longitudinal Valley of northern Chile, while the other (IGS-67: 45, from Potrerillos airport) lies on the surface of the aggradation deposits, and is clearly younger.

Particular care was taken in the separation of the biotite to exclude the minor amphibole of the sparse lithic fragments of andesitic material occurring in the non-welded ignimbrites. Potassium was determined by flame photometry, and argon measured by isotope dilution with enriched argon-38 on an A.E.I. 'MS.10' mass spectrometer.

Several conclusions may be drawn from the results (Table 1). The close correspondence of the two dates obtained for the Quebrada San Andrés ignimbrite flow suggests that they directly reflect the time of consolidation of the flow, and that contamination from all sources<sup>2</sup> has been insignificant. Furthermore, the minor apparent difference between the ages of the two Río Salado flows, although approaching the limit of precision of the analyses, nevertheless probably indicates the period in which the intervening alluvium was deposited.

Table 1. RESULTS OF POTASSIUM ARGON AGE DETERMINATIONS ON BIOTITES FROM IGNIMBRITES, SOUTHERN ATACAMA DESERT, CHILE

Specimen No.	Specimen location	Potassium (wt. per cent)	Vol. of radiogenic argon-40 (c.c./g of sample, S.T.P.) ( $\times 10^6$ yr)	Age and error ( $\times 10^6$ yr)	Mean age and error ( $\times 10^6$ yr)
IGS-67: 19	26° 55' lat. S. 69° 37' long. W.	7.29	$2.781 \times 10^{-6}$	$9.5 \pm 0.5$	9.15 $\pm 0.25$
IGS-67: 44	27° 07' lat. S. 69° 43' long. W.	6.91	$2.493 \times 10^{-6}$	$9.0 \pm 0.3$	
IGS-67: 45	26° 22' lat. S. 69° 29' long. W.	7.25	$3.335 \times 10^{-6}$	$11.5 \pm 0.5$	
IGS-67: 40	26° 21' lat. S. 69° 53' long. W.	6.96	$3.443 \times 10^{-6}$	$12.6 \pm 0.5$	

Isotope abundance: <sup>40</sup>K = 0.0119 atomic per cent

Decay constants:  $\lambda\beta = 4.72 \times 10^{-10} \text{ yr}^{-1}$

$\lambda e = 0.584 \times 10^{-10} \text{ yr}^{-1}$

The new data show that eruption of ignimbrites, presumably from vents in the high Andes to the east of the area, occurred between  $12.6 \pm 0.5$  and  $9.15 \pm 0.25 \times 10^6$  yr B.P., in the Upper Miocene<sup>3</sup>. These volcanics are therefore to be included in Brüggén's 'Formación Liparítica'<sup>4</sup>, the extrusion of which has been shown to have persisted at least from  $18.7$  to  $2.9 \times 10^6$  yr B.P., in northern Chile<sup>5</sup>. Ages within this range have also recently been obtained for acidic volcanics in the region of San Pedro de Atacama, Antofagasta Province<sup>6</sup>.

The ages of the Río Salado ignimbrites show conclusively that aggradation in this area commenced appreciably earlier than  $12.6 \pm 0.5 \times 10^6$  yr B.P., and had terminated by  $11.5 \pm 0.5 \times 10^6$  yr B.P. Previous workers have suggested an Upper Pliocene<sup>7</sup> or Quaternary<sup>8</sup> age for these deposits, but our studies establish a markedly older time of formation. The dates suggest that the rate of aggradation in the Río Salado area was very slow; considerably less than 100 m of detritus was deposited in a maximum period of  $2.1 \times 10^6$  yr. Since the extrusion of the younger ignimbrite, the only apparent topographic changes in this stretch of the Río Salado valley have been the cutting of the canyon, followed by limited aggradation in the canyon floor.

The dates obtained for the Quebrada San Andrés flow indicate that the underlying, youngest pediplain in this area was in existence before  $9.15 \pm 0.25 \times 10^6$  yr B.P. Here it is again evident that the landscape of this region of the Andes has suffered remarkably little modification since the Upper Miocene; the erosion surface has been dissected, however, and restricted alluviation has occurred in the floors of the resulting shallow valleys. Recent topographic changes have, however, taken place in the coastal region which was directly affected by movements of sea level and local tectonic displacements.

On the basis of the deformation of ignimbrite flows in the San Pedro de Atacama region, Rutland *et al.*<sup>9</sup>, in agreement with earlier views<sup>8</sup>, have suggested that the chief Andean uplift occurred over an extended period from the Upper Miocene to the Pleistocene. In the southern Atacama Desert, ignimbrite flows cover a more restricted area, and a comparable ignimbrite stratigraphy is lacking, but our present evidence suggests that tectonic



activity in Pliocene and more recent times has been of little fundamental importance in this area.

On limited stratigraphic evidence, it has been suggested that the supergene enrichment of the Chuquicamata and Potrerillos porphyry copper deposits in northern Chile<sup>9,10</sup> and of deposits in southern Peru<sup>11</sup> occurred beneath an extensive erosion surface, the *Puna*, which developed in the period between the Eocene earth movements and the Andean uplift (of presumed Plio-Pleistocene age). Segerstrom<sup>12</sup>, on the other hand, proposed that enrichment in much of northern Chile resulted from the formation of a mid-Tertiary "matureland", but in our recent work we have been unable to recognize remnants of such a "matureland" in the essentially polycyclic landscape of the Copiapó region. The dating of the ignimbrites, however, yields a minimum age for the supergene alteration which does not contradict the conclusions of earlier workers. The observed inter-relationships of supergene alteration, pediplanation, and ignimbrite eruption in the Copiapó area clearly demonstrate that all important enrichment was completed before the end of Miocene times (before  $9.15 \pm 0.25 \times 10^6$  yr B.P.). Estimation of the maximum age of the enrichment must await the determination of the age of the volcanics which were extruded onto surfaces which existed before the development of the supergene alteration zones.

These new data contribute to the establishment of an absolute chronology of topographic evolution and supergene alteration in the southern Atacama Desert, and, on a broader scale, imply that important enriched zones may be preserved in copper deposits concealed beneath volcanics and aggradation of Mio-Pliocene age elsewhere in northern Chile<sup>13</sup>.

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## The Arctic Mid-Oceanic Ridge

THE Mid-Oceanic Ridge, a broad fractured arch more than 40,000 miles long, is the largest tectonic feature on the surface of the Earth. Associated with the centre

of the ridge over much of its length is an axial fracture or rift which is the locus of shallow earthquakes. An extension of this world encircling ridge into the Greenland Sea and Arctic Ocean was first hypothesized on the basis of a well defined pattern of earthquake epicentres and a few spot soundings<sup>1,2</sup>. The topographic form of the ridge was first revealed by precision depth records obtained during the pioneer polar crossings of the nuclear submarines *Nautilus*<sup>3</sup> and *Skate*<sup>4</sup>. The Arctic Mid-Oceanic Ridge does not bisect the entire Arctic Basin but lies midway between the monolithic, aseismic Lomonosov Ridge and the Eurasian continental margin.

The Arctic Mid-Oceanic Ridge is flanked by abyssal plains. The Pole abyssal plain<sup>5</sup> which lies between the ridge and the aseismic Lomonosov Ridge is deepest near the Pole; this suggests that the primary source of sediment is from the two landward ends near Greenland and off the Lena Delta. Prominent sub-bottom reflectors, usually three in number, are observed on echograms from the deepest portion of the abyssal plain (Fig. 1, profile 4). Sub-bottom layers are usually detected only near the distal ends of abyssal plains where the ponded clays form thick transoral layers.

The existence of the Barents abyssal plain between the Arctic Mid-Oceanic Ridge and the Eurasian continental shelf originally predicted by Heezen and Laughton<sup>6</sup> is confirmed by recent sounding profiles (profiles 3-5). The Barents abyssal plain has an appreciable slope from east to west indicating the dominance of the Ob and Yenisey rivers as sources of sediment<sup>6</sup>.

Within 10° of the Greenwich meridian the ridge has a width of approximately 150 miles (profiles 5 and 6). Flanking topographic highs border a depressed axial valley. This valley is strikingly developed in the area north of Spitsbergen (profile 6) where it is 2,800 fathoms deep in contrast to the adjacent rift mountains which are only 1,600 fathoms deep.

The axis of the Mid-Oceanic Ridge is characteristically offset at intervals by fracture zones which cut across the ridge at approximately right angles to its axial trend. The Arctic Mid-Oceanic Ridge ends abruptly in the continental margin of Greenland. This sudden termination (profiles 6 and 7) suggests the existence of a N.N.W. extension of the Spitsbergen Fracture Zone<sup>4</sup> which may be masked by the sedimentary blanket<sup>7</sup> of the Greenland continental margin.

The Mid-Oceanic Ridge off the Laptev and Kara Seas (profiles 2-4) is a relatively subdued feature. The flat portions of the sea floor (profile 3) might be interpreted as segments of the Pole abyssal plain elevated by the growing ridge. But the more likely interpretation seems to be that the sediment continuously poured into the Arctic Basin by the Lena, Ob and Yenisey rivers has been sufficient in quantity to bury the eastern end of the ridge continuously. The multitude of hyperbolic echoes which are reflected from the uneven surfaces of the ridge along the belts of the epicentre indicates continuing tectonic activity, but the subdued relief suggests that the growth of the oceanic crust is taking place beneath a blanket of sediments (profile 1).

Magnetic anomalies<sup>7,8</sup> over the Arctic Mid-Oceanic Ridge have very small amplitudes as compared with the Mid-Atlantic Ridge. Dementitskaya and Karasik<sup>9</sup>, however, report that a well defined pattern of linear axially oriented magnetic anomalies is associated with the ridge. The thick blanket of sediment which covers the growing ridge may raise the level of the Curie isotherm and diminish the amplitude of the magnetic anomalies. A similar dampening effect has been observed in the Red Sea<sup>10</sup> north of 23° where the axial trough is buried under a thick sedimentary cover; it has also been observed in the northern Gulf of California<sup>11</sup>.

The suggestion of Heezen<sup>12-14</sup> that the Mid-Oceanic Ridge has grown wider with time by the continuing emplacement of mantle derivatives in an ever-widening

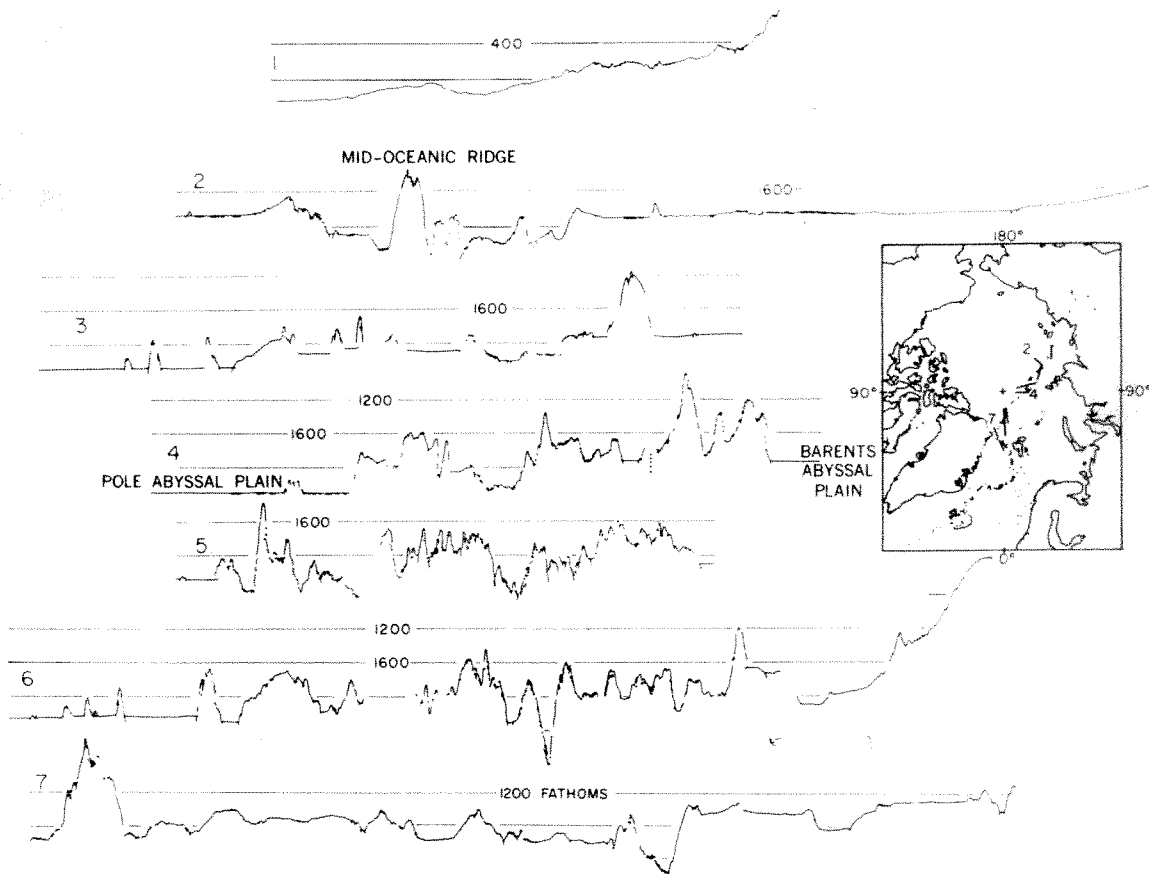


Fig. 1. Tracings of precision echograms (PDR) obtained by submarines in the Arctic. Transducer depth is not computed. Profiles 5 and 7 taken from Skate<sup>4</sup>. Small dots on index chart are earthquakes<sup>14</sup>.

rift valley has received important support from the palaeomagnetic interpretation of geomagnetic traverses of total intensity<sup>15</sup>.

Crustal growth along the present Arctic Mid-Oceanic Ridge has gradually separated the aseismic Lomonosov Ridge from the Eurasian continent. Alpha Ridge—a subdued feature which lies between the Lomonosov Ridge and the continental margin of American and western Siberia—may represent a former axis of crustal growth.

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## Carbonatite and Alkaline Igneous Rocks in the Brent Crater, Ontario

THE Brent crater (46° 04' N., 78° 29' W.) is cut into the Pre-Cambrian gneisses of central Ontario. On the surface the crater is marked by a poorly defined depression 18,000 ft. in diameter, with a sharply defined central crater 11,000 ft. in diameter and about 200 ft. deep. Drilling<sup>1</sup> has shown that the central crater is partly filled with flat lying Ordovician strata underlain by a thick lens of brecciated Pre-Cambrian material. A hole drilled approximately in the centre of the structure<sup>1,2</sup> passed through about 800 ft. of Ordovician strata, 1,900 ft. of breccia of various kinds, and beginning at a depth of 2,716 ft. penetrated breccia with a matrix of igneous textured trachyte for more than 100 ft. Below this "melt zone" brecciation died out very rapidly. Because of petrographic features found in the breccias, Brent has been widely accepted as a holotype of a "fossil" meteorite crater<sup>1-4</sup>. The Brent crater occurs on or near the Ottawa-Bonnechere graben, however, a major structure with a length of some hundreds of miles<sup>5</sup>, along which circular alkaline and carbonatitic complexes were intruded in Cambro-Ordovician time<sup>6,7</sup>. These facts suggest a possible endogenetic origin for the crater. In an attempt to resolve this problem we examined the petrographic and chemical composition of twenty-three samples of drill core from the central hole and nine samples collected on the surface. It was found petrographically that the samples fell into four groups. Underlying the Ordovician were about 100 ft. of breccias with substantial amounts of glass (often of several types), below which were 1,700 ft. of breccias with little or no glass. At a depth of 2,700 ft., 16 ft. of breccia rich in glass was again encountered

Table 1. AVERAGE COMPOSITION OF ROCK UNITS FROM THE BRENT CRATER, ONTARIO

	a	s	b	s	c	s	d <sub>1</sub>	s	d <sub>2</sub>	s	e	a-d	b-d	c-d	a-b
SiO <sub>2</sub>	61.2	1.74	58.5	4.69	63.3	5.44	66.1	3.52	66.4	9.00	25.0	Yes	Yes	No	No
TiO <sub>2</sub>	0.77	0.15	1.04	0.32	0.76	0.53	0.57	0.09	0.56	0.08	4.0	Yes	Yes	No	No
Al <sub>2</sub> O <sub>3</sub>	15.7	0.33	15.3	1.01	16.6	1.85	15.7	0.86	15.8	2.72	6.9	No	No	No	No
Fe <sub>2</sub> O <sub>3</sub>	7.6	0.82	8.3	2.37	7.0	3.07	5.4	1.45	5.0	2.80	12.9	No	No	No	No
MgO	2.08	0.10	3.11	0.68	2.09	0.87	1.08	0.33	1.29	0.61	8.0	Yes	Yes	Yes	Yes
CaO	1.38	0.47	0.78	0.40	0.79	—	1.55	0.74	2.00	—	20.0	No	Yes	No	No
MnO	0.15	0.037	0.100	0.077	0.09	0.08	0.09	0.06	0.07	0.04	0.50	Yes	No	No	Yes
K <sub>2</sub> O	7.60	0.85	9.26	1.39	4.50	0.31	4.08	0.48	4.09	1.93	1.38	Yes	Yes	No	No
Na <sub>2</sub> O	1.86	0.32	0.98	0.85	3.36	0.13	3.60	0.43	3.22	0.57	0.87	Yes	Yes	No	No
Ni	115	51	47	—	9	6	5	4	21	—	330	Yes	Yes	No	No
Cr	43	18	28	—	6	—	11	2	13	7	500	Yes	Yes	No	No
Co	7	4	16	12	< 10	—	< 5	—	6	—	67	Yes	No	No	Yes
Zr	735	210	650	320	545	370	550	200	355	230	300	Yes	Yes	No	No
Ba	800	450	512	360	762	340	930	300	890	565	500	No	Yes	No	No
Cu	7	2	11	—	7	—	7	—	25	—	70	No	No	No	No
Zn	420	370	127	55	98	35	85	30	110	40	1,200	Yes	No	No	Yes
B	10	—	10	—	10	—	10	—	< 10	—	10	No	No	No	No
Be	1	—	2	—	1	—	1	—	1	—	1	No	No	No	No
V	10	0	25	—	3	—	8	—	70	—	300	Yes	No	No	No

a, Trachytic igneous rocks (3 samples); b, glass rich breccia (6 samples); c, glass poor breccia (6 samples); d<sub>1</sub>, country rocks from drill cores (7 samples); d<sub>2</sub>, country rocks from surface (9 samples); e, vein cutting glass poor breccia; s, standard deviation of average analysis.

The last four columns show whether differences between units are significant at the 1 per cent level.

grading into inclusion rich trachyte of igneous aspect. About 50 ft. of breccia rich in glass occurred below the trachyte, passing abruptly into relatively undeformed country rocks. A specimen from one of the dykelets cutting the breccia poor in glass was composed of extremely fine grained carbonates, minute needles of a pleochroic blue mineral and abundant opaque dust.

The average composition and standard deviation of each of these petrographically defined units is given in Table 1. The compositions were obtained by direct reading spectrography for SiO<sub>2</sub>, Al<sub>2</sub>O<sub>3</sub>, total iron, CaO, MgO and TiO<sub>2</sub>, by emission spectrography for beryllium, boron, barium, nickel, cobalt, chromium, copper, vanadium and zirconium, and by wet chemistry for nickel, cobalt, copper, lead and zinc. Potassium and sodium were analysed by flame photometry. Analysis of standards G-1, T-1 and W-1 by the same methods suggests that the average error for the principal elements is about 4 per cent of amount present.

Student's *t* test with the Bessel correction for small samples has been applied to Table 1 to detect the differences between units. The agreement between the two samples of country rocks is excellent, showing that a satisfactory mean composition was achieved by the number of samples used. The breccias free of glass differ significantly from the country rocks only in a greater content of MgO. The igneous rocks and breccias rich in glass are notably similar in composition, but they differ significantly from the other two units for thirteen of the nineteen elements analysed. Both rocks are strongly potassic and characterized by a dominance of MgO over CaO unusual for such silicic rocks. The dyke rock is alnoitic in chemical composition (compare Table 2). The composition prompted a semi-quantitative spectrographic search for rare earths. Niobium and cerium were the only rare earths found in detectable amounts. The dyke

rock has a very high heavy metal content. In Table 2 analyses from Brent are compared with rocks from various alkaline and carbonatite complexes.

To summarize, potassic trachytes associated with alnoite have been found at depth in the Brent structure. The geometric form of these rocks is unknown. Breccias rich in glass and similar in composition to the trachyte are found above and below it at the top of the structure, where rocks of igneous appearance have not as yet been discovered. The composition of these breccias implies strong enrichment of potash and magnesium together with depletion of sodium and calcium, so that the breccias could be fenites.

The Brent structure occurs in an area of known alkaline carbonatite activity, on or near a structure known to be associated with such activity. The age of igneous activity is the same as that of the Brent structure, as nearly as is known<sup>6</sup>. The size and general disposition (breccia funnel with igneous rocks at depth) of the Brent structure is compatible with that of known alkaline carbonatite complexes elsewhere<sup>8</sup>. Petrographic observations<sup>2,3</sup> have hitherto been thought to demonstrate an impact origin for the Brent crater, but the petrochemistry of the rocks strongly suggests that the Brent structure is an alkaline carbonatite complex. The implications drawn from the petrographic data must therefore be reassessed.

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Table 2. COMPARISON OF ROCKS FROM ALKALINE-CARBONATITE COMPLEXES WITH BRENT ANALYSES

	1	2	3	4	5	6	7	8
SiO <sub>2</sub>	61.2	58.89	59.51	58.5	60.93	57.70	25.0	23.54
TiO <sub>2</sub>	0.77	0.88	1.27	1.04	0.44	1.27	4.0	4.18
Al <sub>2</sub> O <sub>3</sub>	15.7	17.61	15.32	15.3	12.64	18.05	6.9	7.97
Fe <sub>2</sub> O <sub>3</sub>	7.6	2.44	4.17	8.3	7.12	5.07	12.5	9.31
FeO	—	3.47	0.71	—	0.88	0.65	—	5.71
MgO	2.08	0.65	1.83	3.11	0.65	0.18	8.0	5.91
CaO	1.38	2.11	3.93	0.78	1.71	0.39	20.0	19.29
MnO	0.15	—	0.10	0.098	0.26	0.06	0.5	0.51
K <sub>2</sub> O	7.52	7.86	10.70	9.26	9.58	13.26	1.34	2.21
Na <sub>2</sub> O	1.86	4.57	0.75	0.98	4.78	0.74	0.87	3.01

1, Average of 3 trachyte samples from the Brent crater.

2, Trachyte, Karisimbi<sup>1</sup>.

3, Trachyte, Kogotokskyia suite<sup>10</sup>.

4, Average of 6 "glass rich breccia samples", Brent crater.

5, Fenite, Chigwakwala Hill, Tundul<sup>11</sup>.

6, Feldspathic breccia, Nkalonje complex<sup>11</sup>.

7, Alnoite from the Brent crater.

8, Carbonitized alnoite, Tundulu complex, Malawai<sup>12</sup>.

9, Alvikite alnoite, Stavreviken, Sweden<sup>13</sup>.

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## PHYSICS

## Timing of Sonoluminescence Flash

It has been pointed out<sup>1</sup> that theories attempting to explain the origin of the sonoluminescent flash, which is sometimes observed in acoustic cavitation, can be divided according to whether they predict the flash to occur at the moment of birth or collapse of the bubble.

Negishi<sup>2</sup> has shown that light is emitted when the bubble volume is at a minimum, and this is taken to favour the second class of theories. It also appears, however, that individual cavitation bubbles may rebound or re-form for many cycles of the acoustic field<sup>3</sup>. In this case, it is not possible to distinguish with certainty between the collapse and birth, or rebirth, time. Furthermore, it was shown that then only one flash is usually emitted which is followed by a series of rebounds lasting for some milliseconds. The only obviously unique occasion when the bubble volume is at a minimum is at the initial birth, which in Hahn's case<sup>3</sup> was nucleated by fast neutrons from an isotope source.

We have carried out similar experiments in which cavitation bubbles are nucleated by fast neutrons from a pulsed source. The time delay between the neutron burst and the emission of the flash was observed, using a 100 channel time sorter on 1  $\mu$ sec channels. The flash was not emitted during the burst, that is, at the time of nucleation and the initial growth of the bubble, but on average more than half a period of the sound wave later.

Two 1 in. photomultipliers were arranged to view the central pressure maximum of a cylindrical glass container of liquid, excited in the 101 mode by a barium titanate ring cemented to the outside of the container. The liquid used was degassed tetrachloroethylene and the exciting frequency about 20 kc/s, the sound pressure amplitude being 10–15 atm. peak. A block diagram of the electronics is shown in Fig. 1. The outputs from the photomultipliers were arranged in coincidence, and provided a stop pulse for the 100 channel analyser. An 8 pulses/sec pulser (not phase locked to the 20 kc/s sound wave) triggered the neutron generator, a modified Elliot K-tube generator, which accelerates deuterons from a plasma onto a tritiated target to produce a 2  $\mu$ sec wide burst of 14.3 MeV neutrons. A magnetic field is used to assist in the production of the deuterium plasma and there is a delay of about 60  $\mu$ sec after the trigger pulse while this field is established and before the neutron burst. A delay unit was arranged to start the sweep of the time sorter approximately 12  $\mu$ sec before the neutron burst. The neutron intensity was adjusted to give approximately two bubbles/sec, that is, only about one neutron burst in four resulted in bubble nucleation.

The time spectrum obtained in this way is shown in Fig. 2. It is clear that there is a long delay between the burst and the emission of the sonoluminescent flash. There are 1,376 counts in the spectrum. When the liquid container was replaced with a cylinder of plastic scintillator, a single sharp peak about 2  $\mu$ sec wide was observed in the spectrum at the point marked "neutron burst time".

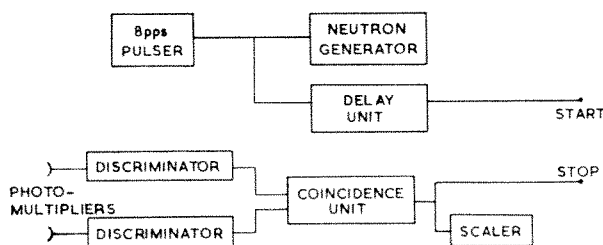


Fig. 1. Block diagram of the electronics of the experiments.

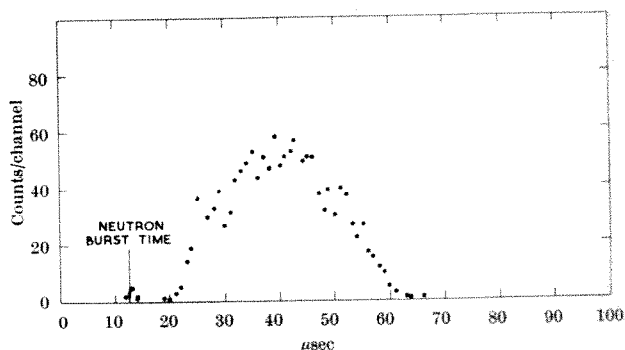


Fig. 2. The time spectrum of sonoluminescence flashes. The spectrum is made up of a total of 1,376 counts.

The rise time of flash, as observed by the photomultipliers, was not greater than 12 nsec.

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### Upper Limit on the Abundance of Anti-protons in the Low Energy Galactic Cosmic Radiation

THE existence of anti-matter in the universe has intrigued astrophysicists for quite some time. Several authors have suggested the existence and creation of anti-matter at various places in the universe<sup>1,2</sup>. One way of attempting to verify such possibilities is to see if cosmic rays, which include nuclei accelerated from various regions of our galaxy (if not the whole universe), contain anti-nuclei. Alfven<sup>1</sup>, however, argues that cosmic rays at the Earth may not contain anti-nuclei for various reasons. Even if this were so, cosmic rays should contain secondary anti-protons produced in collisions of cosmic ray protons with interstellar hydrogen. Estimates of the abundance of secondary anti-protons have been made by several authors: Fradkin<sup>3</sup> gives a value  $(\bar{p}/p) \sim 5 \times 10^{-4}$  for anti-protons of energy  $E_p > 1.7$  GeV; Milford and Rosen<sup>4</sup> estimate that for  $E_p \sim 0.5$  GeV,  $(\bar{p}/p) \leq 3 \times 10^{-3}$  and more recently by calculating the energy spectrum of such anti-protons they state<sup>5</sup> (the numbers are not published to my knowledge) that the ratio  $(\bar{p}/p)$  at low energies may be greater by several orders of magnitudes than that calculated at high energies.

There have been several attempts made in the past to detect anti-nuclei with charge  $Z \geq 6$  in cosmic radiation<sup>6-8</sup>. These experiments consisted of examining the ends of tracks of cosmic ray nuclei, brought to rest in nuclear emulsions, for characteristic annihilation interaction. No such nucleus has been detected so far; therefore upper limits have been set to the ratio of these anti-nuclei to normal nuclei<sup>6-8</sup>, the lowest being  $10^{-3}$ . If anti-nuclei exist in the cosmic rays at balloon altitudes, however, it is likely that they exist as anti-protons, because the mean free path of anti-nuclei ( $Z \geq 6$ ) for annihilation ( $\sim 3$  g/cm<sup>2</sup> of hydrogen) is much smaller than that of anti-protons ( $\sim 18$  g/cm<sup>2</sup>) and the nuclei are observed after crossing several g/cm<sup>2</sup> of interstellar hydrogen. Aizu *et al.*<sup>7</sup> have examined the stopping ends of singly charged nuclei obtained in their experiment, and give an upper limit for the ratio  $(\bar{p}/p)$  as  $10^{-3}$ , assuming that all the singly charged nuclei which they observed were of cosmic origin.



In the present experiment, we used a nuclear emulsion stack exposed at Fort Churchill, Canada, on August 4, 1962, for 13 h 52 min under 4.2 g/cm<sup>2</sup> of residual atmosphere. The stack consisted of nineteen Kodak 'NTB-4' emulsions each 625 $\mu$  thick. The experimental procedure consisted in looking for the "signature" of an anti-proton, which is an interaction or "star" produced by an incoming slow particle in the nuclear emulsion with at least one minimum ionizing singly charged particle track (pi-meson) associated with it. Microscope scanning was first performed for all interactions; each interaction observed was then examined under high power to select those which had at least one minimum ionizing particle track\*. In order to find whether there is any incoming slow particle associated with the selected stars, all tracks in the upper hemisphere with ionization greater than six times that of a relativistic particle were traced through the stack until they either came to rest or left the emulsion stack. If the track left the stack, measurements of the ionization, one near the interaction and a second near the exit, were made. The change in ionization, if any, indicates the direction of motion of the particle. It is easy to see that in the case of a genuine anti-proton annihilation star, the track of the incoming anti-proton will show an unambiguous and easily detectable change of grain density and Coulomb scattering.

A total volume of 4.5 c.c. from three emulsion plates has so far been scanned. About 5,000 stars from this scan have been examined for the anti-proton signature. Only one anti-proton star was found; the anti-proton producing this interaction, however, came from another interaction within the emulsion stack itself. No anti-proton coming from outside the stack was observed.

In order to obtain an upper limit on abundance ratio of anti-protons to protons ( $\bar{p}/p$ ) for the cosmic ray, we have to know the number of primary protons that would have stopped in the volume scanned. This number has been calculated using a flux of 0.4 protons per (m<sup>2</sup>.sec.ster.MeV)<sup>10</sup>. In this calculation protons with zenith angles greater than 60° have been neglected, because in our experiment, tracks with such large angles will be steep in the emulsion plate with a possible reduction of efficiency in tracing and other procedures. The energy of protons that would have stopped in the scan volume is between 100 and 150 MeV and the total number of such protons relevant to our experiment has been calculated to be about 3,500. If one anti-proton had been found in this experiment, the ratio ( $\bar{p}/p$ ) would have been about  $3 \times 10^{-4}$  so that the upper limit can be set as  $(\bar{p}/p) \leq 3 \times 10^{-4}$ .

The upper limit given here, however, is much lower than the value suggested by Rosen and Milford<sup>5</sup>.

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\* About 6 per cent of anti-proton interactions do not produce even one charged relativistic pi-meson (minimum ionizing track), so that these will be missed. The average number of relativistic charged pi-mesons produced in an anti-proton interaction in emulsion is  $2.6 \pm 0.3$  (ref. 9). The number of black prongs associated with a star with one or two pi-mesons is on the average five to six. As the charged pi-meson number increases to five the black prong number falls to about two\*. In either case then, as a result of the large size of the stars, scanning losses are negligible.

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## Short-range Dating of Fresh Nuclear Debris

FRESH nuclear debris particles were detected in ground air and rain water at Niigata, Japan, on May 11, on November 1 and on December 30, 1966. Without doubt, the particles collected on May 11 resulted from a Chinese nuclear test conducted at 1700 h (Japanese time) on May 9, 1966. These particles of known origin provided a favourable opportunity to examine a rapid method for dating of nuclear debris particles shortly after their formation.

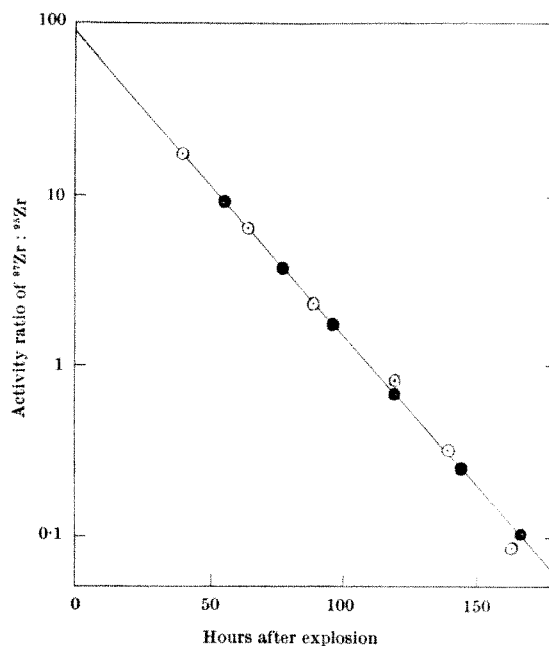


Fig. 1. Variation of ratio of <sup>97</sup>Zr : <sup>95</sup>Zr after the explosion. ○, Explosion on May 9, 1966; ●, explosion on December 28, 1966.

The determination of the activity ratio of a short-lived (1 day) to a relatively long-lived (10–100 days) fission product in a debris particle at a given time after a nuclear fission process can be used as a sensitive test of the age of nuclear debris. Of many available pairs <sup>97</sup>Zr and <sup>95</sup>Zr, <sup>133</sup>I and <sup>131</sup>I, and <sup>143</sup>Ce and <sup>141</sup>Ce are considered most useful for a number of reasons. For example, the fractionation behaviour during the formation of debris between the respective isotopes is similar and radiochemical analyses can be rapidly and simply carried out. We chose the pair <sup>97</sup>Zr and <sup>95</sup>Zr because they exhibit a similar fractionation behaviour; the decay chain of <sup>97</sup>Zr is 17h, <sup>97</sup>Zr → 60s, <sup>97m</sup>Nb → 73m, <sup>97</sup>Nb → stable <sup>97</sup>Mo. Because the half lives of <sup>97m</sup>Nb and <sup>97</sup>Nb are much shorter than that of <sup>97</sup>Zr, a state of radioactive equilibrium is established immediately after the formation of <sup>97</sup>Zr. Some precursors of <sup>97</sup>Zr and <sup>95</sup>Zr are gaseous or volatile elements, for example, bromine, krypton and rubidium, but their half lives are all so short that no significant fractionation effect would be expected between the two isotopes during particle formation<sup>1</sup>. Measurement of the activity ratio of <sup>97</sup>Zr : <sup>95</sup>Zr in a single particle can thus provide a simple method for determining its age.

Some highly fractionated particles of larger size—more than 10 $\mu$  in diameter—consisted mainly of short-lived refractory fission products such as <sup>97</sup>Zr, <sup>99</sup>Mo and <sup>143</sup>Ce with no detectable amount of <sup>132</sup>Te, <sup>132</sup>I and <sup>133</sup>I. In this case, activities of the radiozirconium were directly measured with no chemical separation. To do this we used a 1½ in. × 1 in. sodium iodide scintillation detector with a 200 channel pulse-height analyser, and determined the activities by measuring the area of the photopeaks. Many of the particles, however, contained a considerable

amount of the short-lived volatile fission materials  $^{132}\text{Te}$ – $^{132}\text{I}$  and  $^{133}\text{I}$ , and therefore after the samples had been fused with sodium carbonate they were dissolved in nitric acid. The sample solution was then analysed by a TTA–xylene extraction method<sup>2</sup> and the purified radiozirconium sample submitted to  $\gamma$ -spectrometric measurement.

Fig. 1 shows the measured activity ratio of  $^{97}\text{Zr} : ^{95}\text{Zr}$  (open circles) plotted as a function of time 2–7 days after the explosion, together with an assumed line (solid line) of the time variation in the  $^{97}\text{Zr} : ^{95}\text{Zr}$  ratio. The line is plotted against time on the assumption that cumulative fission yields of  $^{97}\text{Zr}$  and  $^{95}\text{Zr}$  are 5.9 and 6.2, respectively<sup>3</sup>. The single particle used for these measurements was spherical in shape,  $16\mu$  in diameter, reddish brown in colour and had a total  $\beta$ -activity of  $2.1 \times 10^6$  pc. at 1300 h, May 11. As can be seen from the figure, most of the results seem to lie along the assumed line. Thus it would seem that no enrichment of  $^{97}\text{Zr}$  relative to  $^{95}\text{Zr}$  occurred in the particle. As a result, it was possible to calculate an age for it, without making any corrections for fractionation, by comparing a measured activity ratio of the two isotopes with a corresponding value on the assumed line. The results obtained are given in Table 1. It can be seen that most of the estimated ages agree with the values calculated from the known time of explosion for the May 9 nuclear test; they do so especially well within 5 days of the explosion. Thus it would seem possible that when two nuclear explosions are conducted with a time interval of more than 10 h between them, particles released from them can be distinguished by measuring their activity ratio of  $^{97}\text{Zr} : ^{95}\text{Zr}$  for up to a week after their formation.

Table 1. CALCULATED AND ESTIMATED AGES AT DIFFERENT TIMES OF A SINGLE FALL-OUT PARTICLE OF KNOWN ORIGIN

Date of measurement	Age since the formation Calculated* (h)	Estimated (h)
1000 h, May 11, 1966	41	41
1000 h, " 12 "	65	66
1100 h, " 13 "	90	91
1100 h, " 14 "	121	116
1300 h, " 15 "	140	138
1300 h, " 16 "	164	172

\* These figures were calculated on the basis of the time of explosion—1700 h, May 9, 1966 (Japanese time)—given by the responsible Chinese agency.

Table 2. ACTIVITY RATIO OF  $^{97}\text{Zr}$  to  $^{95}\text{Zr}$  AND ESTIMATED AGE OF FRESH NUCLEAR DEBRIS PARTICLES COLLECTED ON DECEMBER 30, 1966

Date of measurement	Activity ratio	Estimated age (h)
2300 h, Dec. 30, 1966	9.0	56 (1500)*
1100 h, " 31 "	5.3	69 (1400)
2100 h, " 31 "	3.7	77 (1600)
1400 h, Jan. 1, 1967	1.7	97 (1500)
1700 h, " 2 "	0.67	120 (1700)
1400 h, " 3 "	0.24	145 (1500)
1400 h, " 4 "	0.09	170 (1200)

\* The figures in parentheses refer to assumed times of explosion on December 28, 1966, calculated from each of the respective ages estimated.

In order to test this method of age determination, we estimated the age of hot fall-out particles collected on December 30, 1966, at Niigata. As the radiozirconium content in a single particle collected on December 30 was too small to determine the activity ratio of  $^{97}\text{Zr} : ^{95}\text{Zr}$  with a sufficient accuracy—less than one-two-hundredth that of the May 9, 1966, test—a composite sample of fifty-five particles was used for measurement. The ratios were determined at different times after collection using a chemically purified sample of radiozirconium. Table 2 shows the activity ratios of  $^{97}\text{Zr} : ^{95}\text{Zr}$  measured during the period December 30, 1966–January 5, 1967 (solid circles in Fig. 1), and the estimated ages at different times of the composite sample. They were also evaluated by a comparison of their  $^{97}\text{Zr} : ^{95}\text{Zr}$  ratios with the corresponding values on the assumed line (Fig. 1). From the ages thus obtained, we conclude that the explosion from

which these hot particles originated was probably between 1200 and 1700 h (Japanese time), December 28, 1966.

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## CHEMISTRY

### Irradiation and the Subsequent Solid State Thermal Decomposition of Silver Nitrite

We have examined the thermal decomposition of solid silver nitrite at  $50^\circ\text{C}$  after it has been exposed to  $\gamma$ -radiation from a cobalt-60 source (dose rate at the sample =  $1.5 \times 10^4$  r./h). The kinetics were followed gravimetrically *in vacuo* using collections of many well formed single crystals (sample size about 10 mg). The results are shown in Fig. 1. In contrast with results found in decomposition studies of other solids, which have shown either an enhancement of rate or negligible effect following irradiation<sup>1</sup>, with silver nitrite there is a marked inhibition in the rate of decomposition with the dose of  $\gamma$ -rays. There was no significant loss in weight of the sample during irradiation. The irradiation was performed *in vacuo*. If the irradiation was carried out in a poor vacuum or at 1 atm. there was no effect on the subsequent thermal decomposition. This finding is of considerable importance because it may apply to the study of radiation damage in other reversible solid state decomposition reactions of the type  $A_{(s)} \rightarrow B_{(s)} + C_{(g)}$ .

The kinetics of the thermal decomposition of unirradiated silver nitrite have been examined from  $35^\circ\text{C}$  to  $90^\circ\text{C}$ . The decomposition of both irradiated and unirradiated silver nitrite continues to completion ( $100 \pm 3$  per cent), that is,  $\text{AgNO}_{2(s)} \rightarrow \text{Ag}_{(s)} + \text{NO}_{2(g)}$ , if the nitrogen dioxide is continually removed during a run. It was found that the rate constants determined from the slope of the extensive linear region (15–45 per cent) in the decomposition curves (Fig. 1) for runs at temperatures above  $60^\circ\text{C}$  fell below the extrapolated Arrhenius plot as determined from data at lower temperatures. This fall-off is believed to result mainly from inhibition by gaseous

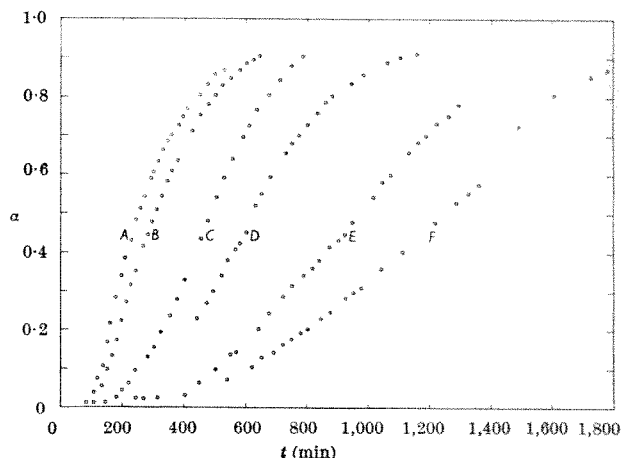


Fig. 1. Fraction decomposed,  $\alpha$ , versus time plots for the decomposition of irradiated silver nitrite at  $50^\circ\text{C}$ . Exposure time in hours: A, 0; B, 1; C, 2.5; D, 5; E, 10; F, 20 (dose rate  $1.5 \times 10^4$  r. h<sup>-1</sup>).

products, that is, even with direct evacuation the gases are evolved too rapidly to be removed. For this reason most effort has been concentrated on slow runs, at temperatures below 60° C; this, of course, also minimizes any errors arising from self-cooling. It should be noted that the lowest temperature at which this reaction previously appears to have been studied is 72° C (ref. 2).

Boldyrev and Eroshkin<sup>2</sup> have recently observed similar behaviour when silver nitrite is irradiated with X-rays before decomposition (80° C). Their results show an inversion effect, that is, at higher doses the rate increases relative to lower doses. This behaviour was not observed in our work. Their inversion effect occurs at a relatively low level of inhibition when compared with the more marked inhibitory effects observed in the present data. Because this reaction is sensitive to the presence of gaseous products both during the irradiation and the subsequent thermal decomposition, we suggest that the inversion which they observed is an experimental artefact and may result from inhibition by gaseous products.

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### Solubility of Cupric Oxide in Pure Water at Temperatures up to 550° C

WE report here work in progress on the measurement of the solubility of metals and metal oxides in pure water, in the temperature range 50°–550° C and at pressures up to 400 bars. The isobaric solubility of cupric oxide at 310 bars is reported here and compared with the 300 bar isobar for silica (quartz).

The solubility measurements were made by passing demineralized, distilled and de-aerated water, at the required temperature and pressure, through a packed bed of solute particles contained in an autoclave. To prevent carry-over of particulate material, the solution leaving the autoclave was filtered through a stainless steel frit (1  $\mu$ m pore diameter). It was then cooled at pressure to reduce deposition in the cooling system and passed through a back-pressure regulator to be collected for analysis. The high pressure water circuit was constructed from 'Nimonic' 80.4 and 90 alloys. For the cupric oxide-water system the samples of solution were collected in dilute hydrochloric acid and were analysed for copper by the solvent extraction-colorimetric method described by Wilson<sup>1</sup>. The random error of the solubility values reported is about  $\pm 5$  per cent, but this excludes the systematic error resulting from the deposition of copper in the sampling system. It has been shown, however, that deposition was only significant at the highest solubility values, above 400  $\mu$ g copper/kg. No dependence of solubility on the rate of flow through the autoclave was found at rates from 1–12 kg water per hour, indicating that solutions leaving the autoclave were saturated.

Pocock and Stewart<sup>2</sup> have also measured the solubility of copper, cuprous and cupric oxides in water in the temperature range 480°–620° C, but their results are almost an order of magnitude lower than those presented here. It is suggested that this discrepancy may result from:

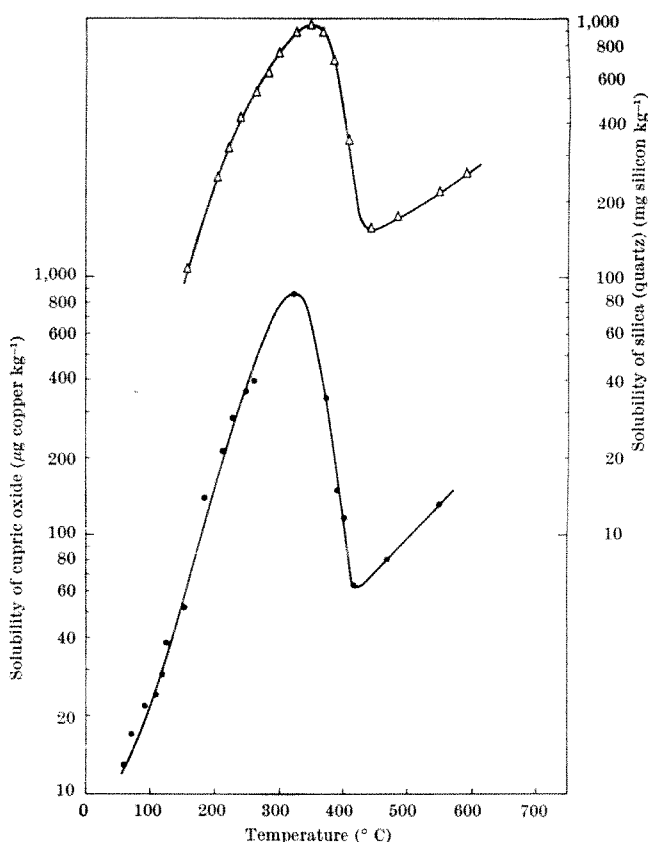


Fig. 1. Isobaric solubility of cupric oxide and silica in water. ●, Present results with cupric oxide at 310 bars; Δ, Heitmann's results<sup>3</sup> with silica at 300 bars.

(a) incomplete acid cleaning of their sampling system; (b) insufficient equilibration time in the solubility autoclave; (c) interaction with the high concentrations of iron in the water, which resulted from their use of stainless steel equipment; (d) interaction with the hydrogen evolved during the corrosion of the stainless steel system.

The isobaric solubility of cupric oxide in water (pH 7.5) at 310 bars obtained in the present work is shown in Fig. 1, which also shows the 300 bar isobar for the silica (quartz) water system<sup>3</sup>. The two curves show a markedly similar variation of solubility with temperature, with the silica solubilities about three orders of magnitude greater than those of cupric oxide. This similarity suggests that the nature of the solvent determines the isobaric temperature coefficient of solubility for systems of this type, while the nature of the solute determines the absolute solubility level. It follows therefore that a generalized solubility equation based on the physical properties of the solvent is feasible. Attempts to formulate such an equation, for example by Martynova<sup>4</sup>, have included only the specific volume of the solvent and have not successfully correlated the experimental data for any given system. Work is in progress in this laboratory on an empirical equation which includes further physical properties of water.

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### Solid State Polymerization at {00.1} Sub-grain Boundaries of Trioxane

THE polymerization of crystalline organic compounds in the solid state is an intriguing process, not least because of the influence of the crystal lattice. A recent review<sup>1</sup> distinguishes two chief categories depending on whether or not the resulting polymer bears a crystallographically defined relationship to the monomer crystal. In a preliminary investigation of this problem, microscopic observations have been made on the most frequently studied system, the polymerization of trioxane ( $\text{CH}_2\text{O}$ )<sub>3</sub> to polyoxymethylene [ $-\text{CH}_2\text{O}-$ ]<sub>n</sub>.

The evidence in the literature appears to be conflicting. On one hand, this process is the example par excellence in which polymer crystals are ordered with respect to the monomer<sup>1</sup>—a view based on X-ray evidence<sup>2</sup> from crystals polymerized to maximum yield and supported by observations of polymer fibres parallel to the trioxane *c* axis. But even here there is an apparent contradiction in that X-rays indicate two twinned orientations of polymer<sup>3</sup>, a conclusion not entirely supported by polarizing microscopy<sup>4</sup>. On the other hand, microscopy of the initial polymer showing assorted fibre directions within one grain and also straight fibres passing through several grains seems to indicate that, in detail, there is in fact no well defined orientational relationship between monomer and polymer crystals. The present experiments not only offer a possible reconciliation between these earlier observations because morphologies similar to both have been observed in distinguishable circumstances, but also they identify, for what is believed to be the first time, the preferred site of polymerization as a particular element of crystal sub-structure—{00.1} sub-grain boundaries in trioxane.

The experimental procedure used differs from established procedures only in having a higher dose rate during irradiation. Thin, melt-grown crystals of trioxane, 40–80  $\mu$  thick, prepared from previously sublimed trioxane and sealed between microscope slide and cover glass were irradiated with a standard dose of 1 MeV electrons ( $2 \times 10^{13}$  electrons/cm<sup>2</sup> incident over 10 sec). In all cases the trioxane *c* axes lay in the surface of slide. Specimens were then heated at 55°C for varying intervals before being quenched to room temperature for polarizing microscopy. Reheating was ineffectual in producing further polymer. It was also possible to examine the polymer in the electron microscope after pumping off residual trioxane.

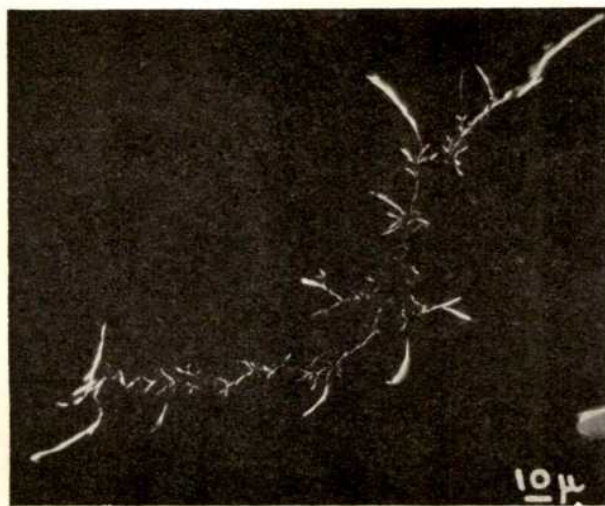


Fig. 1. Branching, fibrous polyoxymethylene formed on the slide-trioxane interface after irradiation. Crossed polars.

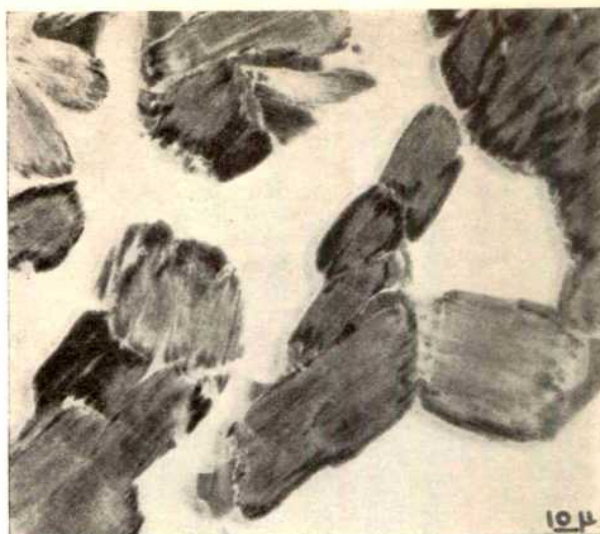


Fig. 2. Polyoxymethylene fibres showing the grain structure of the original trioxane subsequently pumped off. Crossed polars.

Polymer was observed to form in three distinct ways, two before heating to 55°C and one after. In all cases the polymer eventually formed fibrous crystals with *c* along the axis. The first occurrence, which is interesting in that it alone resembles the morphology cited against initially ordered polymer<sup>3</sup>, was as fibrous sheaves often at the junction of, and passing into, several grains of trioxane which appeared to have nucleated on it. This was infrequently observed and is believed to have been present in the trioxane before melting and recrystallization. Because these processes are likely to have disrupted earlier morphologies it does not necessarily indicate here a disordered relationship between monomer and polymer when first formed.

The second type of polymer appeared in small amounts on the slide-trioxane interface, independently of the direction of electron incidence. Very little (<1 per cent) was detected on the cover glass-trioxane interface. Fig. 1 shows an extreme case of the fibrous, branching, incipiently spherulitic morphology, which has no preferred orientation to the underlying trioxane. The habit is suggestive<sup>6</sup> of an origin in a surface diffusion process connected with local heating of the slide during irradiation. Certainly reduced dose rates (which should reduce heating) reduced the amount of polymer.

The third morphology is the important one which gives the high conversion of trioxane to polyoxymethylene. It occurs entirely systematically in crystal interiors after heating at 55°C beyond an induction time of about 10 min. Fibres grow in length along the *c* axis to the limit of the surrounding grain (Fig. 2). In every grain the reaction starts in the interior even though adjacent grains may have undergone maximum conversion to polyoxymethylene. Of special interest is that in all but a few per cent of cases polymer appears first at sub-grain boundaries, but only those in or near to {00.1}. None has been seen in the frequent {*hk*.0} sub-grain boundaries. Fig. 3 shows fibres growing out along *c* from such boundaries; adjacent areas are full of fibres. As first detected the polymer is a birefringent haze<sup>4</sup> which eventually becomes evidently fibrous. At all stages, however, the polymer is aligned with the trioxane, because both extinguish between crossed polars, with their common *c* axes parallel to one polar. Even when trioxane has been pumped off, to avoid ambiguity of interpretation due to its birefringence, there is no evidence in the polyoxymethylene of a second, twinned orientation. This is not necessarily incompatible with X-ray work, however, because the decrease of volume on polymeriza-



tion to which the twinning has been attributed<sup>3</sup> is, under these conditions, taken up by forming cavities at the outer surfaces.

After pumping off trioxane, before electron microscopy, the initial polymer appears as birefringent microcrystals concentrated along {00.1} sub-grain boundaries, although inevitably orientations are disturbed by the pumping. The tiny microcrystals are, initially, thin hexagonal platelets which ultimately join to form hexagonal, prismatic fibres. A few microcrystals together with fibres of a wide range of lengths are seen in Fig. 4, which also illustrates the general observation that lateral dimensions vary little. It is tempting to attribute these dimensions to the microstructure of the sub-grain boundaries, but this factor has still to be investigated.

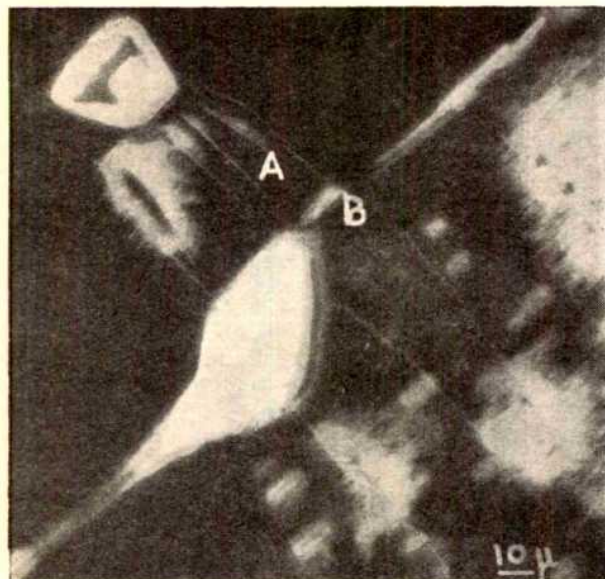


Fig. 3. Polyoxymethylene forming at {00.1} sub-grain boundaries (A) and extending along the c axis of the host trioxane crystal. Note also the pre-fibrous "haze" at B. Crossed polars.

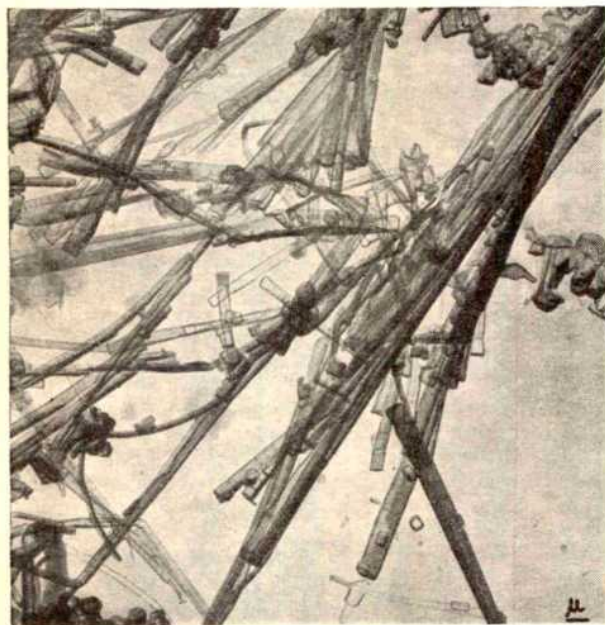


Fig. 4. Hexagonal platelets and prismatic fibres of polyoxymethylene polymerized from trioxane. Electron micrograph.

The importance of crystal sub-structure to solid state polymerization has been suggested in the past to account for the sensitivity of reaction rates to crystal size (see, for example, ref. 1) and to pressure<sup>7</sup>. In acrylamide, which like the other examples forms a glassy polymer, the observation that initial polymer appeared along lines<sup>8</sup> (the precise nature of which was not identified) has been interpreted to mean that dislocations are necessary for the disruption of lattice regularity to allow polymerization to proceed<sup>1</sup>. In contrast, for trioxane, which cannot be polymerized while liquid, it is supposed that lattice perfection favours reaction. These conclusions need modification in view of the experiments reported here, which reveal that in trioxane reaction can occur preferentially at certain defect structures. It would seem reasonable to look for the origins of this here, as in many inorganic systems, in enhanced diffusion along and stresses associated with defects<sup>9</sup>.

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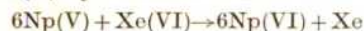
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## Photochemical Oxidation of Neptunium(V) by Xenon Trioxide

XENON trioxide is a strong oxidizing agent, with a potential of  $-1.8$  V (ref. 1) and the kinetics of several of its oxidation reactions, all of them thermal, have been studied<sup>2-4</sup>. We have now obtained evidence of a photochemical reaction in the course of a study of the oxidation of neptunium(V) by xenon trioxide



The neptunium(V) solution was prepared by dissolving neptunium dioxide in concentrated perchloric acid and reduction of the resulting neptunium(VI) to neptunium(V) with hydrogen peroxide. After standing for about 2 weeks, the solution was heated in a boiling water bath to destroy any peroxide present. The ionic strength was maintained constant at 2 molar with lithium perchlorate and recrystallized six times before use. The xenon trioxide solution had been passed through a zirconium oxide-zirconium phosphate bed to remove fluoride. It was standardized by reduction with excess potassium iodide and then by titration of the iodine produced with standard sodium thiosulphate.

The reaction was conducted in the light path of a Cary model 14 recording spectrophotometer, using a water-jacketed 1 cm spectrophotometer cell through which water at a known temperature was passed. All the runs were conducted at 60° C, to ensure that the reaction occurred at a rate which could be conveniently measured. The light source of the spectrophotometer, a tungsten filament projection lamp, was kept on during each run and the solutions were exposed to its full spectrum of light because in the infra-red mode the light passed through the sample before going through the monochromator. The concentration of neptunium(V) was followed at 980 mμ (neptunium(V) molar extinction coefficient 395 l.mole<sup>-1</sup> cm<sup>-1</sup>; neptunium(VI) molar extinction coefficient nil<sup>5</sup>). The several steps of data reduction were accomplished by



means of a computer programme\*. Reduction of the absorbancy readings yielded neptunium(V) concentrations from which xenon trioxide concentrations were calculated using the reaction stoichiometry and the initial xenon trioxide concentrations. Finally, values of  $k$  were obtained by substituting the values of these concentrations and their associated time co-ordinates into the integrated forms of various explicit rate expressions. It was concluded that the reaction rate is first-order in xenon trioxide and zero-order in neptunium(V) because of the relative constancy of the  $k$  values obtained from the corresponding integrated rate expression. Plots of values of the right-hand side of the integrated first-order rate equation

$$k_1 t = \frac{6 \ln [\text{XeO}_3]_0}{[\text{XeO}_3]}$$

versus time yielded straight lines, the slopes of which were equal to  $k_1$ . The least-squares values of  $k_1$  obtained in this manner (Table 1) confirm that the reaction follows the rate expression

$$-d[\text{Np(V)}]/dt = k_1 [\text{XeO}_3]$$

The rate also appears to be independent of acidity in the 0.5–2 molar acid range.

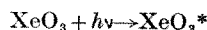
Table 1. FIRST-ORDER RATE CONSTANTS\*  
( $I = 2$  moles/l.,  $60^\circ \pm 0.3^\circ \text{C}$ )

[Np(V)] <sub>0</sub>	[XeO <sub>3</sub> ] <sub>0</sub>	[H <sup>+</sup> ]	Completion (per cent)	$k_1 \times 10^3$ (sec <sup>-1</sup> )
0.003873	0.003880	2	15	6.05 ± 0.03 †
0.003929	0.003880	2	15	6.40 ± 0.07
0.003929	0.003880	2	16	6.38 ± 0.04
0.002056	0.007760	2	59	6.42 ± 0.06
0.003922	0.007760	2	33	6.37 ± 0.02
0.001122	0.007760	2	96	6.23 ± 0.18
0.002051	0.007760	2	61	7.05 ± 0.07
0.002056	0.003880	2	33	6.76 ± 0.03
0.002215	0.007760	0.5	53	6.55 ± 0.08
0.002167	0.007760	1	56	6.38 ± 0.06
0.001324	0.003880	2	47	6.46 ± 0.09
0.001068	0.007760	2	89	5.93 ± 0.14
0.001111	0.001940	2	29	6.82 ± 0.10
0.002061	0.001940	2	12	5.21 ± 0.09
0.002073	0.001940	2	12	4.87 ± 0.03
Average				6.28 ± 0.58

\* Because this is a photochemical reaction and the ultra-violet light flux was not known, the numerical values of  $k_1$  have no real significance. They are included to demonstrate the constancy of  $k_1$  under the conditions of these experiments.

† Uncertainties are standard deviations. In computing the standard deviation of the average, the standard deviations of individual runs were disregarded, because they are small compared with the variation of  $k$  between runs.

The reaction observed appears to be a photochemical reaction caused by absorption of ultra-violet light by the xenon trioxide



The formation of the excited species is the rate-controlling step in the reaction; once formed, this species reacts relatively rapidly with neptunium(V). Such a photochemical reaction is consistent with the high molar extinction coefficient of xenon trioxide at wavelengths below 300 mμ (in acid solution  $\epsilon \sim 50$  at 290 mμ;  $\epsilon \sim 100$  at 280 mμ<sup>1</sup>), the emissivity spectrum of the spectrophotometer lamp, and the transmittance of the spectrophotometer cell walls at these wavelengths.

Other runs at 60° C, conducted outside the spectrophotometer light path, did not obey the rate law given here; the reaction was much slower and exhibited non-integral dependence on the neptunium(V), xenon trioxide, and possibly also the neptunium(VI) concentrations. The slowness and complexity of this thermal reaction tend to support the hypothesis<sup>4</sup> that xenon trioxide preferentially oxidizes by a two electron change mechanism, and that the reaction is hindered in cases—such as the present one—in which only a one electron change is permissible. It would be premature, however, to draw a definite conclusion from these limited data.

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### Photo-oxidation of Magnesium Porphyrins and Formation of Protobiliviolin

As part of a series of experiments on the photo-oxidation of vinylporphyrins<sup>1</sup>, I have studied complexes of protoporphyrin dimethyl ester with various transition metals. Non-fluorescent complexes of protoporphyrin dimethyl ester with iron, nickel, cobalt, copper and silver underwent no alteration in their spectral and chromatographic properties when they were irradiated in benzene with the light of a 500 W lamp. Complexes of lead with the protoporphyrin ester dissociated more rapidly under this treatment than the corresponding cadmium compounds. Zinc protoporphyrin slowly photo-oxidized to a brown unidentified compound.

Magnesium protoporphyrin ester (I) similarly irradiated in benzene, toluene, cyclohexane or chloroform photo-oxidized rapidly to give a greenish brown solution. By chromatography on a column of micro-crystalline cellulose thoroughly preflushed with nitrogen, using mixtures of benzene and light petroleum for elution, a green pigment was obtained. This green pigment contained no magnesium and was very labile: subsequent chromatography on cellulose caused it to decompose to give a biliviolin (II). The green pigment could be stored in deoxygenated benzene at 4° C for several weeks with only slight decomposition, but if frozen at -16° C a biliviolin was obtained overnight.

The green photo-compound also converted to biliviolin when shaken in a benzene or ether solution with normal hydrochloric acid or ammonium hydroxide. Weak organic bases, such as pyridine, α- or β-picoline, or lutidine, slowly converted the pigment to biliviolin. The addition of copper or zinc acetate to benzene solutions of the photo-compound rapidly gave the corresponding metal-complex of biliviolin. The carbonyl reagents, hydroxylamine and potassium borohydride, also reacted with the pigment to give the biliviolin. Identification of the biliviolin as protobiliviolin (divinylbiliviolin), a new compound, was based on a comparison of its spectral properties and those of the hydrochloride and zinc complex, which fluoresced red under ultra-violet light, with the spectra of mesobiliviolin. The spectral shift expected for the replacement of two ethyl side chains by two vinyls was observed. Whether this protobiliviolin is only of IXα structure or is a mixture of isomers<sup>2</sup> is being investigated. Oxidation of the protobiliviolin in methanolic hydrochloric acid with ferric chloride gave a verdin identical with an authentic sample of biliverdin.

The visible spectrum of the green photo-compound in neutral solvents (Fig. 1) connotes an extended series of double bonds, and low extinction ( $10^{-3} \epsilon = 26$  at 426 mμ) in the near ultra-violet is evidence that the ring conjugation of the porphyrin has been interrupted. Moreover, the characteristic fluorescence of the porphyrin is absent. Infra-red spectra of the green photo-compound

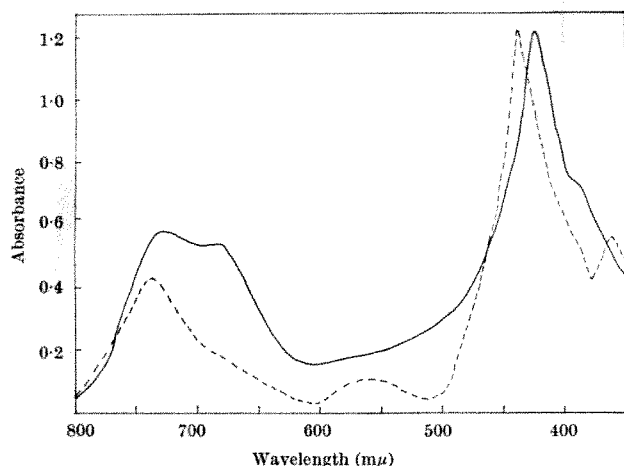


Fig. 1. Spectra of protohydroxyoxophlorin in benzene (—); urophlorin<sup>3</sup> in water at pH 6.8 (---).

in carbon tetrachloride showed a strong band at  $1,680\text{ cm}^{-1}$ ; as the pigment decomposed this band disappeared, to be replaced by a band at  $1,706\text{ cm}^{-1}$  due to the keto-amide carbonyl groups of biliviolin. From this spectral evidence and the reactivity of the green compound towards hydroxylamine and potassium borohydride, it is concluded that one of the methene carbons has been oxidized to a carbonyl. Because magnesium porphyrins without vinyl side chains photo-oxidize to similar green compounds (Table I) with a band at  $1,680\text{ cm}^{-1}$ , this band is evidently not due to the oxidation of a vinyl side chain.

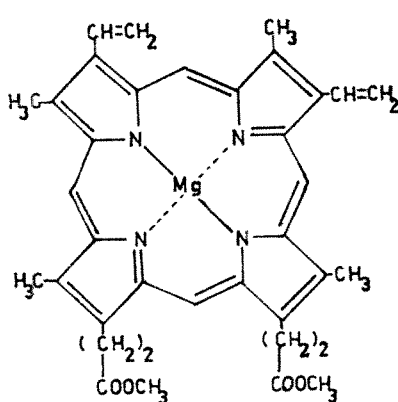
The visible spectra of these green compounds are remarkably similar to those of the phlorins<sup>3</sup> (dihydro-

Table I. MAXIMA OF ABSORPTION OF HYDROXYOXOPHORINS AND PHLORINS ( $\lambda$  in  $m\mu$ )

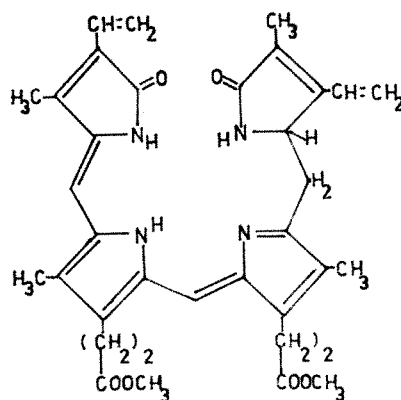
Protohydroxyoxophlorin	738 (668)	426 (395)	330
Meso-hydroxyoxophlorin	722 (660)		
Deuteriohydroxyoxophlorin	716 (662)		
Urophlorin	737	440 (360)	310
Coprophlorin	722	436	
Haematophlorin	705	432	

Solvent: hydroxyoxophlorins,  $\text{CHCl}_3$ ; phlorins<sup>3</sup>, water pH 6.8.

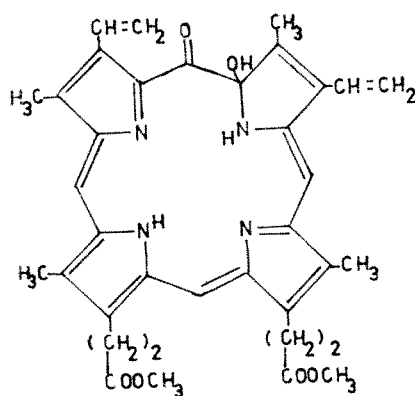
porphyrins having one methine bridge hydrogenated). The spectra of both compounds in the near ultra-violet region show satellite bands to the principal bands and the positions of maxima of absorption are very similar (Table I). The two maxima observed in the band, in the far red of the photo-product of magnesium protoporphyrin, can be ascribed to the asymmetry of the electron field induced by the keto-group. Furthermore, the green pigment is converted to a blue green form, with  $\lambda_{\text{max}}$   $660\text{ m}\mu$  at pH 9.0 (which rapidly decomposes to proto-biliviolin). Urophlorin undergoes a similar spectral transition above pH 9.2. The lability of the green compound has prevented direct analysis of the number of oxygen atoms added to the tetrapyrroles during photo-oxidation. Structure III, or a methene isomer, however, accounts for present spectral and chemical evidence and would explain the instability of the molecule. Libowitzky *et al.* have prepared from natural porphyrins<sup>4</sup>, and Jackson *et al.* have synthesized<sup>5</sup>, oxyporphyrins which have one methine carbon similarly oxidized, but instead of the hydroxyl at the  $\alpha$ -pyrrolic carbon they have an extra hydrogen on a  $\beta$ -pyrrolic carbon or a nitrogen, and thus retain conjugation of the ring system. These oxyporphyrins, their cations and metal complexes are considerably more stable than the hydroxyoxophlorins and can be transformed to porphyrins. Compounds of the class of structure III will be referred to as hydroxyoxophlorins.



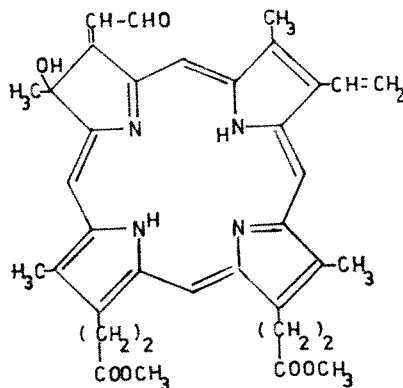
I



II



III



IV

The photo-oxidation of a methene carbon of a magnesium porphyrin is dependent on the electron distribution on the methene bridge carbon. The corresponding hydroxyoxophlorins were obtained by irradiating magnesium deuteroporphyrin and magnesium mesoporphyrin esters. Magnesium monoformyldeuteroporphyrin, magnesium porphyrin *a* esters and magnesium chlorins were not photo-oxidized. An additional factor is the stability of the metal porphyrin bond. In a series of magnesium porphyrins photo-irradiated in benzene, the stability of the metal porphyrin bond paralleled the thermodynamic stability<sup>6</sup> of these complexes. From magnesium-deuteroporphyrin only a trace of free porphyrin was found, but significant amounts of protoporphyrin were obtained from magnesium protoporphyrin, and magnesium dissociated even more rapidly from magnesium monoformyldeuteroporphyrin, magnesium porphyrin *a*, magnesium monovinyl- and magnesium monovinylmonocarbonyl-chlorin.

A strongly electron donating ligand completely suppresses the formation of hydroxyoxophlorins. Magnesium deuteroporphyrin and magnesium mesoporphyrin dipyrindino-complexes in benzene could not be photo-oxidized. A similar effect was obtained with  $\beta$ -picoline, but not with  $\alpha$ -picoline or lutidine. With the dipyrindino-complexes of magnesium protoporphyrin and of magnesium monovinylmonohydroxyethyl deuteroporphyrin, however, photo-oxidation occurred, not at the methene bridge but on one of the pyrroles at the vinyl side chain to give magnesium dioxyporphyrin and magnesium dioxymonovinylmonohydroxyethyldeuteroporphyrin. Free dioxyporphyrin (IV) (ref. 1) is obtained on photo-oxidation of protoporphyrin in either benzene or pyridine. Complexing of the magnesium porphyrins with pyridine or picoline had the further effect of stabilizing the magnesium-porphyrin bond. The pyridine complex of magnesium diformyldeuteroporphyrin and magnesium porphyrin *a* could be photo-irradiated for long periods without dissociation of magnesium.

Only hydrophobic solvents permit the formation of hydroxyoxophlorins. None could be obtained when magnesium protoporphyrin was irradiated in alcohols, ketones or were dispersed in 'Emasol' phosphate buffer at pH 6.8. In either neither photo-oxidation of the porphyrins nor dissociation of the magnesium occurred.

The photo-oxidation of magnesium porphyrins, to give specifically biliverdins on splitting of the ring, contrasts with the biliverdin obtained, which has three methene bridges, as the product of the opening of the porphyrin ring on chemical oxidation of pyridine haemochromes. The transformation of oxophlorin to biliverdin requires not only the addition of oxygen, because the methene carbon probably leaves as CO, or CHO, but also a reduction. Moreover, the photo-oxidation of the disodium complex of aetioporphylin in pyridine<sup>7</sup> gave a mixture of verdins and violinoid pigments.

The specific formation of protobiliverdin from magnesium protoporphyrin raises the possibility that magnesium protoporphyrin is a precursor of the chromophores of *c*-phycoerythrin and *allo*-phycoerythrin, and possibly the chromophore of phytochrome. The absorption spectrum of magnesium protoporphyrin is very close to the photo-action spectra of the formation of phycoerythrin<sup>8</sup>.

Magnesium protoporphyrin has been implicated as a precursor<sup>9</sup> of chlorophyll, and the cell membrane could provide a hydrophobic environment, required for the formation of the oxophlorin.

I have now found<sup>10</sup> that at least one vinyl group appears to be present in the native chromophore attached to the protein, and the other vinyl present in the protobiliverdin could be involved in a covalent linkage to the protein.

The spectral shift observed (725–665 m $\mu$ ) for phytochrome on going from the enzymatically active forms, Pfr, to the inactive form, Pr, closely mimics that found for hydroxyoxophlorins on going from neutral to alkaline

solvents. This strongly suggests that the chromophore is not of the biliverdin type<sup>11</sup>, but still has a bridge carbon present.

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## MOLECULAR STRUCTURE

### Nucleotide Sequence of 5S-ribosomal RNA from *Escherichia coli*

In 1964 Rosset, Monier and Julien<sup>1</sup> described and characterized a low molecular weight ribonucleic acid (5S RNA) that is present in the ribosomes of *Escherichia coli* in addition to the two larger components, the 16S and 23S RNA. It contains 120 nucleotide residues and in contrast to transfer RNA contains no "minor" bases. Using a two-dimensional fractionation procedure for the separation of nucleotides labelled with phosphorus-32 (ref. 2), we have determined the sequences of all the oligonucleotides obtained by complete digestion of <sup>32</sup>P-labelled 5S RNA with ribonuclease T1 and ribonuclease A (pancreatic ribonuclease)<sup>3</sup>. In order to arrange these nucleotides in the unique sequence of the 5S RNA, we

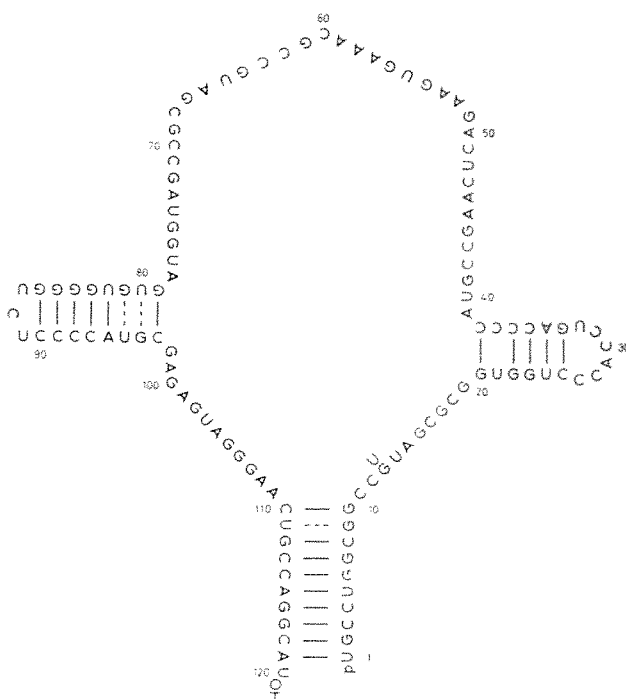


Fig. 1. Sequence of the two chief 5S RNAs of *E. coli* (MRE600) showing regions involved in base-pairing. A solid line indicates a "standard" (G—C or A—U) pair and a dashed line a G—U pair, for example, between residues 81 and 95.



## BIOPHYSICS

## Electron Spin Resonance in Biological Tissues

SEVERAL points arise from the recent communication by Dettmer, Driscoll, Wallace and Neaves<sup>1</sup>.

First, the simple factors affecting electron spin resonance signals in tissues, which they set out to describe, seem to be only the rapid decay of free radical signals, as measured extensively by Commoner and Ternberg<sup>2</sup>, Kerkut *et al.*<sup>3</sup> and mentioned briefly as a source of error by Mallard and Kent<sup>4</sup>. Far from not having appeared in print, as stated in their first paragraph, it is seen that many workers have noted this effect.

Second, the techniques of lyophilization used by Dettmer *et al.* seem to us to be unnecessary and, in certain respects, dangerous. It was shown by Truby *et al.*<sup>5</sup> that lyophilization can create unwanted free radicals in organic materials, and Varian Associates, Inc., even show an example of this in their literature. We think that it is unnecessary to use this technique because adequate techniques now exist for the study of whole tissues at both room temperature and low temperatures<sup>2-4</sup>.

That lyophilization has some effect can be shown from the spectra of lyophilized samples which Dettmer *et al.* show in their communication. These spectra, when observed in the light of the methods of analysis of Searle *et al.*<sup>6</sup> and Lebedev<sup>7</sup>, are seen to represent asymmetric lines, possibly arising from *g*-value anisotropy. The lines have a Lorentzian shape and the anisotropic splitting in terms of individual line width is about 3.0.

On the other hand, the lines observed by other workers, although still asymmetric, can be shown to have a value of anisotropic splitting approximately equal to 2.0, which applies both at room and low temperatures down to 77° K. In addition, the spectra under these conditions result from individual lines of Gaussian shape. Because these Gaussian spectra are in the presence of water (and therefore an environment containing many protons) the Gaussian shape may result from the broadening effect of

have now studied the products of various partial ribonuclease digestions. This has involved the development of a number of new techniques which will be described in detail later. From the large number of partial digestion products obtained, we deduced the unique sequence shown in Figs. 1 and 2.

Fig. 1 is drawn to show the residues which are believed to be involved in base-pairing. These base-paired regions were identified as four sequences which were particularly resistant to digestion by ribonucleases. The longest double-stranded region is believed to be formed by base-pairing between the two ends of the molecule, and there are also two smaller "loops". Base-pairing between the two ends of the molecule is also found in transfer RNA, but otherwise there is less base-pairing in 5S RNA than in transfer RNA.

It will be noted that two residues are shown as occupying position 13. It appears that there are two 5S RNAs, presumably controlled by separate genes, one having a G in position 13 and the other a U. This was found in one strain of *E. coli* (MRE600, obtained from Dr H. E. Wade of the MRE Experimental Station, Porton, Wiltshire), while in another strain (CA265, obtained from Dr S. Brenner of this laboratory) a difference has been found in another position. It is probable that there are also other minor heterogeneities and therefore Fig. 1 illustrates the structure only of the two principal components of 5S RNA in *E. coli*, MRE600.

There are two sequences of ten and eight residues, respectively, that are repeated twice in the molecule. In Fig. 2 the structure is written so that the common sequences are aligned. There is considerable homology between the two parts of the chain, indicated by the boxed regions. This observation suggests that the 5S RNA may have evolved from a smaller RNA by a duplication of a part of the DNA sequence within the gene. There also appears to be some homology between the two ends of the molecule as shown by the underlining in Fig. 2. This could be explained by a separate duplication.

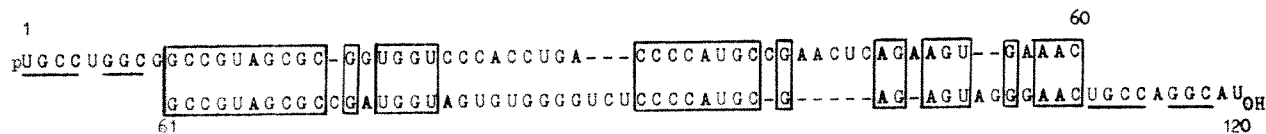


Fig. 2. Homologies between the two halves of the sequence of 5S RNA. The residues are numbered as in Fig. 1. Homologies are shown by the boxed areas. Dashes are where gaps have to be left in the sequence in order to maximize these homologies. The underlining shows similarities between the two ends of the molecule.

Previous work on RNA sequences has been confined to transfer RNAs and a number of complete sequences have been reported<sup>4-7</sup>. The 5S RNA is 120 residues long compared with 75-85 residues in the transfer RNAs, and the absence of "minor" bases makes interpretation somewhat more difficult. This work shows, however, that it is possible, using the small-scale techniques which we have developed, to determine the nucleotide sequence of an RNA labelled with phosphorus-32.

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this. The difference in the apparent anisotropic splitting between the Lorentzian and the Gaussian spectra may arise from a difference in individual line width between the two cases. Because no details of line widths are published by Dettmer *et al.*, however, it is not possible to follow this further.

Alternatively, it is possible that the differences described here arise merely because these authors are observing electron spin resonance signals generated by the lyophilization process, and this in itself represents a serious pitfall.

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## IMMUNOLOGY

## Rapid Activation of Lymphocytes by Phytohaemagglutinin

WHEN human small lymphocytes are incubated *in vitro* with phytohaemagglutinin (PHA) a large proportion of them transform into large, rapidly dividing lymphoblasts<sup>1</sup>. Synthesis of DNA begins about 24 h after the addition of PHA<sup>2</sup>. Nowell<sup>1</sup> and Newsome<sup>3</sup> found that when lymphocytes were in contact with PHA for 1 h or less a lesser degree of transformation took place, and preliminary results in this laboratory suggest that exposure of lymphocytes to PHA for 3 h results in substantial transformation<sup>4</sup>, as judged by the incorporation of <sup>14</sup>C amino-acids into protein. This finding has been re-investigated in the light of recent reports that little transformation or synthesis of DNA occurs if the period of exposure to PHA is less than 24 h (refs. 5-7).

Lymphocyte cultures were prepared and incubated as previously described<sup>1</sup>. After incubation of cultures for the time required, medium containing either tritiated thymidine (3.3  $\mu$ Ci/ml.) or <sup>14</sup>C leucine (1  $\mu$ Ci/ml.) was added and isotope incorporation was determined after incubation of the cultures for a further 2 h. Two preparations of PHA have been used in these experiments, PHA-W (Burroughs Wellcome) and PHA-P (Difco). PHA-W was used at a final concentration of 150  $\mu$ g/ml., and PHA-P at 25  $\mu$ g/ml. These concentrations were found to cause the greatest stimulation of the incorporation of tritiated thymidine and <sup>14</sup>C leucine in lymphocyte cultures incubated with PHA for 30 or 48 h.

Fig. 1 shows that cultures incubated without PHA incorporate little tritiated thymidine. Both preparations of PHA caused a marked stimulation of the incorporation of tritiated thymidine, beginning about 24 h after the addition of PHA. These results are similar to those found by other workers<sup>2</sup>. At all times from 27 to 70 h after the addition of PHA, PHA-W caused more incorporation of tritiated thymidine than PHA-P. In several experiments, it was shown that this result was not due to the use of sub-optimum concentrations of PHA-P. PHA-W was also more effective than PHA-P in stimulating the incorporation of <sup>14</sup>C leucine.

When lymphocytes were incubated with PHA-W for 1 h and then the PHA removed, the rate of incorporation of tritiated thymidine after incubation for 48 h was about 50 per cent of that found when PHA was present throughout the incubation (Fig. 2). When the time of incubation with PHA was increased to 3 h, incorporation of tritiated

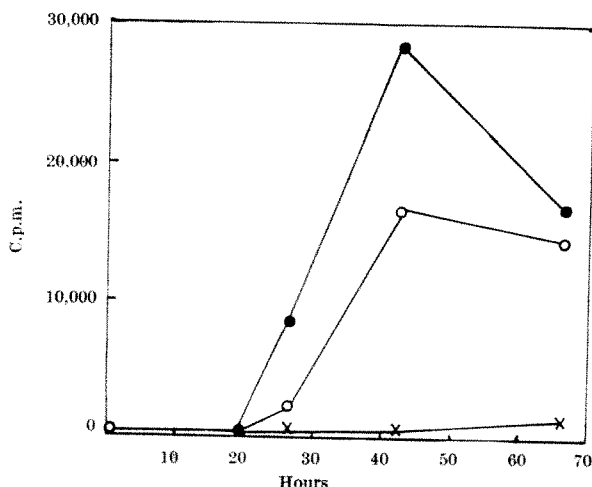


Fig. 1. Incorporation of tritiated thymidine. Cultures were incubated with or without PHA and at the times indicated the medium was replaced by medium containing tritiated thymidine (3.3  $\mu$ Ci/ml.). The incorporation of tritiated thymidine was determined after incubation for a further 2 h. ●, PHA-W; ○, PHA-P; ×, no PHA.

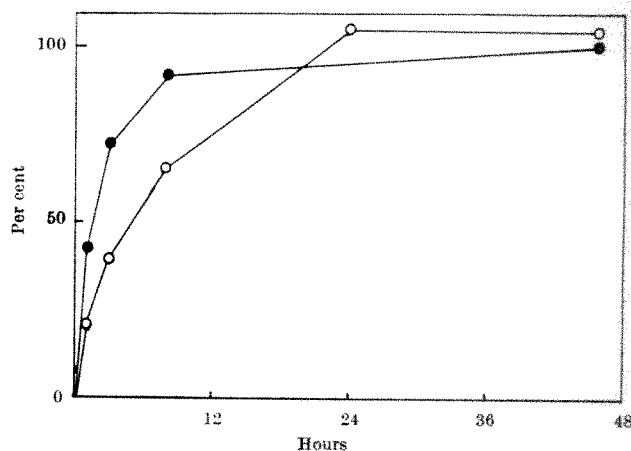


Fig. 2. Effect of limited exposure to PHA on the incorporation of tritiated thymidine. Cultures were incubated with PHA for the times indicated and culture medium was removed after gentle centrifugation. The cells were washed once with culture medium and then resuspended in medium either with or without additional PHA. After incubation of cultures for a total of 48 h the incorporation of tritiated thymidine during a 2 h period was determined. The incorporation found after incubation with PHA for a limited period is expressed as a percentage of the incorporation by cultures incubated with PHA throughout. ●, PHA-W; ○, PHA-P.

thymidine was 70-90 per cent of control levels, and after incubation with PHA-W for 8 h further addition of PHA did not lead to any significant increase in the incorporation of tritiated thymidine. Fig. 2 shows that a longer incubation with PHA-P was required for maximal stimulation of tritiated thymidine incorporation to result, but even when the exposure to PHA-P was for 1 h the incorporation of tritiated thymidine was substantially higher than that found in cultures incubated without PHA. Increasing the concentration of PHA-P to 50  $\mu$ g/ml. did not affect the results, and in all cases the results obtained when the incorporation of <sup>14</sup>C leucine was investigated were similar to those found with tritiated thymidine.

The possibility that PHA might be taken up and later released by phagocytic cells contaminating the cultures was excluded because similar results were obtained when cultures of nearly pure lymphocytes, prepared by the cotton wool column method<sup>8</sup>, were used. To exclude the possibilities that PHA was not removed by the washing procedure, or that it was bound to the red blood cells or to the culture vessels, red blood cells were incubated with and without PHA for 3 h, and after the removal of the PHA the red blood cells were washed and unstimulated lymphocytes added. The incorporation of tritiated thymidine after incubation of the lymphocytes for 48 h was very low whether the red blood cells had been incubated with or without PHA. The red blood cells did not inhibit the incorporation of tritiated thymidine when PHA was added together with the lymphocytes.

The reason for the difference between the results reported here and those of Tormey and Mueller<sup>6</sup> and Yamamoto<sup>6</sup> is not obvious. The washing procedures used by these authors do not appear to be more rigorous than ours. It may be relevant that these authors used a preparation of PHA not used in these studies, PHA-M (Difco), but PHA-M was also used by Newsome<sup>3</sup>, who found some transformation after incubation of lymphocytes with PHA for short periods. The experiments reported here are not necessarily comparable with those of Mellman and Rawnsley<sup>7</sup>. These workers also used PHA-M, but the PHA was inactivated by the addition of antibodies. It is possible that PHA could be bound to the cells in such a way that it was not removable by washing, but was accessible to neutralizing antibodies.

Ling and Holt<sup>9</sup> found that exposure of lymphocytes to a purified preparation of PHA-W for 16 h caused sub-

stantial transformation. Their results are in good agreement with those reported here. A similar purified preparation of PHA-W has been used in these experiments, and the results obtained did not differ significantly from those found with crude PHA-W except that the concentration of PHA-W causing maximal stimulation of incorporation of tritiated thymidine was reduced to 2.5  $\mu\text{g/ml}$ . The transformation of lymphocytes by antibodies to autologous gamma globulins has been shown to follow exposure of lymphocytes to the stimulant for less than 1 h (refs. 10 and 11).

The results reported here suggest that the period of exposure of lymphocytes to PHA required for transformation may be short. Comparison of these results with those obtained by other workers shows that the factors affecting the period required are not yet established, but suggest that they may include the preparation of PHA used. The results suggest that the active components of PHA-W and PHA-P may not be identical, and that the PHA-W preparation is the more active. An alternative explanation would be that the active components of the two PHA preparations were identical, but that the PHA-P preparation contained a substance that inhibited lymphocyte growth.

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### Release of Histamine and Slow Reacting Substance with Mast Cell Changes after Challenge of Human Lung sensitized passively with Reagin *in vitro*

I HAVE examined various tissues for their ability to adsorb reagins from whole serum *in vitro*, and human lung was one of the most effective adsorbents. I tested fresh human lung, removed at operation from a non-allergic individual, for passive sensitization, and release of histamine with mast cell changes after challenge with antigen *in vitro*.

Sera used to sensitize the lung fragments passively were from two patients, allergic to milk, who gave an immediate skin response to milk protein. The sera contained reagins to  $\beta$ -lactoglobulin detected by passive cutaneous anaphylaxis in baboons. Two sera, which contained similar agglutination titres of IgG and IgA antibodies to  $\beta$ -lactoglobulin, were from normal persons and two were from cases of cot death. The sera were tested for IgG, IgA and IgM antibodies to  $\beta$ -lactoglobulin by the very sensitive red cell linked-antigen antiglobulin reaction, employing monospecific antiglobulin sera<sup>1</sup>, and also for potency in preparing skin for passive cutaneous anaphylaxis in baboons, with 28 h between sensitization and challenge (Table 1). None of the sera sensitized guinea-pig skin for passive cutaneous anaphylaxis after a latent period of 4 h. Any release of smooth muscle contracting agents caused by soluble antigen-antibody complexes, although their formation in the test conditions

Table 1. RECIPROCAL OF SERUM ANTIBODY TITRES TO  $\beta$ -LACTOGLOBULIN BY THE RED CELL LINKED ANTIGLOBULIN TEST AND PASSIVE CUTANEOUS ANAPHYLAXIS IN BABOONS

Serum	Titres			Passive cutaneous anaphylaxis
	IgG	IgA	IgM	
Normal 1	32	4	0	0
Normal 2	128	32	2	0
Milk sensitive 1	64	16	0	32
Milk sensitive, heated	64	16	0	0
Milk sensitive 2	64	8	4	16
Cot death 1	128	16	32	0
Cot death 2	32	8	4	0

is unlikely, was controlled by testing in parallel sera from non-allergic and allergic individuals with agglutination titres very similar to the pure  $\beta$ -lactoglobulin.

Fresh lung was cut into sections 1–2 mm thick including fragments of pleura and small bronchioles, and divided into equal portions weighing 200 mg which were quickly enclosed in stainless steel mesh folded flat to expose a large surface of tissue when submerged in fluid. These were washed in Tyrode solution at 37° C for 20 min, drained of surplus fluid, and treated with 1 ml. of the undiluted test serum. The sera from the normal and allergic subjects were tested three times, but the amounts of the sera from the cases of cot death could sensitize only one piece of lung each. After incubation for 20 min at 37° C they were washed twice in warm Tyrode solution with gentle agitation for 20 min and then slowly irrigated three times with 50 ml. of Tyrode from a reservoir 25 cm above the vessel containing the lung to ensure the removal of unbound serum protein.

The lung was challenged by a modification of the method of Mongar and Schild<sup>2</sup>. Each piece was placed for 10 min in 6 ml. of warm, aerated Tyrode. This solution was removed and kept as the control release sample.  $\beta$ -Lactoglobulin (50  $\mu\text{g}$ ) in 6 ml. of Tyrode was added and removed after 10 min as the test release sample. The control and test release samples, and two pieces of lung each in 6 ml. of Tyrode were placed in a boiling water bath for 5 min and the fluids were assayed for histamine and slow reacting substance. Fragments of a third piece of lung were fixed in 10 per cent formalin, in 4 per cent lead subacetate in 10 per cent formalin and in Bouin's fixative to preserve the mast cells. The single cot death samples were used only for histamine assay on guinea-pig ileum in Tyrode containing  $2 \times 10^{-7}$  molar atropine. The results are presented in Table 2. Release of histamine from the piece of lung used for mast cell examination is not shown, because it closely resembled that of the other two pieces in each test.

Table 2. HISTAMINE EXPRESSED AS  $\mu\text{g}$  OF BASE/200 mg OF TISSUE, RELEASED ON CHALLENGE OF HUMAN LUNG PASSIVELY SENSITIZED *in vitro*, AND MAST CELL CHANGE

Serum	Release				Percentage release		Changed mast cells/total counted after challenge
	Con-trol	Chal-lenged	Residual	Total	Con-trol	Chal-lenged	
Normal 1	0.25	0.32	11.05	11.62	2.2	2.8	7/200
Normal 2	0.40	0.37	9.33	10.10	4.0	3.7	11/200
Milk sensitive 1	0.22	2.60	8.72	11.54	1.9	22.5	58/100
Milk sensitive, heated	0.23	0.26	11.11	11.60	2.0	2.2	8/200
Milk sensitive 2	0.24	2.28	9.06	11.58	2.1	19.7	62/150
Cot death 1	0.42	0.49	7.49	8.40	5.0	5.8	Not done
Cot death 2	0.46	0.43	7.14	8.03	5.7	5.4	Not done

Results of normal sera and milk sensitive sera are the mean of two tests.

About 20 per cent of the total extractable histamine was released by antigen from lung sensitized passively with sera from patients allergic to milk; heating the serum at 56° C for 30 min before the tests destroyed the sensitizing activity. The non-specific release of histamine by the cot death sera without addition of antigen may have been caused by post-mortem tissue breakdown products, because histones and possibly other tissue components will disrupt mast cells<sup>3,4</sup>, or it may have been caused by enzymes activated by anaphylaxis, if this was the cause of death. The anaphylactoid effect of soluble complexes is unlikely to have been the cause, because

gel plate precipitation detected no milk antigen in the cot death sera.

One of the normal sera released nearly twice as much histamine as the other in the control release solution, which was probably caused by the cytotoxic effect on tissues of serum from another donor. The cause may have been non-allergic, but, theoretically, could have resulted from incompatible blood group antibodies.

Both histamine and slow reacting substance are released from lungs of actively sensitized persons when tested with antigen *in vitro*<sup>5-7</sup>. Because slow reacting substance increases the amplitude of the contraction of guinea-pig ileum induced by histamine when both are present, four of the test solutions were also examined for slow reacting substance, using  $10^{-6}$  molar mepyramine maleate to inhibit the histamine.

No detectable slow reacting substance was released with the histamine leached from the tissue before the test (Table 3), but because no precautions were taken to prevent its loss by adsorption to proteins or glassware there may have been small amounts in the samples. The contractions of guinea-pig ileum were small and slow with the solutions obtained from lungs sensitized with reagin after challenge, so that the histamine equivalent is probably slightly larger than the true histamine content<sup>8</sup>. The samples from the portions of the lung treated with cot death sera caused weak contractions before and after challenge; as with the histamine released this may have been caused by post-mortem tissue breakdown products in the sera or by activated enzymes releasing the pharmacological agents from the lung.

Table 3. RELEASE OF SLOW REACTING SUBSTANCE FROM HUMAN LUNG PASSIVELY SENSITIZED AND CHALLENGED *in vitro* BY ANTIGEN

Test serum	Release	
	Control	Challenge
Normal serum 1	—	—
Milk sensitive 1	—	—
Same serum kept at 56° C for 30 min	—	—
Milk sensitive 2	—	+
Cot death 1	tr	tr
Cot death 2	tr	tr

—, Absent; tr, trace; +, present.

Tables 1 and 2 show that passive sensitizing ability of the serum is unrelated to the titre of the IgA antibody, which was also found in tests with several antigens by the passive cutaneous anaphylaxis technique in baboons and by Prausnitz Kustner tests in man (my unpublished work). Furthermore, the IgA titre was unaltered in sera kept at 56° C, confirming the findings of Coombs *et al.*<sup>1</sup>, although the tissue passive sensitizing activity was destroyed. Thus either the antigen-binding and cell-binding properties of the antibody molecule are not equally susceptible to heat, or, more likely, IgA is rarely, if ever, reaginic.

The changes in mast cells, stained with 0.5 per cent aqueous toluidine blue and cleared with butanol, resembled those described after *in vitro* challenge of mesentery taken after death from a person actively sensitized to egg-white<sup>8</sup>. There were fewer mast cells in the sensitized lung after challenge, and of those remaining (Table 2) some had shed their granules or had become vacuolated with aggregation of the granules within the cell membrane, and in a few the granules had lysed in the cytoplasm and the nucleus was pyknotic. These changes were evident in lung fixed by all three methods; but the granules were most distinct after fixation in lead subacetate. Changes in mast cells are a useful indication of allergic reactions when release of histamine is also measured, but it provides unreliable evidence of anaphylaxis in tissues which have undergone inflammation and been sampled some time after death because non-specific changes occur; although anaphylaxis-like changes in mast cells have been seen in five out of fourteen lungs after cot death<sup>9</sup>.

Fresh human lung can be passively sensitized *in vitro* with reagins to milk, and when challenged releases hist-

amine concurrently with damage to mast cells. Its use in this way provides an additional method for the investigation of human allergies and particularly for the study of milk sensitivity and its relation to cot death in infants<sup>9,10</sup>. The independence of reagin activity and IgA antibody is confirmed. Two cot death sera did not sensitize the lung passively but both induced a spontaneous release of histamine.

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## Sodium Transport and Specific Antiserum

THE cytotoxic effects of tissue specific antibodies have been studied *in vivo* under conditions of auto-immunity (induced or idiopathic) and *in vitro* using cells in tissue culture<sup>1</sup>. Although much is known about the histochemical and morphological changes induced by specific antibodies, very little is known of the effect of specific antibodies on the active transport mechanisms of the cell membrane. We have used the urinary bladder of the toad (*Bufo marinus*) to test the effect of antibodies on the active transport of sodium.

Antisera to the urinary bladder were obtained from rabbits injected with a homogenized preparation of bladder tissue. The washed urinary bladder was homogenized with 3 ml. of toad Ringer solution (115 mmolar NaCl; 2.5 mmolar KHCO<sub>3</sub>; 1.0 mmolar CaCl<sub>2</sub>) using a hand homogenizer, and the resulting homogenate was diluted to 10 ml. with Ringer solution. Rabbits were injected intravenously with 1 ml. of tissue homogenate on alternate days for a total of three injections. One week after the last injection the rabbits were bled and the sera collected. The antiserum to the toad bladder (TBA) and the control serum, normal rabbit serum (NRS), were dialysed against Ringer solution before being tested.

The bladder was removed and divided into its equal hemispheres. Each hemisphere was mounted between two conical compartments which were then filled with 10 ml. of Ringer solution and continuously aerated (Fig. 1). One hemisphere served as the experimental tissue and the other as the control. The active transport of sodium was measured by the short circuit current (SCC) method of Ussing and Zerahn<sup>2</sup>.

After the tissue had been mounted, it was allowed to equilibrate for 1 h. At 1 h the transmembrane potential was adjusted to zero and the SCC required to maintain the transmembrane potential at zero was recorded every 10 min for the next 60 min to establish the stability of the membrane and to discern whether the sodium transport was increasing or decreasing with time. The membrane in the experiment represented by Fig. 1b was stable and



without an upward or downward trend. At 61 min (that is, 121 min after the tissue was mounted) 1 ml. of normal rabbit serum (NRS) was added to both sides (mucosal and serosal) of the control membrane and to the mucosal side of the experimental membrane; 1 ml. of TBA was added to the serosal side of the experimental membrane. A decrease in the SCC was observed in both cases. The addition of antiserum produced a 24 per cent decrease at 70 min as compared with a 4-5 per cent decrease when NRS was added. The percentage decrease in the SCC of the control was calculated by averaging the SCC values observed during the 30 min immediately preceding the addition of serum (4 per cent decrease) and again by using the value at 60 min (7 per cent decrease).

The experiment was repeated eight times. In seven out of eight experiments the SCC decreased when TBA was added to the serosal side (in one case the SCC remained constant) while no effect was observed when NRS was added (in the one exception the SCC decreased slightly).

The data were normalized in subsequent experiments by the following procedure. First, all SCC measurements were expressed as a fraction by dividing each value by the 60 min SCC measurement. In Figs. 2 and 3 the normalized SCC is referred to as the relative short circuit current (RSCC). Because the RSCC seldom remained constant, a trend in the RSCC was derived from the measurements made at 30, 40, 50 and 60 min and projected into the subsequent time period (see dashed line, Fig. 2). The trend was then equated to 1 and individual RSCC plotted as fractions. It was thus possible to com-

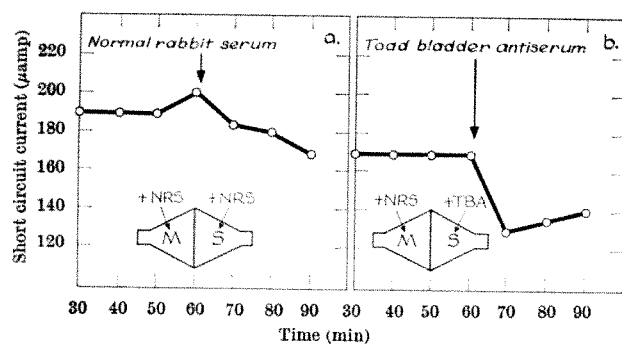


Fig. 1. An experiment demonstrating the effect of specific antiserum on sodium transport. Reagents added to the mucosal (M) and serosal (S) chambers are indicated by schematic drawing of the urinary bladder mounted between the two conical chambers.

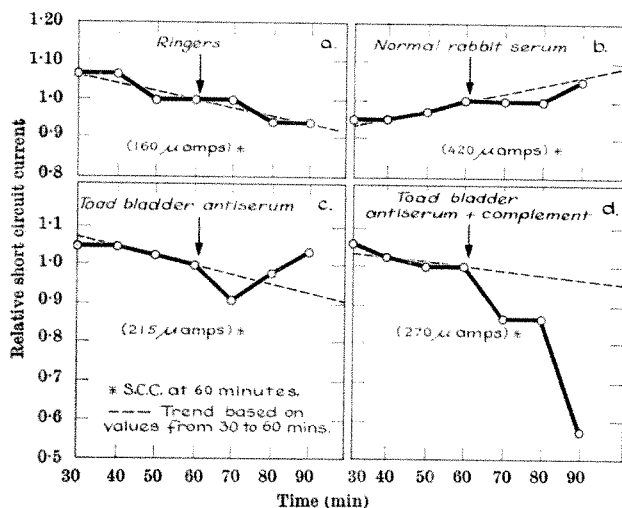


Fig. 2. Experiments demonstrating the effect of various reagents (that is, Ringer solution, normal rabbit serum, antiserum and antiserum plus complement) on sodium transport.

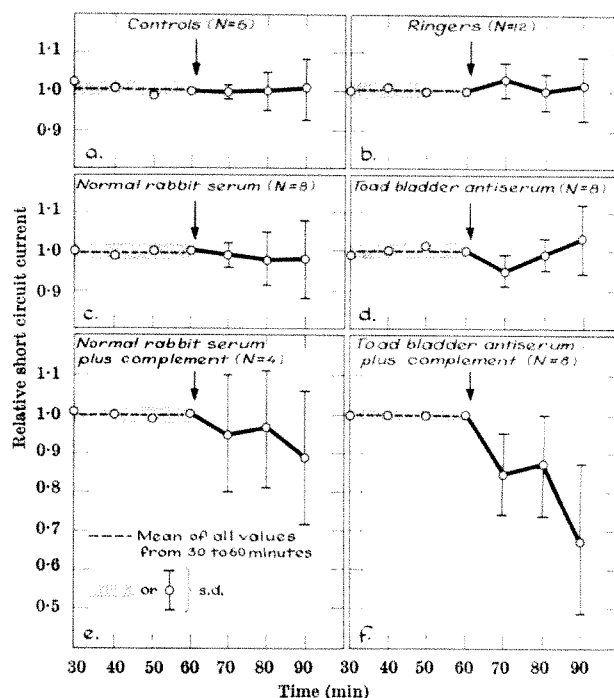


Fig. 3. Summary of combined results.

pare the results obtained from different membranes. Fig. 3 summarizes the results of forty-six experiments which were analysed by this procedure.

The results summarized by Fig. 3c and 3d are consistent with previous results. NRS had little or no effect on the RSCC and the addition of antiserum produced a significant decrease when added to the serosal side. The greatest decrease in the RSCC occurred at 70 min and it was followed by an upward trend, which was not observed in the NRS controls.

Because the decrease in SCC was small when antiserum was added, it was decided to test the system in the presence of complement to determine whether the response could be potentiated. At 61 min, 1 ml. of antiserum and 0.5 ml. of normal guinea-pig serum (complement) were added to both sides of the membrane. The 70 min measurement was 85 per cent of the value obtained at 60 min; at 90 min the RSCC had decreased to 55 per cent (Fig. 2d). In contrast to antiserum alone, the antiserum plus complement caused a dramatic and irreversible decrease in the RSCC (Fig. 3f).

The complement control experiments tested a combination of NRS and complement. This combination caused an average decrease in the RSCC of 5 per cent and caused a marked instability in the activity of the membrane as indicated by the large S.D. at 70 min (see Fig. 3d). The reaction elicited by the NRS complement combination might be because the normal guinea-pig serum was not dialysed against Ringer solution or because of the presence of heterophile antibodies in either or both sera.

The cytotoxic properties of specific antibodies have been extensively studied using mammalian cells in tissue culture. In the absence of complement, ascites tumour cells remain viable even in the presence of specific antibodies<sup>3</sup>. Changes are produced, however, by the antibody alone because in the presence of antibody the growth rate is slower than that of the controls, and if sufficient antibody is used death can occur<sup>3,4</sup>. Examination with the electron microscope of cells treated with ferritin labelled antibodies in the absence of complement revealed that antibodies were attached to the cell membrane and that the antibodies induced a labyrinthine folding of the membrane<sup>5,6</sup>. Thus even though the integrity of the cell



membrane was not destroyed by antibody alone, changes in the ultrastructure of the cell membrane were observed.

In view of work reported with mammalian cells we interpret our results in the following manner. Rabbits injected with toad bladder homogenates produced antibodies which specifically altered some part of the sodium transport mechanism. The small reversible decrease in the SCC elicited by antiserum alone could be explained by postulating that only a small percentage of the antibodies were directed to key transport structures on the cell membrane. The large irreversible effect produced by the addition of complement indicated that the integrity of the cell membrane was disrupted and all antibodies directed to the cell membrane were potential contributors towards its destruction. Thus antibodies specific for those parts of the membrane responsible for sodium transport caused the initial reversible decrease in SCC; antibodies directed to the membrane in general in the presence of complement caused irreversible damage to the cell membrane and a subsequent decrease in the SCC.

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### Renal Transplantation in Cattle Twins

SIMONSEN<sup>1</sup> was the first to demonstrate that in one pair of chimeric cattle twins renal transplants produced urine for 5 months, and Calne<sup>2</sup> confirmed this observation. Recently, Cragle and Stone<sup>3</sup> reported that most of the renal grafts among chimeric cattle were still functioning at 76–295 days. These reports<sup>1–3</sup> did not contain details of histological and functional changes in the grafts. The present communication presents the results of histological study of renal transplants in 3–6 month old calves. Autografts, allografts between single born animals and grafts between non-identical twins were performed. The twins were from like-sexed pairs and were diagnosed as dizygotic by morphological criteria, for example, type and pattern of pigmentation and hair colour, shape of the head, general appearance and conformation. Blood typing was not done. Because of the high incidence (90 per cent) of chimerism in cattle twins<sup>4</sup>, however, it seems likely that these animals were chimeric.

The kidney, usually the left, was transplanted to the left side of the neck after perfusion with heparin and procaine containing cold (4°C) Hartmann solution. The vascular anastomoses were completed within 30 min and the ureter was anastomosed to the oesophagus. In each animal only one of its own kidneys was retained *in situ*. Percutaneous needle biopsies of the graft were taken at various times.

In biopsies from the four allografts, taken at daily intervals, there was progressive lymphocytic infiltration from day 3 onward indicating graft rejection. In three of the grafts there were also changes of superimposed pyelonephritis, that is, some of the tubules contained pus cells. All the grafts became necrotic between days 10 and 14. On removal, the arterial and uretero-oesophageal

anastomoses were patent but the renal veins contained fresh thrombi.

Biopsies were taken at 2, 3 and 4 weeks from the two autografts. The grafts were removed at 4 months. In one graft no pathological changes were observed. In the other autograft the biopsy taken at 2 weeks showed acute pyelonephritis which subsided during the following two weeks. On removal the kidney was scarred. Histologically there were wedge shaped areas of fibrosis in the cortex which contained a moderate lymphocytic infiltration. There were no changes indicating graft rejection. Samples of urine produced by the graft and *in situ* kidney were collected immediately before removal of the graft; both were free of protein and contained normal concentrations of sodium, potassium, chloride and urea.

From the four grafts between co-twins the biopsies obtained at 2, 4 and 8 weeks showed progressive lymphocytic infiltration, tubular degeneration and atrophy and thickening of blood vessels. In three grafts, there was also evidence of pyelonephritis. These three grafts were removed at 4 months. The fourth graft was excised at 8 months. In all grafts, except one removed at 4 months, the anastomoses were patent. In this graft the renal artery contained a recanalized thrombus and the uretero-oesophageal orifice was occluded by fibrous tissue. In the graft removed at 8 months there was marked perivascular and periglomerular infiltration with small lymphocytes, plasma cells and "large pyroninophilic cells", localized tubular atrophy and necrosis, periglomerular fibrosis, intimal and medial fibrosis of arteries and some veins (Fig. 1), massive periureteral lymphocytic infiltration sharply terminating at the uretero-oesophageal junction (Fig. 2), and ingrowth of muscular arteries from the renal capsule (Fig. 3). These observations suggest an immunologically mediated graft rejection process. Comparison of chemical analyses of urine samples from the graft and from the animal's own kidney, collected immediately before removal of the graft, showed that their concentrations of sodium and chloride were similar. The urine from the graft contained less potassium (44:130 m.equiv./l.) and urea (150:800 mg per cent) and more lactic acid dehydrogenase (242:29 U/ml.). Protein was present only in the urine from the graft (110 mg per cent).

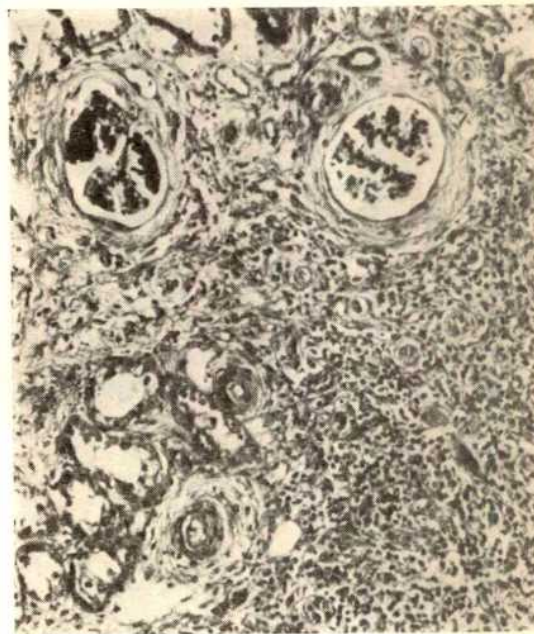


Fig. 1. Co-twin renal graft at 8 months. Section of kidney showing massive lymphocytic infiltration, parenchymal damage and vascular thickening. (Haematoxylin and eosin,  $\times 42$ .)



In the other three co-twin grafts, the complicating infection had not affected all segments of the kidney. In the areas without suppuration, the histological changes were similar to those shown in Figs. 1-3.

The sequence of events and the histological findings in our allografts are similar to those described for immunological rejection of renal grafts in man and dog<sup>5,6</sup>. The time of rejection is similar to that described by Cragle and Stone<sup>3</sup> for cattle. Although all co-twin grafts survived for long periods of time, histological features associated with immunological rejection were already present in biopsies taken at 2 weeks. The rejection process progressed very slowly and in many respects resembled the changes that occur in allografts maintained by immuno-

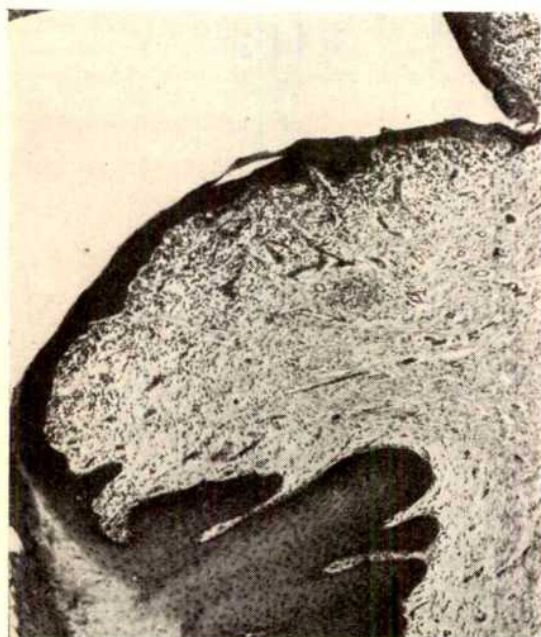


Fig. 2. Same animal as Fig. 1. Section of uretero-oesophageal junction showing periureteral lymphocytic infiltration sharply terminating at the junction. (Haematoxylin and eosin,  $\times 17$ .)



Fig. 3. Same animal as Fig. 1. Section showing ingrowth of arteries from the renal capsule into the kidney. (Haematoxylin and eosin,  $\times 17$ .)

suppressive therapy<sup>7</sup>. The raised lactic acid dehydrogenase content of urine from the co-twin graft—a serious prognostic sign according to Prout *et al.*<sup>8</sup>—also indicates that the graft was being rejected. The slow mild rejection process in the kidney grafts which we observed is in agreement with findings of Billingham and Lampkin<sup>9</sup> and Stone *et al.*<sup>10</sup> on skin grafts between dizygotic cattle twins. The slow rejection of co-twin tissues and organs by chimeric cattle twins suggests that these animals are in a state of split tolerance<sup>11</sup>, that is, they are tolerant to only some of the antigens from each other. These experiments also help to explain the clinical observations that long term survival of renal allotransplants can occur when donor and recipient are unrelated but are largely compatible for principal erythrocyte and leucocyte antigens.

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### Concentration of Immunoglobulins in Lethally X-irradiated Mice

SEVERAL reports have been published concerning the immunoglobulins of sublethally and lethally irradiated mice<sup>1-4</sup>. These have relied on the techniques of electrophoresis and immunoelectrophoresis. The former, although it can yield some quantitative information, does not adequately distinguish between different classes of immunoglobulin, and the latter, while offering excellent resolution of immunoglobulin classes, is only in the roughest way quantitative. These studies have indicated that a profound drop occurs in the concentration of gamma-globulins within a few days after lethal irradiation. The availability of unispecific antisera to each of four mouse immunoglobulins<sup>5</sup>—IgA, IgM, IgG1 and IgG2a (ref. 6)—and the development of the technique of single radial diffusion in agar whereby such antisera can be used to yield quantitative measurements of their corresponding antigen<sup>7</sup> have now made it possible to obtain more precise information concerning the effects of radiation on the concentration of immunoglobulins.

Twenty male CBA/H mice, 5 months of age, were used. Seven received 1,000 rads (250 keV, 14 m.amp., half value thickness 1.2 mm copper), seven received 800 rads, and

the remaining six served as non-irradiated controls. Irradiation was administered ventro-dorsally to the whole body. The twenty mice were caged in four boxes, among which irradiated and control mice were distributed at random.

All mice were bled from the tail immediately before irradiation, and on the fifth, seventh and ninth days thereafter. Control mice were bled also on the twelfth, sixteenth and twenty-second days. The amount of blood taken on each occasion was about 0.05 ml.

Mortality among the irradiated mice was as follows:

	Time of death (days)
800 rads	8 (3 mice), 9, 10, 12
1,000 rads	6 (3 mice), 7, 8 (2 mice), 9

Each serum sample was individually titrated in single radial diffusion<sup>7</sup> against each of the four unispecific anti-Ig sera. Diffusion was allowed to continue for 14 days under paraffin oil, because shorter periods of diffusion had been found to give inconsistent results.

Pure reference preparations were made of IgM and IgG2a. IgM was prepared from normal mouse serum by separation of euglobulins on 'Sephadex G-25' (superfine grade), followed by filtration on 'G-200', as described elsewhere<sup>8</sup>; IgG2a was purified from paraprotein of mouse myeloma 5563 by the technique of Askonas<sup>9</sup>. Use of these reference preparations enabled us to express the results of titrations for IgM and IgG2a in mg protein/ml. of serum. No reference preparations of IgA and IgG1 were available, and the results are therefore related to a large pool of normal mouse serum the values for which are taken as unity.

There were no significant differences between the two irradiated groups in respect of any of the four immunoglobulins, and the results have therefore been pooled in order to simplify presentation and facilitate statistical comparison with the controls.

The concentrations of each of the four immunoglobulins in the serum are shown in Fig. 1. Each shows a progressive and significant fall in concentration from that before irradiation and this is most pronounced with IgA.

The percentage falls were as follows:

	IgM	IgA	IgG1	IgG2
Seventh day	11	86	41	56
Ninth day	32	85	68	56

The non-irradiated controls showed substantial fluctuations possibly due to the repeated bleeding; one consequence of this is that at no time can the concentrations of IgG1 and IgG2a in the experimental groups be shown to differ significantly from the controls of the same day. The statistical comparisons, based on Student's *t* test, are shown in Table 1.

Table 1. LEVELS OF SIGNIFICANCE, ESTIMATED BY STUDENT'S *t* TEST, OF THE DIFFERENCES IN THE CONCENTRATIONS OF Ig (A) BETWEEN IRRADIATED AND CONTROL MICE, AND (B) BETWEEN IRRADIATED MICE BEFORE AND AFTER EXPOSURE

	A Significance of difference from controls of same day			B Significance of difference from concentrations before irradiation		
	5 days after irradiation	7 days after irradiation	9 days after irradiation	5 days after irradiation	7 days after irradiation	9 days after irradiation
IgM	NS	<0.01	<0.01	<0.02	NS	<0.01
IgA	<0.05	<0.01	<0.01	<0.01	<0.01	<0.01
IgG1	NS	NS	NS	<0.05	<0.05	<0.01
IgG2	NS	NS	NS	NS	<0.02	<0.05

NS, Not significant.

These results do not support the widely held view that the concentration of immunoglobulins in the serum are profoundly reduced, some to the point of virtual disappearance, after lethal doses of radiation. There appears to be a particularly marked effect on IgA, but even this protein by no means disappears. The difference between the present and previous conclusions is probably attributable in large measure to the greater sensitivity of the

technique of single radial diffusion, which, with the antisera used, yielded quantitative estimates of antigen down to lower concentrations than were required for this study (about 0.10 mg/ml. for IgM; 0.01 mg/ml. for IgG2a; 0.05 unit for IgA; 0.05 unit for IgG1). Other factors which may have influenced the results are the age, strain and immunological status of the mice. These were older than those commonly used for such experiments and had relatively high initial concentrations, at least of IgM and IgG2a. According to Sassen *et al.*<sup>4</sup>, some kinds of infection can influence the concentration of Ig under similar experimental conditions, and it would be difficult to exclude the operation of such a factor.

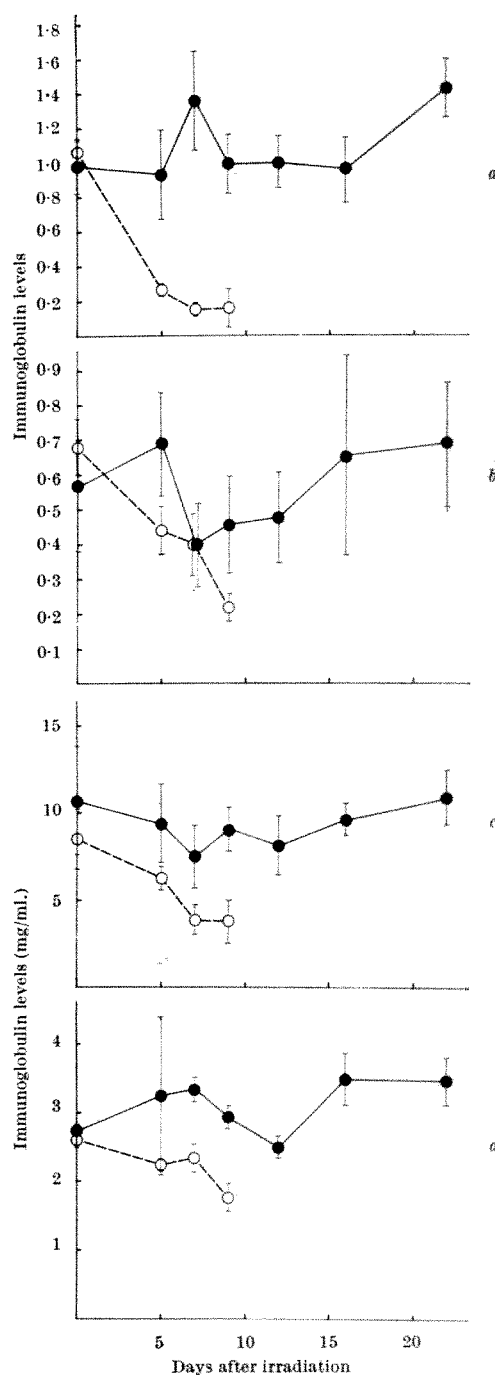


Fig. 1. Changes in the concentration of immunoglobulins after lethal total-body X-irradiation (mean  $\pm$  S.E.). a, IgA; b, IgG1; c, IgG2a; d, IgM. Values for IgA and IgG1 are expressed as units, the concentration in a large pool of normal serum being taken as 1 unit. Values for IgG2a and IgM are in mg/ml. ●, Non-irradiated mice; ○, irradiated mice.



The reduction in the concentration of Ig after irradiation probably results largely from interference with synthesis after damage to lymphoid tissue and particularly from proliferating cells of the plasmacytic series. The possible intervention of other factors, such as loss of protein through the intestinal wall<sup>10</sup>, and changes in the water-content of the body, however, make it impossible, on the basis of our results, to say exactly when and to what extent the synthesis of Ig is disrupted.

This work was begun while the authors were colleagues at the MRC Radiobiological Research Unit, Harwell. One of us (H. B.) was aided by a grant from Euratom.

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## Hepatic Suppression of Sensitization to Antigen absorbed into the Portal System

THE position of the liver between the gastrointestinal tract and the general circulation provides certain metabolic advantages. If, in addition, the liver functions to prevent sensitization to potential antigens contained in food and absorbed into the portal system, then this interposition is also immunologically important.

Specific immunological unresponsiveness to picryl chloride has been induced in adult guinea-pigs by feeding this hapten to the animal before application to the skin<sup>1</sup>. Picryl chloride administered to mesenteric veins of guinea-pigs produced effects similar to oral feeding<sup>2</sup>; this phenomenon is referred to as the Chase-Sulzberger effect and remains unexplained. The underlying mechanism, however, seems to depend on the fact that antigen is carried to the liver before it reaches either the lymphatic system or the general circulation. In this communication we describe experiments which indicate that repeated oral feeding of 1-chloro 2,4-dinitrobenzene (DNCB) to adult dogs before subcutaneous injection suppresses the formation of specific circulating antibodies and that diversion of portal flow from the liver abolishes this effect.

Experiments were carried out on twenty healthy adult (10–15 kg) mongrel dogs. The animals were divided into four equal groups, and the following procedures were carried out. In group 1 concentrations of anti-dinitrophenol (DNP) antibody were measured in samples of blood collected from five dogs which had no known exposure to DNCB. In group 2 a solution of 8 mg of 1-chloro 2,4-dinitrobenzene (DNCB) in 1 ml. of olive oil was injected subcutaneously into each of five dogs in quantities of 400γ on day 1, and 200γ on days 5, 8, 11, and 15. On the twenty-sixth day after the first injection samples of blood were collected and levels of anti-DNP antibody were determined.

In group 3 DNCB dissolved in corn oil (20 mg/ml.) was mixed with ground beef and fed to five dogs. A pellet of beef containing 10 mg of the hapten was placed on the back of the dog's tongue with a spatula and swallowing was induced, according to the following schedule: days 1–6, 10 mg of DNCB/day; days 7–14, no DNCB; days 15–20, 10 mg DNCB/day; days 21–33, no DNCB. On the thirty-fourth day DNCB was injected subcutaneously according to the same schedule followed in dogs in group 2. Twenty-six days after the first subcutaneous injection samples of blood were collected for determinations of antibody.

In group 4 under intravenous 'Nembutal' anaesthesia complete portacaval transposition was carried out in five dogs through a midline abdominal incision. After dividing the portal vein and inferior vena cava just inferior to the hilum of the liver, the proximal cut end of each vein was connected to the distal cut end of the other by two short segments of thick walled silastic tubing measuring 8 mm in internal diameter. With the exception of one animal that died during the first 24 h after operation the post-operative courses were uneventful. On the seventieth post-operative day oral feedings of DNCB were started and thereafter the regimen was identical to that carried out in animals in group 3.

Samples of blood were centrifuged immediately at 15g for 10 min at 37° C. The serum was incubated at 56° C for 30 min to inactivate complement and was then absorbed with washed human erythrocytes to remove non-specific agglutinins. The titre of anti-DNP antibodies was then determined by the haemagglutination technique of Bullock and Kantor<sup>3</sup>. For this purpose serum was diluted with saline buffer and tested in concentrations which ranged from 1 to 2–1 to 1,024.

Titres of antibody in the nineteen dogs tested are shown in Fig. 1. Serum from the five animals with no known contact with DNCB (group 1) contained no measurable antibody. Titres of antibody in animals that received only subcutaneous injections of DNCB (group 2) ranged from 1 to 64–1 to 512. In contrast were the dogs repeatedly fed antigen before subcutaneous injections (group 3). The sera of two contained no measurable amounts of antibody, in two the titres were 1 to 4 and in one the titre was 1 to 16.

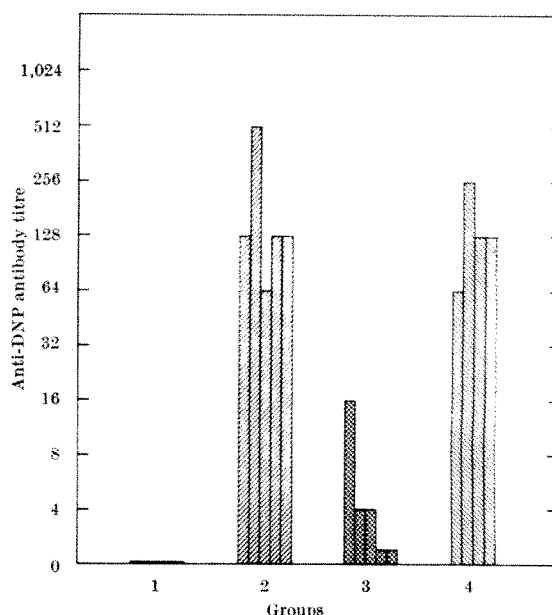


Fig. 1. Titres of serum antibodies to DNP in each dog. Group 1, no known exposure to DNCB. Group 2, DNCB injected subcutaneously. Group 3, DNCB feedings followed by subcutaneous injection. Group 4, portacaval transposition followed by oral feedings and subcutaneous injection.

Sera of dogs undergoing portacaval transposition (group 4) contained antibody in practically identical amounts to sera of dogs in group 2, ranging from 1 to 64-1 to 256. Three to four weeks after operating, femoral venograms showed that contrast material entered the hepatic end of the portal vein from the inferior vena cava. After an interval of 4-5 months large collateral veins appeared on the abdominal wall. Injection of X-ray contrast material into mesenteric veins at this time showed that flow had ceased from the portal vein into the inferior vena cava. No blood from the portal vein entered the liver because all collaterals from the obstructed portal vein bypassed the liver and flowed into the caval or azygos systems.

These experiments indicate that when DNCB is fed to dogs the expected increase in serum antibody after subcutaneous injection of this antigen does not occur. With respect to circulating haemagglutinating antibody therefore the Chase-Sulzberger effect applies in dogs as in guinea-pigs. The effectiveness of preliminary oral feedings was abolished when portal blood was diverted from the liver and this supports the view that circulation of antigen to the liver before entry into lymphatics or into the general circulation impairs ability to recognize or react with the antigen on subsequent contact. Earlier experiments in rats indicate that when complex products of cell disruption were injected into the portal vein, skin grafts from donors of disrupted cells survived four or five times longer than similar grafts in control animals in which disrupted cells were injected into a systemic vein<sup>4</sup>.

Studies of the hepatic reticuloendothelial system have suggested a mechanism of immunological unresponsiveness after circulation of antigen in portal blood to the liver<sup>5</sup>. Immunological conjugates formed on absorption of hapten into portal blood course through liver sinusoids where the complexes are phagocytosed by Kupffer cells. As a result, the particulate or "phagocytosable" portion of the complex which is usually taken up by immuno-competent macrophages to induce the immunological response is separated instead and perhaps permanently deposited in Kupffer cells<sup>6</sup>. Evidence indicates that Kupffer cells specifically and the liver generally are incapable of producing antibodies<sup>7,8</sup>. The soluble portion of the antigen, on the other hand, which is unable to induce antibody production by itself, enters small lymphocytes, rendering them immunologically incompetent, and unresponsiveness results.

Considered in these terms the strategic interposition of liver between the gastrointestinal tract and the general circulation insures against sensitization to simple antigens in food which are absorbed into the portal system. On the other hand, bacteria, lipids containing ten or more carbon atoms and protein are absorbed into lacteals and are transported through mesenteric lymph nodes to the blood<sup>9-11</sup>. It may be significant in this respect that the mesenteric lymph node system reaches its highest development in mammals, a species distinguished by reliance of the newborn on lipid absorbed from the gastrointestinal tract.

Increased concentrations of gamma-globulin characterize patients with advanced cirrhosis and the increase in gamma-globulin consists almost entirely of immunologically active protein<sup>12</sup>. It is tempting to speculate that derangements in the wall of the sinusoid, described by Schaffner and Popper, or spontaneous portacaval shunts, or both, may so alter the disposition of antigen that the effectiveness of the liver in suppressing sensitization to absorbed antigen is lost<sup>13</sup>.

Dogs developed high titres of serum antibody after subcutaneous injection of the simple chemical DNCB. When this antigen was repeatedly fed to dogs for 4 weeks and the animals were then challenged by subcutaneous injections, there was no antibody response. The effectiveness of oral feeding was abolished in dogs undergoing portacaval transposition.

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## HAEMATOLOGY

### Non-identity of the $\alpha$ -Granules of Human Blood Platelets with Typical Lysosomes

PLATELETS are known to be important in blood coagulation, in haemostasis and in thrombosis, and during this process they undergo a series of profound morphological and biochemical changes termed viscous metamorphosis. Platelets contain a wide variety of subcellular organelles<sup>1-5</sup>, and some of them are altered during viscous metamorphosis<sup>6,7</sup>. The most prominent organelles are the very osmiophilic  $\alpha$ -granules, which disappear shortly after the onset of viscous metamorphosis<sup>8</sup>. The  $\alpha$ -granules are believed by some to be the chief source of platelet factor 3, the pro-coagulant phospholipoprotein of platelets<sup>4,8,9</sup>; others regard them as storage organelles for serotonin<sup>10,11</sup> and adenosine triphosphate (ATP)<sup>12</sup>. On the other hand, several authors<sup>13-15</sup> have believed that platelets contain granules with lysosomal activity, and recently Marcus *et al.*<sup>16</sup> claimed to have identified as lysosomes  $\alpha$ -granules isolated from platelet homogenates. Evidence from electron microscopy has encouraged<sup>17</sup> a comparison of the  $\alpha$ -granules with the enzyme-secreting vesicles of the pancreas, and similar particles have been described with the same typical striated internal structure in endothelial cells<sup>18</sup>. Thus there are discrepancies in the interpretation of the nature and function of the  $\alpha$ -granules and a re-investigation of the problem seemed justified.

Human blood platelets were isolated from 15-25 l. of acid citrate dextrose-blood and washed twice, as described before<sup>9</sup>, within 24 h of the collection of the blood.

A suspension containing about  $3 \times 10^6$  platelets/mm<sup>3</sup> was prepared in isotonic imidazole buffer, pH 7.2, containing 2.7 mmolar ethylenediamine tetraacetic acid (EDTA), and homogenized in the cold using an MSE mechanical blender at 10,000 r.p.m. for 6 min. To remove cell fragments and aggregated material the homogenate was centrifuged first for 10 min at 1,200g, then for 20 min at 6,800g, in the swing-out head of a refrigerated centrifuge; the sediments were discarded. The organelles were then sedimented (30 min, 12,000g), and resuspended in the original imidazole buffer. An aliquot of 1.2 ml. of this suspension was layered onto a sucrose density gradient prepared 10 h earlier in a 5 ml. centrifuge tube. For a typical gradient the following concentrations and amounts of sucrose solution were used (from bottom upwards):



0.25 ml. of 45 per cent (g of sucrose/100 g solution), 0.5 ml. of 40 per cent, 0.75 ml. of 37.5 per cent, 0.5 ml. of 35 per cent, 1.0 ml. of 27.5 per cent, and 0.5 ml. of 25 per cent. Separation of the organelles was accomplished by centrifugation at 100,000*g* for 90 min at 2° C (Spinco, model L, with an 'SW-39L' rotor). The zones obtained were displaced from the centrifuge tube by sucrose solution of high density, which was run into the bottom of the tube through a fine capillary. Fractions corresponding to the visible zones were collected. Densities were determined by refractometry, with the aid of a sucrose calibration curve. Characterization of the isolated zones was carried out either on washed material (sedimented from a larger volume of added imidazole buffer at 15,500*g* for 30 min, and resuspended in the same buffer) or directly on the fractions collected from several separations carried out simultaneously. Protein was determined by the biuret method, and phosphate by the procedure described by Bartlett<sup>20</sup>. Acid phosphatase was estimated with *p*-nitrophenylphosphate as substrate, using the Boehringer method; for  $\beta$ -glucuronidase the method in bulletin 105 by the Sigma Chemical Company was used, and the test was carried out in 0.1 molar acetate buffer, pH 4.5. Cathepsin was determined by the method of Gianetto and de Duve<sup>21</sup>. To estimate the activity of platelet factor 3 the method of Egli<sup>22</sup>, and/or the "Stypven"-time<sup>23</sup>, were used. In order to liberate enzyme activities, "Triton-X-100" was added to the organelle fractions.

By the method described, five well defined zones were obtained after density gradient centrifugation. (With a slightly modified procedure a sixth zone, located above the others and consisting of membrane material, is found; this is absent when the procedure described here is followed.)

The characteristics of the five different zones, in the order of increasing densities, are as follows: zone *a* consists mainly of soluble components, and zone *b* is composed mainly of the  $\alpha$ -granules (see Fig. 1). This fraction is rich in acid phosphatase, but has negligible cathepsin and  $\beta$ -glucuronidase activities; it has the greatest content of phosphorus of all the five zones, and the most (absolute and specific) platelet factor 3 activity, which is further increased on disruption of the granules.

Zone *c* follows zone *b* closely. It consists of microvesicles of variable diameter (Fig. 2), most of them surrounded by a single membrane, interspersed with still smaller and darker bodies. This fraction is characterized by cathepsin and  $\beta$ -glucuronidase activities. Acid phosphatase is also present, although the activity is less than that observed in zone *b*. As already reported<sup>24</sup>, immunoelectrophoresis of the disrupted material reveals several plasma proteins, indicating the presence of micropinocytosis vacuoles. No soluble plasma proteins were detected by this method in the  $\alpha$ -granule fraction.



Fig. 1. Electron micrograph of fraction *b* containing  $\alpha$ -granules (glutaraldehyde-osmium tetroxide fixation). ( $\times 33,840$ .)

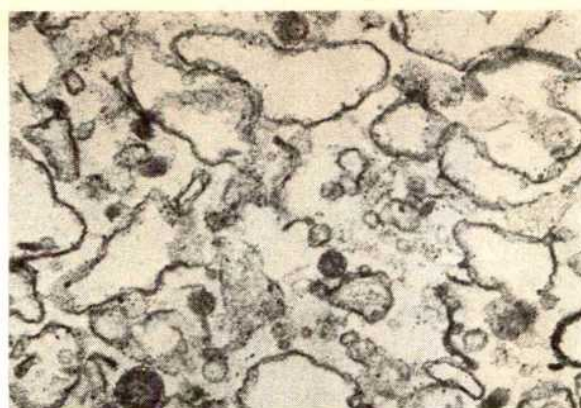


Fig. 2. Electron micrograph of fraction *c* containing microvesicles (glutaraldehyde-osmium tetroxide fixation). ( $\times 33,840$ .)

Zone *d* consists mainly of mitochondria, with some ill-defined membrane material and glycogen grains. Zone *e*, from the bottom of the centrifuge tube, contains all the material with densities equal to or higher than the most concentrated part of the gradient.

A more detailed description of this and the other fractions will be published elsewhere.

These results confirm the idea that blood platelets contain lysosomes; they do not agree, however, with the concept that the  $\alpha$ -granules are typical lysosomes, if such are defined as sedimentable structures containing several acid hydrolases<sup>25</sup>. Table 1 shows the activities of three typical lysosomal enzymes, that is, acid phosphatase, cathepsin and  $\beta$ -glucuronidase, in zones *b* and *c* of the density gradient. It is evident from these values that the structures contained in fraction *c* fulfil much better than the  $\alpha$ -granules the requirements of the definition of lysosomes just given; however, it should be noted that several types of lysosomes have been described, which differ in their morphology as well as in their content of enzymes<sup>26</sup>.

Table 1. ACTIVITIES OF ACID HYDROLASES IN ZONE *b* ( $\alpha$ -GRANULES) AND ZONE *c* (MICROVESICLES)

Zone (localization, expressed as density of sucrose in gradient)	Acid phosphatase (Bessey units/10 $\mu$ g N)	$\beta$ -Glucuronidase ( $\mu$ g phenolphthalein/10 $\mu$ g N/h)	Cathepsin ( $\mu$ g tyrosine/10 $\mu$ g N/h)
<i>b</i> (1.108-1.118)	156	15	17
<i>c</i> (1.156-1.163)	78.2	118	434

The organelles were disrupted with "Triton-X-100". Without this pretreatment no catheptic and only insignificant amounts of  $\beta$ -glucuronidase activities were discernible, while acid phosphatase was less in both zones. The reported values are taken from one of fifty-four separation experiments.

Does the high acid phosphatase content of the  $\alpha$ -granules justify their classification as lysosomes? It must be pointed out that the presence of an acid hydrolase alone is not enough to identify an organelle as a lysosome (compare the high  $\beta$ -glucuronidase activities of microsome<sup>27,28</sup>). It would be important to know more about the specificity and the functional significance of the granular phosphatase. Very little is known, however, about the function of the  $\alpha$ -granules themselves. Although they have a high phospholipoprotein content, it remains uncertain whether they, or their breakdown products, remain confined to the intracellular space during viscous metamorphosis or whether they are released to take part in plasmatic blood coagulation.

In summary, it has been demonstrated that the  $\alpha$ -granules of human blood platelets can be separated from a typical, though morphologically heterogeneous, lysosomal fraction characterized by its comparatively high content of acid phosphatase, cathepsin and  $\beta$ -glucuronidase. Of these acid hydrolases, only acid phosphatase is present in large amounts in the  $\alpha$ -granule fraction. The question of whether this observation, along with the



characteristic morphology of these organelles (compare also ref. 18), permits the classification of the  $\alpha$ -granules among the lysosomes needs further clarification.

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## Immune Lysis of AET-treated Normal Red Cells (PNH-like Cells)

NORMAL human red cells treated with the sulphydryl compounds AET (2-aminoethylisothiuronium bromide) or cysteine in suitable conditions develop some of the characters of the red cells of paroxysmal nocturnal haemoglobinuria (PNH)<sup>1</sup>. In particular, they lyse in slightly acidified fresh compatible normal serum (pH 6.5). Mengel *et al.*<sup>2</sup> later reported that they had obtained similar results by incubating normal red cells with another sulphydryl compound, reduced glutathione. Lysis in acidified serum (a positive Ham test) has been considered characteristic of PNH, and appears to be an expression of the peculiar sensitivity of such cells to lysis by complement. This has been investigated with a complement lysis sensitivity test based on the use of red cells sensitized with a constant, optimal amount of an anti-I antibody and then treated with graded amounts of fresh compatible normal human serum as a source of complement. PNH cells were found to consist of two populations, one markedly sensitive to lysis by C' (about twenty-five times more sensitive than normal cells) and the other usually only slightly more (about twice) sensitive than normal cells. The very sensitive population of cells and the existence of two populations of cells are distinguishing characteristics of PNH which have not so far been found in health or in any other disease state.

Because of the apparent similarities between PNH cells and normal cells treated with AET (AET cells), it seemed worth while to investigate the sensitivity of the latter to immune lysis and to elucidate the relative roles of C' and antibody in bringing about their lysis. The techniques

used were slightly modified versions of those already described<sup>3,4</sup>. The same anti-I antibody of high haemolytic potency, however, was used.

Normal red cells, AET cells and the red cells from two PNH patients, both suffering from a comparatively mild form of the disease, were tested. The AET cells were prepared<sup>1</sup> using an 8 per cent (w/v) (0.285 molar) solution of the chemical.

The complement lysis sensitivity titre (C'L.S. H<sub>50</sub> units) of AET cells (exposed to 8 per cent (w/v) AET for 9 min) was approximately twenty times more than normal (range 15–25), that is, AET cells were found to require only about one-twentieth the amount of C' for 50 per cent lysis compared with normal cells sensitized with the same amount of antibody. Thus in this respect AET cells resemble the more sensitive population of PNH cells. In contrast to PNH blood, however, the haemolysis of anti-I-sensitized AET cells by increasing amounts of C' occurs as a simple function of the concentration of C'; that is, when the logarithm of  $y/(1-y)$  is plotted against the logarithm of concentration of complement (where  $y$  is the fraction of cells lysed), a straight line is obtained and there is no evidence of two populations of cells as is characteristic of PNH (Fig. 1). The degree to which AET cells are sensitive to C' lysis was found to be related to the concentration of AET and to the time to which the normal cells were exposed to its action (Fig. 2). As with normal cells<sup>3</sup>, the line may curve at high C' concentrations.

The amount of lysis which took place after incubation of AET cells in acidified compatible normal serum was related to their complement sensitivity (Table 1). Analysis of the sensitivity to complement lysis of AET cells before and after exposure to acidified serum showed that the cells surviving this treatment were less sensitive to complement lysis than the original cell population.

It is worth noting that AET cells which survived the Ham test were agglutinated by a potent anti-whole-serum antiglobulin serum, while they were not agglutinated by the antiglobulin reagent before exposure to acidified compatible normal serum. Similar observations have been made with PNH cells<sup>5</sup>.

The relative ability of AET and PNH cells to adsorb antibody compared with that of normal cells was calculated by determining the ratio of the percentage of antibody adsorbed by a given number of each type of cell. The ratio for AET cells was 2.7 and that for PNH

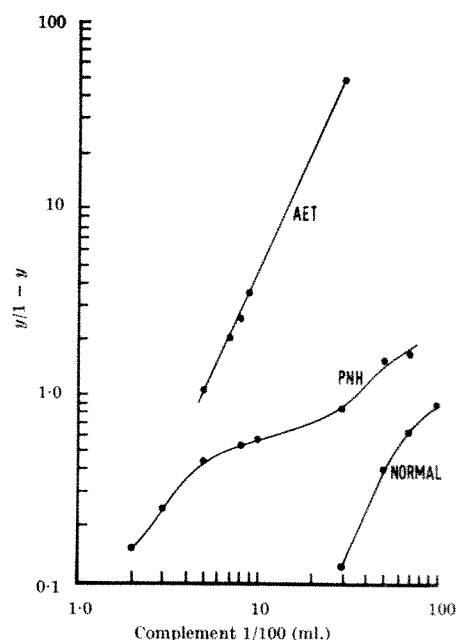


Fig. 1. Representative complement lysis sensitivity curves of normal, PNH and AET-treated cells.



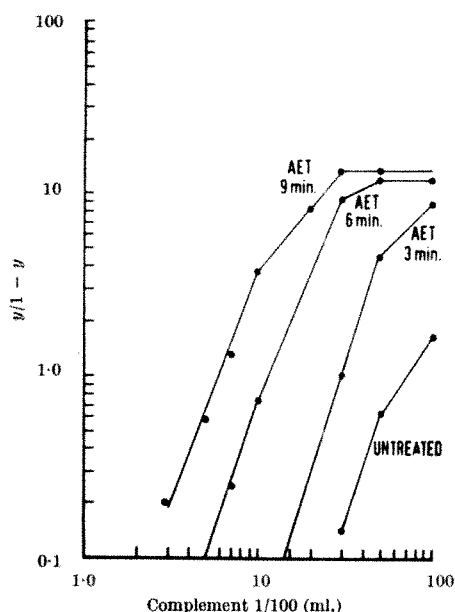


Fig. 2. Complement lysis sensitivity curves of normal cells and of normal cells exposed for different periods of time to 8 per cent (w/v) AET.

cells was 2.0 (ratio for normal cells 1.0). This indicates that the behaviour of the two types of cells is not very different in this respect. The ratio for papainized normal cells has been found to be as high as 7.9 (ref. 4).

The amount of complement and the amount of antibody were measured in a test system before and after reaction with normal cells and with AET cells, respectively, and the ratio of complement fixed/antibody adsorbed was calculated. The amount of complement fixed for each unit of antibody adsorbed seemed to be the same for both types of cells.

Table 1. RELATION OF COMPLEMENT LYSIS SENSITIVITY OF NORMAL CELLS EXPOSED TO 8 PER CENT (W/V) AET TO LYSIS IN THE HAM TEST

Time of exposure (min)	Ham test percentage of lysis (a)	C.L.S. $H_{50}$ units (b)	Ratio (a)/(b)
3	5	3.33	1.50
6	24	8.70	2.75
9	36	16.66	2.16

These experiments indicate that AET cells are very sensitive to the lytic action of complement. They adsorb about 2.7 times as much antibody as normal cells, but this difference in adsorption of antibody does not explain the approximately twenty-fold increase in complement sensitivity of AET cells compared with normal cells. In this respect therefore AET cells behave in a manner similar to the sensitive population of PNH cells. It is tempting to believe that in both cases there is an abnormality of the red cell membrane which renders the cells more sensitive than normal to the action of the late-acting components of the complement sequence.

Mengel *et al.*<sup>2</sup> reported that both PNH cells and normal cells treated with reduced glutathione are more sensitive than normal to lysis in hydrogen peroxide and that in both cases lipids extracted from the cells form increased and comparable quantities of lipid peroxides during exposure to ultra-violet light. The authors concluded that the effect of reduced glutathione in producing PNH-like cells is oxidative, ultimately involving the red cell lipids, and they suggested that in PNH the corpuscular defect which renders the cells susceptible to lysis is related to peroxidation of red cell lipids. Our data indicate that chemical damage can increase a cell's sensitivity to lysis by complement.

Irrespective of the mechanism by which sulphhydryl compounds alter the normal red cell membrane, either by a modification of their lipids or proteins (or both), it seems reasonable to conclude that normal red cells treated

with sulphhydryl compounds such as AET develop damage to their surfaces which may be similar to that occurring spontaneously in PNH. Further study of AET cells should throw light on the nature of the PNH cell defect; it could also provide clues to the way in which the late-acting components of complement bring about haemolysis.

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## BIOCHEMISTRY

### Enhancing Effect of Insulin on the Tuberculin Reaction in the Albino Rat

SEVERAL authors have suggested that insulin is involved in inflammatory and hypersensitivity reactions<sup>1,2</sup>. It is known<sup>2,3</sup> that tuberculin-type skin sensitivity reactions to purified protein derivative (PPD) in the guinea-pig can be depressed by alloxan, and this depression can be partially reversed by the administration of insulin<sup>2</sup>. In the rat<sup>1</sup> insulin intensifies inflammatory and immunological reactivity. It was thus of interest to determine whether tuberculin reactions in the rat (a relatively poor reactor to delayed-type hypersensitivity) could be intensified when insulin was given at the same time.

Purified protein derivative (Parke Davis and Co. second strength tablets, 0.05 ml./tablet) was used at a concentration of 0.1 mg/ml. The buffer used in the injections was supplied with the tablets. 'Gelatin' (Difco) was used at a concentration of 180 mg/ml. of buffer. Regular insulin ('Iletin', Lilly) was injected 1 h before and again 3 and 6 h after challenge (or, alternatively, incorporated into the 'Gelatin' mixture at a concentration of 2 U/ml.). PPD, by itself or in combination, was administered under light ether anaesthesia to the dorsal skin, the lip or the foot pad of the rear paw in a volume of 0.1 ml. A control injection of buffer or other carrier without PPD was given to a similar site, usually on the opposite side of the animal. Rats (Wistar derived, Greenacres Controlled Flora) were challenged throughout these experiments at a weight of 140–160 g.

Delayed-type hypersensitivity was induced by injecting 70–80 g rats subcutaneously in each of two sites with 0.25 ml. of Freund's complete adjuvant containing killed, dried *M. tuberculosis* (Lederle) to give a total of 1 mg of the injected rat. PPD challenge was carried out 35 days after sensitization.

Table 1 shows that paw challenge in rats treated with 2 U of insulin gave a high percentage of positive reactions, while untreated rats were always negative. The use of

Table 1. PPD REACTIONS IN THE RAT

Challenge site	Treatment	Result No. reacting/No. used
Paw	1 U of insulin	1/6
Paw	2 U of insulin	20/29, 9/29
Lip		0/29
Skin		0/29
Skin	None	0/8
Lip		0/8
Paw		0/8

the lip and skin as a site of challenge in either treated or untreated animals also gave negative results.

A mixture of 'Gelatin', buffer and insulin, containing the usual 0.01 mg of PPD, intensified the gross reaction to PPD in rats sensitive with *M. tuberculosis*. After 24 h the skin showed lesions with an overall average size of 15–18 mm, as compared with control sites containing only 'Gelatin' and insulin which averaged 6–10 mm.

Although the combination of insulin and 'Gelatin' with PPD intensified the gross skin reaction, it was impossible to grade such lesions microscopically. Within a few hours the 'Gelatin' caused polymorphs to aggregate at the site of injection; this was increased in the presence of insulin.

The induction of a tuberculin reaction in the sensitized rat treated with insulin, in contrast to the lack of reaction in the untreated sensitized rat, emphasizes the importance of insulin or concentrations of glucose in hypersensitivity reactions. Such results are consistent with observations of systemic anaphylaxis in the mouse and rat<sup>1,2</sup> and tuberculin reactions in the guinea-pig<sup>2,3</sup>. The relative importance of insulin (glycaemic levels or the related energy pathways involved) has still to be established. The present experiments, however, extend the use of rats in delayed-type hypersensitivity experiments.

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### Inhibition of DNA-dependent RNA Polymerase by 4-Nitroquinoline-N-oxide in Isolated Nuclei

4-NITROQUINOLINE-N-OXIDE (4-NQO) is a potent carcinogen<sup>1</sup> and mutagen<sup>2</sup>, which has been shown to induce tumours in mammals<sup>3</sup>, cause nuclear abnormalities in *Tetrahymena*<sup>4</sup> and induce alterations in tissue culture cell nuclei<sup>5</sup>.

The activity of DNA-dependent RNA polymerase (E.C.2.7.7.6) was assayed on intact isolated nuclei from Chang liver cells grown in spinner cultures<sup>6</sup>, using a modified Eagle's suspension medium with foetal calf serum. 4-NQO was added in a concentration of 10<sup>-3</sup> molar in Hanks balanced salt solution. Experimental cultures were exposed to 4-NQO for 1 h followed by media for 1 h. The cells were collected by centrifugation at 1,000g for 10 min at 4° C and the resultant pellet was resuspended twice in 10 ml. of Eagle's medium and centrifuged at 1,000g for 10 min after each suspension. After the final centrifugation the cells were suspended in 2.0 ml. of 0.5 molar sucrose containing 2.5 mmolar magnesium chloride and homogenized in a Potter-Elvehjem homogenizer intermittently at 1,000 r.p.m. for 5 min at 4° C. The homogenate was centrifuged at 5,000g at 4° C for 15 min and the crude nuclear fraction was collected and suspended in 0.5 molar sucrose in the presence of 2.5 mmolar magnesium chloride and centrifuged at 80,000g for 5 min at 4° C.

The purified nuclear fraction was suspended in an assay mixture containing 100 mmolar *tris* buffer, 3.0 mmolar mercaptoethanol, 20 mmolar sodium fluoride, 3 mmolar manganese chloride, 60 mmolar potassium chloride and 160  $\mu$ molar ammonium sulphate. To the assay medium, 0.5 mmolar of each of the RNA precursors (ATP, GTP, UTP and CTP) were added to the assay mixture at a final volume of 0.5 ml. at pH 7.4. Similar assay mixtures have been used in previous studies of nuclear RNA polymerase activity<sup>7,8</sup>. The carbon-14 label (0.5  $\mu$ c.) was introduced in labelled ATP (specific activity, 30.7 mc./

mmole) or labelled CTP (specific activity, 20.6 mc./mmole). After incubation for 20 min at 37° C, 1 ml. of a saturated sodium pyrophosphate solution and 1 mg of crude yeast RNA were added and the reaction was terminated by the addition of 4 ml. of cold 0.5 molar perchloric acid. The precipitate was collected on a 'Millipore' filter (pore diameter, 0.2  $\mu$ ) and washed with two 10 ml. volumes of cold perchloric acid, two 10 ml. portions of cold ethanol and two 10 ml. portions of ethanol:ether (1:1, v/v). The filter and precipitate were dissolved in 0.4 ml. of 'Hyamine' and counted in a liquid scintillation counter.

The incorporation of carbon-14 into acid insoluble RNA in the controls required the presence of all four precursors as has previously been shown<sup>9</sup>. Table 1 summarizes the data in terms of  $\mu$ moles of labelled ribonucleotide triphosphate incorporated into the acid insoluble fraction/mg of DNA estimated by Burton's method<sup>10</sup>. Incorporation of carbon-14 into the acid insoluble fraction (RNA) was inhibited by low temperatures, RNase, DNase and actinomycin D as has been shown by other investigators<sup>7</sup>. Inhibition was also caused by the deletion of one or more of the ribonucleotide precursors.

Table 1

Assay condition (at 37° except as noted)	$\mu$ Moles of acid insoluble <sup>14</sup> C/mg of DNA	
	Control nuclei	Pretreated (10 <sup>-3</sup> molar 4-NQO)
Complete (0.0 min)	0.07	0.04
Complete (20 min)	3.85	1.90
Complete (40 min)	6.09	2.32
Complete (60 min)	8.56	4.40
Complete (120 min)	10.06	5.40
Complete (120 min, 0° C)	0.14	0.07
No <sup>14</sup> C-ATP ( <sup>14</sup> C-CTP substituted) (20 min)	0.43	1.05
No CTP (20 min)	0.61	0.95
No UTP (20 min)	0.38	0.78
No (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> (20 min)	5.63	1.45
Complete + 50 mg/ml. of DNase (20 min)	0.43	0.33
Complete + 200 mg/ml. of RNase (20 min)	0.57	0.39
Complete + 25 mg of actinomycin D (20 min)	0.23	0.17

An unexpected finding was the lowered dependence of groups treated with 4-NQO on the presence of all four precursors. Control groups in assay mixture, with ATP, UTP or CTP omitted, showed smaller contents of RNA polymerase than groups pretreated with 4-NQO when assayed with one nucleotide omitted.

Sucrose density gradient centrifugation of the cytoplasmic RNA produced after exposure to 4-NQO has shown that this RNA species exhibits a sedimentation constant of less than 8S with most of it less than 4S. RNA sedimenting in this region was greater by a factor of 3 to 5 than the controls. Cultures treated with 4-NQO showed an almost complete absence of RNA components of higher S-coefficients. Base sequence studies on this fraction are now in progress. This fraction was lacking in amino-acid accepting ability.

The lower dependence of RNA polymerase activity in 4-NQO treated nuclei may be caused by increased utilization of nuclear RNA as a ribosetriphosphate pool or to the partial substitution of another ribonucleotide in the assay medium for the one that is absent from the assay medium. We believe the second alternative to be the correct one, because it is conceivable that 4-NQO when complexed with the DNA molecule<sup>11</sup> may cause misreading of the template with a concomitant increase in RNA polymerase activity above as a result of base substitution.

The ability of 4-NQO to inhibit RNA polymerase was not as marked as that seen with actinomycin D. This may be due to the relatively small size of the 4-NQO molecule as compared with actinomycin D<sup>12</sup> and is thus less effective in blocking RNA transcription, but may cause misreading of the template during RNA synthesis. A recent morphological observation<sup>4</sup> suggests that 4-NQO may also induce errors in DNA replication as it was only effective by the criteria used if present during DNA replication.

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## Proteins, Persulphate and Disc Electrophoresis

POLYACRYLAMIDE electrophoresis of purified chick interferon at pH 8.9 by methods similar to that of Davis<sup>1</sup> gave an average recovery of activity of about 30 per cent<sup>2,3</sup>. The recovery could be improved by omitting the spacer and sample gels. Electrophoresis at pH 4.3 by a method similar to that of Reisfeld *et al.*<sup>4</sup> invariably abolished all or nearly all interferon activity<sup>5</sup>.

One of the materials used by both Davis and Reisfeld for polymerizing the small-pore gels is ammonium persulphate, and we suspected that this substance, at least under acid conditions, destroyed interferon. We therefore incubated (18 h at 4° C) crude chick interferon at pH 4.3 and pH 8.9 (in 0.3 molar acetate and 0.3 molar *tris* buffer, respectively) with and without persulphate (0.0064 molar, the concentration used by Reisfeld for polymerization). Activity completely disappeared in the acidic sample containing persulphate but not at all in the other three preparations (Table 1). Highly purified interferon was stable to incubation at pH 4.3 but only in the absence of persulphate. Similar incubation with ammonium chloride caused no loss of activity, proving that destruction was not due to the cation. When riboflavin (0.0005 per cent) was used instead of persulphate to catalyse the polymerization of the gels, good recoveries of interferon were also obtained after electrophoresis at pH 4.3.

Persulphate is widely used in polyacrylamide gel electrophoresis and therefore it seemed worth while to investigate its effect on three other biologically active materials containing protein, poliomyelitis virus, ribonuclease and insulin.

Live, concentrated<sup>6</sup> poliomyelitis virus (type 1, Brunenders), containing 880 "D-units"/ml. (ref. 7), crystalline pancreatic ribonuclease (100 µg/ml.), and crystalline insulin (50 µg/ml., 23.16 U/mg) were incubated in the same way as chick interferon. The "D" antigen content

of the re-neutralized poliomyelitis virus samples was assessed by the method of Beale and Mason<sup>8</sup>, and the potency of the ribonuclease solutions by that of Anfinsen *et al.*<sup>9</sup> (Table 1). Poliomyelitis virus "D" antigen was completely destroyed by persulphate at pH 4.3, and perhaps to some extent also at pH 8.9, but no significant loss occurred at either pH in the absence of persulphate. Ribonuclease, on the other hand, was destroyed extensively in the presence of alkaline persulphate, though some destruction may also have been caused by acid persulphate.

The effect of persulphate on insulin was investigated by subjecting it to polyacrylamide gel electrophoresis under various conditions and then staining the gels with amido black. Alkaline and acid electrophoresis was carried out by methods similar to those of Davis<sup>1</sup> and Reisfeld *et al.*<sup>4</sup>, except for the omission of sample and spacer gels. Insulin samples intended for electrophoresis at pH 4.3 or pH 8.9 were first adjusted to pH 7.4 or pH 6.0, respectively, and applied to the gels in 0.02 molar phosphate buffers at these values. At pH 4.3 the band in the persulphate-catalysed gel was definitely less intense than the corresponding one in the riboflavin-catalysed gel (Fig. 1a and b). When the samples were pre-incubated at pH 4.3 without persulphate and then subjected to electrophoresis at pH 4.3 in both types of gel, no band at all could be detected in the persulphate gel, but the pretreatment did not seem to have diminished the band intensity in the riboflavin gel (Fig. 1c and d). Pretreatment at pH 4.3 in the presence of persulphate again revealed the absence of any band in the persulphate gel but it also reduced the intensity of the band in the riboflavin gel. Electrophoresis at pH 8.9 produced stained bands of approximately equal intensity in both gels, but the band in the persulphate-catalysed gel had a lower *R<sub>Br-phenol blue</sub>* value than the sample in the riboflavin gel (Fig. 1g and h). Pre-incubation pH 8.9 without persulphate did not influence these results, but pre-incubation in the presence of persulphate completely prevented formation of any band in either type of gel (Fig. 1k). Results of the work with insulin are summarized in Table 2. (It should be noted that the fact that the biological activity of insulin is not stable at pH 8.9 (ref. 10) has no bearing on the main theme of this communication.)

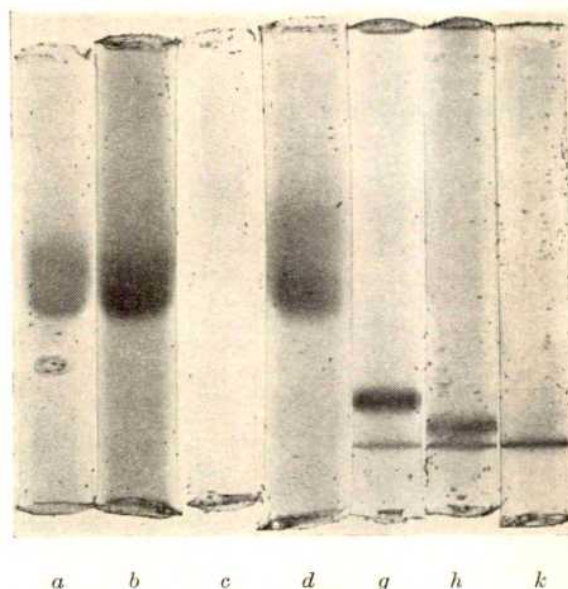


Fig. 1. Polyacrylamide electrophoresis of insulin. Direction of travel was from the top downwards. The sharp band at the bottom of gels g, h and k is bromophenol blue. Electrophoresis in gels a, b, c and d was carried out at pH 4.3, in gels h, g and k at pH 8.9. The current per gel was kept at about 0.6 m.amp. For details see text and Table 2.

Table 1. EFFECT OF AMMONIUM PERSULPHATE AT pH 4.3 AND pH 8.9 ON ACTIVITIES OF CHICK INTERFERON, POLIOMYELITIS VIRUS AND RIBONUCLEASE

Treatment (18 h at 4° C)	Interferon (U/ml.)	Poliiovirus ("D"-units/ml.)	Ribonuclease (µg/ml.)	
None	160	880	100	100
pH 8.9	160	880	103	102
pH 8.9 + 0.0064 molar persulphate	160	580	36	28
pH 4.3	160	790	60	69
pH 4.3 + 0.0064 molar persulphate	< 15	0	47	55



Table 2. EFFECT OF AMMONIUM PERSULPHATE AT pH 4.3 AND pH 8.9 ON THE PROTEIN BAND OBTAINED ON GEL ELECTROPHORESIS OF INSULIN

Pretreatment (18 h of 4° C)	Catalyst in gel pH of elec- polymerization trophoresis	Intensity of band
(a) None	Persulphate	4.3 ±
(b) None	Riboflavine	4.3 +
(c) pH 4.3	Persulphate	4.3 -
(d) pH 4.3	Riboflavine	4.3 +
(e) pH 4.3 + 0.0064 molar persulphate	Persulphate	4.3 ±
(f) pH 4.3 + 0.0064 molar persulphate	Riboflavine	4.3 ±
(g) None	Persulphate	8.9 + *
(h) None	Riboflavine	8.9 +
(i) pH 8.9	Persulphate	8.9 + *
(j) pH 8.9	Riboflavine	8.9 +
(k) pH 8.9 + 0.0064 molar persulphate	Persulphate	8.9 -
(l) pH 8.9 + 0.0064 molar persulphate	Riboflavine	8.9 -

\* Retardation of band, presumably indicating a change in the protein.

These findings show that a number of quite diverse proteins are affected by even a low concentration of either acid or alkaline ammonium persulphate, present either in the solvent or in the polyacrylamide gel. Attempts to remove persulphate from a gel column by pre-electrophoresis at pH 4.3 (6 m.amp./tube, 2 h) were not successful because insulin (after incubation at pH 4.3 without persulphate) still gave no band after electrophoresis in a persulphate gel, but, as already mentioned, one was produced in a riboflavine gel.

Although the mode of action of persulphate is unknown, its use as a catalyst for the polymerization of acrylamide gels does lead to changes of some proteins and can thus give rise to artefacts. It may therefore be advisable to re-examine some of the results reported from electrophoresis in persulphate catalysed gels and to polymerize gels with riboflavine instead of persulphate.

*Note added in proof.* Since the submission of this communication for publication, J. M. Brewer (*Science*, 156, 256; 1967) has shown that enolase is degraded by persulphate, but only in the presence of 8 molar urea.

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## Urinary Crystals and their Relation to Stone Formation

A VARIETY of crystalline substances, particularly urates, oxalates and phosphates<sup>1,2</sup>, have been described in urine, but the relation of these crystals to the pathogenesis of calculi of the urinary tract, which commonly contain some or all of these materials, has never been established. Nor is it known which of these crystals (if any) form in the urinary tract and which develop in the urine outside the body. We have therefore studied the crystalline material in freshly voided urine from normal and stone-forming subjects to determine whether any material is present which might be regarded as the nucleus of a calculus.

The initial study involved ten male hypercalciuric stone-formers and ten male normal controls on unrestricted fluid intake and a variety of diets. Urine was collected at hourly intervals during the day in double-walled, silvered Dewar flasks at 37° C until crystals were observed. The specimens were centrifuged in a warm apparatus for 10 min at 1,500 r.p.m. and the sediment was immediately examined under the microscope. The

pH of the samples varied from 5.8 to 7.0 and none of them was grossly infected. Crystalline substances were identified and distinguished by their habit, their appearance in polarized light, and their solubility in acetic and hydrochloric acids on the microscope stage. These procedures were found to be sufficient for the range of crystalline substances occurring in urine.

The only crystalline substances observed in these samples of fresh urine were calcium oxalate and one form of calcium phosphate. Other compounds previously described in urine, notably uric acid, ammonium urate, stellar calcium phosphate (calcium hydrogen phosphate, brushite) and magnesium ammonium phosphate, were only seen when the slides or incubated samples were examined several hours after voiding. The calcium oxalate was invariably present as tetragonal dipyramids (octahedra, "envelopes") known to be the dihydrate or weddellite salt. At the limits of our light microscopy (about  $\times 2,000$ ) the calcium phosphate precipitate appeared irregular. It did not extinguish well in polarized light, gave no evolution of carbon dioxide with acetic acid, and though presumably formed of basic calcium phosphate, could not accurately be described as crystalline hydroxyapatite. All the subjects passed one or both of these materials at some time during the day and there was no clear difference between stone-formers and controls in this respect.

Urine samples which contained no crystalline material were next cooled to room temperature and their pH raised to 6.8 by the addition of 0.1 normal sodium hydroxide, in order to observe the formation of crystals. Again, calcium oxalate and calcium phosphate were the only crystalline substances observed to precipitate, although the calcium oxalate now began to assume various habits (hexagon or "tablet", oval or "biscuit"). Both these and later observations in this qualitative study showed no significant differences between the two groups.

The samples treated with sodium hydroxide were then refrigerated overnight at 4° C in order to obtain a fresh crop of crystals. Under these conditions the previously discrete calcium oxalate crystals aggregated, as shown in Figs. 1 and 2, to form fused and semi-fused masses of appreciable size ( $> 100\mu$ ). This was the only point in the study at which true crystal aggregates (as opposed to twinning effects) were noted. No similar phenomenon was observed for the irregular deposits of calcium phosphate. Although the urine state was ill-defined we nevertheless thought that this aggregation might be a stage in the formation of a stone, and a further experiment was performed to check this theory.

Twelve calcium oxalate stones, all predominantly or partly weddellite, were finely ground individually in an agate mortar, using a slow, lateral shearing motion. The powdered samples were taken up in a minimum of dis-

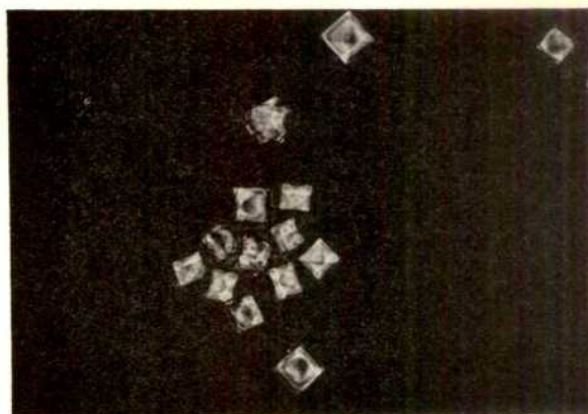


Fig. 1. Calcium oxalate tetragonal dipyramids in early state of aggregation ( $\times 450$ , polarized light).



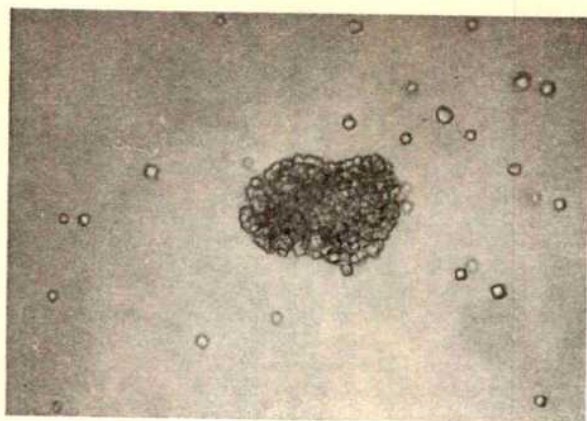


Fig. 2. Semi-fused mass of calcium oxalate crystals in later aggregation stage ( $\times 200$ , plane light).

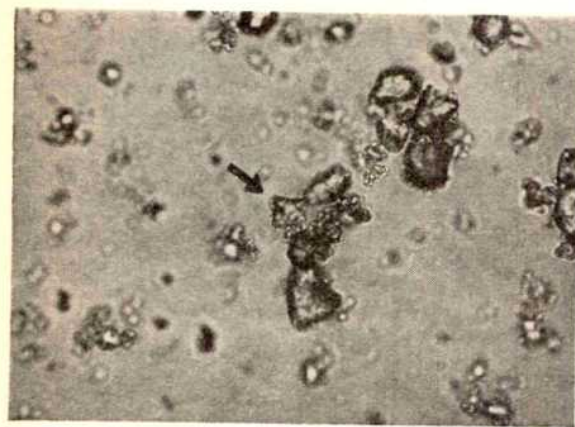


Fig. 3. Debris from ground calcium oxalate stone, with (centre) well formed tetragonal crystal ( $\times 200$ , plane light).

titled water and microscopically examined. In each case, against a background of morphologically unrecognizable debris, many roughly shaped but unmistakable calcium oxalate crystals of the tetragonal dipyramidal type were visible (Fig. 3). These crystals were invariably  $5\text{--}10\mu$  in size, that is, of a size comparable with those seen in fresh urine. Recognition in plane and polarized light was supplemented by phase contrast examination, when the crystal edges became visible as dark lines radiating from the pyramidal apex.

These observations suggest that many or most of the crystalline materials hitherto described in urine<sup>3,4</sup> develop after voiding when the urine is left to cool and the pH is allowed to rise. They may therefore be regarded as artefacts. The only crystalline substances consistently found are calcium oxalate and calcium phosphate, but the latter is seen only as irregular deposits with the light microscope. The calcium oxalate crystals are much smaller than those previously described in urinary calculi by Prien<sup>5</sup>, Murphy and Pyrah<sup>6</sup> and others, but the crystals which we observed after grinding a calculus were of a size comparable with those seen in the fresh urine and indistinguishable from them. It is therefore possible that aggregation of crystals is more important in stone formation than crystal growth. We were able to produce aggregation very early outside the body but did not see any aggregates in freshly voided urine. Aggregates have been described in urine by Sengbusch and Timmermann<sup>7</sup>, but in view of the method of collection of their samples it is not possible to decide whether they were present before the urine was voided.

On the present evidence no conclusions can be drawn as to whether the larger crystals found in calculi are

formed solely by the aggregation or by the selective dissolution and reprecipitation of the smaller urinary crystals. What does seem probable is that the initial step in the formation of urinary calculi involves the precipitation from supersaturated solution of the crystalline salts which will eventually form the great bulk of the concretion.

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### Long-term Behavioural Aberration produced in Mice by a Pharmacological Agent

HARMALA alkaloids which are derived from plants all contain a  $\beta$ -carboline moiety. These compounds have been of interest because they are hallucinogenic<sup>1-3</sup>, in addition to possessing other pharmacological properties<sup>4</sup>. Because of the ease of formation of tetrahydro- $\beta$ -carbolines from the reaction of tryptamines and aldehydes in mild, aqueous conditions, they have been suggested to be endogenously formed psychotropic agents and causative factors in some psychoses<sup>5</sup>. In order to investigate further their effect on animal behaviour, and other pharmacological responses, a series of new tetrahydro- $\beta$ -carbolines (tetrahydroharmans) has been synthesized<sup>4</sup>. One of these was prepared by the Pictet-Spengler reaction from 5-methoxytryptamine and pyridoxal (vitamin B<sub>6</sub>). The structure of the compound, 1-(3-hydroxy-5-hydroxymethyl-2-methylpyridyl-4)-6-methoxy-1,2,3,4-tetrahydro- $\beta$ -carboline (compound 1), is shown in Fig. 1. Thus it contains indole, 5-substituted tryptamine,  $\beta$ -carboline and vitamin B<sub>6</sub> moieties combined in one molecule. When compound 1 was injected intraperitoneally into trained rats at dosages useful for behavioural studies of other tetrahydro- $\beta$ -carbolines (1.5–3 mg/kg) all animals were slowly intoxicated and died within 24–48 h.

The toxicity of the compound was therefore examined in less valuable animals (untrained mice). Six male Sprague-Dawley mice (weighing 30–40 g) kept in separate cages were injected with doses of 1.2, 2.4, 4.6, 8, 12 and 14 mg/kg of compound 1 in water. Only the animals at the two larger dosages showed acute responses consisting primarily of hind leg paralysis shortly after injection lasting about 20 min. All animals were alive 48 h later but seemed to suffer a lethargy or catatonia compared with uninjected mice. These six animals were kept in separate cages for several weeks, and at the end of this time they seemed to recover completely and were transferred to

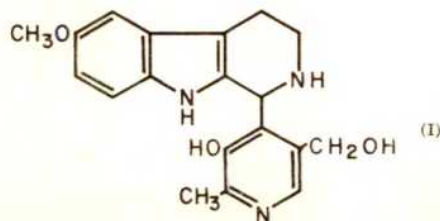


Fig. 1.

cages containing four or five mice, not treated previously with any agents.

The four mice which received 4.6–14 mg/kg (13.6–41.3  $\mu$ mole/kg) reacted identically in this situation. First, there seemed to be a short period of 3–20 min of "induction", when the newly introduced mouse began to exhibit signs of great agitation characterized by trembling and erection of its tail and rapid rotation of it. These traits persisted as long as the treated mouse was in the presence of the others and terminated when it was replaced alone in its cage. After several minutes in the cage with the others, the single mouse began repeated biting attacks on one or two of the other mice, and eventually it was in conflict with every member of the new group. As long as the animals were awake fighting continued. The great intensity of the combat was indicated by several animals suffering from deep bleeding wounds. The animals, when allowed to remain together overnight, were found in the morning with the untreated mice sleeping together and the previously medicated animal sleeping on the opposite side of the 22 cm cage. As soon as they awakened, the intense attacks by the previously treated animal resumed. In several instances the smaller animals of the group died during the fighting. Although, initially, the largest mouse of the group of animals attempted to thwart the aggression of the treated animal, it was eventually dominated in the same way as the others in the group and huddled with them for mutual protection.

The experiment was repeated with another group of six mice taking each animal from a group of its associates, injecting it, keeping it separate, and placing it back in the same cage 24 h later. The same pattern was seen as before, with the injected mice undergoing a dramatic transformation of their behavioural patterns. The mice were removed and kept separately for 3 weeks. On placing each one into a cage containing the group it came from, again there was a high level of aggressiveness and biting attacks by the new animals. In this later experiment six control animals were injected in the same manner with saline and caged separately in the same way as the other mice. After 3 weeks, when they were placed back with their original groups, fighting occurred with only two out of the six control animals. This abated usually within 20 min and the newly introduced animal was then assimilated by the group. With five out of the six animals introduction into the cage was characterized by either intense fighting, great agitation without fighting or polarization from the group where the animal stayed on the opposite side of the cage from the grouped animals; this was especially noticeable during sleep.

When solutions of the carboline were allowed to stand overnight or heated while they were being prepared, all or part of their potency was lost. Examination of these solutions on silica thin-layer or glass chromatography (9 : 1 chloroform-methanol) showed the original xanthrydrol positive spot changing to two new ones. Thus varying degrees of chemical transformation occurred in these instances.

These observations indicate that a long range aberration of the animals' normal behaviour occurred after one injection of compound 1. Explanation of this long-term phenomenon seems to be dependent on the formation of a lesion in the central nervous system. Vitamin B<sub>6</sub> is the co-factor for the enzyme formation of various brain amines<sup>7</sup>. Tetrahydro- $\beta$ -carbolines apparently cross the barrier between blood and brain in animals readily, because they have psychotropic effects. When pyridoxal is reduced in position 4 to desoxypyridoxine, it is converted to the most potent antagonist of that vitamin<sup>8</sup>.

Because compound 1 combines both a tetrahydro- $\beta$ -carboline moiety and a form of pyridoxine where the 4 position is modified, it may be an antagonist of vitamin B<sub>6</sub>, especially capable of crossing the blood-brain barrier. Possibly compound 1 acts through an inhibition of synthesis and complete depletion of an important amine

in some part of the mouse brain which could produce a lesion. The possibility of the involvement of ribonucleic acid cannot be excluded. These results suggest that the tetrahydro- $\beta$ -carboline would be a useful tool for studying the role of vitamin B<sub>6</sub> in the brain or for manipulating concentrations of amines in the central nervous system. It may also be applicable to the production of lesions in specifically localized sites of the brain.

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### Crystalline Aflatoxin M<sub>1</sub> from Urine and Milk

Allcroft and Carnaghan<sup>1</sup> and de Jongh *et al.*<sup>2</sup> reported the appearance in milk of a compound related to aflatoxin B<sub>1</sub>, and Allcroft *et al.*<sup>3</sup> showed by thin-layer chromatography and ultra-violet studies that a similar factor appeared in the urine of sheep given mixed aflatoxins. Holzapfel *et al.*<sup>4</sup> isolated the urinary compound and established that it was aflatoxin M<sub>1</sub> (Fig. 1).

That the factor found in milk, which has not hitherto been crystallized, is the same as urinary aflatoxin M<sub>1</sub> has been assumed solely on the basis of chromatography and ultra-violet spectra. We report here the isolation of crystalline M<sub>1</sub> from sheep urine and cows' milk after a dose of mixed aflatoxins.

A 'Holstein' cow was given the equivalent of 80 mg of aflatoxin B<sub>1</sub> daily, contained in rice that had been inoculated with *Aspergillus flavus*, NRRL 2999 (ref. 5). The rice was mixed in the feed but, because the cow refused it, was given by capsule in the third week. The cow succumbed rapidly: her appetite diminished, she developed acute emphysema, and in the fourth week, when her lactation ceased, she was killed. Urine was collected from a ewe and a wether given aflatoxin B<sub>1</sub> (200 and 100 mg, respectively) on rice, which again had to be given by capsule in the fourth week. The ewe died several days later.

Lyophilized milk from the cow (11 kg) was defatted with *n*-hexane and the supernatant from the basket centrifuge extracted with methanol (46 kg, plus 6.5 kg wash). The methanol extract was concentrated *in vacuo* (3 l.); water (200 ml.) was added and then extracted with low boiling petroleum ether. More water (1.7 l.) was added to the aqueous methanol and three chloroform extracts, each of 3 l., were obtained. The residues from the chloroform extracts weighed 14, 1.2 and 0.8 g. Thin-layer chromatography showed that the first two chloroform extracts contained most of the aflatoxin M<sub>1</sub> and so only these were used. A crude residue was obtained from 15 l. of the sheep urine, collected in the fourth week, by extracting twice with equal volumes of chloroform (methanol was added to prevent emulsions) after neutralization with hydrochloric acid.

The purification and isolation of aflatoxin M<sub>1</sub> from both the milk and urine extracts were essentially the same, so only the procedure for the milk will be described. The residue from the first chloroform extract in 0.5 l. of methanol-water (1 : 1, v/v) was extracted with petroleum



ether. The residue from the second chloroform extract in 0.5 l. of fresh methanol-water (1:1) was combined with the defatted aqueous methanol of the first chloroform extract. The resulting 1 l. of methanol-water was extracted with two 200 ml. portions of xylene from which a recoverable amount of aflatoxin  $M_1$  was back-extracted with an aqueous methanol wash. The aflatoxin  $M_1$  was then extracted with two 1 l. portions of chloroform. The residue from the combined chloroform (2.6 g) was chromatographed on a silica gel column developed with chloroform, then successively with 0.5, 1, 1.5 and 2 per cent (vol.) methanol in chloroform. Crystals of aflatoxin  $M_1$  (8 mg) appeared in the eluate after evaporation and were washed with chloroform, followed by methanol. Crystals of aflatoxin  $M_1$  (15 mg) were obtained in a similar way from a column eluate of the urinary extract.

The identity of crystalline aflatoxin  $M_1$  from urine and milk was established on the basis of melting point (310° C, decomp.<sup>4</sup>); ultra-violet spectra in methanol ( $\lambda_{\max}$  357, 265 and 226 m $\mu$ <sup>4</sup>); formation of acetyl derivatives which were indistinguishable by thin-layer chromatography and ultra-violet spectrum and which had the same ultra-violet spectrum as the unreacted material but were separable from it by thin-layer chromatography; crystallography (shape and refractive indices); X-ray diffraction powder patterns (Fig. 2); infra-red spectra (potassium bromide) similar to that of aflatoxin  $B_1$  but showing a hydroxyl signal at 3.430 cm<sup>-1</sup>; and paramagnetic resonance spectra.

The paramagnetic resonance absorption and integral spectra of the isolated compound from urine were taken at 100 Mc/s in dimethylformamide- $d_7$  (DMF- $d_7$ ) and are fully compatible with the structure of aflatoxin  $M_1$  (Fig. 1). The resonances of all protons can be assigned

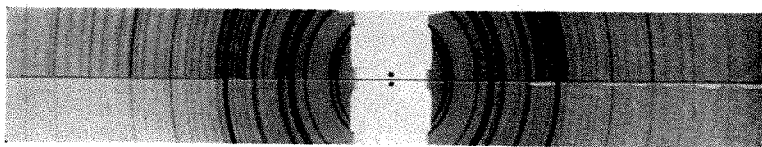


Fig. 2. X-ray powder patterns of aflatoxin  $M_1$  crystals from milk (upper) and urine (lower).

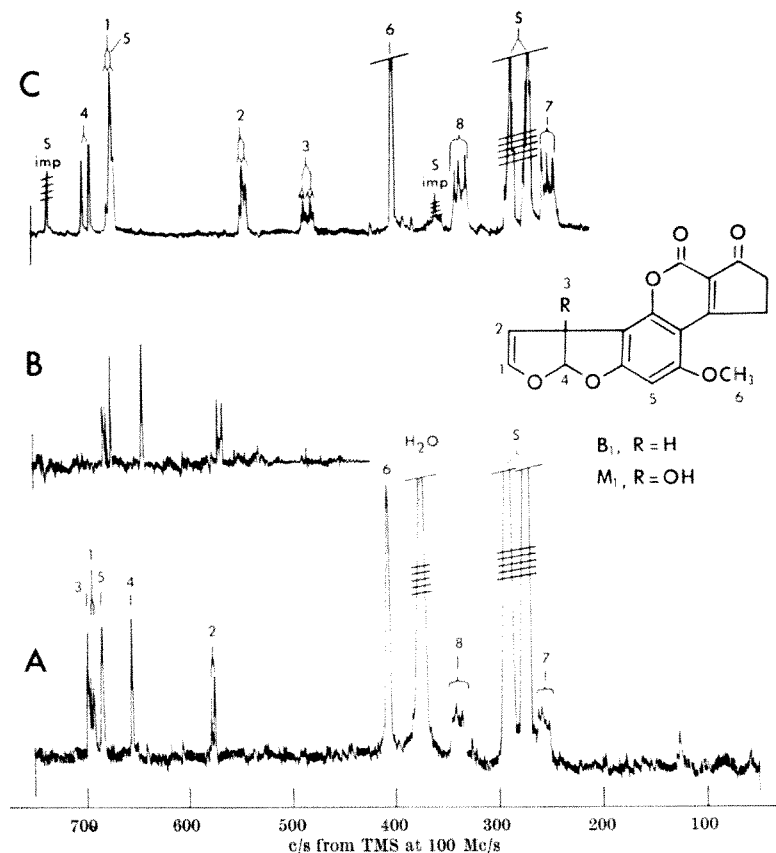


Fig. 1. 100 Mc/s paramagnetic resonance spectra with DMF- $d_7$  as solvent of: A, urinary aflatoxin  $M_1$  at -20° C; B, urinary aflatoxin  $M_1$  at 33° C with trace of TFA vapour added to shift hydroxyl peak; C, aflatoxin  $B_1$  at 31.5° C with TFA vapour added.

(Fig. 1A) by comparison with the spectrum of aflatoxin  $B_1$  (Fig. 1C). The signal assigned to the hydroxyl proton shifted upfield with increasing temperature and disappeared when a trace of trifluoroacetic acid (TFA) vapour was added to the sample as a result of exchange with the carboxyl proton (Fig. 1B). The location of the hydroxyl group is confirmed by the absence in the spectrum of aflatoxin  $M_1$  of any signal corresponding to the three-proton signal of the spectrum of aflatoxin  $B_1$  and by the lack of any coupling of a three-proton to the remaining protons. The spectrum of aflatoxin  $M_1$  from milk in DMF- $d_7$  at 31.5° C was identical to that of urinary aflatoxin  $M_1$  except that the 3-hydroxyl peak was absent because of exchange with traces of water plus acid in the solvent. The DMF used for the aflatoxin  $M_1$  from milk displayed an exchange-broadened (120 c/s) hydroxyl band centred at 540 c/s rather than the sharp peak at 344 c/s observed with the DMF used for the urinary aflatoxin  $M_1$ , which was obtained from another source.

The identification by Holzappel *et al.*<sup>4</sup> of aflatoxin  $M_1$  in the urine was based on comparison of a purified, though not crystalline, urinary concentrate with crystalline aflatoxin  $M_1$  from peanuts. Our identification of urinary aflatoxin  $M_1$  confirms both its structure and appearance in urine.

It has been suggested<sup>1,2</sup> that the milk factor is identical with urinary aflatoxin  $M_1$ , and that aflatoxin  $M_1$  is metabolically derived from aflatoxin  $B_1$  in the mammalian organism. Our results substantiate the first suggestion. As regards the second, de Iongh *et al.*<sup>2</sup> showed chromatographic evidence that administration of purified aflatoxin  $B_1$  to the rat resulted in the excretion in milk of a compound probably identical with the milk factor. Holzappel *et al.*<sup>4</sup> isolated aflatoxin  $M_1$  not only from sheep urine but also from mouldy peanuts. In addition, we have tentatively identified aflatoxin  $M_1$  (by thin-layer chromatography, ultra-violet spectrum and acetyl derivative formation) in the inoculated rice used in our experiments. These observations lead us to suggest the possibility that the appearance of aflatoxin  $M_1$  in urine and milk is related to its presence preformed in the concentrates of aflatoxin  $B_1$  rather than exclusively to its *de novo* synthesis from  $B_1$ . In other pertinent studies in the literature, "mixed aflatoxins" rather than crystalline aflatoxin  $B_1$  have been used. Furthermore, an estimate of aflatoxin  $M_1$  in the dry milk appears to be only 2-3 per cent of the ingested dose of  $B_1$ ; and the excretion of  $M_1$  in the milk is completed within a few hours of ingestion of a  $B_1$  concentrate. The question of preferential excretion of aflatoxin  $M_1$ , if present in extracts of *A. flavus*, into milk had been raised by de Iongh *et al.*<sup>2,6</sup>

Finally, aflatoxin  $B_1$  itself was identified (thin-layer chromatography, ultra-violet spectrum and formation of characteristic derivatives<sup>7</sup>) in the milk of this cow and is estimated at 190 parts per 10<sup>9</sup> in the dried milk, representing only about 0.3 per cent of the ingested dose of aflatoxin  $B_1$ .

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### Metabolism of Nuclear RNA in the Rat Kidney after Injection of Folic Acid

WE have previously reported that a single injection of folic acid induces a response in the rat kidney which is characterized by large increases in the synthesis of both RNA and DNA<sup>1</sup>. Twenty-four hours after an injection, the RNA content of each kidney is increased by approximately 50 per cent, although at this time the increase in DNA content is very small<sup>2</sup>.

We have now examined the mechanism of the increase in the synthesis of RNA in the rat kidney during the first 18 h after the injection of folic acid. We measured the incorporation of labelled orotic acid into kidney nuclear RNA *in vivo* and used these results to follow the changes in the rate of synthesis of RNA. In order to ascertain whether the changes were related to a modification of RNA polymerase activity and/or a modification of the efficiency of the DNA template, *in vitro* measurements were made of the "aggregate" RNA polymerase activity of the kidneys<sup>3</sup>.

Male rats were intravenously injected with folic acid (250 mg/kg body weight) as previously described<sup>2</sup>. Injected and control rats were killed at intervals up to 18 h after injection.

**RNA synthesis *in vivo*.** Each animal was intravenously injected with 6-<sup>14</sup>C orotic acid (1  $\mu$ c./100 g body weight) 7 min before it was killed. At each time interval the kidneys from eight animals were pooled and the nuclear pellet prepared as for the "nuclear preparation B" of Widnell and Tata<sup>4</sup>. The pellet was extracted by phenol<sup>5</sup> to obtain the nuclear RNA fraction. The radioactivity of this fraction was determined using a liquid scintillation counter and the RNA content was determined by the orcinol reaction<sup>6</sup>. The results are expressed as c.p.m./mg of RNA.

**RNA polymerase activity.** For each experiment the kidneys from groups of three animals were pooled and the nuclei prepared according to the method of Chauveau *et al.*<sup>7</sup>. A large quantity of the injected folic acid is concentrated in the kidneys (as much as 5 per cent of the total dose per kidney) and is recovered in the form of a precipitate associated with the nuclear pellet. In a preliminary experiment it was found that the presence of large amounts of folic acid was strongly inhibitory to the *in vitro* enzyme activity. In order to separate the nuclei and folic acid precipitate, the pellet was suspended in 2.2 molar sucrose, layered over 2.5 molar sucrose and centrifuged at 40,000g for 15 min in a swinging bucket

rotor: the nuclei remained at the interface whereas most of the folic acid was deposited at the bottom of the tube. The remaining folic acid had only a slight effect on the activity, as shown by the addition of comparable amounts to incubations of "aggregate" enzyme from control rats prepared as previously described for rat liver<sup>8</sup>. The nuclei of control kidneys were treated in the same way. The activity was measured in media of both low and high ionic strength<sup>9</sup>. Because there is no increase in the synthesis of kidney DNA up to 20 h after folic acid injection<sup>10</sup>, in order to facilitate comparison of the enzyme activities the DNA was determined in each nuclear preparation using the method of Burton<sup>11</sup> and the results expressed per mg of DNA. The results shown in Table 1 indicate that there is no increase in the rate of incorporation of labelled orotic acid into nuclear RNA of the kidney up to 6 h after injection of folic acid. By 12 h, however, there is an increase of 75 per cent and at 18 h the increase in specific activity of the isolated nuclear RNA is 90 per cent higher than the control value.

Table 1. EFFECT OF INJECTION OF FOLIC ACID ON THE RATE OF INCORPORATION OF 6-<sup>14</sup>C OROTIC ACID INTO NUCLEAR RNA OF THE RAT KIDNEY *in vivo*

Time after folate injection (h)	0	2	6	12	18
Specific activity (c.p.m./mg RNA)	1,304	1,532	1,218	2,296	2,480

Table 2. VARIATION OF "AGGREGATE" RNA POLYMERASE ACTIVITY OF THE RAT KIDNEY 12 H AFTER FOLIC ACID INJECTION

Source of tissue	Enzyme activity expressed as $\mu$ moles <sup>32</sup> GMP incorporated/mg DNA in 10 min at 37° C	
	"Aggregate" enzyme (low ionic strength medium)	"Aggregate" enzyme (high ionic strength medium)
Normal rats	55	282
Folic acid treated rats	119	461
Ratio	2.16	1.63

Values in the table are the average of three experiments with very similar results. The reaction mixture (0.25 ml.) contains: 160 mmoles *tris* buffer pH 7.0, 4 mmoles MnCl<sub>2</sub>, 8 mmoles 2-mercaptoethanol, 1 mmole each of ATP, CTP, UTP, 1 mmole  $\alpha$ -<sup>32</sup>P GTP and approximately 1 mg DNA of the enzyme extract. High ionic strength medium contains 0.025 ml. of a saturated solution of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>.

Increases were also found in the activities of the "aggregate" RNA polymerase 12 h after injection of folic acid, as shown in Table 2. The increase of activity at high ionic strength is less than that at low ionic strength. On incubating equal amounts of enzyme extracts from control and injected rats, the average of the values for the two activities was found to indicate that the increase in activity was not related to the disappearance of an inhibitor.

Our results show that there is an early increase in the rate of synthesis of nuclear RNA which is evident several hours before the onset of increased synthesis of DNA in the kidney. This observation agrees with results previously reported for the total RNA during renal compensatory hypertrophy<sup>12</sup> and for the nuclear RNA of regenerating liver<sup>13,14</sup> and of the salivary glands after injection of isoproterenol<sup>15</sup>, and suggests that an increase in nuclear RNA synthetic activity is an essential requirement for the subsequent increase in synthesis of DNA in these systems. It is difficult to compare precisely the kinetics of the increased RNA synthesis *in vivo*, because this is dependent on the specific activity of the labelled precursor pools<sup>14,16</sup> which may differ from tissue to tissue. It appears, however, that both the magnitude of the increases (about double) and the times at which the maximum increases are attained (between 6 and 12 h) are about the same for both regenerating liver and the kidney after folic acid treatment when "pulse" labelling with <sup>14</sup>C orotic acid is used to measure the synthesis of RNA.

The difference in the increase of "aggregate" RNA polymerase activity of the kidney after injection of folic acid measured at both high and low ionic strength was also found in regenerating liver<sup>17,18</sup> and in the prostate gland of castrated rats after injection of androgen<sup>19,20</sup>. The situation was different from that found in rat liver after injection of growth hormone<sup>21,22</sup>, however, when only the "aggregate" RNA polymerase at low ionic strength is increased.



From previous studies it seems that "aggregate" RNA polymerase activity measured at low ionic strength is related both to the enzyme activity and to the "template efficiency" of the DNA, whereas the activity measured at high ionic strength is proportional only to the quantity of active RNA polymerase previously bound to the DNA (ref. 3). Soluble RNA polymerase activity which is also related only to the quantity of active enzyme has been observed in various animal tissues including the kidney<sup>2a</sup>, but unfortunately it has been impossible to measure the changes in activity of soluble RNA polymerase as a result of the loss of the enzyme during the process of removal of folic acid from the nuclei. Thus the increase of RNA synthesis in the kidney after injection of folic acid appears to be related both to an increase in the activity of RNA polymerase and to a better "template efficiency" of the DNA.

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### Repression of DNA Synthesis in Mouse Liver Cells after Unilateral Nephrectomy

It is well known that the renal tissue which remains after unilateral nephrectomy is effective in excretion and there is no increase of excreted metabolites in the blood serum a few hours after the operation. Changes in the other body functions (apart from the excretory function of the remaining kidney) after unilateral nephrectomy are still little known. A decrease in the dry weight of the liver<sup>1</sup>

Table 1. INDEXES OF HEPATOCYTE NUCLEI LABELLED WITH TRITIATED THYMIDINE

Series	Time of killing (h after operation)	Index of labelled hepatocytes (%)	
		After nephrectomy	After sham nephrectomy
1	43	0.03 0.10	0.60
2	49	0.10 0.20	2.00
3	67	0.05 0.03	0.60
4	79	0.23 0.09	1.80
5	91	0.30 0.26	0.80

and heart<sup>2</sup> has been observed 1 month after unilateral nephrectomy in mice. This suggests that kidneys take part not only in the regulation of erythropoiesis by erythropoietin but also in the regulation of the growth of other tissues.

I have further investigated this subject in the following experiments. The left kidney was removed from ten C57 mice under ether narcosis, and sham nephrectomy was performed on five of the mice. The animals were divided into five groups with two nephrectomized and one sham operated in each group. The animals were killed 43, 49, 67, 79 and 91 h respectively after nephrectomy. Tritiated thymidine was given intraperitoneally to each animal in a dose of 0.1  $\mu$ Ci/100 g of body weight; the dose was given four times at intervals of 6 h, with the last dose 1 h before the animals were killed. Liver slices were fixed in Carnoy solution and autoradiography was carried out, using liquid P emulsion. After exposure the preparations were stained with haematoxylin and eosin.

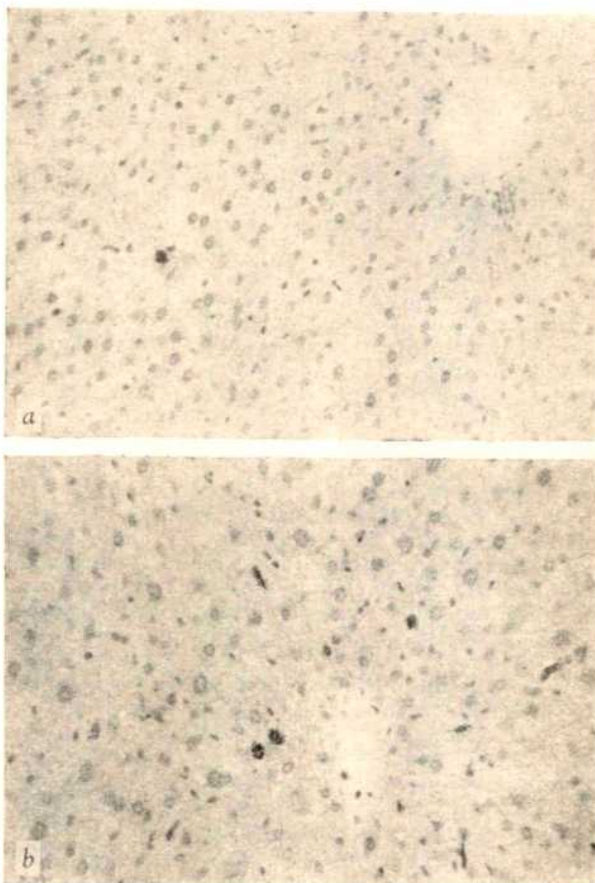


Fig. 1. Autoradiography of liver slices from a mouse given tritiated thymidine. a, Mouse at the sixty-seventh hour after unilateral nephrectomy; b, mouse after a sham nephrectomy.

The liver from an animal killed at the sixty-seventh hour after nephrectomy is shown in Fig. 1a; only one hepatocyte and one fibroblast were labelled. In the liver from a sham nephrectomized mouse (Fig. 1b) many labelled nuclei were visible. Indexes of hepatocyte nuclei labelled with tritiated thymidine are given in Table 1. In all cases the indexes of labelled hepatocytes were significantly lower after nephrectomy than after sham nephrectomy. The repression of DNA synthesis in the liver cells after unilateral nephrectomy suggests a regulatory influence of renal tissue on proliferation in other tissues.

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### Strontium-Calcium Selectivity of Alginates

THERE has been considerable medical interest in the ion exchange properties of alginates. Inhibition of the uptake of strontium by rats given doses of sodium alginate has been reported<sup>1-3</sup> and there was a similar experiment with a human adult<sup>4</sup>. The absorption of calcium was not significantly affected. The results were considered to have potential value in connexion with therapy of overdoses of strontium-90 (ref. 5). The effect of two commercial alginates with different chemical compositions, that is, different ratios of mannuronic and guluronic acid residues in the alginate, on strontium uptake in rats was compared<sup>6</sup>. The alginate with the greater content of guluronic acid residues reduced the uptake of strontium from the diet more effectively.

Alginates rich in guluronic acid are known to have more affinity for divalent metals in the ion exchange reactions calcium-sodium, copper-sodium and barium-sodium than more mannuronic acid-rich alginates<sup>7,8</sup>. In the ion exchange reaction between calcium and magnesium, alginates rich in guluronic acid also have been shown to have more affinity for calcium than have alginates rich in mannuronic acid<sup>9</sup>.

The potential therapeutic applications of alginates make their calcium-strontium ion exchange reaction particularly interesting. The ion exchange properties were determined by dialysing 2 ml. of 1 per cent sodium alginate against 50 ml. of a solution containing a mixture of calcium chloride and strontium nitrate. The ionic strengths of the solutions were 0.66, and the equivalent ratio of the two salts in the solution was chosen to give approximately equal amounts of the two cations bound to the alginate. The salt solution was changed three times, and the alginate gels were dialysed thoroughly against distilled water, and finally against 0.1 normal hydrochloric acid (three samples of 20 ml.). The amount of the two cations in the combined acid dialysates were determined by atomic absorption. The results were calculated as the selectivity coefficient

$$k_{\text{Ca}}^{\text{Sr}} = \frac{[\text{Sr}_g][\text{Ca}_i]}{[\text{Ca}_g][\text{Sr}_i]}$$

where  $[\text{Ca}_i]$  and  $[\text{Sr}_i]$  refer to concentrations in the original salt solution and  $[\text{Ca}_g]$  and  $[\text{Sr}_g]$  to the concentrations in the acid dialysates. Two alginate samples prepared from *Laminaria digitata* (Huds.) Lamour. (61.5 per cent and 68 per cent mannuronic acid) and two samples prepared from the stipes of *L. hyperborea* (Gunn.) Fosl. (30.5 per cent and 27.5 per cent mannuronic acid)

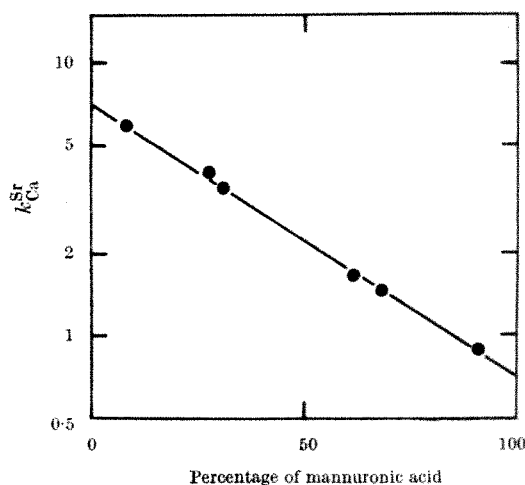


Fig. 1. Strontium-calcium selectivity coefficients for alginates and alginate fragments of varying uronic acid compositions.

were used. In addition, two oligouronides with more extreme uronic acid compositions (91 per cent and 8 per cent mannuronic acid, respectively) were investigated. The oligouronides were prepared by partial acid hydrolysis<sup>10</sup>, and their average degree of polymerization was twenty. The results are given in Fig. 1. By extrapolation of the curve the selectivity coefficients of the two homopolymers can be estimated.

The correlation between the selectivity coefficients and the composition of the uronic acid of the alginates is very clear and indicates that the strontium selectivity of the alginates is caused by their content of L-guluronic acid residues. A polyguluronic acid would seem to be the alginate preparation with the most selectivity for strontium compared with calcium. So far, however, no polyguluronic acid has been isolated from brown algae or from other sources. Among the larger brown algae, the stipes of *L. hyperborea* give the alginate with the greatest content of guluronic acid<sup>11</sup>. This was the source of the commercial alginate with a high guluronic acid content used by Harrison *et al.*<sup>6</sup>. Alginates can be separated into fractions with different uronic acid compositions by precipitation<sup>9,11</sup>, but in no case have fractions with more than 75 per cent guluronic acid residues been obtained. Oligouronides with a very high content of guluronic acid residues (85-93 per cent), however, can be prepared by partial acid hydrolysis of alginates and fractionation of the hydrolytic products<sup>10</sup>. When an alginate rich in guluronic acid is used for the preparation, a high yield may be obtained without fractional precipitation of the hydrolytic products<sup>12</sup>.

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### Induction of Malate Dehydrogenase by Oestradiol-17 $\beta$ in the Human Endometrium

HISTOCHEMICAL and biochemical investigations of the human endometrium have revealed variations in the activity of a number of enzymes throughout the normal menstrual cycle<sup>1-3</sup>. Marked differences in the concentration and localization of enzymes have been found between endometrial gland cells and stromal cells. It is commonly assumed that these variations in enzyme activity are mediated by oestrogens and progesterone. I thought that a more detailed knowledge of hormone action at a cellular level might be obtained by combining organ culture of human endometrium with quantitative histochemical methods. Maurer has, for example, recently demonstrated the value of organ culture techniques for the study of the effects of steroid sex hormones on target tissues in animals<sup>4</sup>. By the combined techniques I have now detected an increase in malate dehydrogenase activity in the human endometrium stimulated *in vitro* by oestradiol-17 $\beta$ . The increase in enzyme activity occurs mainly in the cells of the endometrial glands and is suppressed by actinomycin D and I think that it is due to the formation of new enzyme.

Regenerative phase endometrium was obtained at routine curettage from five patients with a normal menstrual history. One specimen was obtained on the seventh day of the cycle, three specimens on the twelfth day and one on the thirteenth day. A strip of tissue was immediately divided on removal into four portions. One portion was frozen at  $-70^{\circ}\text{C}$  by the cold tube method<sup>5</sup> and the others were transferred to culture chambers. The tissue was set on lens paper on a stainless steel grid in a silica dish containing Trowell tissue culture medium T 8. Culture was carried out at  $37^{\circ}\text{C}$  in an atmosphere of 95 per cent oxygen and 5 per cent carbon dioxide for 5 h. One culture was used as a control. Oestradiol-17 $\beta$  was added to the second culture at a concentration of  $100\text{ }\mu\text{g/l.}$  ( $3.7 \times 10^{-7}$  moles/l.). The third culture contained actinomycin D at a concentration of  $600\text{ }\mu\text{g/l.}$  in addition to the oestradiol-17 $\beta$ . After culture the tissue was frozen according to the above method and stored in a cryostat for a maximum of 12 h.

Sections of the tissue  $10\text{ }\mu$  thick were prepared in the cryostat with a knife cooled to  $-60^{\circ}\text{C}$  and with the chamber between  $-25^{\circ}\text{C}$  and  $-35^{\circ}\text{C}$ . These were then mounted on warm slides and covered with the histochemical medium without allowing them to dry. Pearse's<sup>6</sup> method was used to determine the malate dehydrogenase, except that 0.05 molar glycylglycine buffer at pH 7.5



Fig. 1. Regenerative endometrium, seventh day, after culture for 5 h with oestradiol-17 $\beta$  and actinomycin D, to show failure of actinomycin to inhibit mitosis. (Stained with haematoxylin and eosin,  $\times$  c. 260.)

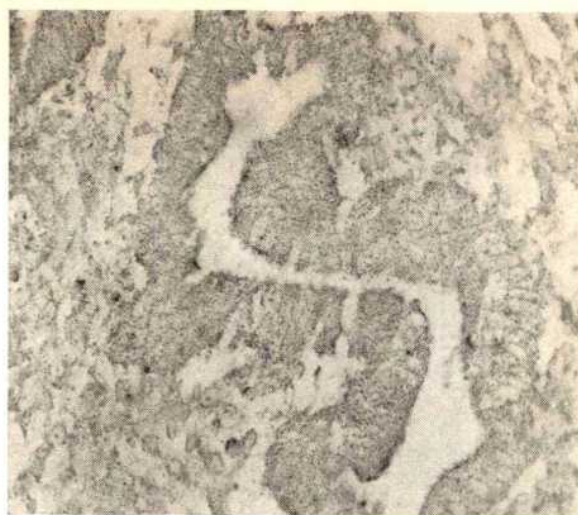


Fig. 2. Specimen No. 5. Control tissue after culture for 5 h, showing malate dehydrogenase activity. Nitro blue tetrazolium reduction. ( $\times$  c. 300.)

was used instead of phosphate buffer. Neo-tetrazolium chloride was used as the electron acceptor for quantitative purposes because of the insolubility of the formazan formed from nitro blue tetrazolium. The sections were incubated for 1 h in air at  $37^{\circ}\text{C}$  and afterwards washed in water and dried in air. Five sections were taken from each block for quantitative study. Sections for permanent mounting were fixed in neutral 10 per cent formalin before mounting and some were fixed in Masson's fixative and stained with haematoxylin and eosin for histological examination. From time to time sections were incubated in the medium without substrate and these acted as controls. Formazan was not formed in any of these controls.

The area of the sections was measured and formazan was eluted in 0.5 ml. of 10 per cent *n*-heptanol in tetrachloroethane<sup>7</sup>. The optical density of the eluent was read at  $550\text{ m}\mu$ .

Histological examination confirmed that all specimens were in the regenerative phase of the cycle and showed no abnormal features. Tissue survival during culture was excellent as judged histologically (Fig. 1) and continuing mitotic activity was seen in most cases. The tissue from

Table 1. INCREASE IN MALATE DEHYDROGENASE ACTIVITY IN OESTROGEN STIMULATED TISSUE

Tissue and section No.	Area (cm <sup>2</sup> )	Optical density (550 m $\mu$ )	Total formazan ( $\mu\text{g}$ )	Formazan ( $\mu\text{g/cm}^2$ )
Fresh tissue				
1	0.113	0.016	0.209	1.849
2	0.115	0.018	0.235	2.043
3	0.119	0.018	0.235	1.975
4	0.114	0.017	0.222	1.947
5	0.105	0.015	0.196	1.867
Mean	0.113		0.219	1.936
S.D.				0.069
Culture control				
1	0.109	0.015	0.196	1.798
2	0.111	0.015	0.196	1.766
3	0.117	0.018	0.235	2.008
4	0.106	0.015	0.196	1.849
5	0.115	0.017	0.222	1.930
Mean	0.112		0.209	1.870
S.D.				0.093
Oestrogen treated				
1	0.087	0.026	0.340	3.908
2	0.089	0.027	0.353	3.966
3	0.089	0.028	0.366	4.112
4	0.091	0.028	0.366	4.022
5	0.081	0.024	0.314	3.877
Mean	0.087		0.348	3.977
S.D.				0.084
Actinomycin and oestrogen				
1	0.069	0.010	0.131	1.899
2	0.066	0.010	0.131	1.985
3	0.064	0.008	0.105	1.641
4	0.075	0.011	0.144	1.920
5	0.065	0.008	0.105	1.615
Mean	0.069		0.123	1.812
S.D.				0.153

Results for specimen No. 2. Formazan eluted in 0.5 ml. 10 per cent *n*-heptanol in tetrachloroethane.



Table 2. RESULTS OF FIVE SPECIMENS OF ENDOMETRIUM TREATED TO DEMONSTRATE MALATE DEHYDROGENASE AFTER *in vitro* STIMULATION BY OESTRADIOL-17 $\beta$ 

Specimen	Fresh tissue	Culture control	Oestrogen treated	Actinomycin and oestrogen
1	2.51	2.87	4.76	2.54
12th day S.D.	0.08	0.11	0.25	0.32
2	1.94	1.87	3.98	1.81
13th day S.D.	0.07	0.09	0.08	0.15
3	0.66	0.89	1.89	0.80
7th day S.D.	0.07	0.10	0.07	0.15
4	2.21	2.20	4.07	
12th day S.D.	0.09	0.14	0.11	
5	1.85	1.92	3.57	1.57
12th day S.D.	0.05	0.11	0.15	0.13

Tetrazolium reduction expressed as  $\mu\text{g}$  formazan/cm $^2$  of section. Means of five sections.

specimen No. 4 treated with actinomycin became impregnated with ice crystals during mounting and is not included in the present results (see Table 2).

Figs. 2-4 show the appearances of staining for malate dehydrogenase in a typical specimen. There is a substantial increase in the amount of formazan deposited in the tissue treated with oestrogen and the increase is largely in the gland cells. The changes in the specimen treated with oestrogen are not evident in the other three preparations which have a similar appearance to one another.

Table 1 gives the full results of formazan elution for specimen No. 2 while Table 2 gives the combined results of formazan elution for the series. It can be seen that the results for the tissues treated with oestrogen are consistently almost double those for the control tissues and for the tissues treated with actinomycin D. The specimens from late in the regenerative phase show concentrations of enzyme of the same order while the specimen from seven days shows a much lower concentration.

The techniques used in this investigation provide a rough method of estimating enzyme activity in a heterogeneous tissue. Measurement of the area of a section does not take into account variations of thickness and this should be regarded as a possible source of error. The number of measurements in the series is small, but the results are sufficiently consistent to allow some general conclusions.

Malate dehydrogenase is known to increase in activity throughout the regenerative phase of the menstrual cycle

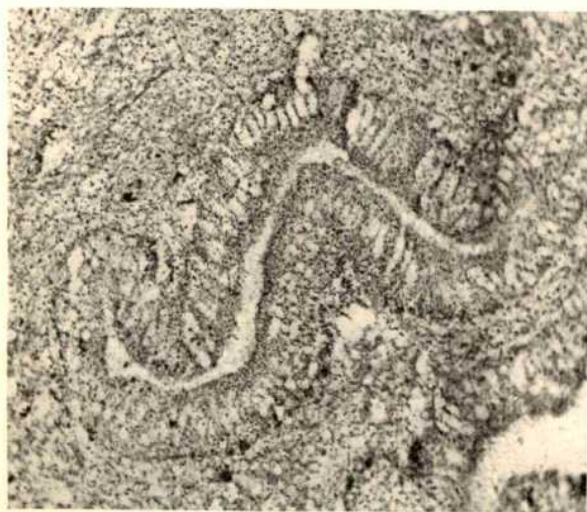


Fig. 3. Specimen No. 5. Increase in malate dehydrogenase activity after culture for 5 h with oestradiol-17 $\beta$ . Nitro blue tetrazolium reduction. ( $\times$  c. 300.)



Fig. 4. Specimen No. 5. No increase in malate dehydrogenase activity after culture for 5 h with oestradiol-17 $\beta$  and actinomycin D. Nitro blue tetrazolium reduction. ( $\times$  c. 300.)

and reaches a peak early in the secretory phase $^2$ . The results presented here show that a similar effect can be observed when regenerative phase human endometrium is stimulated *in vitro* by oestradiol-17 $\beta$ . One criterion of a successful *in vitro* technique is that known *in vivo* processes should be simulated.

The suppression of the oestrogen effect by actinomycin D suggests that the increase in enzyme activity occurred as a result of the synthesis of new RNA followed by the synthesis of new enzymes. Such a pattern conforms with current theories of enzyme induction by steroid hormones $^8$ . Although no information concerning the detailed mode of action of oestrogens can be derived from these results, the overall pattern of action in the human is shown to be similar to that described for other animals $^9-10$ .

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### Permeability Factors of Rabbit Skin associated with Delayed Vascular Response to Thermal Injury

INCREASED vascular permeability is an important feature of thermal injury, as it is of all inflammatory processes. The phenomenon is often biphasic: the early transient response is mediated by a substance inhibited by antihistamine drugs, while the more intense and prolonged delayed response is mediated by factors which are not influenced by antihistamines. A permeability factor isolated by us from lesions during the delayed response to thermal injury was found to be distinct from previously



described permeability factors which are either proteases (PF/dil, kallikrein), proteins (lymph node permeability factor), or peptides (leukotaxin, bradykinin, kallidin)<sup>1-4</sup>. This communication deals with further purification of the burns permeability factor.

Thirty sites in an area of 10×12 cm on the clipped flanks of albino rabbits (2-2.3 kg) were subjected to 56° C for 20 sec<sup>2,5</sup>. The permeability effects in the burn lesions, and the potency of extracted permeability factors injected intradermally, were measured in terms of exuded dye extractable from the lesions in animals with pontamine blue in the circulation<sup>6</sup>. The delayed response to the heating began within about 40 min, reached a peak in about 2 h and disappeared in 6-8 h. From the extracts of skin acetone powder prepared from 2 h old lesions, pseudoglobulin fractions were prepared with ammonium sulphate<sup>2</sup> and chromatographed. Concentrations of protein were recorded as the absorbancy, *E*, at 280 mμ, and increase of specific permeability was expressed as μg of dye extracted/absorbancy at 280 mμ. Before the test on rabbit skin all samples were dialysed against M/15 phosphate buffer (pH 7.4).

When pseudoglobulin fractions (15 ml.; absorbancy 20-30) in M/15 phosphate buffer (pH 7.4) were desalted through a column of 'Sephadex G-50' medium<sup>7</sup> (with a flow rate of seven drops/min and 6 g effluent fractions collected) one chromatographic peak of absorbing material was obtained, and the potency for increasing the permeability of the effluent fractions clearly paralleled the absorbancy. The mean specific activity of the active fractions was about 3.1.

The active fractions (absorbancy 20-22) were fractionated on DEAE-cellulose (Brown)<sup>8</sup> by successive elution with phosphate buffer of the following molarity and pH: 1/150, pH 6.8; 1/150, pH 7.4; 1/100, pH 7.4; 1/50, pH 7.4; 1/15, pH 7.4; 1/15 plus M/5 sodium chloride, pH 7.4; and finally with molar sodium chloride. The rate of flow was 12 ml./h and 3 g effluent fractions were collected. Of the four absorbing peaks obtained the first three had specific activity of 1.3 (eluted in M/150, pH 6.8), 6.8 (in M/15) and 3.2 (in M/15 plus M/5 sodium chloride). The fourth (in 1.0 molar sodium chloride) was inactive. The materials in the active fractions were designated factors I, II and III, respectively.

The factor II fractions were further fractionated on DEAE-'Sephadex A-50' medium<sup>9</sup> by successive elution with phosphate buffer as follows; 1/50, pH 7.4; 1/30, pH 7.4; 1/15, pH 6.8; 1/15, pH 7.4; 1/15 plus M/5 sodium chloride, pH 7.4; and finally with 1.0 molar sodium chloride. The flow rate was 8 ml./h and 3 g fractions of effluent were collected. There were six absorbing peaks, of which that in buffer, M/50, pH 7.4, was the most potent, with a specific activity of about 73.3.

Also on DEAE-'Sephadex A-50', factor III fractions were eluted by phosphate buffer at pH 7.4 as follows: 1/50, 1/15, 1/15 plus M/10 sodium chloride; 1/15 plus M/5 sodium chloride; and finally with 1.0 molar sodium chloride. The fourth eluate, in M/15 plus M/5 sodium chloride, was active, with a low specific activity of 9.5. This eluate was fractionated on hydroxylapatite<sup>10</sup> by successive elution with buffers at pH 6.8, as follows: M/200 phosphate, 3 M/100 phosphate, 3 M/20 phosphate, 3 M/10 potassium phosphate and 1.0 molar potassium phosphate. The flow rate was 8 ml./h and 6 g effluent fractions were collected. The mean specific activity of the first component (in M/200) was about 74.2. No permeability effect was produced by four other components.

The purified factors II and III gave a positive biuret reaction and behaved as homogeneous substances on electrophoresis and ultracentrifugation. They were non-diffusible and thermostable. They would seem to be a family of peptides.

These observations indicate that the burns permeability factor in the pseudoglobulin fraction is a complex of factors I, II and III. The *in vivo* effects of the burns permeability

factor and of each of the separate factors are long-lasting<sup>11</sup>, and so it seems reasonable to suppose that the delayed vascular permeability response to thermal injury is caused by the combined action of the three factors. The long-lasting effect of the factor distinguishes it from the permeability factors mentioned in the first paragraph, all of which produce only a short-lived increase in vascular permeability on experimental injection. In the same way it appears that the delayed vascular response in the Arthus reaction is mediated by an Arthus permeability factor made up of three similar factors, I, II and III<sup>12,13</sup>.

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### Inhibition by Purines of the Production of Fronds in *Lemna perpusilla*

WHILE examining the herbicidal properties of a variety of compounds using a modified duckweed test<sup>1</sup>, we noted that several purine derivatives showed marked inhibition of the production of fronds of *L. perpusilla* growing in a chemically defined medium<sup>2</sup>. In these experiments 10 ml. of sterile Hillman's medium<sup>2</sup> (with sucrose) was added to 1×4 in. test-tubes (capped with Morton stainless steel closures). Dilutions of the test substances were added to the tubes and three fronds of *L. perpusilla* were then added. The inoculated tubes were placed on slanting boards in a room with a constant temperature of 75° F which was illuminated for 14 h each day. The number of fronds in each tube was determined after 3, 5, 7 and 9 days of incubation. Four replicate tubes were started for each concentration of test compound, and the average number of fronds in each tube was plotted in the dose-response curve. The concentration of inhibitor needed to reduce growth to 50 per cent of that of the untreated controls was determined graphically. In these conditions a ten-fold increase in the number of fronds was noted in the control tubes during the 9 days.

Table 1. INHIBITION OF *Lemna perpusilla* BY PURINE

Purine tested	Concentration causing 50 per cent inhibition of frond production (p.p.m.)
Purine	2
Kinetin	2
6-Methylpurine	0.001
6-Chloropurine	10
6-n-Heptylamino purine	> 10
6-n-Amylamino purine	> 10
6-β-Naphthylamino purine	> 10

The data collected in a series of experiments are summarized in Table 1. Purine and kinetin inhibited the production of fronds at 0.1–0.2 p.p.m., and 2 p.p.m. ( $1.5 \times 10^{-5}$  molar) was sufficient to cause 50 per cent reduction in frond production. Kinetin did not stimulate the production of fronds at any concentration tested (0.001–0.2 p.p.m.), and had an inhibitory effect at concentrations greater than 0.1 p.p.m. 6-Methylpurine was even more toxic, 0.001 p.p.m. ( $1 \times 10^{-8}$  molar) causing 50 per cent inhibition, and significant inhibition was noted with 0.00001 p.p.m. 6-Chloropurine was much less effective, 10 p.p.m. being required for inhibition of multiplication of fronds, and the other purines examined had no inhibitory effects at concentrations of less than 10 p.p.m.

The inhibition caused by 6-methylpurine, kinetin, and purine could be reversed by addition of adenine to the growth medium; 2 p.p.m. of adenine completely reversed the inhibition caused by 2 p.p.m. of purine and 0.001 p.p.m. of adenine reversed the inhibition caused by 0.001 p.p.m. of 6-methylpurine.

These experiments show that the production of fronds by *L. perpusilla* is quite sensitive to exogenous purines. 6-Methylpurine may have possibilities as an agent to control duckweed in ponds. It probably acts by interfering with purine metabolism, because adenine is an effective reversing agent.

We thank Dr W. S. Hillman for the culture of *Lemna perpusilla* and Professor F. M. Strong for the kinetin analogues.

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## Genetic Variant of Human Erythrocyte Malate Dehydrogenase

MALATE dehydrogenase exists in two distinct forms in a variety of mammalian tissues, one of which is in the cytoplasm as a soluble or supernatant enzyme and the other is tightly bound to the mitochondria<sup>1</sup>. These two forms have different chemical, physical and kinetic properties<sup>2–4</sup> and each has a characteristic electrophoretic pattern<sup>5</sup>. Unlike most tissues erythrocytes possess only cytoplasmic malate dehydrogenase, as would be expected in the absence of mitochondria. This report concerns an inherited electrophoretic variant of erythrocyte (supernatant) malate dehydrogenase which was found in a survey of haemolysates prepared from 2,910 individuals, of whom 1,440 were white and 1,470 were North American Negroes. All were unrelated so far as is known, and consisted of unselected patients and personnel of three Buffalo hospitals. In the family investigations, both haemolysates and leucocyte extracts were electrophoresed.

Haemolysates were prepared by mixing one volume of packed, twice washed red cells with two volumes of water. Leucocyte extracts were prepared by removing the buffy coat with a Pasteur pipette, after which the cells were washed briefly in distilled water and then in saline. The white cells were resuspended in an equal volume of distilled water and subjected to sonication for 1 min.

The haemolysate survey was carried out on horizontal starch gel electrophoresis using a 0.01 molar phosphate buffer in the gel and a 0.2 molar phosphate buffer in the end trays, both at pH 7.0. Good results were obtained both when run at 10 V/cm for 5 h and 4 V/cm for 16 h.

Improved definition of the mitochondrial enzyme was obtained with vertical starch gel electrophoresis in a phosphate-citric acid buffer at pH 7.0 (ref. 5). In each case the gels were sliced horizontally after electrophoresis and the developing solution consisted of 725 mg of DL-malic acid, 5 mg of nicotinamide-adenine dinucleotide (NAD), 5 mg of phenazine methosulphate and 5 mg of MTT tetrazolium in 50 ml. of 0.1 molar phosphate buffer solution at pH 7.0.

Partial purification of the enzyme was obtained by starch block electrophoresis or DEAE cellulose chromatography. For starch block electrophoresis the method was a modification of that described by Kunkel<sup>6</sup>. Starch blocks measuring 75 × 30 × 1.5 cm were prepared in a glass mould. A trough 25 cm long and 0.15 cm wide was cut across the centre of the block. Haemolysate (15 ml.) was placed in the trough after which electrophoresis was carried out at 4° C. A 0.008 molar phosphate citric buffer, pH 7.0, was used and a gradient of 300 V was applied to the block for 20–48 h. To avoid changes in pH on the block, the outer end trays were exchanged approximately every 8 h during the electrophoresis. At the end of electrophoresis 2.5 cm sections were removed from each end of the block and discarded and the remaining 70 cm was cut into 2.5 cm sections and numbered 1–28 from the cathodal end. Each section was eluted in a coarse fritted disk funnel with 7.0 ml. of a 0.008 molar phosphate citric buffer, pH 7.0. The eluates were tested for activity of malate dehydrogenase by spectrophotometric assay<sup>7</sup> and they were subjected to starch gel electrophoresis.

For DEAE cellulose chromatography 10 g of DEAE cellulose was equilibrated with 0.01 molar *tris* hydrochloride buffer, pH 8.2, in a column 2.5 cm in diameter. Haemolysate (5 ml.) was washed into the column and a linear gradient to 0.3 molar *tris* hydrochloric acid, pH 8.2, was used for elution. Fractions (7 ml.) were collected and analysed for malate dehydrogenase activity, and subjected to starch gel electrophoresis.

Eluates from starch block or cellulose chromatography which had been proved to contain only the major normal band or the slowest variant band were concentrated by vacuum filtration to 0.5–1.0 ml. and dialysed in 0.1 molar sodium phosphate buffer, pH 7.0. A 0.5 ml. sample of each concentrate was mixed and subjected to reversible inactivation in acid, a process which involves the dissociation of the enzyme into inactive sub-units and then recombination of the sub-units to yield active enzyme<sup>8,9</sup>. The reactivated enzyme was subjected to starch gel electrophoresis and assay for malate dehydrogenase.

The usual red cell malate dehydrogenase pattern after starch gel electrophoresis consists of a major anodal band and two minor bands which migrate slightly faster and which are very variable in staining intensity (Fig. 1). One variant was found in an apparently normal, healthy Negro female among 2,910 samples from Negroes and Whites. The variant pattern consists of three distinct major bands, one of which corresponds to the normal major band; the other two have slower migratory rates as depicted in Figs. 1 and 2 and in the diagram in Fig. 3. The minor anodal bands are the same for the proposita as for a normal individual. The supernatant leucocyte enzyme also shows the variant pattern, but the mitochondrial pattern is normal, as seen in Fig. 2. The variant pattern was observed in the erythrocyte malate dehydrogenase of the two sons of the proposita but not in that of the daughter or her mother. Her father was not available for testing. Six half sibs of the proposita had the normal erythrocyte malate dehydrogenase pattern.

Concentrated eluates from both starch block and DEAE cellulose columns were found which contained only the slowest moving band of the variant erythrocyte enzyme (Fig. 3, slot 4). Slot 3 contained a similarly isolated and concentrated eluate of the normal enzyme. An untreated mixture of these isolated slow and normal bands was placed in slot 5. The result of a mixture of the same two



bands subjected to the reversible inactivation and recombination procedure is shown in slot 6. Here the intermediate band was created from the fast and slow bands.

This seems to be the first reported genetic variant of human erythrocyte malate dehydrogenase. The three bands suggest that the supernatant enzyme is a dimer, because the random association of two sub-units would be expected to produce such a pattern. The dissociation and subsequent recombination of the slowest moving variant band and the normal band to produce three bands represents *in vitro* support for the dimer hypothesis. Presumably there has been a mutation of the allele which controls one of the sub-units of the molecule. The family and electrophoretic data show that the genetic control of supernatant malate dehydrogenase is autosomal. Although the pedigree does not rule out sex linkage, because the variant pattern is the same in males and females, the mutant

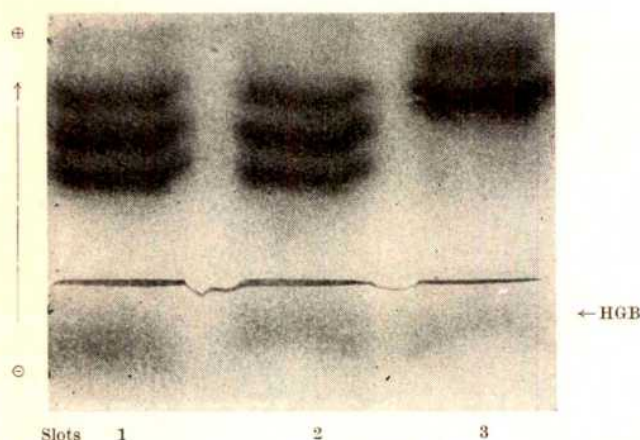


Fig. 1. Photograph of a horizontal starch gel showing the normal erythrocyte pattern of malate dehydrogenase in slot 3 and the variant pattern in slots 1 and 2. In this picture only one of the minor bands is visible, and in the case of the variant it is very indistinct.

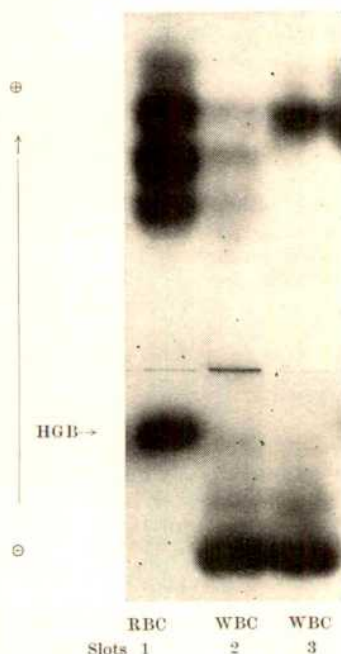


Fig. 2. Photograph of a vertical gel in a phosphate-citrate buffer as described in the text. In slots 1 and 2 are erythrocyte and leucocyte preparations from the proposita and in slot 3 is a normal leucocyte extract. The variant pattern is confined to the anodal or supernatant malate dehydrogenase, while the cathodal or mitochondrial malate dehydrogenase is unaffected.

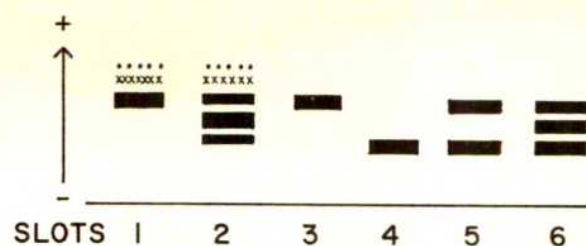


Fig. 3. A diagram of erythrocyte patterns of malate dehydrogenase. In slot 1 is the normal pattern, and in slot 2 the variant. Slot 3 represents a partially purified normal band, and slot 4 the partially purified slowest moving band of the variant. In slot 5 is a simple mixture of 3 and 4, and in slot 6 this mixture following dissociation and recombination of sub-units.

allele could not be on the X chromosome. If it were, the erythrocyte malate dehydrogenase of hemizygous affected males would appear as a single band with the same electrophoretic mobility as the slowest band of the variant pattern. The observation that the mitochondrial malate dehydrogenase is indistinguishable from normal in an individual with a genetic variant of the supernatant enzyme provides additional evidence suggesting that the two cellular forms of this enzyme are controlled by separate genetic loci.

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## PHYSIOLOGY

### Further Investigations into the Function of the "Mirror" in Tettigonioidae (Orthoptera)

FROM the calculations of Pierce<sup>1</sup> nearly two decades ago, students of the production of sound in bush crickets have assumed the area of thin cuticle on the right tegmen known as the "mirror" to be acoustically important<sup>2</sup> (Fig. 1). Pierce's attribution of a resonant function to this membrane was challenged by Broughton<sup>3</sup> and Dumortier<sup>4</sup>. Dumortier suggested that the whole tegmen is responsible as an acoustic coupler, not only the mirror. Broughton, working on the decticine, *Metrioptera roeselii* (Hagenbach), found that the frequency spectrum of one individual was not obligatorily changed by coating the mirror with a deadening film of latex. His results have stimulated further work of the same nature on two

members of the Conocephalidae, *Conocephalus discolor* (Thunberg) and *Homorocoryphus nitidulus nitidulus* (Scopoli). Both these animals, like *M. roeselii*, have a well-defined spectrum, but their peak frequencies are at 28 kc/s and 15.5 kc/s, respectively.

The experiments closely followed those of Broughton: recording, applying latex to the mirror, recording, removing latex from the mirror and recording. It was assumed that if the mirror is of acoustic importance latex would (a) lower general intensity and (b) change any natural frequency of vibration. In contrast to Broughton's experiments, however, the latex was not confined to the mirror membrane alone but often covered part of the frame of more or less heavily sclerotized veins surrounding it.

Application of latex in *C. discolor* spread the spectrum over a wider range of frequencies and in most cases caused the peak frequency to occur higher than that of the un-

operated animal (Fig. 2), although in some it was below and in even fewer cases it remained the same. The peak frequency in *H. n. nitidulus* was lowered. While intensity could not be measured in absolute terms it was found to be usually lower for both species.

If the mirror membrane alone were responsible for the amplification of certain frequencies by resonance, to produce a peak in the spectrum, then this property should be modified by the application of the relatively thick film of latex. I suggest that the touching of the frame in my experiments (and perhaps in some of Broughton's) may have been the operative factor in disturbing the spectrum. This implies that the frame is of primary importance in sound production and the mirror membrane of secondary importance (a finding amply confirmed by more recent work on African material).

This work was carried out with the aid of a grant from the French Government at the Laboratoire de Physiologie Acoustique, I.N.R.A., C.N.R.Z., Jouy-en-Josas, S. et O., France, during 1964-65. I thank Dr R.-G. Busnel and Mr W. B. Broughton for their direction of this work, and Mme M. C. Busnel, Mr B. Dumortier and Dr W. Loher for their advice and criticism.

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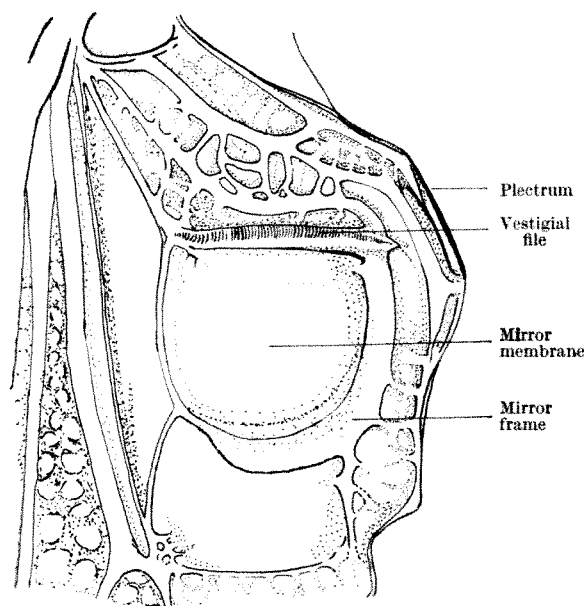


Fig. 1. *Homorocoryphus nitidulus nitidulus* (Scopoli): cubito-anal area of the right tegmen, viewed ventrally.

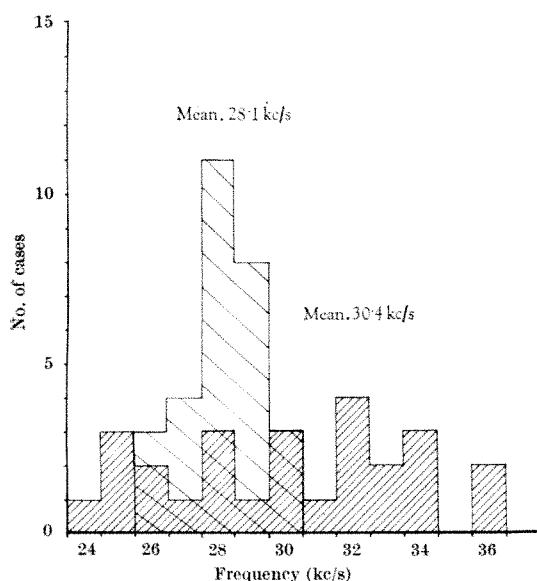


Fig. 2. *Conocephalus discolor* (Thunberg): comparison of peak frequency in unoperated animals (I), and animals with mirror area coated with latex (II).

### Gastrin-like Effects of Cholecystokinin-Pancreozymin

WE have studied the effects of "pure" cholecystokinin-pancreozymin (CCK-PZ, kindly supplied by Professor Jorpes) on the human stomach and pancreas, comparing them with the response of these same viscera to a synthetic gastrin-like peptide (a tetrapeptide made by Leo, 10 µg/kg of body weight inducing a maximal response when given subcutaneously).

In all our experiments, the CCK-PZ was given intravenously at the constant rate of 2 Crick-Harper-Raper units/kg body weight/h. The gastrin analogue was given in a similar manner at the rate of 0.5 µg/kg body weight/h. Responses are submaximal at these concentrations.

CCK-PZ induces an acid response in the resting stomach, increasing both the volume and the concentration of the acid and leading to a steady level of secretion (average 12 m.equiv./h) in about an hour. A very similar response was obtained with the gastrin analogue (average 14 m.equiv./h). In both instances a rise in the hydrogen ion concentration was accompanied by a fall in sodium ion concentration.

When both substances were given together at these rates, the one on a background infusion of the other, whether the analogue preceded or followed the CCK-PZ, the acid response of the stomach showed an additive effect with no evidence of potentiation (average 16.5 m.equiv./h). Thus the arithmetic summation of their separate effects is much greater than their combined effect, suggesting a non-linear relationship in their additive effect at submaximal stimulation.

On the pancreas, both substances induced a similar pattern of output of amylase and trypsin.

We feel therefore that CCK-PZ displays gastrin-like effects. It may well be that CCK-PZ and not gastrin accounts for the gastrin-like activity found in extracts of duodenal mucosa. The similarity of action of these two hormones suggests that they may have important amino-



acid sequences in common. The confirmation or refutation of this suggestion will have to await the detailed chemistry of CCK-PZ. The work carried out at the Caroline Institute by Professor Jorpes and Dr Mutt<sup>1</sup> seems to support this point of view, however, because the two compounds have been shown to have C-terminal sequences in common.

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### Cerebellum of Mormyrids

In teleost fishes the cerebellum is well developed and consists of two principal parts: the corpus cerebelli and the valvula cerebelli. The former is massive and usually arches backward; the latter is a pouch-like structure which projects forward under the tectum in the mesencephalic ventricular cavity. It has long been known<sup>1,2</sup> that in one group of teleosts, the Mormyrids, the cerebellum attains amazing dimensions. In these forms the valvula consists of an intermediate part and two lateral lobes, and it is the latter which has greatly hypertrophied. These lobes have grown out of the ventricle of the mid-brain, to become superficial structures which cover all other parts of the brain. Fig. 1 shows that the lateral lobes consist of an arched and folded basal layer on which are numerous small and regularly arranged ridges. The intermediate part of the valvula is composed of two lobules which are situated in front of the corpus. The corpus cerebelli itself is bent forward and is of average size (Fig. 1).

The relationships of the fibre tracts<sup>3,4</sup> suggest that the hypertrophy of the valvula in the Mormyrids is related to the high degree of development of the lateral line system, which to a large extent has become transformed into an electroreceptor organ<sup>5,6</sup>.

Microscopically both corpus and valvula contain the three characteristic cerebellar layers: granular, Purkinje and molecular<sup>1,2</sup>, but it is important to note that the granular layer does not extend into the valvular ridges. The latter contain a thin, central fibre zone, on either side of which are found a Purkinje and a molecular layer (Fig. 2). The Purkinje layer also contains medium-sized cells. The molecular layer of corpus and valvula shows characteristic "stripes" perpendicular to the surface (Fig. 2). This so-called palisade pattern has been ascribed to the orientation of the glial fibres<sup>1,2</sup>.

The fibre connexions of the Mormyrid cerebellum have been extensively studied by the Weigert method<sup>3,4,7</sup>, but little attention has been paid to its constituent neurones. We studied serially sectioned brains of *Gnathonemus petersi* and *Petrocephalus bovei*, which were stained according to the Bodian and the Golgi rapid methods. The Golgi material showed that the Purkinje cells in the

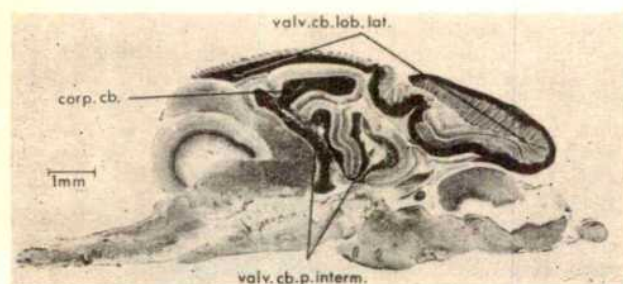


Fig. 1. Sagittal section through the brain of *Petrocephalus bovei*; the anterior is on the right. Corp. cb., Corpus cerebelli; valv. cb. p. interm., valvula cerebelli, pars intermedia; valv. cb. lob. lat., valvula cerebelli, lobus lateralis.

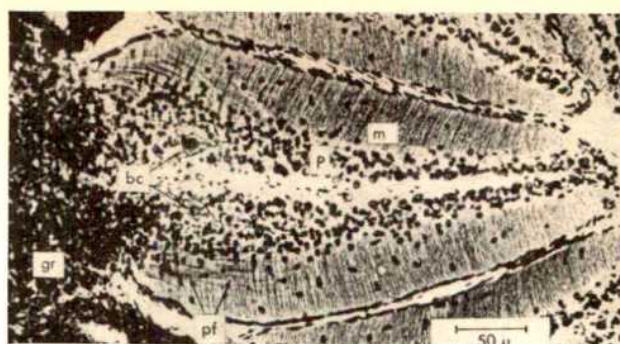


Fig. 2. Sagittal section, passing transversely through one of the ridges of the lateral lobe of the valvula. Bodian method. bc, Large basal cells; gr, granular layer; m, molecular layer; P, Purkinje layer; pf, parallel fibres.

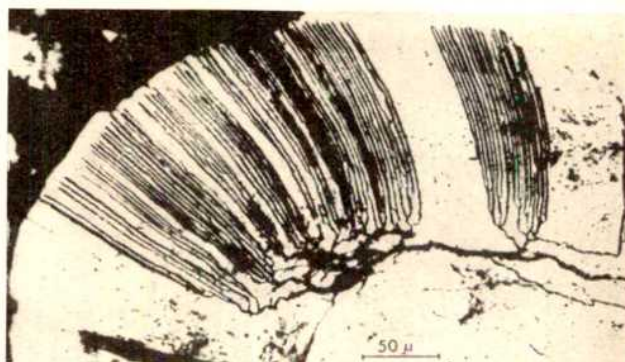


Fig. 3. A composite photomicrograph of a Purkinje cell in a sagittal section through the corpus cerebelli of *Gnathonemus petersi*. Golgi method.

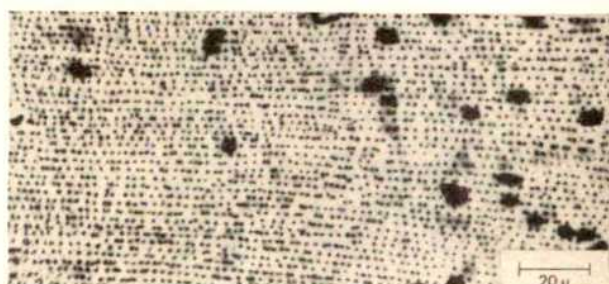


Fig. 4. Section through the lateral lobe of the valvula. The molecular layer is cut parallel to the surface. The rows of dots represent transversely cut dendrites of Purkinje cells. Bodian method. In this and Fig. 5 the dendritic trees of the Purkinje cells spread in a plane oriented perpendicular to that of the rows of dots.

corpus and in the intermediate part of the valvula are large and possess a widely spreading dendritic tree, which, as in other teleosts<sup>2,8</sup>, is clearly oriented in the sagittal plane. The initial dendritic branches tend to spread parallel to the surface of the cerebellum. From these primary dendrites issue numerous branches which eventually, after some bifurcation, ascend parallel to each other towards the external surface (Fig. 3). These parallel dendrites are studded with numerous tiny projections and confine themselves to the molecular layer. The Purkinje cells in the lateral lobes of the valvula are smaller than those in the central parts of the cerebellum, but are also provided with straight dendrites. The typical palisade pattern of the molecular layer of the Mormyrid cerebellum is clearly derived from the Purkinje cell dendrites. This vertical orientation of the Purkinje dendrites has not been reported in other teleosts<sup>2,8</sup>, nor in any other group of vertebrates<sup>9</sup>. Sections through the molecular layer, parallel to the cerebellar surface, revealed, furthermore, that in corpus and valvula the Purkinje dendrites are arranged in regular rows (Fig. 4). In the intermediate part of the valvula the parallel fibres are coarse and



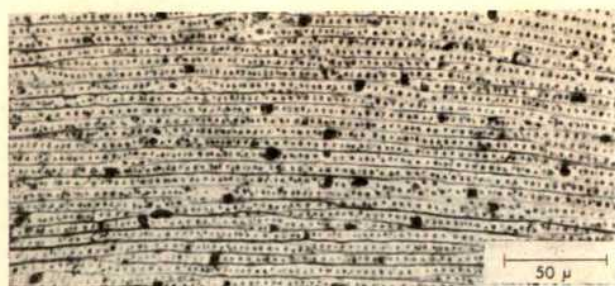


Fig. 5. Tangential section through the molecular layer of the intermediate lobe of the valvula. The transversely cut Purkinje dendrites are separated by bundles of parallel fibres.

distinctly impregnated. In this region bundles of parallel fibres alternate with the rows of Purkinje dendrites (Fig. 5). (The granular cells, the axons of which give rise to the parallel fibres, resemble in every respect those of other teleosts<sup>2,8</sup>.)

In the lateral lobes of the valvula the parallel fibres pass directly from the granular layer into the molecular zone, and pass straight to the top of the ridges (Fig. 2). There is no bifurcation. The dendritic trees of the Purkinje cells are oriented perpendicular to the direction of the parallel fibres. It is noteworthy that the cerebellum of birds and mammals shows a transversely oriented folding, whereas the valvula of Mormyrids, judging from the orientation of its parallel fibres and Purkinje dendrites, is folded sagittally. Also remarkable is the fact that the most basal parts of the two cell layers of each valvular ridge contain a row of large cells, the perikarya of which are enveloped by a conspicuous axonal plexus. Two of these elements are visible in Fig. 2. Golgi preparations have shown that the dendrites of these cells extend into the molecular layer but are both thinner and more widely spaced than those of Purkinje cells. The destination of their axons has not yet been determined.

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### Monolayer Films of Retinal<sub>1</sub> and the Effects of Light on Them

SEVERAL stereoisomers of retinal<sub>1</sub> (vitamin A<sub>1</sub> aldehyde) are known and their interconversions can easily be promoted by the action of light<sup>1</sup>. The photoconversion of 11-cis to all-trans retinal<sub>1</sub> takes place with the highest efficiency<sup>2,3</sup> and is involved in the first step of the visual process<sup>4</sup>. Studies of vitamin A and its derivatives have suggested that their surface-active properties are closely related to their physiological functions<sup>5,6</sup>. The purpose of the present investigation was to study the properties of monolayer films of all-trans and 11-cis retinal<sub>1</sub> at air-water interfaces, and to determine the effect of light on the films.

Vitamin A oil (15 × 10<sup>6</sup> unit) was obtained from Riken Vitamin Oil Co., Ltd. All-trans and 11-cis retinal<sub>1</sub> were prepared by a modification of Wald's method<sup>7</sup> and re-

crystallized three times from light petroleum ether. The spreading solutions were prepared by dissolving 3 mg of the respective retinal<sub>1</sub> in 10 ml. of ethanol. The solutions were spread on a clean surface of water by means of a micrometer syringe. Surface pressures were measured for various film areas at room temperature using the hanging plate method.

To minimize oxidation of retinal<sub>1</sub>, the measurements for every force-area curve were completed within 20 min of spreading the solution. For the radiation experiments a 500-W iodine lamp, placed 45 cm from the surface, was used. The light passed through a 10-cm thick water cell to avoid heating the films. Surface pressures were measured before and after irradiation, and the area of the film was held constant at 15 × 14.5 cm<sup>2</sup>. All experimental procedures were carried out under a dim red light.

Fig. 1 shows force-area curves of all-trans and 11-cis retinal<sub>1</sub>. Both isomers give liquid expanded films, that of the 11-cis isomer being more expanded than that of the all-trans. The collapse pressures and limiting areas of each molecule of the isomers are shown in Fig. 1. The 11-cis isomer has a larger limiting area and lower collapse pressure than the all-trans one.

Retinal<sub>1</sub> has a bulky lipophilic β-ionone ring attached to a side chain with four conjugated double bonds and terminating in a hydrophilic aldehyde group. The side chain of the all-trans isomer is considered to be flat and straight, while that of the 11-cis one is bent and twisted at the central carbon atom<sup>1</sup>. The relatively straight and flat form of the all-trans isomer allows a closer and more ordered packing on the water surface than with the cis-isomer. The intermolecular forces, which make the film able to withstand the increasing lateral compression, would therefore be stronger than in the other case.

When all-trans and 11-cis films were irradiated, changes were observed in the surface pressures required to keep the film area constant. The changes were maximal after irradiation for 30 sec. In Fig. 2 the changes (percentage increase or decrease) in the surface pressure of films after irradiation for 30 sec are plotted against the initial surface pressure. The surface pressure of the film of all-trans retinal<sub>1</sub> increases in the region below 9 dynes/cm, changes little between 9 and 12 dynes/cm and decreases in the region above 12 dynes/cm. The increase of the pressure on irradiation may be explained by supposing that any other isomers formed will tend to expand the film, or

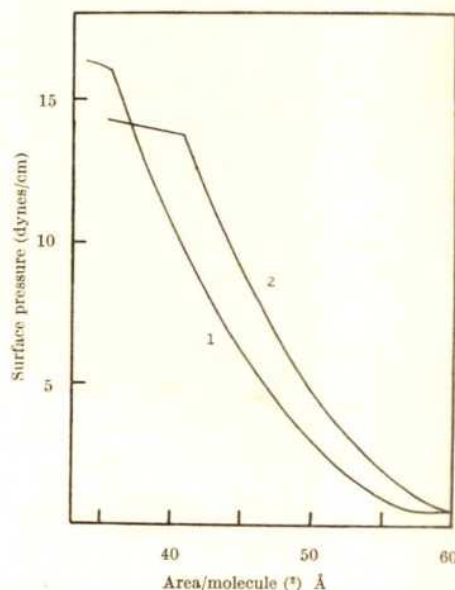


Fig. 1. Force-area curves of all-trans and 11-cis retinal<sub>1</sub>. Curve 1, all-trans retinal<sub>1</sub>; curve 2, 11-cis retinal<sub>1</sub>. The curves show that all-trans and 11-cis retinal<sub>1</sub> have collapse pressures of 16.1 and 13.8 dynes respectively, and limiting areas of 55.7 and 41 Å<sup>2</sup>/molecule respectively.

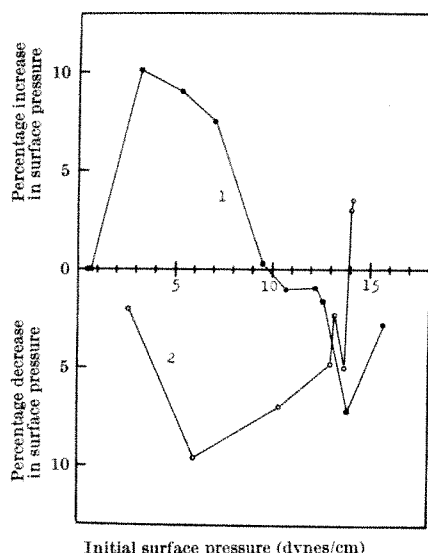


Fig. 2. The changes (percentage increase or decrease) in the surface pressure of films of retinal, after irradiation for 30 sec. Curve 1, all-trans retinal; curve 2, 11-cis retinal.

because the area of the film is kept constant, to increase the surface pressure. The decrease in pressures at regions above our initial pressure of 12 dynes/cm could be caused by the squeezing of some part of the retinal<sub>1</sub> out of the film. Conversely, irradiation of the films of 11-cis retinal<sub>1</sub> results in decrease of surface pressure. This similarly may be explained by supposing that the 11-cis isomer is photoisomerized to the lower pressure all-trans one. These properties of retinal<sub>1</sub> films contrast with those of other vitamin A derivatives (our unpublished results).

The change of molecular packing in the film may also be expected to occur in the native physiological membrane when conformational change of the retinal<sub>1</sub> molecule occurs. The molecular packing of rhodopsin in rod outer segments is not well known and the orientation of 11-cis retinal<sub>1</sub> in the membrane is still more obscure. It is probable, however, that the retinal<sub>1</sub> moiety of the rhodopsin molecule exists in the lipid-rich part of the disk-like membrane in the rod outer segment, because retinal<sub>1</sub> is itself a member of the lipid family. Photoisomerization of the retinal<sub>1</sub> would affect the permeability of the membrane, or at least the conformation of the rhodopsin in that membrane<sup>8-10</sup>. Further experiments are in progress along this line.

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## Pituitary-Adrenal Regulation of Ceruloplasmin

INVESTIGATIONS of the effect of sex hormones on copper metabolism in man and animals have shown a sharp rise in ceruloplasmin after hormone injection<sup>1</sup>; however, the mechanism of this elevation has not previously been discussed. Hormonal control of the synthesis of RNA has been demonstrated (reviewed by Davidson<sup>2</sup>), and other investigations have shown that both testosterone and oestradiol can bind to DNA and stimulate RNA synthesis<sup>3</sup>. Ceruloplasmin is a protein and as such depends on RNA synthesis for its production. Molecules which act as inhibitors or stimulators of RNA synthesis could therefore control ceruloplasmin production.

In this laboratory, studies of the effect of copper metabolism on the rat have shown that hypophysectomy and bilateral adrenalectomy cause a significant rise in concentrations of ceruloplasmin. In contrast, ACTH administered to intact animals caused a significant decrease in the concentration of ceruloplasmin, as did corticosterone given to adrenalectomized rats. Correlating these findings with recent reports on factors regulating RNA, we have attempted to discover a mechanism to explain the influence of hormone on ceruloplasmin.

Hypophysectomized rats (Hormone Assay) were purchased at 30 days of age and raised to maturity on 'Metrecal', 'Purina' laboratory chow, wheat and water. The remainder of the animals were adult Wistar strain rats, fed 'Purina' laboratory chow, wheat and water. Bilateral adrenalectomy was performed by the trans-abdominal approach via midline incision. After surgery, the animals were continued on the normal diet supplemented with 5 per cent dextrose in saline, and experiments were begun two weeks later.

Blood was drawn by cardiac puncture into a heparinized syringe after the animals had been lightly anaesthetized with sodium pentobarbital (2.5 mg/100 g). Plasma ceruloplasmin concentrations were determined by the method of Houchin<sup>4</sup>, which measures the oxidase activity of the enzyme. Plasma blanks were used with each determination. Oxidase activity was converted to mg/100 ml. ceruloplasmin by multiplying optical density times 157, the constant we have determined for the rat.

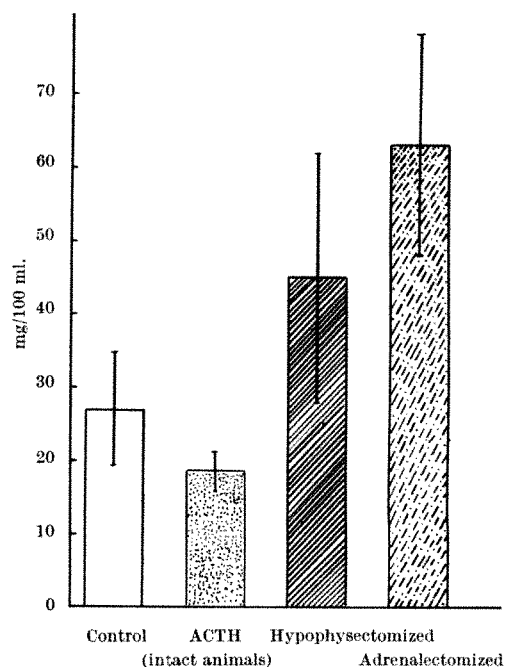


Fig. 1. Concentration of ceruloplasmin for the four groups studied. There were twelve animals in each group. The bar represents the mean and the centre line indicates standard deviation.



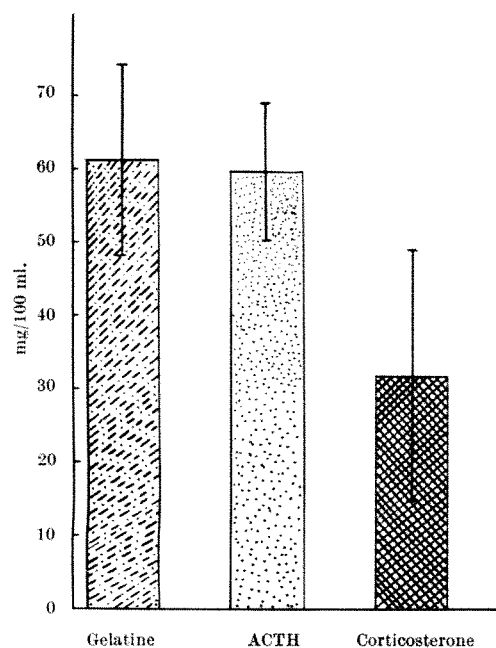


Fig. 2. Concentration of ceruloplasmin in the adrenalectomized animals which received the supplements indicated below each bar. Each supplement was given for 15 days to twelve animals. The bar shows the mean and the centre line indicates standard deviation.

ACTH in gelatine, 10 IU per day, was administered subcutaneously to intact animals for 15 days. The controls received 0.1 ml. gelatine per day. The ceruloplasmin concentrations for hypophysectomy, adrenalectomy, ACTH, and controls are shown in Fig. 1. Injections of ACTH significantly decreased the concentration of ceruloplasmin ( $P < 0.01$ ), while hypophysectomy and adrenalectomy significantly increased it ( $P < 0.01$ ).

To substantiate the findings of the first experiment, we studied groups of adrenalectomized rats given 0.1 ml. gelatine/day, 10 IU ACTH in gelatine/day or 2.5 mg corticosterone/day, for 15 days (Fig. 2). The ceruloplasmin concentrations of the groups treated with gelatine and ACTH showed no significant difference ( $P > 0.05$ ) but the ceruloplasmin of the animals treated with corticosterone was significantly lower ( $P < 0.01$ ) than either of the other groups.

Extirpation of the adrenal glands may have removed an inhibitor of ceruloplasmin synthesis as suggested by the higher concentration of ceruloplasmin in the adrenalectomized rats. ACTH administered to the intact animal caused a decrease in ceruloplasmin but brought about no change in the adrenalectomized animals. Corticosterone provoked a decrease in ceruloplasmin when administered to the adrenalectomized animals, indicating that the adrenal glands, mediated by the pituitary, play a key part in maintaining ceruloplasmin concentrations.

Steroid hormones alter the synthesis of protein by acting at the transcription level—RNA synthesis<sup>5</sup>. This would imply that the concentration of circulating steroids could influence the concentration of ceruloplasmin. During investigations of copper metabolism in the neonatal rat, we have found that the concentration of ceruloplasmin is low until the twentieth day (0–15.0 mg/100 ml.), and thereafter rises to adult concentrations. In comparison, Halmeyer has shown the concentration of circulating corticosterone in the neonatal rat to decrease from birth until adulthood. On the first day the level is 17.4  $\mu$ g/100 ml., and decreases to an adult level of 3.45  $\mu$ g/100 ml.<sup>6</sup> This indicates an inverse relationship between the concentrations of corticosterone and ceruloplasmin.

The concentrations of ceruloplasmin in the hypophysectomized animals corresponded well with the proposed

mechanism. Without pituitary stimulation, the steroid concentration was lower than normal, which allowed a rise in ceruloplasmin. Because the adrenals were intact, however, the ceruloplasmin did not rise to the high concentrations found in the adrenalectomized animals.

These results indicate the importance of the pituitary-adrenal axis in regulating the concentration of ceruloplasmin. By controlling the level of circulating hormones, the pituitary-adrenal system monitors the production of protein at the site of RNA synthesis.

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## Thermal Conductivity of Normal and Infarcted Heart Muscle

ONE of the curiosities of pathology concerns the nature of infarction. Why, when heart muscle dies as part of the total death of an animal, does it produce a histological picture which we term normal, yet when it dies locally in the midst of functioning muscle as a result of a coronary occlusion, it produces a totally different picture called "infarction"?

The present work is not directly concerned with histology, but it does present another physical parameter—thermal conductivity—which is different in normal and infarcted muscle.

Thermal conductivity of heart muscle was measured using a heat exchange method and internal calorimetry<sup>1</sup>. The probe was inserted in the muscle mass of the anterior wall of the left ventricle of the dog or the monkey. This records the temperature increment produced by a current of 0.115 amp<sup>1</sup>. In an infinite mass of muscle the relation between thermal conductivity,  $k$ , and the temperature increment,  $\theta$ , is given by Carslaw's relation  $I^2R = 4\pi r\theta K$ , where  $I$  is the fixed current referred to here,  $R$  is the resistance of the heater,  $r$  is a constant, being the radius of the thermodynamically equivalent sphere. The relation can therefore be re-expressed as  $k = F/\theta$ , where  $F$  is an instrumental constant.

In the present work,  $F$  was determined for each instrument used by fusing the probe into a mass of 10 per cent gelatine in water gel (200 ml.) and determining  $\theta$ . Grayson<sup>1</sup> showed the thermal conductivity of such a gel to be  $12.2 \times 10^{-4}$  e.g.s.u. Accordingly  $F$  could be easily determined.

Thermal conductivity for the heart under observation was obtained by implanting the heated thermocouple during life. The animals were killed and the hearts removed. The section of the ventricle with the heated thermocouple *in situ* was embedded in a mass of 10 per cent gelatine in water gel. The thermal conductivity of such a gel has been shown to be  $12.2 \times 10^{-4}$  e.g.s.u.<sup>1</sup> which approximates to that of a normal protein tissue, and so this procedure minimized the effects of any slight heat losses across the gel-muscle interface.

The results are given in Table 1. It will be seen that the scatter for normal heart muscle is slight and that the figures obtained,  $12.1 \times 10^{-4}$ , approximate closely to those normally quoted for skeletal muscle<sup>2</sup>.



Table 1. THERMAL CONDUCTIVITIES RECORDED FROM HEARTS EMBEDDED IN 10 PER CENT GELATINE/WATER GELS. MEAN TEMP. = 23° C

Normal and infarcted dogs—the difference of the means is highly significant ( $P < 0.001$ )

Normal dog	Infarcted dog Time after ligation	Infarcted monkey Time after ligation
12.1	3 min	11.9
12.2	1 h	7.9
12.2	2 h	11.3
11.6	3 h	9.4
12.2	3 h	11.0
11.8	3 h	10.5
11.4	4 h	10.2
12.8	5 h	10.7
12.2	6 h	10.3
11.9	7 h	8.9
12.2		
Mean 12.1 ± 0.2	10.2 ± 0.4	10.3

Table 1 also gives results for heart muscle removed from the animal at varying periods following occlusion of a coronary artery. In all, evidence of infarction was present; electrocardiograph records before death, macroscopic evidence post mortem and usually histological confirmation. Table 1 shows the thermal conductivity findings. There is an early change and in all cases the thermal conductivity was lower than for normal muscle, the mean value being  $10.2 \times 10^{-4}$  c.g.s.u. for the dog and  $10.3 \times 10^{-4}$  c.g.s.u. for the monkey.

The physical significance of these findings is by no means clear. One suggestion which was considered was that they reflected alterations in the water content of the infarct. Direct measurement of water content was not possible, but relative density was measured by a flotation method. Small portions of muscle were placed in water, in which they sank. A solution of ferric chloride having a relative density of 1.4 was added until the muscle just floated. The procedure was then reversed—beginning with ferric chloride and adding water until the muscle just sank. The density of the solution was measured directly with a hydrometer. The mean relative density for normal heart muscle was 1.06, an identical figure to that quoted for frog skeletal muscle<sup>2</sup>. The mean relative density for infarcted muscle was also 1.06. This suggests that the explanation for the change in thermal conductivity in infarction has a more complex basis than a simple alteration in water content.

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### Dermal Photosensitivity and the "Hen and Egg" Problem

GREGORY<sup>1</sup> has viewed the evolution of the visual sense as a "hen and egg" problem involving eyes and brains. It is only reasonable to infer that eyes and brains have mutually exerted evolutionary pressures, but setting aside the somewhat arbitrary distinction between the two, because some eyes incorporate part of the integrative mechanism, partnership between an eye (whether of the simple or compound type) and an integrative apparatus is not the only prescription for a successful photosensory system.

Unfortunately Gregory overlooks the so-called dermat-optic sense which has been widely, repeatedly and successfully exploited in the course of evolution. Although its manifestations have long been known and several times reviewed<sup>2-4</sup>, their implications have been repeatedly

overlooked in considering the evolution of photosensory systems<sup>5</sup>.

Much that has recently been revealed concerning the dermal light sense is directly relevant to Gregory's thesis and initially there appears to be no difficulty in matching his ideas of primordial pattern touch with the development of a diffuse photosensitive surface, which in echinoids such as *Diadema* extends over the whole skin. Integrative centres are already there but eyes are not. The existence of such a photosensory system blurs, but by no means invalidates, Gregory's sharp distinction between photic and tactile or chemo-sensory systems. Thus light not only signals distant events for computation; in some echinoids it also acts as a nociceptive stimulus, signalling an environmental state that causes the animal to cover itself with a pattern of opaque objects corresponding with the pattern of light that falls on the skin<sup>6</sup>. Initially such diffuse photosensitivity appears wholly primitive and a fulfilment of the premise that the first eyes were merely photosensitive areas of skin from which more successful photosensory systems were developed by concentrating and elaborating the photoreceptive areas and their attendant nerve pathways and centres. It is widely assumed that in this process the dermal light sense was left behind as an evolutionary survival. Such a view is over-simplified, because optic and dermatoptic sensory systems may not only co-exist, fulfilling complementary functions<sup>7,8</sup>, but either one or both may be elaborated in accordance with evolutionary potentiality or environmental needs.

Consideration of recent work on bivalves and echinoids is salutary in this connexion. An impressive photosensitivity may be attained without eyes<sup>9</sup> and sophistication may appear not only in the integrative mechanisms associated with the diffuse photoreceptive surface but also in attendant photomechanical pigment movements and light scattering elements<sup>3,10</sup>.

Experimental analysis<sup>9,11,12</sup> has revealed that in these animals the so-called "dermal" photoreceptors are nerve elements devoid of obvious structural adaptation at the microscopic level. Moreover, search for the anticipated sub-microscopic structural differentiation has so far yielded nothing unequivocal<sup>8,13</sup>, though studies of the spectral sensitivity<sup>9,14</sup> and pigmentation of the photosensitive nerve have been more rewarding<sup>15</sup>.

Neural photoreception and its potentialities are therefore to be reckoned with. Its importance is indicated by the means which have been evolved to eliminate what are presumably disadvantageous manifestations, by screening photosensitive nerve with pigment<sup>16</sup>, or by elaborate covering behaviour<sup>6</sup>. The importance is more strikingly revealed in the echinoid *Diadema* where neural photosensitivity has been turned to great advantage. Here light and shadows falling on the skin with its underlying felt of photosensory nerve elicit vigorous movements of the poisonous spines. The parameters of the response depend not only on those of the photic stimulus, but also on integrative and computing activity in superficial and deeply seated nerve centres<sup>17-19</sup>.

The changes in the intensity and pattern of lighting signal the distant events prescribed by Gregory, such as the arrival or movement of a predator, against which the formidable armament of spines is directed. But all this is done without eyes. Moreover, the nervous interaction involved may enhance the perception of movement and contrast<sup>10,20</sup>. There can be no doubt about its effectiveness, judging from the elaborate means of assailing the defences evolved by a predator *Cassia*, the helmet conch, which resorts to spraying the photosensitive skin of its victim with a salivary neurotoxin<sup>21</sup>.

The same sentient surface responds to touch, but the events which follow and the neural pathways involved are different<sup>21,22</sup>. The condition is therefore not primitive in the manner envisaged by Gregory whereby photic stimuli are mediated by the touch neural system.

Shadow reactions are widespread among bottom-dwelling organisms so that recent studies of the dermal light sense have added a new dimension to the significance of photic "off responses", which are now revealed not only as an endowment associated with complex eyes<sup>20</sup> but also as the basis of a widespread characteristic behaviour that may be essential for survival<sup>21</sup>.

Any speculation concerning the evolution of visual systems must therefore take into account what has been achieved by the use of photosensitive nerve and an integrating system, much of which may remain spread out over the entire body surface.

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### Habituation of the Crayfish Escape Response during Release from Inhibition induced by Picrotoxin

If the more posterior segments of the crayfish body are disturbed, it will often escape by darting backwards. This escape manoeuvre, in which the appendages are thrust forward and the tail is flexed, is mediated by two lateral giant axons which lie dorsolaterally in the nerve cord<sup>1</sup>. A most intriguing aspect of the reflex is its extreme lability; it invariably fails if an attempt is made to elicit it repeatedly (unpublished results of Krasne and Woodsmall). It is this phenomenon, so reminiscent of habituation in higher forms, which concerns us here.

The escape reflex can be elicited from the isolated crayfish tail by a single shock to one of the segmental nerves which carry sensory fibres to the central ganglia. This response, like that of the intact animals, fails as a result of repeated stimulation. A more direct expression of the same events can be obtained by measuring the transmembrane potential of the lateral giant fibre which lies on the side of the stimulated nerve. An intracellular micro-pipette inserted near the point where the fibre sends a large process into the neuropile of the stimulated ganglion will record a long depolarization (Fig. 1A) each time the segmental nerve is stimulated with a brief (0.1 msec) current pulse. The depolarization can be divided arbitrarily into three components (Fig. 1A:

alpha, beta and gamma). Action potentials which propagate and cause the escape movements arise from the beta component when it exceeds about 7 or 8 mV depolarization at the recording electrode (Fig. 1B). When the segmental nerve is stimulated more often than about once a minute, however, the beta and gamma components show marked decrements which bring the depolarizing response below the threshold for spike initiation (Fig. 1B and C). Direct electrical stimulation of the giant axons as often as once each second elicits tail flexion reliably. The failure of the reflex with repetitive stimulation at the frequencies reported here (1–12/min) is therefore essentially the result of the lability of the beta component of the sensory-evoked response.

The mechanism underlying this lability is an open question. It has been popular to suggest that habituation, at least in higher forms, is caused by active suppression by growing inhibition as opposed to failure of synaptic efficacy as a result of repetitive use<sup>2–7</sup>. This view has been engendered mainly by evidence that recovery (or protection) from habituation can be caused by drowsiness<sup>8</sup>, presentation of novel stimuli (dishabituation)<sup>9</sup>, reduction in intensity of the stimulus to which habituation has occurred<sup>6</sup>, barbiturate anaesthesia<sup>2</sup>, and ablations of various neocortical<sup>3,9</sup> and brain stem<sup>2</sup> structures in mammals or of the vertical lobes or inferior frontal system in octopus<sup>10</sup>. Such evidence, although certainly suggestive,

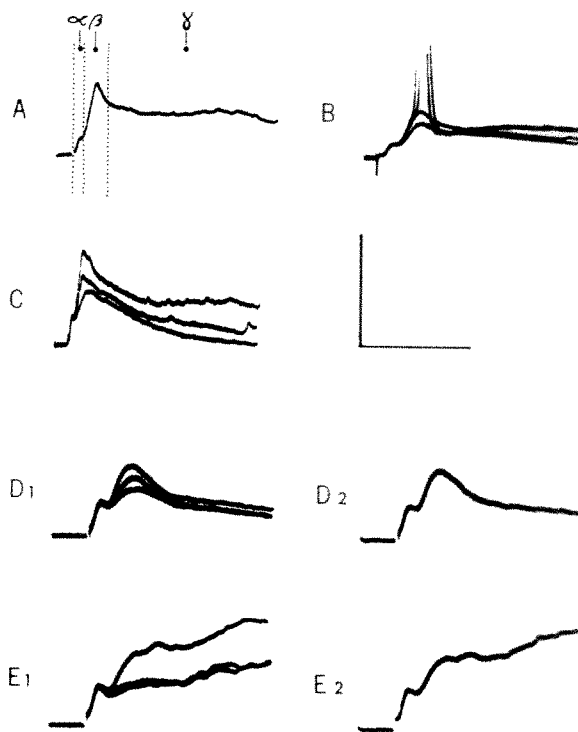


Fig. 1. Intracellular responses of lateral giant fibres to ipsilateral second root shocks at the level of the third or fourth abdominal ganglia. The nerve cord is exposed dorsally and left *in situ*. A, B, and C are from different preparations; D and E are from a single preparation. In each case the stimulus voltage was held constant during the experiment. (A) A typical response with the normal components labelled. Calibration: 10 mV and 25 msec. (B) Superimposed responses to the first, second, third, and fifteenth shocks at a rate of 1/min in a rested preparation. Spikes arise from the rising limb of the beta component of the first two responses. Calibration: 25 mV and 10 msec. (C) Superimposed responses to the first, second, and twenty-ninth shocks at a rate of 1/5-7 sec. The stimulus intensity was set low enough to avoid reflex induced spikes. Both beta and gamma but not alpha components suffer decrements. Calibration: 10 mV and 25 msec. (D1) Superimposed responses to the first, second, and eighth shocks at a rate of 1/5-7 sec. The stimulus voltage was adjusted so that the beta component did not evoke a spike. (D2) Response after a 5 min rest. Calibration: 10 mV and 10 msec. (E1) Superimposed responses to the first, second, and eighth shocks at a rate of 1/5-7 sec applied 62 min after replacing normal van Harreveld's solution with  $10^{-4}$  molar picrotoxin in van Harreveld's solution. (E2) Response after a rest of 5 min. Calibration: 10 mV and 10 msec.

by no means demands the postulation of augmenting inhibitory processes as the cause for habituation<sup>11</sup>.

A relatively direct evaluation of the role of inhibition in the present case is made possible by the action of the drug picrotoxin on the crayfish nervous system. The drug is a potent convulsant<sup>12,13</sup> and its specific effect has been examined at the crayfish neuromuscular junction<sup>14</sup>, cardiac ganglion<sup>14</sup>, abdominal stretch receptor<sup>15</sup> and lateral giant fibre (unpublished work of Roberts). In each case a selective blockage of known inhibitory processes has been found. The effect of picrotoxin on the response of the lateral giant fibre to second root stimulation is shown in the top trace of Fig. 1, *E1*. Earlier parts of the response are little affected, but the gamma component, which ordinarily does not measure more than about 5 mV, is much augmented and spiking occurs at about 15 mV depolarization. It seems reasonable to attribute this augmentation to a release of the system from inhibition.

If the habituation phenomenon seen in the giant fibre is caused by an inhibitory process, then the present disinhibited preparation might be expected to respond more reliably than a normal preparation to repeated stimulation. A comparison of the effects of stimulus repetition in the normal (Fig. 1, *D1* and 2) and picrotoxin poisoned (Fig. 1, *E1* and 2) preparations shows that this is decidedly not the case; the effect, if any, of the picrotoxin is to make the response more labile than normal.

We conclude that there is at least one class of central nervous inhibition which cannot be held responsible for the decrement of the beta component and the consequent habituation of the crayfish lateral giant fibre escape reflex. Spencer, Thompson and Neilson<sup>16</sup> similarly failed to prevent habituation of the flexion reflex in the spinal cat by application of either picrotoxin or strychnine. While there may be some inhibitory processes of the crayfish nervous system which are not attacked by picrotoxin, we feel that the best hypothesis is that habituation of the crayfish escape reflex is caused by a depression intrinsic to the reflex's excitatory pathways. Desensitization of postsynaptic membrane, depletion of transmitter, and depression of electrogenic processes in axonal or dendritic arborizations are salient as potential mechanisms of action.

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## Regulation of Noradrenaline Biosynthesis in Nerve Tissue

THE noradrenaline (NA) content of sympathetically innervated organs remains remarkably constant, at a concentration characteristic of each individual organ, during widely varying states of nerve activity<sup>1</sup>. Because an increase in the frequency of nerve impulses is associated with an increased loss of NA from the neurone, its capacity to maintain an essentially unchanged NA content has been regarded as evidence that the synthesis of NA is accelerated by nerve activity<sup>1</sup>. The opposite view—that the synthesis of NA in the neurone proceeds at a constant “supramaximal” rate, independent of activity—has, however, also been proposed<sup>2,3</sup>.

Several recent reports indicate that the synthesis of NA is indeed accelerated when the nerve impulse frequency is increased<sup>4-9</sup>. We have found that the mechanism of this regulation of synthesis does not seem to be related to the depolarization phenomenon itself, or to variations in the activity of the effector organ<sup>9</sup>. Our results show that the factor triggering acceleration of synthesis, or removal of its inhibition, appears rather to be depletion of some critical NA “pool” in the tissue.

This implies that the rate of NA synthesis may be controlled by variations in the NA content of the neurone. This concept is supported by recent *in vitro* evidence. Thus on incubation with different tyrosine hydroxylase preparations Nagatsu, Levitt and Udenfriend<sup>10</sup> found that a DL-NA concentration of  $2 \times 10^{-4}$  moles/l. in the incubation medium caused a 25–50 per cent inhibition of the ring hydroxylation of tyrosine, which is considered to be the rate-limiting step in the formation of NA.

Although the inhibition of the synthesis of NA induced by NA described by these authors was very incomplete and required high concentrations of NA in the medium, we have recently been able to show that the synthesis of NA from tyrosine in a fraction from a homogenate of bovine splenic nerves can be inhibited by as much as 85 per cent by concentrations of noradrenaline as low as  $1.2 \times 10^{-5}$  moles/l. in the medium. We also found that the inhibition operated not only at the first step in the synthesis sequence but also at the last step, the  $\beta$ -hydroxylation of dopamine (Fig. 1).

The tissue used for these experiments was a preparation of bovine splenic nerve granules<sup>11</sup> obtained by ‘Ultra-Turrax’ homogenization of de-sheathed splenic nerves in ice-cold isotonic potassium phosphate, pH 8.2, and subsequent removal of nuclei, mitochondria and coarse tissue fragments by centrifugation at 9,000g for 10 min. The supernatant fraction from this centrifugation, containing the specific NA-storing granules, was passed over a column of aluminium oxide in order to remove non-bound extra-

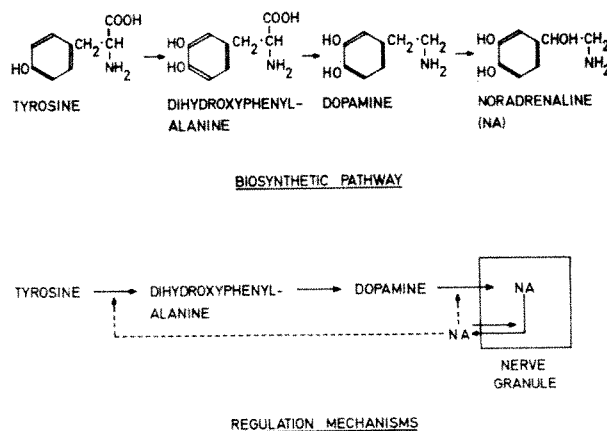


Fig. 1. The principal biosynthetic pathway leading to the formation of NA from tyrosine. Diagrammatic representation of the two mechanisms for the regulation of the synthesis of NA, described in the text.

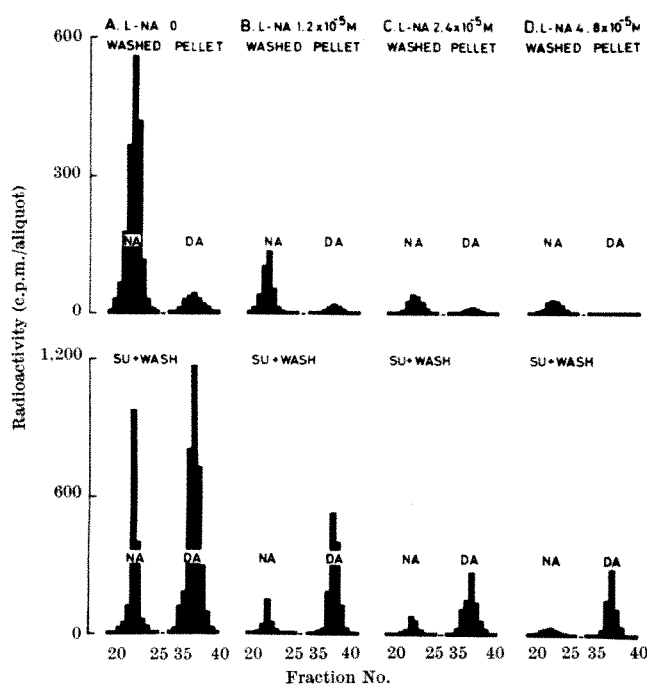


Fig. 2. Inhibition of the NA and dopamine formation from tyrosine by addition of NA to the incubation medium. Sections from ion exchange chromatograms of the washed pellet and of the combined wash and supernatant fractions.

granular NA (ref. 12). The effluent was adjusted to pH 7.5, divided into fractions of 8 ml. and incubated in stoppered plastic centrifuge tubes at 20° C for 60 min in the presence of  $3 \times 10^5$  c.p.m./ml. of tyrosine labelled with tritium (New England Nuclear Corp., L-tyrosine-3,5- $^3$ H, specific activity 4 c./mmole).

Non-labelled NA was added to the medium to give final concentrations of from 0 to  $9.6 \times 10^{-5}$  moles/l.

After the incubation period the granule fraction was separated from the medium by centrifugation at 50,000*g* for 30 min. The sediment obtained was washed and re-centrifuged. The formation of NA and of the two intermediates, dihydroxyphenylalanine and dopamine, was determined by cation exchange column chromatographic analysis of extracts of the washed high speed sediment and of the pooled supernatant and wash fractions. Tyrosine was removed from the extracts before chromatography, by passing them at pH 8.4 over a column of aluminium oxide, which selectively retains catechol compounds, while allowing non-catechol material to pass into the effluent and water wash. Catechols were then eluted with perchloric acid, and the eluate analysed by chromatography. The positions of dihydroxyphenylalanine, dopamine and noradrenaline in the chromatograms were determined by reading the spontaneous fluorescence (activation at 285 mμ, fluorescence at 335 mμ) in an Aminco-Bowman spectrophotofluorometer. The radioactivity of the different fractions was determined by counting 0.5 ml. aliquots in a 7:3 toluene-absolute ethanol solution containing 4 g of 2,5-diphenyloxazol and 100 mg of 1,4-bis-2(4-methyl 5-phenyloxazolyl) benzene per litre of toluene, by means of a Packard liquid scintillation spectrometer. (The overall recovery of noradrenaline put through the entire procedure ranged from 70 to 90 per cent.)

The results obtained are presented diagrammatically (Fig. 2) by a series of ion exchange chromatograms from a typical experiment. Radioactive dihydroxyphenylalanine never accumulated to any considerable extent and was thus not included in the figure. About 2.5 per cent of the tyrosine added to the control tube, in which the extragranular medium was essentially free of NA, was converted to catecholamine. About one-third of the

dopamine formed was β-hydroxylated to NA. Because the tyrosine content of these preparations, determined by the fluorimetric method of Waalkes and Udenfriend<sup>13</sup>, was found to range from 0.3 to  $0.5 \times 10^{-5}$  moles/l., the NA formed amounted to 50–70 ng/g splenic nerve/h, corresponding to about 5 per cent of the total endogenous NA content of the granules. In the presence of increasing concentrations of NA in the medium the formation of catechol compounds was inhibited to a progressively increasing extent. The synthesis of dopamine was blocked to a lesser extent than that of NA. In the experiment shown in Fig. 2 the inhibition of dopamine and of NA formation was 57 and 85 per cent, respectively, at a concentration of NA in the medium of  $1.2 \times 10^{-5}$  moles/l., and 77 and 97 per cent at  $4.8 \times 10^{-5}$  moles/l.

Our results confirm and extend the finding of Nagatsu *et al.*<sup>10</sup> that the synthesis of NA from tyrosine can be inhibited by high concentrations of NA in the medium. In agreement with their results, our experiments indicate that the enzyme tyrosine hydroxylase is inhibited by an accumulation of the end-product of the biosynthesis pathway, NA. In the present experiments, however, a much more complete inhibition of the formation of catechol was obtained at much lower concentrations of NA.

Moreover, the block of synthesis of NA in our experiments occurred not only at the first but also at the last step in NA biosynthesis. According to previous results in this laboratory, the β-hydroxylation of dopamine appears to be the only step in NA synthesis requiring the presence of granules. The concept that dopamine is formed from tyrosine extragranularly is supported by the observation in the present experiments that more than half the NA formed was found in the washed high speed sediment, while less than 2 per cent of the dopamine formed was granule-bound. This finding also suggests that drugs known to antagonize the uptake and retention of amines in the nerve granules<sup>10</sup> interfere with the synthesis of NA exclusively by blocking the conversion of dopamine to NA (ref. 11), while not affecting the formation of dopamine from tyrosine. This implies that dopamine must become granule-bound before it can be β-hydroxylated<sup>12</sup>. Because NA in the medium competitively inhibits the uptake and retention of dopamine in the granules<sup>12</sup>, the block of synthesis at the dopamine β-hydroxylation step caused by NA in the medium is most likely to result from competitive inhibition of the uptake of dopamine into β-hydroxylation sites.

These *in vitro* results show the feasibility of control of the synthesis of NA by changes in the concentration of this product outside the granules. Thus the test-tube results obtained with subcellular fractions strongly support the conclusions proposed on the basis of experiments with intact tissues that depletion or accumulation of NA in strategic parts of the neurone are the factors which trigger the homeostatic adjustment of the rate of synthesis of NA, which results in the maintenance of a constant transmitter level in the neurone. Our results have established that control of NA synthesis can be exerted at the first step of the biosynthetic pathway. This finding provides an example of regulation of the synthesis of a neurotransmitter by the classical principle of feed-back control of the rate-limiting step of the biosynthetic pathway, by variations in the accumulation of the end-product.

We also found, however, that control of synthesis can be exerted by a different mechanism, operating at the DA β-hydroxylation step. Observations in this laboratory suggest that under normal conditions regulation of synthesis occurs primarily by the first mechanism (that is, by variation in the degree of inhibition of the tyrosine hydroxylation step). It is conceivable, however, that in special circumstances, such as after treatment with drugs like reserpine, the synthesis of NA may also be affected by interference with the transport and/or binding of



dopamine<sup>1,16</sup>, formed extragranularly to the dopamine hydroxylating sites in the granules.

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## RADIOBIOLOGY

### Haematopoietic Repopulation after Intestinal Shielding from X-irradiation

THE two methods most commonly used to produce repopulation after irradiation are: (1) exogenous colonization, or "grafting", by the injection of healthy haematopoietic tissue into a heavily irradiated recipient<sup>1-3</sup>; (2) endogenous colonization accomplished by the shielding of haematopoietic tissue<sup>4,5</sup>. In comparing these two techniques, Porteous and Lajtha<sup>6</sup> concluded that, after radiation, exocolonization—involving considerable cellular handling—gives a different pattern of recovery from that observed with endocolonization.

Whereas grafted lymph node and foetal thymus suspensions<sup>7</sup> and thoracic duct lymphocytes<sup>8</sup> have failed to elicit "post-irradiation" recovery, Jacobson *et al.*<sup>9</sup> demonstrated that 26.6 per cent of the mice, the exteriorized gut of which was shielded from 1,025 r., survived for 30 days and their haematopoietic recovery was nearly complete in 8 days. The present experiments were performed in an attempt to resolve this apparent discrepancy and they consisted of examining the haematopoietic potential of the shielded intestine in the irradiated mouse.

CF-1 virgin female mice were anaesthetized with an intraperitoneal injection of 'Diabital' (1.8 mg/0.5 ml. sterile saline/mouse) and randomly divided into four groups: (1) about 75 per cent of the intestine and mesentery was exteriorized and shielded with lead while the rest of the body was being irradiated; (2) a similarly treated group received whole-body irradiation while the gut and mesentery was exteriorized but not shielded; (3) whole-body irradiation of intact animals; and (4) a normal, non-irradiated control group. The irradiation was carried out using a 250 kVp. 'Maximar' unit operating at 15 m.amp with a half-value thickness of 2 mm copper at

a source to mid-line distance of 45 cm. Each irradiated animal received 700 r. at 60 r./min and when shielding was used the thickness of the lead was sufficient to attenuate the dose to less than half of 1 per cent.

Six days after irradiation, all animals were injected with 0.5 µc. of iron-59 (specific activity: 15.5 mc./mg iron) as ferrie chloride in 0.2 ml. of buffered solution, by way of their tail vein. Forty-eight hours later (that is, a total of 8 days after irradiation) all animals were bled and their peripheral erythrocyte and whole spleen radioactivities were determined in a well-type scintillation counter. Macroscopic splenic colonies were counted and then routinely sectioned and stained for histological examination.

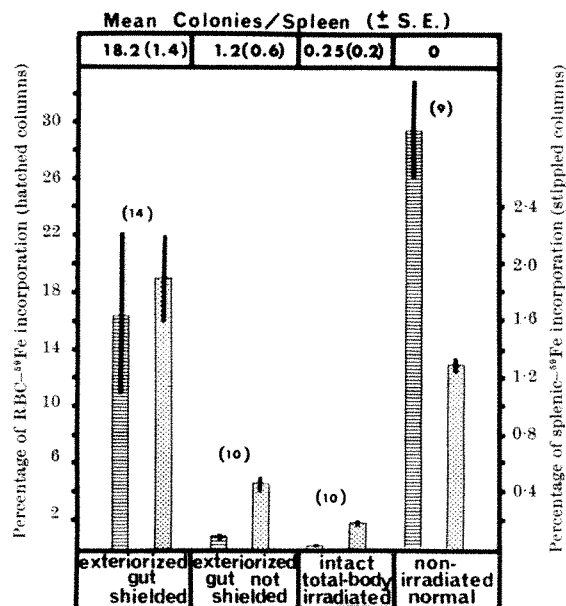


Fig. 1. Effects of 700 r. X-irradiation and gut shielding on splenic colonization and erythropoiesis as indexed by erythrocytic and splenic incorporation of iron-59. The number of animals in each group is enclosed in brackets.

The results are summarized in Fig. 1. Animals which had their intestines shielded during irradiation showed higher incorporation of iron-59 into peripheral red blood cells and spleens than those groups of animals which received whole-body irradiation while their gut was either exteriorized or intact and not shielded ( $P < 0.0005$ ). The number of splenic colonies in group 1 animals was greater than that of the control groups. Histological examination of serially sectioned spleens from group 1 mice revealed intensive areas of haematopoiesis with numerous mitotic figures. Erythrocytic, myelocytic and megakaryocytic foci were observed as individual colonies, as well as intermixed. In addition to these, aggregates of lymphocyte-like cells were noted. Spleens from animals in groups 2 and 3 did not contain any observable haematopoietic foci.

The most likely origins of the repopulating cells in the shielded region are the mesenteric lymph nodes and Peyer's patches, although the possibility cannot be precluded that migratable stem cells also exist in the non-lymphoid tissue or blood of the protected area. Failure of these cells to show colonizing capacity on transplantation may be the result of their sensitivity to handling, whereas similar cells *in vivo* may retain this property. The findings in this experiment of increased erythropoiesis and colonization in the group of animals of which the gut was shielded probably result from the seeding of such cells into the spleen and bone marrow.

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## PATHOLOGY

### Nuclear Pockets in Normal Leucocytes

RECENTLY there have been several reports on nuclear blebs found in human leukaemic cells<sup>1</sup> and in human bone marrow cells of leukaemic patients on drug therapy<sup>2</sup>. Similar nuclear structures have also been found in developing human<sup>3</sup> and guinea-pig<sup>4</sup> thymocytes, and possibly in Burkitt's lymphoma<sup>5</sup>. Anderson<sup>6</sup> noted identical nuclear structures in myeloblasts from patients with treated and untreated acute myelocytic leukaemia and referred to them as nuclear loops.

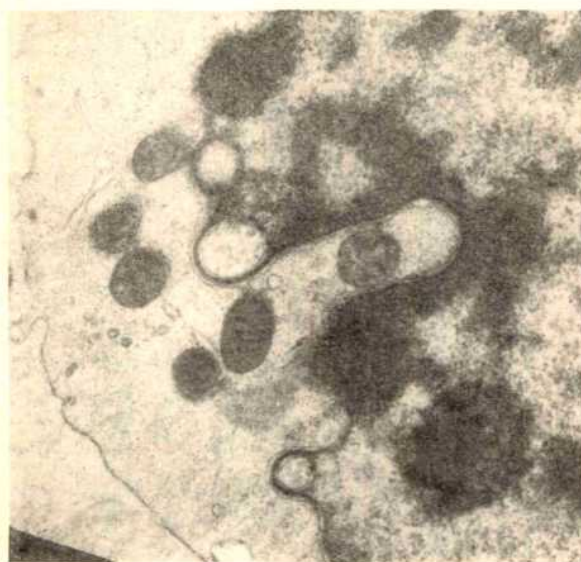


Fig. 1. Nuclear pockets in nucleus of lymphocyte ( $\times 15,750$ ).

Electron microscopy of leucocytes from eight normal people showed that nuclear blebs, which we prefer to term "nuclear pockets" (see Fig. 1), were present in the lymphocytes and neutrophils of all eight. We consider the term "nuclear pockets" to be more accurate than "nuclear blebs" as we have demonstrated by serial sectioning. The significance of nuclear pockets in leukaemia

cells has to be evaluated in relation to frequency and ease of demonstration in leucocytes from normal people.

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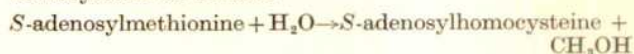
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### Formation of Methanol by an Enzyme in an Ectopic Pinealoma

THE tissue of origin of a tumour is conventionally determined by histological examination. It has been possible to identify some tumours by their secretion of substances specific to tissue into the general circulation. Thus pheochromocytomata—tumours which originate in chromaffin tissue—secrete large amounts of catecholamines, hormones normally manufactured by chromaffin tissue. Malignant carcinoids, derived from the enterochromaffin cells of the intestine, may secrete serotonin, a normal product of enterochromaffin cells. Pheochromocytomata and malignant carcinoids respectively contain high concentrations of catecholamines and serotonin. These tumours presumably also contain the enzymes required to synthesize these compounds. Similar unique biochemical characteristics have not yet been described for tumours within the central nervous system. Recent studies from this laboratory have identified at least two enzymes which are highly localized in specific sites in the central nervous system. It was found that hydroxyindole-*O*-methyl transferase (HIOMT), the enzyme which *O*-methylates *N*-acetylserotonin to form melatonin<sup>1</sup>, is present almost exclusively in the pineal gland of several mammalian species<sup>2</sup>. HIOMT activity has been found to be of comparable magnitude in the pineal glands of human beings from 3 to 70 years of age, suggesting that calcification of the human pineal gland during adulthood does not interfere with this biochemical function<sup>3</sup>. Melatonin, the product of the enzyme action, has been found to exert an inhibitory influence on gonadal growth and on the oestrous cycle in rats<sup>4</sup>. Wurtman *et al.*<sup>5</sup> have identified melatonin and HIOMT activity in a metastatic parenchymatous pinealoma and suggested the utility of this enzymatic assay in the diagnosis of this tumour.

Axelrod and Daly<sup>6</sup> have described an enzyme which forms methanol and is localized in the pituitary gland of several mammalian species including man. The reaction involves either the methylation of water by *S*-adenosylmethionine to form methanol or a hydrolytic cleavage of *S*-adenosylmethionine to form methanol and *S*-adenosylhomocysteine as follows:



The methanol forming enzyme in the anterior pituitary has one-fourth the activity of the posterior pituitary, and negligible activity is present in all other tissues examined, including brain, pineal gland, liver, lung, kidney and adrenal.

The present report will describe biochemical investigations of an ectopic pinealoma. Ectopic pinealomata are frequently located in the region of the optic chiasma. Although the histology of these tumours resembles that of pineal parenchymal tissue, their tissue of origin is not well established. It is thought that such tumours can arise in the pineal gland and metastasize to the floor of the third ventricle and the optic chiasma. They may also originate in the



Table 1. METHANOL-FORMING ACTIVITY IN PINEALOMA AND NORMAL TISSUES

Tissue	Labelled methanol formed (mμmoles/g/h)
Ectopic pinealoma	70
Whole human pituitary	18*
Bovine pituitary	
Posterior	77*
Anterior	25*

\* These values were obtained in previous experiments<sup>4</sup>.

Soluble supernatant fractions obtained from 17 mg of the tumour were incubated with 10 mμc. of <sup>14</sup>C-S-adenosylmethionine (40 mμc./mole) and the mixture was assayed for formation of <sup>14</sup>C-methanol as described earlier<sup>4</sup>.

third ventricle, the pineal gland being intact, or they may arise directly from the chiasmal region. It has been suggested that these tumours arise from misplaced primordial germ cells and should be considered germinomata<sup>7</sup>. In the present investigation the activities of enzymes which are specific to the pineal or pituitary glands in an ectopic pinealoma have been measured to ascertain the tissue of origin of this tumour.

Tissue was obtained from a spinal cord metastasis of an ectopic pinealoma which had been removed at craniotomy from the region of the third ventricle of a patient who had the symptoms of a left temporal haemianopsia and diabetes insipidus. Histologically the tumour resembled a germinoma and was very similar to that described by Wurtman *et al.*<sup>5</sup>.

The tumour from the spinal area was first examined for HIOMT (melatonin forming activity) by a sensitive assay which can detect the formation by an enzyme of as little as 1 μmole of melatonin<sup>8</sup>. The tumour was found to be devoid of HIOMT activity. In marked contrast, a metastatic parenchymatous pinealoma<sup>9</sup> and normal human pineal tissue<sup>3</sup> have considerable HIOMT activity. Moreover, Wurtman and Kammer<sup>9</sup> detected HIOMT activity in an ectopic pinealoma. These results would suggest that the tumour was not derived from functional pineal parenchymal tissue.

To examine the possibility that the tumour might be of pituitary origin, it was assayed for methanol forming enzyme activity, which has been found to be localized to the pituitary gland, by a method previously described<sup>6</sup>. The tumour formed 70 mμmoles methanol/g tissue/h (Table 1). This amount was more than that formed by human pituitary gland.

These observations indicate that the biochemistry of this tumour resembles that of pituitary gland tissue and might therefore be of pituitary origin. The especially high activity suggests that it might originate from the posterior pituitary gland which has the highest capacity to form methanol in the pituitary.

Another enzyme activity which is highly concentrated in normal pituitary gland tissue is the histamine methylating enzyme, histamine-methyl-transferase. The highest histamine-methyl-transferase activity of all parts of the central nervous system and all peripheral organs examined has been found in the posterior pituitary gland<sup>10</sup>. Although posterior pituitary has the highest histamine-methyl-transferase activity, this enzyme is present in other tissues<sup>11</sup>. The ectopic pinealoma was assayed for histamine-methyl-transferase activity by a method described earlier<sup>12</sup>. The tumour was capable of synthesizing at least 2.34 μmoles of methylhistamine/h/g tissue. This amount of histamine-methyl-transferase activity is greater than that demonstrated previously for any other tissue except for the posterior pituitary gland of the monkey<sup>2</sup>. The high histamine-methyl-transferase activity of the tumour is consistent with an origin in the pituitary gland. The mammalian pineal gland, however, also has fairly high concentrations of this enzyme<sup>2</sup>.

The ectopic pinealoma has been thought to represent a germinoma<sup>7</sup> or teratoma<sup>13</sup>; both these sometimes contain high concentrations of gonadotropins. Accordingly, the ectopic pinealoma was assayed for gonadotropin content (by Dr Roy Hertz). The tumour was found to be devoid of gonadotropin activity as measured by the mouse

uterus weight method<sup>10</sup>. This method can detect as little as 0.1 units of gonadotropin activity.

The presence of the pituitary specific methanol forming enzyme in the ectopic pinealoma and the absence of the pineal specific melatonin forming enzyme, HIOMT, indicate that the tumour came from the pituitary rather than the pineal. The tumour appeared, at operation, to originate near the pituitary stalk. Because the specimen examined for enzyme activity was metastatic to the spinal cord, it is unlikely that contamination from pituitary gland tissue provided the methanol forming enzyme activity. Wurtman and Kammer<sup>9</sup> have independently examined an ectopic pinealoma which contained considerable HIOMT activity and had little or no methanol forming capacity. Histologically, the ectopic pinealoma investigated by Wurtman and Kammer<sup>9</sup> closely resembled the tumour examined here. The markedly different enzyme characteristics of these two tumours would indicate that they constitute separate and distinct pathological entities.

The histological appearance of a tumour is frequently not sufficiently distinctive to indicate its pathological derivation. The ectopic pinealoma is an example. Histologically it resembles seminomata of the testis, dysgerminomata of the ovary, teratomata, and a variety of mediastinal tumours. The results of the present study and the results of Wurtman and Kammer<sup>9</sup> suggest that there are two different biochemical classes of ectopic pinealomata.

Histological examination is sometimes unable to predict fully the malignant potential of certain tumours. It is possible that enzyme characterization of tumours might aid in revealing the pathological derivation of tumours and might be useful in assessing their malignant potential.

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## CYTOLOGY

### Longitudinal Differentiation of the Small Intestine

THE inner surface of the small intestine is covered by a "monolayer" consisting of simple epithelium and a smaller number of goblet cells. The epithelial cells differ in the various parts of the small intestine. Their vertical differentiation was suspected<sup>1</sup> and confirmed by Dahlquist

and Nordström<sup>2</sup>. This differentiation arises from the fact that in the crypts of Lieberkühn the cells can undergo mitosis, but are incapable of digesting and absorbing nutrients. According to Grobstein's terminology<sup>3</sup>, this state corresponds to covert differentiation. Towards the apex of the intestinal villi, the cells gradually develop the enzyme pattern necessary for digestion and absorption, but lose their ability to undergo mitosis. This state would, according to Grobstein, correspond to overt differentiation.

A longitudinal differentiation of small intestinal epithelium has also been demonstrated in morphological and functional respects. Nutrients enter the small intestine from an oral direction. In general, the capacity of the intestinal epithelium to absorb (transport) important foodstuffs (glucose, sodium ions, water, fats) tends to decrease in the aboral direction, that is, towards the lower part of the intestine<sup>4</sup>. The activity of certain enzymes<sup>5,6</sup> and cell turnover<sup>7</sup> show a similar longitudinal distribution in the intestine.

Much less frequently the lower (aboral) part of the small intestine displays either greater or exclusive capacity to absorb a given nutrient. Good examples are the lactose or carbohydrate-dependent absorption of calcium<sup>8</sup>, the absorption of conjugated bile acids<sup>9,10</sup> and of the  $B_{12}$ -intrinsic factor complex. The last capacity has been

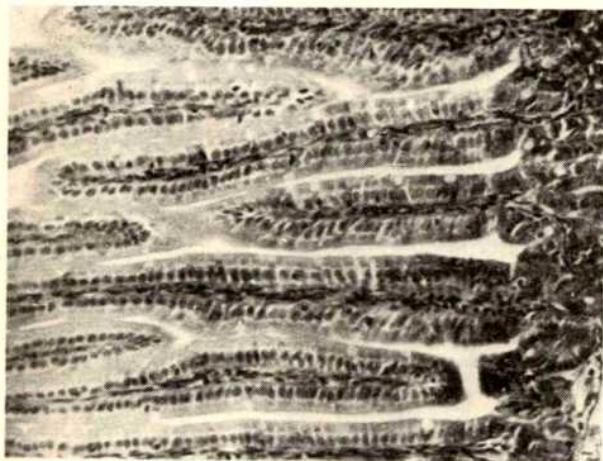


Fig. 1. Duodenum of suckling rat. Paraffin embedding, with haematoxylin and eosin stain. The epithelial cells resemble those of adult animals.

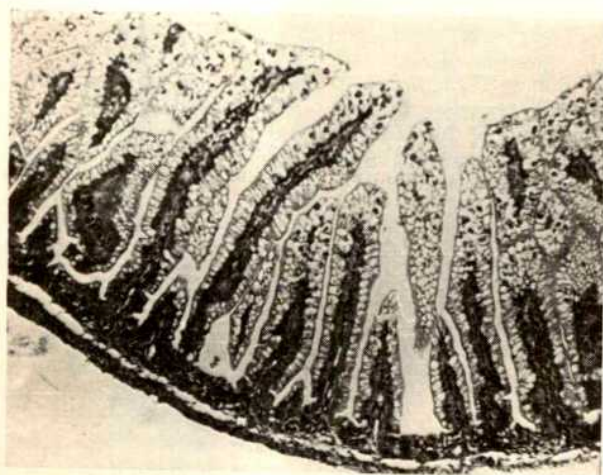


Fig. 2. Ileum of suckling rat. Paraffin embedding, with haematoxylin and eosin stain. The epithelium consists of "hollow" cells. In the apical region of the villi inclusion bodies are apparent in the vacuoles.

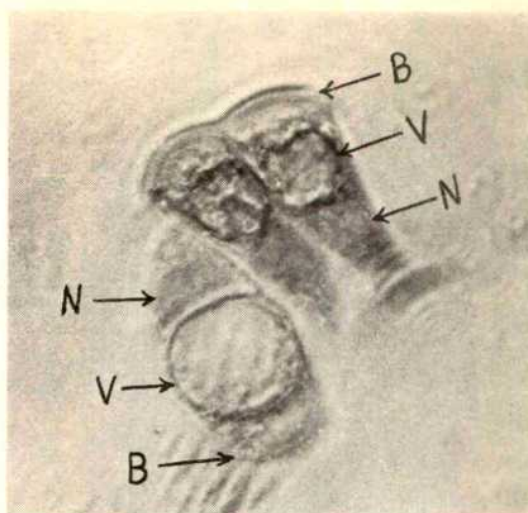


Fig. 3. Unfixed and unstained cell suspension from ileum of suckling rat. The cell at the bottom is swollen. The vacuole (V) and the brush border (B) are clearly visible on each cell. The nucleus (N) is ghost-like.

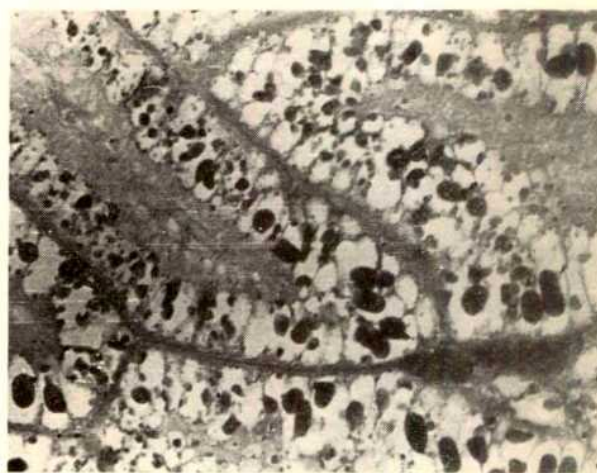


Fig. 4. Ileum of suckling rat. Paraffin embedding, with periodic acid-Schiff reaction. "Hollow" cells with inclusion bodies are apparent.

demonstrated in the ileum of man, dog, guinea-pig, golden hamster and in the jejunum of the rat<sup>11</sup>.

We became interested in morphological differences between the upper and lower parts of the small intestine through a histological study of the absorption of gamma-globulin. The duodenal epithelial cells of suckling rats (Fig. 1) were found to resemble those of adults, whereas their ileal cells were of a different type, showing large vacuoles giving the impression of being "hollow" in paraffin-embedded sections (Fig. 2). These vacuoles were also clearly visible in the unstained suspension of living cells (Fig. 3) and contained fluid but no fat or mucus. On staining with mucicarmine, only a few goblet cells were seen among the "hollow" cells. The vacuoles of the latter frequently contained periodic acid-Schiff-positive inclusion bodies, yellow in colour in unstained and eosinophilic in stained preparations (Fig. 4). The relationship of these bodies to gamma-globulin absorption will be described in detail in another paper. Transition between the "hollow" and normal epithelial cell was gradual. Both cell types, as well as their transitory forms, have been recognized by us on electron micrographs by Clark<sup>12</sup> and Kraehenbuhl *et al.*<sup>13</sup>. These authors, however, have not distinguished between the large vacuole as a marker of a special cell type and the small



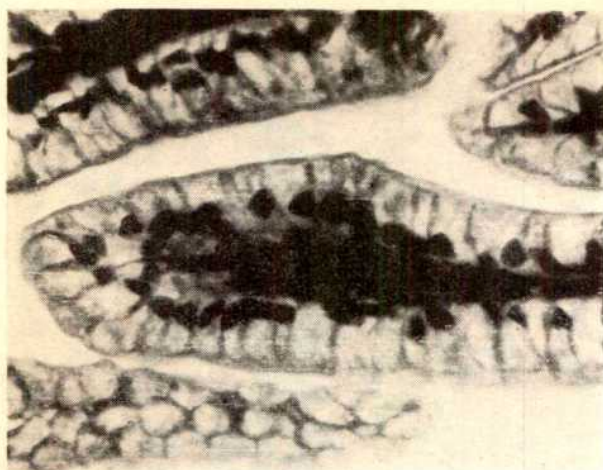


Fig. 5. Ileum of newborn guinea-pig. Paraffin embedding, with haematoxylin and eosin stain. The epithelium consists of "hollow" cells, but no inclusions are apparent.

ones as that of a physiological condition, the absorption of gamma-globulin.

Macroscopically the upper part of the small intestine of the suckling rat is milky white, whereas its lower part, where the "hollow" cells are located, is yellowish brown in colour. The inclusion bodies are probably responsible for this coloration. This part of the suckling rat's intestine stains slightly with orally administered vital stains<sup>14</sup>.

The ileal cell type described is not specific for the suckling rat. We have found it also in mouse and guinea-pig, but failed to detect it in dog, cat, goat and pig. Unlike the rat, guinea-pigs had no inclusion bodies in their "hollow" cells (Fig. 5).

The parenchymal cells of different organs usually differ in morphology, function and localization. In view of this fact the small intestine of the suckling rat can be visualized as two tube-like organs in sequence, incompletely separated from each other by the transitional cellular region. This model is not so well defined for adult mammals as for the suckling rat, and is confined to functional characteristics in the former case.

These observations suggest a new aspect which should be taken into account when describing the characteristics of the epithelial lining of the small intestine.

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## Mast Cells free of Histamine in *Rana catesbiana*

THE theory that mast cells are the source of histamine is based exclusively on investigations carried out on mammals and it is not known whether it also holds true in non-mammalian vertebrates. It is not even known whether histamine is contained in the tissues of these animals.

In the course of a study of the mast cells in the tongue of the bullfrog, *Rana catesbiana*<sup>1</sup>, it was noted that intracardial or local injections of a large dose of the histamine releaser compound 48/80 did not cause any distinct morphological changes in the mast cells nor did it bring about a general shock reaction. Although Arvy<sup>2,3</sup> has reported that frog mast cells are sensitive to the histamine releaser, stilbamidine, careful reading of his reports reveals that he found cytological changes in the mast cells only in the vicinity of the local injections. Another experiment which we have carried out has also shown that a large dose of histamine administered intracardially provokes no detectable general change in the bullfrog. These observations caused us to doubt the relation between histamine and mast cells and also to doubt the biological significance of histamine in this animal.

In a histological survey of sections and spreads of various organs (fixed in 10 per cent formalin containing 4 per cent basic lead acetate and stained in acidic toluidine blue), it was found that the tongue, the mesenteries and liver are suitable material for an analysis of the relationship between the histamine value and the mast cell population. The chief reason for this is that the tongue and the mesenteries are very rich in mast cells, whereas the liver is almost completely free of them. Histamine was extracted according to the method of Code<sup>4</sup> and assayed by the contraction of guinea-pig ileum in a Tyrode bath containing atropine. Neocatergan was used to confirm that the effective substance was histamine.

The concentrations of histamine in the various organs obtained from five bullfrogs of both sexes, weighing 250–600 g, are given in Table 1. For comparison, we include values for histamine in the abdominal skin and mesenteries assayed in 4 male (140–170 g) rats of the Wistar strain. The table shows that in striking contrast to those of the rat, the tissues of the bullfrog contain only a trace of histamine. This becomes more evident if one compares the concentrations in the mesenteries of the two animals. The concentration of histamine in the mesenteries of the bullfrog is as little as 1/130th of that in the rat, although the distribution of mast cells in the former is about four times as great as in the latter. Taking into account the fact that the mast cells of the frog are smaller in size, the total volume of mast cells contained within one unit of frog mesentery is at least equal to that contained within the same quantity of rat mesentery.

The liver of the bullfrog, which was, as found by Arvy<sup>3</sup> in *Rana esculenta*, lacking in mast cells, showed concentrations of histamine not essentially different from those in the organs rich in mast cells. This suggests that in the bullfrog histamine is not carried by mast cells as in the mammals. This is supported also by histochemical observations. Fluorescent *o*-phthalaldehyde stain for histamine<sup>5</sup> was applied to the spreads of the mesenteries and tongue of the bullfrog. Although a bright yellow fluorescence was detected in the mast cell cytoplasm in the rat mesenteries examined as a control, it could not be found in the mast cells of the bullfrog.

The possibility that the mast cells of the bullfrog carry serotonin—another biogenic amine known to be contained in the mast cells of the rat and mouse—instead of histamine was rejected by histochemical examination. Fluorescence microscopy of the spreads of bullfrog tongue and mesenteries exposed to paraformaldehyde vapour<sup>6</sup> revealed that the mast cells did not fluoresce; Padawer<sup>7</sup> has recently reported similar findings with *Rana temporaria*.



Table 1

		Histamine value ( $\mu\text{g/g}$ wet tissue)	Mast cell count
Bullfrog	Mesentery	0.19 $\pm$ 0.06	+++
	Tongue	0.04 $\pm$ 0.01	+++
	Liver	0.07 $\pm$ 0.01	—
Rat	Mesentery	24.8 $\pm$ 4.4	++
	Abdominal skin	29.8 $\pm$ 3.1	++

Preliminary results obtained in the course of a comparative study of various animals in our laboratory suggest that mast cells are free of histamine throughout the teleosts and amphibia and that it is not present below the level of the reptilia.

We thank Professor Hidemasa Yamasaki, Department of Pharmacology, Okayama University Medical School, for his advice and encouragement during this work.

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## GENETICS

### Haptoglobins, Transferrins and Serum Gamma-globulin Types in Malayan Aborigines

THE Malayan aborigines form a distinct ethnic group which is socially and anthropologically different from the three main population groups in Malaya—the Malays, the Chinese and the Indians. The aborigines live mostly in the jungle, follow their own customs and beliefs and have little contact with non-aborigines. They are plagued by diseases, the most common of which are malaria and tuberculosis. Genetically the aborigines are of particular interest. Studies have revealed high frequencies in this population of certain haematological abnormalities: haemoglobin E ranges from 8 to 50 per cent in different groups<sup>1</sup>, glucose-6-phosphate (G-6-P) dehydrogenase deficiency from 8 to 23 per cent<sup>1</sup>, and hereditary ovalocytosis is found in 12.3 per cent of aborigines examined<sup>2</sup>. These high frequencies may be due to malaria or inbreeding or both. We now report the results of a preliminary study of haptoglobins, transferrins and serum gamma-globulin types in Malayan aborigines.

Blood samples obtained from various aborigine groups were frozen and shipped by air to San Francisco. Haptoglobin types were determined by starch-gel electrophoresis by the method of Smithies<sup>3</sup>. A highly sensitive benzidine solution was used to stain the haemoglobin-haptoglobin complex. In those instances when haptoglobin was not detected, the serum sample was run three times before ahaptoglobinaemia was diagnosed. Transferrin types were determined from the same starch gel by the autoradiographic method of Giblett *et al.*<sup>4</sup>, in which they are identified by the use of radioactive iron-59. At a later stage a method of micropurification, followed by electrophoresis, was also employed<sup>5</sup>. Typing for hereditary gamma-globulin (Gm) groups was performed by the inhibition of agglutination reactions using standard tube systems described by Fudenberg and Kunkel<sup>6</sup>.

Blood from 266 Malayan aborigines, 72 jungle fighters and 194 villagers and aboriginal hospital personnel was examined for haptoglobins. The aborigines were from different jungle areas, and 64 villagers had to be excluded from the study because they were related to persons included. Type 1-1 was found in six aborigines, 2-1 in

79, and 2-2 in 101, and haptoglobin was not detectable in sixteen. The gene frequencies for Hp<sup>1</sup> and Hp<sup>2</sup> were respectively 0.24 and 0.76. The frequency for haemoglobin E in the whole group was 34.0 per cent, for G-6-P dehydrogenase deficiency, 15.7 per cent, and for ovalocytosis, 12.0 per cent. Of the sixteen haptoglobin-negative persons, six carried haemoglobin E and ten did not; two were deficient in G-6-P dehydrogenase and fourteen had normal amounts in the erythrocytes; two had ovalocytosis and fourteen had not; one had malarial parasites and fifteen were uninfected at the time of study. From these data, malaria, haemoglobin E and G-6-P dehydrogenase deficiency did not seem to be the cause of the high frequency of haptoglobin-negative persons in this population group.

The same serum samples were also studied for transferrins. Among 202 sera from unrelated subjects, 196 contained only Tf C and six contained Tf CD. The Tf D resembled Tf D<sub>Chi</sub> and Tf D<sub>1</sub>. The exact identity of the Tf D is being studied at the Department of Zoology, University of Texas, Austin. The Tf D<sub>Chi</sub> has been found to differ in amino-acid substitution from that of Tf D<sub>1</sub> (ref. 7).

The attention of population geneticists has recently been attracted to the examination of hereditary gamma-globulins. The Gm types, because of their typical Mendelian inheritance, have proved useful in both anthropological and genetic studies, and the frequencies of different phenotypes vary greatly from one population to another. The systems for the detection of hereditary Gm types have steadily increased in number and, like the blood group system, they have become more and more complex. We examined various Gm factors in the sera from Malayan aborigines, and results were as follows: results of tests for Gm (a) and (b) on 147 sera from unrelated persons were 99 per cent positive; of 72 tested for Gm f, 97 per cent were positive. Gm x was uniformly negative in 107 sera from unrelated persons, and negative results were also uniformly obtained for Gm-like (Gm-c) activity in 131 sera. This pattern differs from the patterns found in Caucasians, Negroes, Australian aborigines, Japanese and Chinese.

We thank Dr H. E. Sutton for providing a standard Tf CD<sub>Chi</sub> and Dr E. R. Giblett for standard samples containing Tf B<sub>2</sub>C and Tf CD<sub>1</sub>.

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*Note added in proof.* Since completion of this manuscript, Dr. H. E. Sutton of the Department of Zoology, University of Texas, has reported that our Tf D is Tf D<sub>Chi</sub>. We are grateful for his help.

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## BIOLOGY

## Population Growth and Blood Groups

THE population explosion throughout the world is an important problem for governments and a topic much debated among demographers and economists. Any hint about the natural factors which might exercise control over population growth would seem to be worthy of further investigation by experts. The ranges of fertility in different communities vary and the variation would seem to be heritable. Mourant<sup>1</sup> states that "blood group studies are probably at present more valuable as a source of genetical information about human populations than studies of all other factors combined, and blood groups will surely remain for many years more important than any other class of factors".

Sperm counts in millions/ml.	Table 1 Blood groups				Total
	O	A	B	AB	
> 40	57 48%	50 41%	10 8.3%	3 2.5%	120
20-40	13 53%	9 37.5%	2 8.3%	0	24
0-20	10 35.7%	14 50%	3 10.7%	1 3.6%	28
Nil	15 37.5%	19 47.5%	6 15%	0	40
Blood group (donors) distribution this area	50.7%	36.0%	10.8%	3.0%	212

As part of our clinical study of male infertility we record the blood groups of the patients. The results in 212 such subjects are set out in Table 1. The most striking feature is the relative increase of group A and the relative decrease of group O among the azoospermic and oligospermic men. (We use the term azoospermic to mean complete absence of sperms in the samples of semen examined.) Preliminary statistical examination of these data on the basis of the standard error of the difference when compared with the normal distribution for this area of Scotland (shown in the lowest line of Table 1) suggests that the excess of group A is just at the level of significance. The proportion of A and O in our series of patients has not changed appreciably in the present group of 212 compared with our analysis of the first 116 some time ago. In view of this observation, we wonder whether there is any correlation between the frequency of blood group A in a community and its rate of population growth. A superficial examination suggests that there is. For example, in India, which has a rapid growth of population, the frequency of blood group A is of the order of 25 per cent, whereas in Europe with a lower rate of population growth the frequency of blood group A is of the order of 40 per cent.

Perhaps an expert in population studies would like to comment on or pursue this point.

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## Westerly Extent of the Range of Three African Lorisoid Primates

IN 1958 Booth<sup>1</sup> reviewed the zoogeography of West African primates and followed Rosevear<sup>2</sup> in placing the Cross River as the western boundary to the range of three Central African lorisoids. These were the angwantibo, *Arctocebus calabarensis* (Smith), Allen's bushbaby, *Galago alleni* Waterhouse, and the needle-clawed bushbaby, *Euoticus elegantulus* (Le Conte). Schwarz<sup>3,4</sup> and Hill<sup>5</sup> had previously speculated on the Niger as the western

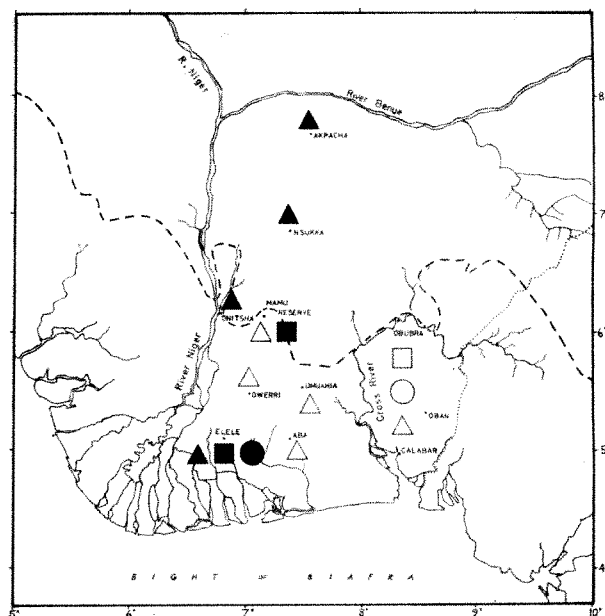


Fig. 1. Map of south-eastern Nigeria, showing new and past records of three lorisoid primates.  $\Delta$ , Previous Nigerian record of *Arctocebus calabarensis*;  $\blacktriangle$ , new locality for *Arctocebus calabarensis*;  $\square$ , previous Nigerian record of *Euoticus elegantulus*;  $\blacksquare$ , new locality for *Euoticus elegantulus*;  $\circ$ , previous Nigerian record of *Galago alleni*;  $\bullet$ , new locality for *Galago alleni*. ---, Northern boundary of high forest zone, where it meets the Guinea savannah; ..... western border of Cameroon.

boundary, but Rosevear's knowledge of this particular area has given much weight to the theory of the Cross River as a faunal barrier. Apparently unknown to Booth, however, angwantibos had been recorded before 1958 (refs. 6 and 7) from Owerri, Umuahia and Aba, which lie far to the west of the Cross River, and Rosevear himself<sup>8</sup> had recorded the animal in a collection brought to him from the Mamu Forest Reserve (see Fig. 1). Through field work in Eastern Nigeria\* we have been able to produce new and positive evidence on the distribution of the angwantibo and the bushbabies.

During the course of many night walks with head-torches in Mamu Reserve we have seen eleven angwantibos, two of which were captured and examined. Angwantibos were recorded in field excursions to two other key points, Akpaka Forest Reserve on the left bank of the Niger near Onitsha (6° 11' N.; 6° 47' E.) and Elele, further south (5° 6' N.; 6° 48' E.). During three nights in Akpaka Reserve nine individuals were seen, one being captured and examined, and at Elele, close to the most easterly distributaries of the Niger Delta, six angwantibos were seen, two of which were captured and examined.

Nsukka, where the university is situated, has a northerly position (6° 51' N.; 7° 24' E.) well inside the Guinea Savannah Zone. Here, during the past 8 months, twenty-four live angwantibos have been brought to the zoo by local natives. In a single expedition further north, to the village of Akpacha (7° 41' N.; 7° 34' E.) in the Igala Division of Northern Nigeria, we made a certain identification of one angwantibo in secondary woodland near the village. This is the most northerly record for the species, and because it is only 23 miles south of the River Benue it seems probable that this large tributary of the Niger forms the northern boundary of the angwantibo's range.

Our records also indicate how abundant the angwantibo is, at least in Eastern Nigeria. The reputation it has of being "very rare"<sup>9-11</sup> is apparently based largely on the accounts of two expeditions to the Cameroons<sup>12,13</sup>, and certainly is not upheld in our area.

\* Note: On May 30, 1967, the former Eastern Region of Nigeria proclaimed itself the Republic of Biafra.

The range of both *Euticus elegantulus* and *Galago alleni* has been found to extend as far west as that of the angwantibo. Specimens of both these animals were already known<sup>2</sup> from within the Cross encirclement, but there were apparently no records from the region between the Niger and the Cross. We have frequently seen *Euticus* in the Mamu Reserve, and have collected two specimens there. It is also common at Elele, where one specimen has been collected. Two Allen's bushbabies were also seen at Elele, and one of these was collected. As yet, we have no evidence to show that these two species of bushbaby range as far north as the angwantibo, and they are probably restricted to the high forest zone.

These observations were made during the course of field work supported by the Wellcome Trust.

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### Feeding Behaviour of the Larval Rat Flea *Nosopsyllus fasciatus* Bosc

DURING investigations on the possibility of inter-vector transmission of the rat trypanosome, *Trypanosoma lewisi* Kent, by the rat flea *Nosopsyllus fasciatus* Bosc, new observations have been made on the feeding mechanism and behaviour of the larva.

Flea larvae are claimed to feed on debris<sup>1</sup>. The mandibles are, according to Sharif<sup>2</sup>, used for rasping and scrubbing the food and then pushing it into the mouth. It is well known that blood, which is passed through the body of the adult flea, is an important and in some cases essential factor for the nutritional requirements of the larvae<sup>1</sup>. One species of flea is said to live in the fur of the Arctic hare, in both the larval and adult stages<sup>3</sup>, and there are occasional records of larvae being found on the bodies of their hosts<sup>4</sup>.

The rat flea has been reared in the laboratory after the method of Leeson<sup>5</sup>. It has been observed that the activity of the larvae in the nest debris frequently brings them into close proximity with adult fleas also present in the debris. Such larvae display a definite response to adults of both sexes and will actively pursue and attempt to seize the adults. If an adult flea is stationary among the debris the larva will attach itself by the mandibles, usually to the posterior end, particularly in the pygidial region. The exact point of attachment does not seem to be important provided that it brings the larva close to the anal opening. When the adult defecates the larva releases the grip with the mandibles and imbibes the faecal blood passed out by the adult flea. The blood is rapidly ingested by the larva with a definite sucking action. The speed with which defecation may occur after the attachment of the larva to the adult flea suggests that possibly its presence stimulates defecation. It is

surprising that sucking by flea larvae has not previously been noted, because the musculature of the pharynx suggests that this method of feeding is usual. Rapid sucking of fresh blood, water and rat urine has been observed under experimental conditions.

Flea larvae collected in nests, especially bird nests, are often recorded as having bright red blood in the gut. This suggests that species other than *N. fasciatus* also obtain blood directly from the anus of adult fleas rather than from the droppings which fall in the nest and rapidly dry.

It has also been found that injured adult *N. fasciatus*, especially if the cuticle is punctured, will be attacked and killed by the larvae, and the body contents eaten. There are apparently only two records in the literature of flea cannibalism<sup>6,7</sup>, but observations suggest this must be a fairly common occurrence. It has also been shown experimentally that larval fleas are attracted to injured mites and ticks which they attack and consume.

It would seem therefore that current views regarding the biology of larval fleas require some modification. The mandibles serve in certain circumstances as organs of temporary attachment and the feeding behaviour involves the sucking of fluids and ingestion of semi-liquid blood at the moment of adult defecation. Furthermore, larval fleas are semi-predatory, readily attacking and killing any slightly injured fleas, mites, ticks and other arthropods present in the nest. In view of these observations it is not surprising to find that flea larvae ingest living trypanosomes with blood from the adult, and experiments have shown that the trypanosomes survive up to 14 h in the gut of the flea larva, and in cultures of fleas that are fed on rats infected with *T. lewisi* up to 18 per cent of the larvae examined from these cultures have trypanosomes present in their gut. Such infections are of a transitory nature and apparently they do not play a part in inter-vector transmission. The intimate relationship between larva and adult flea, however, indicates a method by which bacterial or viral organisms infesting the adult flea either on the surface of the body, as in the case of the myxoma virus<sup>8</sup>, or present in the internal organs could be transmitted to the larvae.

I thank Dr P. Tate and the Hon. Miriam Rothschild for constant advice and encouragement and Dr F. G. A. M. Smit for his help in searching for references. The financial support of the Agricultural Research Council is gratefully acknowledged.

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### Influence of pH on the Uptake of Phosphate by Barley Plants in Sterile and Non-sterile Conditions

I HAVE shown<sup>1,2</sup> that the uptake of phosphate by intact barley plants, particularly from solutions of low concentration, is greatly influenced by the presence of micro-organisms on and around the roots. The complete exclusion of micro-organisms reduces the quantity of phosphate incorporated into nucleic acids and other com-



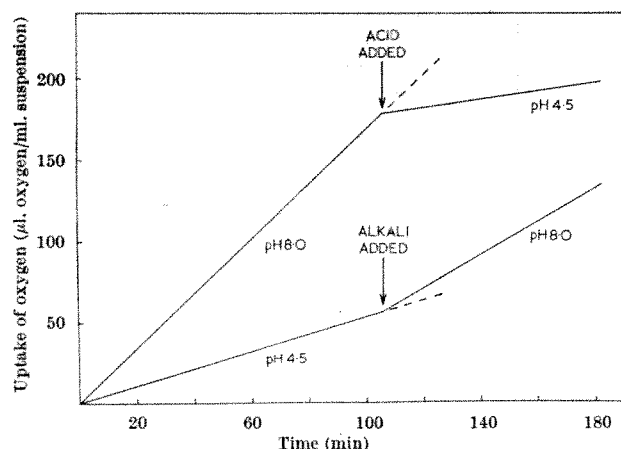


Fig. 1. Effect of pH on the uptake of oxygen by micro-organisms cultured from the roots of barley plants and resuspended in 1.0 per cent glucose.

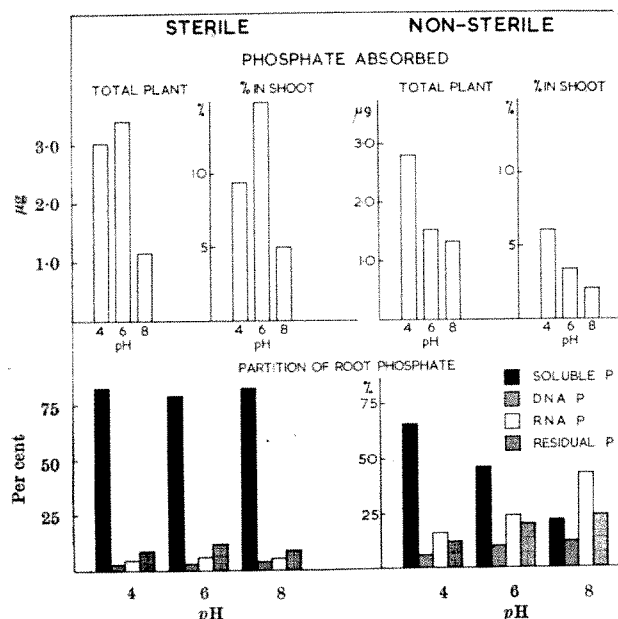


Fig. 2. Effect of pH on the uptake and distribution of phosphate in young barley plants supplied for 3 h with solutions of potassium phosphate under sterile and non-sterile conditions.

plex organic compounds in the root and causes a much greater transfer to the shoot. Similar effects of micro-organisms on the incorporation of phosphate have been shown by other workers<sup>3,4</sup> although the distribution of phosphate within the plants may vary depending on the conditions of the experiments<sup>5</sup>.

The extent to which this influence of micro-organisms varies with pH is here considered. The question is of particular interest in relation to the absorption of phosphate because of the effect of pH on the ionic species which is in solution; whereas at pH 4, 99 per cent is present as  $H_2PO_4^-$ , only about 13 per cent is in this form at pH 8, the divalent ion  $HPO_4^{2-}$  being dominant.

While van den Honert<sup>6</sup> and McGeorge<sup>7</sup> concluded that the absorption of orthophosphate by higher plants was primarily a function of the concentration of  $H_2PO_4^-$ , Hagan and Hopkins<sup>8</sup> deduced from a kinetic study of the uptake of phosphate by excised barley roots from solutions ranging in pH between 4 and 8 that both ions are accumulated, different mechanisms being involved. Because these experiments were not carried out under sterile conditions, however, micro-organisms could have

contributed to the results obtained and the extent of this contribution is likely to have varied widely at the different pH concentrations examined because of the known sensitivity of bacteria to this factor. The effect of pH on the uptake of oxygen by micro-organisms cultured from roots grown under normal laboratory conditions is shown in Fig. 1; their metabolic rate is much reduced at low pH.

The uptake and distribution of phosphate in young barley plants over a period of 3 h from solutions of potassium phosphate containing 0.01 p.p.m. phosphate at pH 4.0, 6.0 and 8.0 were therefore examined under both rigidly sterile and non-sterile conditions by the procedures previously described<sup>2,9</sup>. Although in the absence of micro-organisms the total quantity of ions taken up by the plants and the relative amounts transferred to the shoots varied with pH, the partition of the absorbed phosphate to the roots was similar in all three cases (Fig. 2). In contrast, under non-sterile conditions less phosphate was transferred to the shoots and the partition of the absorbed phosphate in the roots was highly dependent on pH. With increasing pH there was a marked decrease in the incorporation of phosphate into the soluble fraction in the roots and a concomitant decrease in its transfer to the shoots.

It is clear therefore that when the effect of pH on the uptake of phosphate is studied the results obtained reflect not only changes in the relative concentrations of the  $H_2PO_4^-$  and  $HPO_4^{2-}$  ions but also variations in the activity of the attendant microflora. Thus unequivocal conclusions regarding the accumulation of the two ion species by the roots of plants can be drawn only from experiments carried out in the absence of micro-organisms.

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### Distribution of Dry Matter to Flowers in *Chrysanthemum morifolium*

THE horticultural chrysanthemum (*Chrysanthemum morifolium*) is a short-day plant and normally requires long uninterrupted dark periods for flower induction and development<sup>1</sup>. Plants can be induced to flower in summer if the natural daylength is artificially shortened to less than 12 h by covering the plants with black cloth for part of the day. This practice forms the basis for the production of "all-year-round" pot plant chrysanthemums. Flower quality is of great importance with this type of crop and we have examined the effect of both carbon dioxide concentration and daily light total on the growth and flowering performance of *Chrysanthemum morifolium* c.v. 'Golden Princess Anne', when grown in controlled environments. We have also investigated the distribution of dry matter about the plant, with particular reference to its diversion to flowers.

In the first experiment (January 18–April 13, 1966) growth was followed in an orthogonal experimental design using three levels of light (30, 60 and 90 cal/cm<sup>2</sup>/day—30 cal/cm<sup>2</sup>/day is equivalent to the average daily total of visible radiation received in a glasshouse in the south of England in late October and mid February) and three

concentrations of carbon dioxide (300, 600 and 900 p.p.m.). In the second experiment (September 22–December 13, 1966), the same three light levels were combined with four levels of carbon dioxide concentration (300, 600, 900 and 1,500 p.p.m.). In both experiments, the day temperature was maintained at a constant 65° F and the night temperature at 60° F. Rooted cuttings were grown in a vermiculite/sand/gravel mixture (2 : 1 : 2 by volume) in pots irrigated daily with a complete nutrient solution. The normal daily cycle consisted of 8 hours of light from "warm-white" fluorescent lamps followed by 16 h of darkness, although for the first week of growth the plants also received 5 h of low intensity light from tungsten filament lamps given in the middle of the dark period—a non-inductive treatment. One week after the beginning of short day treatment, the growing point and its surrounding leaf initials were removed from each plant to induce the formation of lateral branches. After a further three weeks, the weaker lateral growths were removed and the axillary flower buds on the remaining laterals were also removed to leave a single terminal flower on each branch. The several flowers on any one plant all developed at similar rates. Samples were removed from each treatment at frequent intervals<sup>2</sup> and lamina area and dry weight, and the dry weights of the stems plus petioles, roots, and flowers were determined. The number of flowers per plant was also recorded and the average stage of flower development per plant was assessed using the arbitrary scale given in Table 1. On average the plants passed through three stages of development every two weeks.

Table 1. SCALE OF FLOWER DEVELOPMENT

0. Vegetative apex
1. Flowering apex
2. Flower bud less than 10 mm diam.
3. Flower bud greater than 10 mm diam.
4. Florets visible on the capitulum
5. Florets coloured
6. Florets expanding
7. Few outer florets fully expanded
8. Flower open
9. Flower fully open

Flower weight and the rate of flower development were both affected by treatment and these responses will be discussed elsewhere. In order to assess the effect of treatment on flowering performance and to compare plants of different sizes we made use of the flower weight ratio—the weight of flowers divided by the total plant dry weight. This ratio gave a measure of the proportion of the resources of the plant which had been diverted to reproductive organs. The treatments which produced the heaviest plants also produced the highest flower weight ratios at any moment in time. These treatments, however, also tended

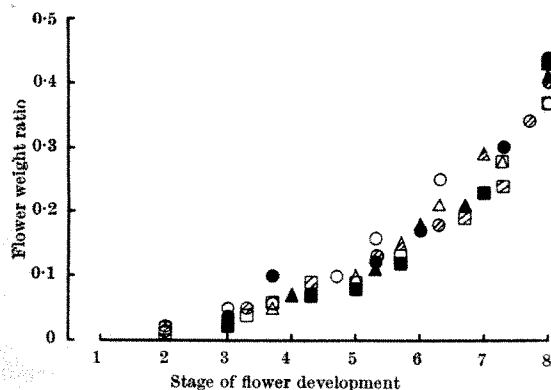


Fig. 1. The relationship between flower weight ratio and stage of flower development in *Chrysanthemum morifolium* c.v. 'Golden Princess Anne'. Experiment began January 18, 1966. Each point represents the mean of three plants. Open circle, open triangle, open square, 30 cal/cm<sup>2</sup>/day; hatched circle, hatched triangle, hatched square, 60 cal/cm<sup>2</sup>/day; solid circle, solid triangle, solid square, 90 cal/cm<sup>2</sup>/day; open circle, hatched circle, solid circle, 300 p.p.m. CO<sub>2</sub>; open triangle, hatched triangle, solid triangle, 600 p.p.m. CO<sub>2</sub>; open square, hatched square, solid square, 900 p.p.m. CO<sub>2</sub>.

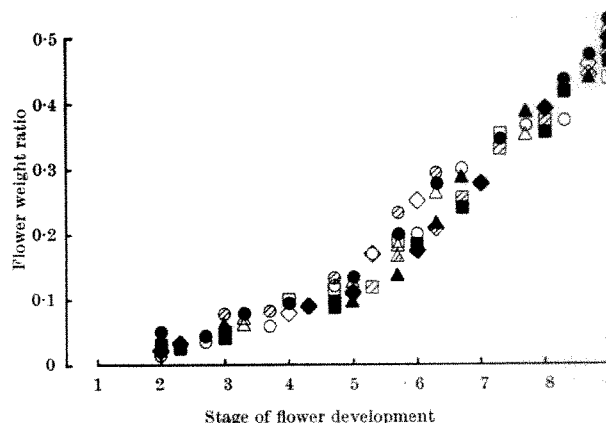


Fig. 2. The relationship between flower weight ratio and stage of flower development in *Chrysanthemum morifolium* c.v. 'Golden Princess Anne'. Experiment began September 22, 1966. Symbols as in Fig. 1. Open diamond, hatched diamond, solid diamond, 1,500 p.p.m. CO<sub>2</sub>. Each point represents the mean of three plants.

to produce the most rapid rates of flower development and, when this factor was taken into consideration by plotting the flower weight ratios against the average stage of flower development, the relationship shown in Figs. 1 and 2 was obtained. Thus the proportion of total dry matter that was diverted to flowers as a whole was highly correlated with their average stage of development. This relationship was independent of treatment and of total dry weight, which was in the range 5–20 g at the final harvest and appeared to be independent of flower number, which ranged from three to eight per plant.

This correlation has relevance to horticultural practice. If it is generally applicable, then it is apparent that the weight of "open flowers" per plant can only be increased by increasing total plant dry weight. Although at the "open flower" stage the flower weight will represent the same proportion of the total weight in a small plant as in a large plant, small plants can only have a small weight of flowers. Because the relationship was apparently independent of flower number, improved weight or quality of individual terminal flowers can be obtained if all the axillary flower buds which would compete for the proportion of dry matter available are removed as early as possible. This provides the basis of the disbudding treatment used to produce large blooms for commerce or showing. The further reduction of flower number by the removal of strong lateral branches would significantly reduce total dry weight, but we have no information regarding the effect of removing some terminal flowers and leaving the leaves and stems.

The two figures correspond exactly at flower stage 8 but not at the lower values; the flower weight ratio is marginally lower for any flower stage in the first experiment. This discrepancy may either reflect a true difference between occasions or a small change in the subjective assessment of these intermediate stages of development. It was apparent, however, that close control over the diversion of dry matter to flowers existed in the chrysanthemum when grown under these conditions.

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## Interaction of CCC and Water Deficit on Wheat Yield

2-CHLOROETHYLTRIMETHYLAMMONIUM chloride (CCC) shortens wheat straw and is used to lessen losses caused by lodging, but there is good evidence that it sometimes also increases the yield of wheat in other ways. For example, Humphries, Welbank and Witts<sup>1</sup> reported an increase of 5 per cent in the grain yield of 'Phoebus' spring wheat after spraying with either 2.5 lb. or 5 lb./acre of CCC at the five leaf stage, although plants in the untreated plots did not lodge. They tentatively attributed the increase to the better penetration of light through the crop enabling more ear-bearing shoots to survive.

This explanation, however, seemed less likely in view of our experience in 1965 when CCC did not increase yield, except perhaps in plots with closely spaced rows (4 in.), although the straw was shortened by CCC as much as in 1964.

In 1966, increases in yield were obtained in two experiments although again lodging in untreated plots was negligible. In the first, CCC in amounts of 2.5 lb./acre at the five leaf stage was applied to 'Kloka' wheat in the irrigation experiment at the Woburn Experimental Station. A wheat section of this experiment consisted of three blocks of two plots with one plot irrigated in each block. CCC was applied to half plots and four amounts of nitrogen fertilizer (0.4, 0.8, 1.2, 1.6 cwt./acre) partially confounded with CCC on quarter plots. During a dry period of 2 weeks from May 25, 2 in. of irrigation water was applied. This was beneficial especially in combination with large nitrogen dressings and increased grain yield estimated from sample areas of 2.8 m<sup>2</sup>/plot by 10 cwt./acre on plots receiving 1.2 or 1.6 cwt. of nitrogen (Table 1). CCC had little effect on the yields from the irrigated plots, but it increased yield from the unirrigated plots by 6 cwt./acre. These increases in yield were associated with more ear-bearing shoots (estimated from a weighed sub-sample from the area of collection) and greater grain weight/ear (Table 1). Plants treated with CCC seemed to lose fewer shoots than untreated plants during a dry spell in the period around ear emergence and so yielded more. More grains on each ear of plants treated with CCC more than offset the smaller weight of 1,000 grains, and so gave a greater grain weight for each ear. The regression of yield on shoot number showed that survival of an additional twenty shoots/m<sup>2</sup> increased grain by 1.9 cwt./acre. In an experiment at Rothamsted in 1966 with 'Kloka' wheat given four quantities of fertilizer (0, 0.8, 1.6, 2.4 cwt. of nitrogen/acre), although untreated plots did not lodge, CCC again increased yield by an average of 2 cwt./acre—a result very similar to that obtained in 1964.

Observations that CCC increases root growth have mostly been made on plants growing in pots<sup>2</sup>, but Hanus<sup>3</sup>

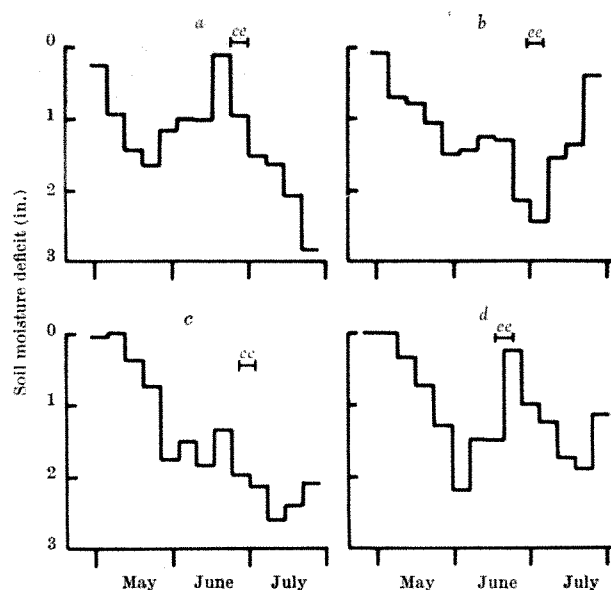


Fig. 1. Mean weekly soil deficits (in.) from May to July. a, Rothamsted, 1964; b, Rothamsted, 1965; c, Rothamsted, 1966; d, Woburn, 1966; ee, time of ear emergence.

points out that root growth in pots may be restricted and the true effect of CCC on root growth can be judged only in the field. We found in both 1964<sup>1</sup> and in 1965 (Table 2) that the weight of root recovered by hand-pulling, that is roots in the top layers of soil, is larger from plants treated with CCC than from untreated plants. Hanus<sup>3</sup> showed that whenever it was applied CCC increased root growth at all depths, and he claimed that the larger root systems were associated with larger yield. Many drought-resistant plants are characterized by relatively large root systems<sup>4</sup> and CCC may have increased yield in our experiments because it increased the amount of roots and so enabled more shoots to survive dry periods when shoot number was declining, especially about the time ears were emerging.

Figures supplied by Dr H. L. Penman show that moisture deficits after ear emergence increased in 1964 and 1966 but decreased in 1965 (when CCC did not increase yield in a normally spaced crop), see Fig. 1. This suggests that drought is most critical near the time of ear emergence and that CCC counteracts it by increasing the size of the root system. The greater effect of CCC at Woburn may be because the sandy soil there is more subject to drought than the clay soil at Rothamsted.

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Table 1. SHOOT NUMBERS, GRAIN NUMBER/EAR AND GRAIN WEIGHT/EAR (Mean of 1.2 and 1.6 cwt. of nitrogen/acre)

	'Kloka'				
	Un- treated	CCC	Irri- gated	Irri- gated and CCC	Stan- dard error
Yield of grain (85 per cent dry matter) cwt./acre	37.5	43.5	47.8	49.6	1.67
Ear-bearing shoots (millions/acre)	1.91	1.99	2.07	2.10	0.07
No. of grains/ear	23.8	28.0	28.4	30.8	1.07
Dry weight of grains/ear	0.85	0.95	1.01	1.02	0.04

Table 2. DRY WEIGHT OF WHEAT ROOTS (G/M<sup>2</sup>) RECOVERED BY PULLING 'Phoebus' 1964

		Phoenix 1964					
	May 14	June 3	June 24	August 24			Mean
Untreated	17	30	44	25			29.0
CCC	16	32	52	26			31.5

		'Opal' 1965						
	May 24	June 15	June 29	July 13	July 27	August 10	August 24	Mean
Untreated	23	61	69	61	64	40	64	54.6
CCC	25	81	83	77	75	51	68	65.7

## Selective Phytotoxicity of 2,4-Dichloro 6, (o-chloroanilino)-s-triazine ('Dyrene') to *Cirsium arvense*

WHILE carrying out a fungicide field experiment for the control of *Botrytis cinerea* on strawberries in an area heavily infested with creeping thistle (*Cirsium arvense*), it was noted that approximately 50 per cent of the thistles in certain plots died within 2-3 days of making the first



fungicide application. The plots concerned appeared to be those treated with 'Dyrene' (2,4-dichloro 6, (o-chloroanilino-*s*-triazine). Neither the crop nor any other weed growing in this area showed phytotoxicity. The effect on the thistles, which were 6 in. tall at the time of spraying (June 1966), consisted of a rapid browning, general discoloration and necrosis of all emerged parts.

This extreme degree of selectivity eventually led to a series of greenhouse and field experiments being conducted in the United Kingdom and by various collaborators in Canada. The results of these field screening operations, all treated at the arbitrary rate of 2 lb. active ingredient/acre, are summarized in Table 1.

Table 1. PERCENTAGE SUSCEPTIBILITY OF GIVEN CREEPING THISTLE POPULATIONS AT DIFFERENT SITES IN THE UNITED KINGDOM AND CANADA

No. of experiment	United Kingdom (Essex)	Canada (Alberta)
	July-Aug. 1966 Percentage of susceptible plants	July, Aug.-Sept. 1966 Percentage of susceptible plants
1	63	100
2	76	88
3	47	4
4	31	43
5	50	75
6	3	80
7	100	50
8	99	10
9	1	0
10	20	7
11	100	5
12	100	35
13	14	20
14	40	81
Average	53	42

The pattern of results given in Table 1 shows that the activity of 'Dyrene' on thistles is an "all or nothing" effect and that the ratio of resistant:susceptible individuals varied immensely from one site to another in both the United Kingdom and Canada. This ratio was found to vary within a few yards on a single field—a pattern which obviously suggested the existence of strains of *Cirsium arvense* which responded differently to 'Dyrene' treatment. Attempts to relate resistance and susceptibility to morphological differences between thistles have so far not been successful although it is possible that in Canada the response may parallel the two varieties of *Cirsium arvense* described by Saidak<sup>1</sup> as 'Horridum' and 'Mite'. These varieties are morphologically distinguishable and accordingly the response of a given population of thistles to either 'Dicamba' or 2,4-DB can be predicted.

A preliminary survey was made in an attempt to correlate resistance or susceptibility to the sex of the specimen concerned, but this proved negative and seeds harvested from both resistant and susceptible specimens were found to be viable. Neither the dosage of 'Dyrene' used nor the growth stage when the chemical was applied had any effect on this pattern in that resistant populations tolerated dosages of up to 2 lb. of active ingredient/acre, while 1 oz./acre killed the susceptible specimens and even spray drift was sufficient to show a marked scorching effect.

Initial observations suggested that effects could be detected outside the sprayed plots even when drift was practically eliminated by the use of screens and this might imply that translocation or systemic transfer of the chemical occurred by way of the rhizome. Such possibilities are obviously of interest, but any attempt to analyse this problem was hampered by the fact that susceptible and resistant specimens could not be distinguished before treatment. By careful dosing, however, it has now been possible to isolate two clones which respond differently to this chemical.

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<sup>1</sup> Saidak, W. J., in *Soils and Crops: Addresses and Proceedings*, 69 (1966).

## Particulate Localization of Acid Phosphatase in Fungi

CYTOCHEMICAL demonstrations of the activity of acid phosphatase have been made in fungi<sup>1-3</sup> and it is generally considered that the distribution of this enzyme in these organisms is of a particulate nature. The lysosomes of animal cells are believed to contain all the cytoplasmic acid phosphatase<sup>4</sup>. Although these granules have not yet been conclusively demonstrated in fungi the localization of this enzyme in the mitochondrial fraction of freeze-substituted centrifuged hyphae<sup>2</sup> is consistent with its distribution in the light mitochondrial or lysosomal fraction of animal cell homogenates.

*Botrytis cinerea* Fr. was grown on coverslips for 2 days at 25° C on disks of malt extract agar. The disks were removed and the unfixed tissue adhering to the coverslips was used directly or after fixation for 1.5 h at 0°-4° C in cold acetone.

The Gomori<sup>5</sup> lead nitrate method gave excellent results with incubation periods of 1.5-2 h at 37° C. The modified lead nitrate method<sup>6</sup> gave similar results with incubation times of 15-30 min, but the particles were paler and more finely delimited. The standard coupling azo dye technique (Fig. 1) using a substrate of sodium  $\alpha$ -naphthyl phosphate and incubation times of 10-20 min at 37° C gave good localization in the presence of 7.5 per cent polyvinyl pyrrolidone when coupled with fast garnet GBC salt. The post-coupling azo dye method<sup>7</sup> gave good results with sodium 6-benzoyl-2-naphthyl phosphate as the substrate and an incubation time of 1-1.5 h using fast blue B salt as the coupling dye. The appropriate controls for these methods gave negative results.

These results were indicative of the particulate localization of acid phosphatase within the cytoplasm. After short periods of incubation no staining or only faint staining was obtained; prolonged incubation resulted in diffuse staining and some nuclear staining. With optimum incubation times spherical particles 0.2-1.0  $\mu$  in diameter were visible and these varied in numbers in each cell from less than ten to more than 100. It was also shown that the cytoplasm contained particles of the same size and numbers as the acid phosphatase particles which absorbed neutral red, fluoresced an orange colour in acridine orange at neutrality and possessed low contrast against the cytoplasm when examined with the phase contrast microscope.

Similar results were obtained using a number of other fungi including *Neurospora crassa* Shear and Dodge, *Sistotrema brinkmannii* (Bres.) Rogers and Jackson, *Cunninghamella elegans* Lendner, *Pyronema omphalodes* (Builliard) Fuckel and *Penicillium notatum* Westling.

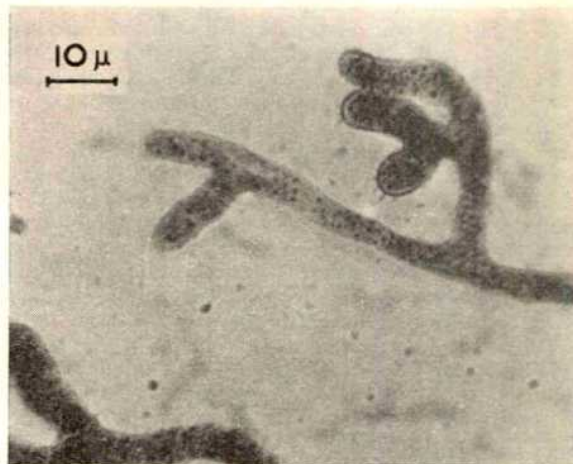


Fig. 1. Particles containing acid phosphatase in *B. cinerea*, stained by the standard coupling azo dye method.



Although it is now generally considered that the lysosomes contain all the acid phosphatase of animal cells, it is apparent that the spherosomes of bulb scales of *Allium*<sup>8</sup> and of root tips of a number of grasses<sup>9</sup> and cytoplasmic particles in root tissues of *Vicia faba*<sup>10</sup> contain acid phosphatase and as such are possibly related to the lysosomes. The present work clearly demonstrates that fungi also possess particles containing acid phosphatase within the cytoplasm. Although the various types of particle shown in this work were not identifiable with each other, it is apparent that fungi possess particles with certain attributes in common with the lysosomes.

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## ANIMAL BEHAVIOUR

### A Maturation Factor in Spontaneous Alternation

WHEN it is given consecutive trials in a *T* maze, the rat will typically enter one arm of the maze on the initial trial and the opposite arm on the second. This phenomenon has been termed "spontaneous alternation"<sup>1</sup>. Lesions to the hippocampus or the administration of cholinergic blocking drugs, processes which have been found to interfere with learning and remembering, also impair spontaneous alternation<sup>2,3</sup>. This suggests that the rate at which an animal spontaneously alternates could be a function of its ability to acquire and retain information. The present investigation was concerned with spontaneous alternation as a function of age. It is well established that the ability to learn and remember develops with maturation<sup>4-6</sup>. We postulated a parallel development for alternation performance.

Four groups of ten Sprague-Dawley albino rats served as subjects. At the commencement of testing the subjects in each group were respectively 20, 40, 60 and 80 days old. Three *T* mazes, proportioned to the sizes of the rats, were used. The 20 day old group was tested in a maze with arms 24 cm long, 10 cm deep and 7.5 cm wide; the 40 day old group in a maze with arms 30 × 12.5 × 10 cm, and the 60 and 80 day old rats in a maze with arms 45 × 15 × 15 cm. In each case the stem of the *T*, separated from the rest of the maze by a guillotine door, served as a starting box.

The subjects were given two trials daily for 3 days. A typical trial was as follows: the rat was placed in the starting box, the guillotine door was raised and the rat was allowed to enter either arm of the *T*. A four-footed entry into one side of the maze completed the trial. Within 5 sec of completion of the first trial the rat was replaced in the starting box for the second trial. If a rat failed to enter an arm within 2 min the trial was terminated and results of that day discounted.

The alternation rates for each group, corrected for initial response tendencies<sup>1</sup>, were tested against chance by

chi square. Younger rats alternated randomly (20 day old group, adjusted rate, 53.3 per cent and  $\chi^2$ , 0.02; 40 day old group, adjusted rate, 65.4 per cent and  $\chi^2$ , 0.94). The 60 day old group (adjusted rate, 74.7 per cent and  $\chi^2$ , 3.25,  $P < 0.05$ , one-tail) and the 80 day old group (adjusted rate, 86.7 per cent and  $\chi^2$ , 5.40,  $P < 0.02$ , one-tail) alternated significantly above chance. The rate of alternation was found to be significantly associated with age ( $C = 0.263$ ,  $P < 0.02$ , one-tail). Percentages of failures to leave the starting box within the 2 min limit were calculated for each group: 20 day old, 0 per cent; 40 day old, 16.7 per cent; 60 day old, 10.0 per cent; 80 day old, 13.3 per cent. These percentages did not differ significantly, which suggests that the differences in alternation were not caused by differences in emotionality as reflected in the "freezing response". Investigations with a variety of species have indicated that exploratory behaviour decreases with age<sup>7,8</sup>; thus it seems unlikely that the lower alternation rates of the younger animals were the result of a lessened exploratory drive. It has been found that rats reared in an "enriched environment" perform better on learning and problem solving tasks than rats reared in a "deprived environment"<sup>9</sup>. Unpublished results of mine have indicated that "enriched environment" rats alternate significantly more than "deprived environment" littermates. These findings, together with those of the present investigation, support the suggestion that the ability of the rat to alternate spontaneously depends on its ability to learn and remember.

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### Tolerance and Dependence in the Planarian after Continuous Exposure to Morphine

MORPHINE addiction is a phenomenon thought to be limited to higher mammalian species<sup>1</sup>. Recent work, however, suggests that it may be possible to produce an addiction syndrome in organisms lower on the phylogenetic scale.

Wikler<sup>2</sup> demonstrated a model of addiction and relapse in rats based on two-factor conditioning theory. Similarly, Nichols and Davis<sup>3</sup> and Weeks<sup>4</sup> have used instrumental techniques to maintain morphine self-administration in rats.

A number of investigators have reported conditioning in the planarian by both classical and instrumental methods<sup>5-8</sup>. These results, while controversial<sup>9</sup>, suggest that if the flatworm can be conditioned, it can be addicted. If addiction can be produced in the planarian, this organism, with its simple ganglionic nervous system and profound capacity for regeneration, would provide a useful system in which to study the cellular changes that accompany the addicted state.

This article presents the results of attempts to produce and measure two aspects of the addiction syndrome, tolerance and dependence, in the planarian, *Dugesia dorotocephala*.

Adult *D. dorotocephala* were kept in individual Petri dishes in 30 ml. of reconstituted distilled water<sup>10</sup>.

The time taken to swim from the centre of a circle of light to the circumference was used as a measure of the worm's performance. A planarian is negatively photophobic and will swim rapidly and directly to the edge of the circle to avoid light. In concentrations of morphine sufficient to depress the animal, the transit time is increased by slow swimming or an erratic path. Tolerance can be defined, then, by the return of function towards normal in a previously depressed animal, as measured by decrease in transit time. Dependence is defined as the deterioration of swimming performance when morphine is withdrawn.

A circle with a radius of 5 cm was drawn on a table top. A tensor-lamp was placed 18 in. above this. A shutter was interposed between the lamp and the target. Worms to be tested were placed individually in a 12 in. 'Pyrex' plate filled with 150 ml. of the test solution and allowed to incubate for 15 min.

The worm was then moved to the centre by moving the plate, the shutter opened, and the transit time to the circumference of the circle recorded. Five consecutive trials were run on each subject. If a worm failed to reach the circumference after 120 sec, the trial was scored as 120 sec.

All test solutions were adjusted to pH 7 with dilute sodium hydroxide.

**Experiment I: Tolerance.** Before exposure to the drug, the transit times of all worms in water were recorded. The mean transit time was 29 sec ( $\pm 15$ ). Trials were then carried out in morphine, 0.5 mg/ml. The experimental animals were then returned to their home dishes into which 30 ml. of morphine solution, 0.4 mg/ml., had been placed. Control worms were handled identically but returned to water between trials. After 8 days' exposure to morphine, the subjects were run again under the same conditions.

**Experiment II: Dependence.** Nine experimental worms were raised in morphine, 0.3 mg/ml., for 15 days; then in 0.4 mg/ml. for 8 more days. Their transit times in morphine, 0.5 mg/ml., were then measured. Subjects were then returned to morphine, 0.4 mg/ml., for 20 more days.

After 43 days' exposure to morphine, the worms were abruptly withdrawn by placing them in water for 60 min. After withdrawal, their transit times in water were measured. Control worms were treated identically, but were raised in water.

**Tolerance:** Table 1 presents the transit times in morphine of eight experimental and four control worms before and after 8 days' exposure to morphine, 0.4 mg/ml. A significant decrease in transit time after exposure to morphine is apparent, suggesting the development of tolerance. Tolerance is also demonstrated after 23 days' exposure in experiment II.

**Dependence:** On withdrawal from morphine, experimental subjects showed a significant increase in transit time. Before withdrawal, all worms were observed to be swimming rapidly and smoothly in their dishes. Of the

nine experimental worms run after withdrawal, only two showed any spontaneous swimming movements in water. Seven adhered to the dish and became crinkled in appearance, refusing to move even when stimulated gently. This behaviour persisted for periods of time varying from 7 to 20 h before normal swimming was observed. Control worms continued to show normal swimming behaviour throughout the period of observation.

It appears that planarians, under the proper experimental conditions, are capable of developing tolerance to, and dependence on, the narcotic properties of morphine.

An alternative explanation is that the experimental subject responds to morphine as a strong, non-specific novel stimulus, and that the subject's improved performance after continuous exposure is a non-specific adaptation to that stimulus. While this position can also be used to account for the deterioration of performance on withdrawal from morphine, the striking effects of withdrawal make this an unlikely assumption in the latter case.

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### Imprinting: Reversal of a Preference established during the Critical Period

Two distinctive features of "imprinting" originally emphasized by Lorenz<sup>1</sup> were that there was a critical period in which the preference for a particular species was established and that the preference established during this period was permanent and could not be changed by subsequent experience. These two features have been reiterated by Hess<sup>2</sup>, who has added a primacy-recency feature, claiming that the first imprinting experience has priority over a subsequent one. These features of imprinting have been questioned by Sluckin and Salzen<sup>3</sup>, who treated imprinting as a perceptual learning phenomenon in which the sensitive period is experience dependent and the stability of an imprinted preference is dependent on the amount of experience. More recent reviews by Sluckin<sup>3</sup> and Bateson<sup>4</sup> have supported this view. In particular the perceptual learning view of imprinting has been developed into a neuronal model hypothesis of imprinting by Salzen<sup>4</sup> and it predicts that object preferences established by the imprinting process should be subject to reversal given sufficient exclusive and enforced experience of new objects after the end of the so-called critical period. The present experiment demonstrates a reversal of this kind.

The experiment used Cornish  $\times$  White Rock chicks hatched in separate boxes and transferred when 12-18 h old to isolation rearing cages. In the centre of each cage separate from the sources of food, water and heat, there was either a dark blue or a green cloth covered paper ball about 5 cm in diameter and hanging approximately 3 cm

Table 1. TRANSIT TIMES (SEC) IN BUFFERED MORPHINE, 0.5 MG/ML., OF PLANARIANS BEFORE AND AFTER 8 AND 23 DAYS OF EXPOSURE TO MORPHINE AT LESSER CONCENTRATIONS AND IN SPRING WATER AFTER 43 DAYS OF CONTINUOUS EXPOSURE TO MORPHINE

	No. of trials	Mean transit time in water before exposure	Mean transit time in morphine (0.5 mg/ml.)	Mean transit time in water after
		Before exposure	After 8 days	After 23 days
Experimental	40	29 $\pm$ 15	120 $\pm$ 0	45 $\pm$ 26.1
Controls	20		120 $\pm$ 0	107 $\pm$ 28.0
Experimental	45		120 $\pm$ 0	65 $\pm$ 3
Controls	15		120 $\pm$ 0	103 $\pm$ 32

a,  $P < 0.001$ ; b,  $P < 0.001$ . Student's *t* test.

\* Seven animals had mean times of 120 sec. Two had mean times of 44.4 and 42.4 sec respectively.

above the floor. The chicks quickly became strongly attached to the balls, spending much time beside them, and interacted with the balls by pushing, pulling, and pecking them. After three days (12 h light/dark cycle) the chicks were tested for their preference between blue and green balls. The balls were hung midway on opposite long sides of a box (45 × 90 cm). The chick under test was placed in the dark at the mid-point of a short side, a lamp was switched on above the box, and the chick was given 2 min in which to go to and stay with one of the balls. A preference was recorded if the chick after reaching a ball either contacted it, pecked and pulled it, and/or gave pleasure calls, or stayed close (1 in.) beside the ball and was silent or pleasure calling. The trial was ended when either of these criteria was reached or at the end of 2 min. The light was switched off, the chick was returned to a holding box and the positions of the objects were reversed ready for the next trial. Ten trials were made with each chick. After testing the chicks were returned to their cages and the balls exchanged so that each chick now had a new and different coloured ball. The chicks began to respond to the new balls on the same day. Three days later the chicks were again tested for their preference between green and blue balls. Then they were returned to their cages for a further three days but this time without any balls present. Finally, a third preference test was given. In this way twelve chicks were imprinted with a blue ball and then given reversal training with a green ball, while ten chicks were tested in the opposite manner.

The results are shown in Fig. 1 in terms of the mean number of responses made by the chicks to each ball in each preference test. The two colour treatments are combined because they were nearly balanced and gave similar results. The results show that at the end of the first 3 days the chicks had an almost exclusive preference for their familiar coloured ball ( $p < 0.01$ , Wilcoxon test). After the second three day period, this preference had been reversed ( $p < 0.01$ , Wilcoxon test). At the third test the new preference was maintained or even slightly increased because the responding to the preferred ball had increased significantly since the second test ( $p < 0.02$ , Wilcoxon test). Twenty-one of the twenty-two chicks made more responses to their familiar ball on the first test. On the second and third tests eighteen and seventeen of these twenty-one chicks made more responses to the newly experienced ball.

There can be no doubt that the period in which the first ball was experienced included any possible critical period for imprinting in chicks. The experience began 18 h after hatching, and Hess<sup>5</sup> has claimed that this period reaches its peak at this time for chicks. Furthermore, the second ball was experienced well after the end

of the critical period of 24 h determined by Hess. Many studies<sup>3</sup> have shown that domestic chicks imprint with objects when exposed to them within the first 3 days after hatching. Thus the experiment shows that a strong preference established during this imprinting period can be completely reversed by subsequent experience. It also shows that this reversal was maintained intact after a period in which forgetting of both objects could have occurred. It cannot be said therefore that learning which takes place after the imprinting period is more rapidly forgotten than the learning during the imprinting period. Thus it would seem that the most recently learned preference predominated contrary to Hess's<sup>2</sup> claim of a primacy effect in imprinting. The recent test by Kaye<sup>6</sup> of a primacy effect gave an equivocal result, probably because of the very short training periods involved. The present test used stimulus objects that differed only in colours that were known from pilot studies to be equally easily learned and discriminated by chicks. The design was balanced for colour and similar results obtained with either colour. The training periods were long and the resulting preferences very strongly developed. Finally, the behaviour in the tests involved patterns of social interaction as well as the simple approach response. This study therefore represents the first closely controlled laboratory demonstration of reversibility of imprinting in precocial birds. It confirms the predictions of the neuronal model hypothesis of imprinting and agrees with the field observation of Steven<sup>7</sup>. In the field study by Schein<sup>8</sup> there appeared to be evidence for an irreversible preference for humans among human imprinted turkeys. It should be noted that these turkeys were always able to see humans as well as their later flock companions. Under these circumstances the neuronal model hypothesis would not necessarily predict a change in preference. The present demonstration of reversibility of imprinting in chicks agrees with the demonstration of a reversible social attachment in lambs<sup>9</sup>. Similar results have been obtained with a shape discrimination and a full account will be published elsewhere.

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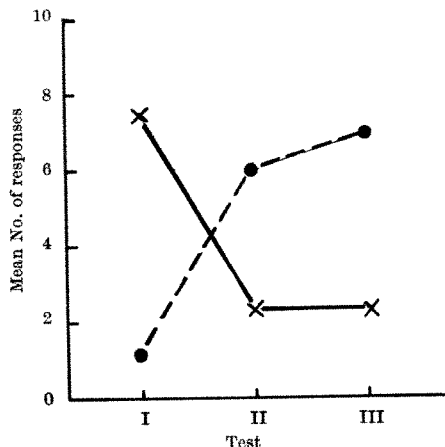


Fig. 1. Mean numbers of responses to the first-learned (x) and second-learned (●) coloured balls in the two-choice discrimination tests. Each of the twenty-two chicks had ten 2 min trials in each of three tests which were given at intervals of three days.

## PSYCHOLOGY

### Frustration Tolerance and Incidental Learning as Determinants of Extinction

Two theories currently exist to explain the retarding effect of partial reward on the extinction of a learned behaviour. Amsel<sup>1,2</sup> has suggested a theory based on the thesis that the shortcomings (non-reward) of a partial reward schedule generate frustration, experience of which reduces the reaction to the frustration induced by extinction. We shall here refer to this as the "frustration tolerance" theory. Sutherland<sup>3,4</sup>, on the other hand, has proposed a theory based on the assumption that partially rewarded animals switch attention more often than con-

sistently rewarded animals and therefore learn about more cues. During extinction the responses to all the cues which the animals have learned about during training have to become extinguished. This suggests that the more cues learned about the longer it takes to extinguish the responses to them. We shall refer to this as the "incidental-learning" theory.

Recent work<sup>5</sup> suggests that the two theories are not incompatible. Thus if frustration diverts attention, animals experiencing frustration during acquisition will learn about more cues, with consequent effects on extinction rates that are compatible with both theories. This view is supported by the finding<sup>5</sup> that when drugs which reduce frustration are administered during acquisition, they can inhibit incidental learning in partially rewarded rats. Extinction of the learned behaviour was faster in these rats than in the placebo controls, but this result does not indicate whether the effect of the drug on extinction results from a reduction of frustration tolerance, or from an inhibition of incidental learning. The experiment reported here was designed to distinguish between these two possibilities.

The subjects were forty adult hooded male rats, which were 90 days old at the start of the experiment. They were maintained on a schedule in which they were deprived of food for 22 h per day but allowed *ad libitum* access to water. The animals were subjected to a pre-training course during which they were trained to make the instrumental response in a Grice box from which the stimuli had been removed. The subjects were then allocated to four groups, matched for performance in pre-training. During training each rat was run for ten trials per day, with a mean inter-trial interval of 10 min. Rewards consisted of access to food for 10 sec.

The training procedure for the four groups is summarized in Table 1. The subjects were transferred from part 1 to part 2 when they reached an individual criterion of eighteen out of twenty successive correct choices. Groups 2 and 4 were then run on a second problem until they reached the same criterion. Groups 1 and 3 were overtrained on the original problem until groups 2 and 4 reached their second criterion. Thus all groups had the same number of training trials. The cues which the animals had learned during training were extinguished and the animals run until they reached a criterion of two "no-runs" in succession. When the animals took more than 2 min from start to choice criterion a "no-run" was scored.

The mean number of trials to the extinction criterion is shown in Table 2. These results show that there was no significant difference in resistance to extinction between the one and two cue consistently rewarded groups, or between the one and two cue partially rewarded groups, but that there was a very large difference (that is to say, there was no overlap in the range) between consistently

rewarded and partially rewarded groups. Moreover, the percentage of correct choices made during the last twenty extinction trials (Table 2) indicates that there is no significant difference between the one and the two cue groups on this criterion, but a very large difference between consistently rewarded and partially rewarded groups.

It is difficult to see how the results can be accounted for on Sutherland's hypothesis because there are no apparent differences in resistance to extinction between groups which learned about one or two cues during training. On Amsel's hypothesis no such difference would be expected, except that the two cue groups might have experienced some frustration in learning the second problem. Because training Part 1 took 180 trials and Part 2 took only eighty trials, however, it is evident that few incorrect choices can have been made in learning the second problem. Whatever extra frustration was experienced may be reflected in the non-significant difference between the magnitude of the scores for the one and two cue groups (Table 2).

The finding that the partially rewarded groups were much more resistant to extinction than the consistently rewarded groups can be accounted for by Amsel's theory, on the basis of the differing degrees of frustration experienced during training. On Sutherland's theory, it can be accounted for on the basis of frustration induced irrelevant learning. It seems difficult, however, to maintain the latter view in face of the lack of differentiation between the one and two cue groups. If Sutherland's<sup>3,4</sup> hypothesis were correct, the more cues learned about during acquisition, whether or not frustration is involved, the greater would be the resistance to extinction, on the assumption that all cues learned about have to be extinguished separately, and that this process takes longer even if each cue is learned about to a lesser extent. The most reasonable conclusion is that resistance to extinction is determined primarily by frustration tolerance, and that the demonstrable<sup>5-7</sup> correlated incidental learning is a side effect which plays no part in extinction. Such a view would be consistent with the finding<sup>6</sup> that competing responses, artificially facilitated during extinction, do not affect the extinction rate.

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Table 1. DISCRIMINANDA USED DURING ACQUISITION AND EXTINCTION

Reward schedule	Group	Training Part 1	Training Part 2	Testing Extinction
CR 100 per cent reward on correct choices	1a	BW	BW	BW
	b	HV	HV	HV
	2a	BW	HV	BW + HV
	b	HV	BW	BW + HV
PR 50 per cent reward on correct choices	3a	BW	BW	BW
	b	HV	HV	HV
	4a	BW	HV	BW + HV
	b	HV	BW	BW + HV

CR, Consistently rewarded. PR, Partially rewarded. BW, Black versus white x 'Perspex' squares on a grey background. HV, Horizontal versus vertical 1 cm wide black and white stripes. BW + HV, BW squares on HV background.

Table 2. EXTINCTION DATA

Training	Group	Mean no. trials to criterion	Mann-Whitney	Mean per cent correct in last 20 ext. trials
CR, 1 cue	1	101.1	N = 19	58.0
CR, 2 cues	2	115.6	U = 25 N.S.	57.5
PR, 1 cue	3	205.7	N = 20	64.5
PR, 2 cues	4	207.8	U = 44 N.S.	62.0

## Attempts to improve Perceptual Clarity in an Aircraft Display

A VISUAL display recently developed for aircraft<sup>1</sup> offers three principal improvements for pilots. First, it is projected at windshield eye-level so that the pilot need make fewer changes in the direction of visual gaze (from "head-up", outside the cockpit, to "head-down", inside). Second, it is collimated, so that the pilot can fixate both display and external view with his eyes accommodated to physiological infinity. Third, the display is capable of presenting the simplest visual picture compatible with the current task of the pilot, so that it can exploit the unique ability of man to follow pictorial information without burdening him with rapidly changing numerical data. Fig. 1 shows the three basic elements of such a display.

A dot at the apex of a triangle consisting of four lines tells the pilot, usually from computer sources, where he is being asked to fly. The centre of a small circle informs him



where he is flying at the moment. The two halves of a broken line give him information about his aircraft's attitude relative to the horizon. His basic task in directed flight is thus to capture a dot at the centre of a circle while paying attention to aircraft rolling and pitching. The display is appreciated so naturally that pilots with no previous relevant experience can readily control both displacements in height and heading as well as rotations in roll and pitch simultaneously.

To evaluate possible changes in display element geometry, four experiments were run, each asking between eight and twelve jet pilots to control simulated flight missions in an aircraft cockpit mockup. Display changes were presented according to statistical designs aimed at balancing out any sequence effects such as learning and fatigue. As well as normal measures of system error, pilot opinion was also obtained based on the assumption, previously justified in aircraft handling studies, that trained pilot opinion is an indication of the effort expended in achieving a measured accuracy<sup>2-4</sup>. Response times to occasional warning light signals gave a measure of the general attention level of the pilots. Fig. 2 shows the display element varieties and conditions.

By any operational criterion, a dot to indicate flight commands was entirely satisfactory, but a small circle allowed even greater precision. Clearly, it has less possible displacement inside the larger circle which denotes the current flight situation. By analogy, one would expect a perfectly fitting cross to be still superior, but this was not the case. Pilots complained that when demands were satisfied, the cross combined perceptually with its containing circle, leaving them in some confusion as to which of the two elements was in their control.

Adding stub wings to the circle representing the current flight situation allowed pilots to identify control more easily with this element, because it now resembled a simplified aircraft sketch. In addition, such stub wings permitted roll control with less effort, by improving roll angle perception. The length of wing, however, turned out to be inconsequential.

Removing the four-line triangle which moves in formation with the flight command dot proved detrimental to performance and unpopular with pilots. Replacing this element with any one of the three illustrated was without effect on accuracy or opinion, however. Here is a second

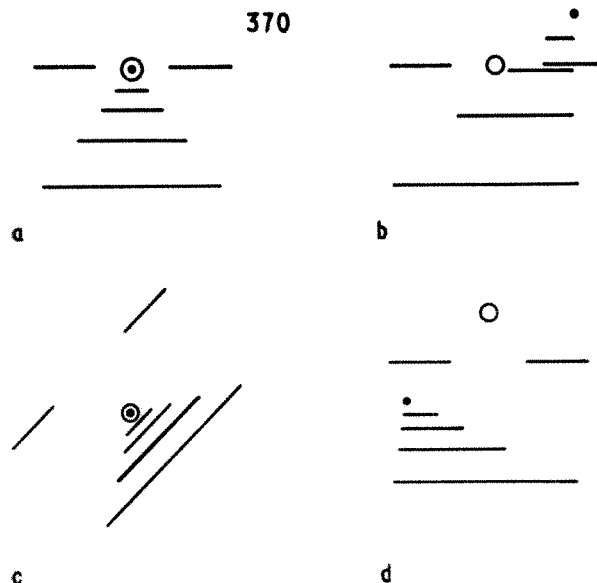


Fig. 1. The basic display. *a*, The aircraft is in straight and level flight with all demands satisfied. The position of the numerical height scale, when added, is at the top right-hand side. *b*, The aircraft is straight and level. The situation requires a climbing turn to starboard. *c*, Correctly following a demand to execute a descending turn to starboard. *d*, Climbing and straight. Demand to execute a descending turn to port.

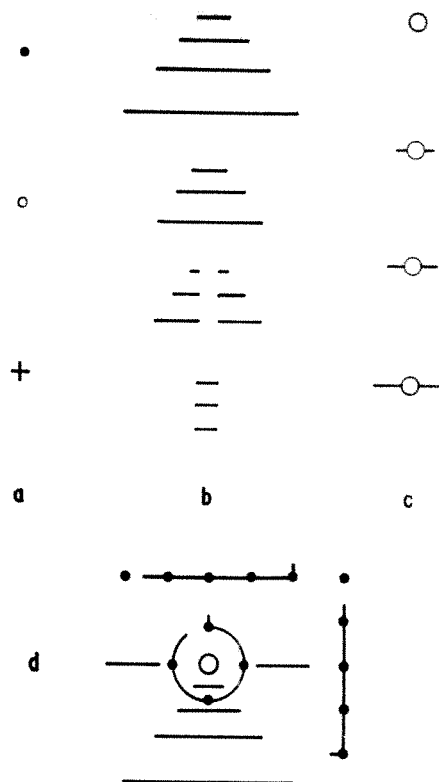


Fig. 2. Varieties of display elements and additions. *a* and *b*, The two halves of the element denoting "where to fly". 12 possible combinations. *c*, Elements denoting "where you are flying". *d*, Range scales, showing distance to go (up to 12 miles) to runway, radio beacon, or target.

case of a symbol change producing no observed effect on the perceptual quality of the whole figure.

None of the three range scales emerged as overall best for pilots trying to control distance to go or closing speed. The circular one was preferred, probably because it surrounds the central tracking task at a set distance whatever value it indicates, and allows pilots to capitalize on clock familiarity if, as here, a maximum indication of 12 miles is appropriate.

The addition of a numerical height scale (at the top right of the display) did not appreciably detract from tracking accuracy, and allowed pilots to control as directed while retaining the ability to check actual aircraft height as a safety precaution without looking down at conventional instruments. Such a finding supports the impression that a pictorial display is easily assimilated, and puts relatively small informational demands on the perception of the pilot.

Clearly, this is a difficult experimental area. The lack of a quantified perceptual theory means that the psychological work required of pilots by the addition or modification of a given visual stimulus cannot be predicted at all well. There remains the distinct possibility that several perceptually equivalent displays exist, each one perhaps offering some electronic or servicing advantage over the present one.

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<sup>1</sup> Naish, J. M., *Nature*, **202**, 641 (1964).

<sup>2</sup> Cooper, G. E., *Aeronaut. Eng. Rev.*, **16**, 47 (1957).

<sup>3</sup> Summers, L. G., and Ziedman, K., *NASA, Washington D.C., CR-125* (1964).

<sup>4</sup> Wasicko, R. J., and Magdalen, R. E., *USAF Aerospace Med. Res. Lab., W-P AFB, Ohio, AMRL-TR-65-158* (1965).

## BOOK REVIEWS

### TEACHING ENGINEERING

#### **Engineering at Cambridge University, 1783-1965**

By T. J. N. Hilken. Pp. xi + 277. (London: Cambridge University Press, 1967.) 45s. net.

IN recent years there has been much public discussion concerning engineering education in this country. Important decisions have been taken by the government and are still being taken. Universities, too, are faced with important decisions. It is therefore fortunate that Captain Hilken, administrator of the Cambridge engineering laboratory, has given us a history of the development of engineering at Cambridge which shows us that many of the problems facing us today have already existed in the past, and how they have been solved in what is probably the most important and successful centre of instruction in England.

Captain Hilken traces the pre-history of engineering in Cambridge back to the Jacksonian Chair. It was the intention of the founder that the holder of this chair should not only enquire into "those things which we cannot be without and on which not only our well being but our very existence in the world depends", but also engage in "something in the way of experiment on the subject undertaken". In particular, he wants him "to have an eye more particular to that opprobrium medicorum called the Gout" and hopes that he will find a cure. Hilken gives a detailed history of the foundation of science chairs in Cambridge, showing how it arises out of the scientific climate created by Newton. It was the first holder of the reformed Jacksonian Chair, Isaac Milner, who started the study of what would now be called engineering, and installed lathes, air pumps, furnaces, electrostatic appliances and so on, and the tradition was continued by William Farish and Robert Willis.

In the eighteenth century the university had fallen into a state of decay, and Hilken traces the history of reform from within, culminating in the appointment of Prince Albert as Chancellor in 1847, which came, however, too late, so that, to the disappointment of the latter, Parliament intervened.

This was perhaps the most crucial period for engineering in Cambridge; the subject had been taught in King's College, London, since its foundation in 1831, likewise in University College, London, where a chair in Civil Engineering was established in 1841. The country had fallen behind Germany, where the creation of technical universities had laid the foundation of German pre-eminence not only in technological education but also in scientific engineering. Hilken's book forms a most useful factual guide to the history of the developments in Cambridge, opening the way for further study of the social history of the pattern of decisions made in England. There were always many in the older universities who argued that engineering education should be developed in industrial areas, for the benefit of the region; many thought that engineering was not an academic subject worthy to be taught at Cambridge or Oxford.

There was great resistance to the creation and operation of workshops. The controversies around James Stuart, 1843-75, first professor of mechanism and applied mechanics, who established mechanical workshops in Cambridge make fascinating reading. Stuart supplied the money needed from his own private means. The workshops were run not only for the instruction of students but also

for profit, and this led to difficulties. It was the strong Newtonian tradition in Cambridge and the sound judgments of the great physicists and mathematicians there that saved the cause of engineering in Cambridge. It may be possible to gather even more information about this than Hilken has done, who does not aim at a work of history in the wider sense but rather at a "biography" of the engineering department.

Right up to the present time the case for engineering teaching and research in Cambridge and Oxford had to be argued; for example, in 1956, the general board of the University of Cambridge published a report containing the suggestion that the teaching of applied sciences might best be left to universities in industrial areas. Professor Baker at that time called this statement a gratuitous blow to those in this country who were working with an eye to national needs and to the development of a proper balance in university education. The remarkable growth and flowering of engineering in Cambridge owe much to private munificence, not only from outside sources ranging from Lord Nelson of the English Electric Company to Sir Dorabji Tata, the Indian industrialist, but also to private funds expended by professors and teaching staff.

The question as to what should be taught in engineering courses has been vigorously discussed for a long time. From the days of Stuart, who laid great emphasis on workshop training, to the present time, the syllabus has undergone great changes. The great distinction of Cambridge has been the close connexion of these studies with mathematics and, after a while, to a lesser degree with physics. It is only recently that the development has come to a full circle, and that modern developments again need the grass roots of pure physics. Electrical science has only recently been established as a separate discipline in Cambridge through the efforts of Professor Oatley. The groundwork had been laid largely on the initiative of the Institution of Electrical Engineers.

Despite this development, however, it is characteristic of the Cambridge engineering laboratory that it remained unspecialized, at a time when other universities awarded separate degrees in many proliferating branches of engineering and when technical universities abroad went in for an ever increasing degree of specialization. This trend is now considered in many places here and abroad to have over-reached itself, and a difficult path of return to a broader training, more closely based on physics, is sought. C. E. Inglis, Professor Baker's predecessor, admirably stated in 1941 that "premature specialization cramps the imagination, that a university course of engineering should concentrate on teaching those essentials which, if not acquired at that stage, will never be acquired. The good of education is the power of reasoning and the habit of mind which remains, when all efforts of memorization have faded into oblivion."

It is, however, clear from Hilken's book that a bar to progress was removed when the engineering school freed itself from the mathematical tripos on the suggestion of Bertram Hopkinson in 1912, establishing an examination in mathematics, Part I, suitable for engineers. The problem of how to fit a reasonable degree of specialization into a general engineering course was solved in ever changing ways. The problem is linked with the sorting of men into streams with different abilities.

Hilken's book tells the story of the evolution of Parts I and II of the tripos, of the papers set for the ordinary degree taken by candidates who were not strong enough for the tripos, the splitting into *A* and *B* papers, and the establishment of a slow and fast course, when the subject of engineering studies designed for weaker men was abolished. There is a lot to be learned from Cambridge experimentation with these problems.

The actual content and quality of the teaching are known to me somewhat indirectly. When I came to teach at Oxford after a ponderous German style engineering education, I found the place colonized by such Cambridge

men as R. V. Southwell and E. B. Moullin, and through them became acquainted with the tradition of Cambridge teaching only partly embodied in texts such as Ewing's *Heat Engines* or Lamb's notes on *Electricity*. It seemed like light champagne compared with the dark German beer of my own upbringing.

Even before the First World War the number of engineers in Cambridge was large (about 260), and although the expansion after the Second World War seems very large, it is only a smooth continuation of the pre-war curve through the war gap. The growth of postgraduate studies and research is perhaps more spectacular.

The practice of engineering is always in danger of falling back on crude empiricism when problems get difficult. There does not exist a pre-formed science of physics which needs only to be applied to solve such problems. The physical phenomena underlying the engineering devices have their own distinct laws which, although they are no doubt a sub-set of the laws of physics, are not readily deductible from the basic set. Particularly now, when physics is so much concerned with elementary particles, the nucleus and fine details of spectroscopy, much research is needed to serve industry—research in engineering science which is nothing else but the physics of devices. In Cambridge such men as G. I. Taylor, C. E. Inglis, J. A. Ewing, B. Hopkinson and many others have developed and maintained the tradition of a fundamental approach to engineering problems. We are indebted to Captain Hilken for making available to us many interesting facts from their biographies in the form of a consistent story.

H. MOTZ

## TALKING BY SATELLITE

### Communication Satellite Systems Technology

By Richard B. Marsten. (Progress in Astronautics and Aeronautics, Vol. 19.) Pp. xx+1,051. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1966.) 96s.

THE historically minded may wish to compare this volume, made up of papers given at a Communications Systems Conference of the American Institute of Aeronautics and Astronautics held in Washington in 1966, with the report of an international symposium on space radio communications held in Paris (under the auspices of URSI) in 1961<sup>1</sup>. The problems remain much the same, but progress has been substantial.

Because I could not judge all the diverse contents of this book, I asked colleagues for opinions. They found the sections on Early Bird excellent, the sections on attitude control good, the sections on electronics and antennae informative to those not familiar with the state of knowledge, and a section on deep space optical communication misleading. One colleague concluded: "This is the best book of its kind I have seen, but it could be improved greatly if reduced to about half its present bulk by leaving out the filler sections on 'systems analysis' and the various propaganda articles which introduce nothing that is new and come to no useful conclusions."

To telephone users, the adverse effect of the delay inherent in a synchronous satellite system is important. Barstow's paper gives the impression that this is small while Helder implies that it is considerable. Both papers are based on tests with Early Bird. Psychologists have tackled this problem through a variety of experiments which indicate the effect and its source, not in the delay itself, but in the operation of echo suppressors<sup>2</sup>.

Communication satellites are important because electrical communication is already so important in our lives. They cannot (except through direct broadcast to home receivers) provide a communication service in themselves. Yet, among the authors, agencies and companies

which actually supply communication service to individual users are scarcely represented—indeed, such representation was greater in the 1961 URSI symposium. I believe that this puts discussions of the profitable uses of satellite communication out of perspective.

Marsten asserts that a Titan III-C can launch 3,500 lb. into synchronous orbit, yet he talks of Comsat's plans for 1,200 voice channels; a 3,500 lb. satellite could supply 100 times as many. Pardoe and Steines concentrate on international communication. In the foreseeable future there is bound to be more national than international communication, and 100,000 economical voice channels could have a profound effect in the communication network of a geographically extensive country (or region). In the same paper they find Russia "an enigma", yet say that the Soviet minister of communications "sees that a national system of telecommunications and television can be economically profitable, especially over long distances, and that any new system would not be opposed to existing terrestrial communication systems".

Technologically, satellite communication has progressed greatly between 1961 and 1966. But it remains difficult to integrate this resource with others in providing man with effective communication, national and international. *Communication Satellite Systems Technology*, good as it is, is in many ways a monument to the insularity of that art.

J. R. PIERCE

<sup>1</sup> *Space Radio Communication* (edit. by Brown, G. M.) (Elsevier, Amsterdam, New York, London, 1962).

<sup>2</sup> Kraus, R. M., and Bricker, P. D., *Acoustical Soc. Amer.*, **41**, 286 (1967).

## PHYSICAL GASDYNAMICS

### Physics of Shock Waves and High-Temperature Hydrodynamic Phenomena

By Ya. B. Zel'dovich and Yu. P. Raizer. Edited by Wallace D. Hayes and Ronald F. Probstein. Vol. 1. Pp. xxiv+464. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1966.) 144s.

THE second Russian edition of this book was published in 1966. The first volume of the English translation containing rather more than half of the Russian text appeared almost simultaneously. The second half is due to appear in English translation shortly. This is a most commendable feat and is largely due to the close collaboration between authors and editors.

Books on physical gasdynamics are appearing at a steadily increasing rate, but the present text stands out as a particularly valuable contribution. Little time is spent on the elementary background and the authors quickly move into the advanced parts of their subject. This does mean, of course, that few readers can absorb the material without having to consult other texts, and it is therefore difficult to define the audience for which the book is suited. I can, however, fully endorse the editors' remark that it is "well suited for researchers, engineers, students and professors", and might add that they will all find it thoroughly enjoyable.

The first chapter reviews the fundamentals of one-dimensional (one space co-ordinate) unsteady gasdynamics and shock waves, including the effects of heat conduction and viscosity but without going into the detailed effects of varying molecular properties of the fluid.

The second chapter is an excellent introduction to the macroscopic theory of radiation gasdynamics, discussing radiative transfer and its effects on moving fluids. The third chapter is a concise introduction to the equilibrium properties of gases at high temperatures and is followed by a brief chapter on shock tubes and their instrumentation.

The fifth chapter returns to the subject of absorption and emission of radiation with a detailed discussion of spectra and the molecular aspects of the interaction between radiation and matter. Included is an up to date discussion of lasers.

The final chapter in this volume deals with the rates of relaxation processes in gases, including vibration, dissociation, chemical reactions, electronic excitation, and ionization.

Throughout the text the physical approach to the subject dominates, and the theory, although at times necessarily complex, is kept to the simplest level consistent with the requirements of rigour. Particularly pleasing is the continuous supply of numerical examples. This is most important in a subject in which it is difficult to keep track of the orders of magnitude of the quantities involved. There are very comprehensive references to both Russian and western literature.

The text follows very closely the Russian original, except for changes in notation, and the translation is excellent. Any minor modifications appear to have been agreed between authors and editors and in addition a number of editors' comments appear as separate footnotes.

I look forward to the second volume, which will contain chapters on shock wave structure, radiation in shock waves, chemical kinetics, thermal waves, and shock waves in solids, and conclude with a chapter on self similar processes in unsteady gasdynamics.

N. H. JOHANNESEN

## UNIFIED METALLURGY

### An Introduction to Metallurgy

By A. H. Cottrell. Pp. x+548+8 plates. (London: Edward Arnold (Publishers), Ltd., 1967.) 60s. net.

I DID not have the good fortune to be a university student under Dr Cottrell, but I did "sit at his feet" for more than three years while he was at Harwell. During this time I never ceased to be amazed at the clarity of his exposition of complex inter-related ideas and the way, as with fine tweezers, he drew out the significant factors in a problem. After a Cottrell explanation one felt that one understood the whole problem as though it were a simple piece of arithmetic. Unfortunately, a few hours later, one's understanding of the problem became hazy and a tremendous amount of hard work was required to get to the basis of the explanation. After reading this book in a long full day session I am left with the same kind of feelings. The subject is beautifully presented in a clear logical way and for a brief time one understands all those complex problems, but there is a lot of very hard work required to really understand these subjects in depth. To take one example: the idea of lattice defects called dislocations is introduced in a simple, clear and fascinating way, but within the space of a few paragraphs mention is made of slip and twinning dislocations, elastic energy of dislocations, Peierls-Nabarro stress, movement of edge, screw and mixed dislocations, Burgers vectors, Frank-Read source and multiple cross glide, dislocation networks, partial dislocations, and sessile and stair-rod dislocations. In my experience all these topics have to be described in great detail to undergraduate students and the three dimensional concepts may take many months to "sink in". In this sense I do not think that this book provides a satisfactory basic text for students of metallurgy.

The book is, however, an excellent expanded syllabus for a properly balanced metallurgy course. Dr Cottrell has been responsible for many great changes in the teaching of metallurgy and, in particular, has emphasized the need for a basic scientific understanding of the subject. His earlier book *Theoretical Structural Metallurgy* laid

some of the foundations for this approach and, as Dr Cottrell notes in his preface, the science has now become well established and triumphant. He goes on to emphasize the need to reassert the unity of all metallurgical knowledge and to link up this science with the more traditional aspects of the subject. This is not just a pipe dream, for in much of his own work Dr Cottrell has demonstrated the technological and economic potentialities of bringing together the understanding provided by a scientific approach and the broad metallurgical knowledge developed through hundreds of years of experience.

The basic plan of the book is to outline successively scientific principles relevant to metallurgical practice and, in the same chapter, whenever this is appropriate, to relate these principles to practice. To give one example, the chapter on heat and energy starts with some simple thermodynamics, continues by applying these ideas to the combustion of fuels used in metallurgical processes and ends with a purely descriptive account of furnaces used in metallurgical practice. Of necessity some chapters are almost entirely scientific while others are entirely descriptive.

The essential unity mentioned earlier is demonstrated continuously and all metallurgists will find the interplay of disciplines refreshing and illuminating. Once again I must pause to emphasize that in my view it is difficult to teach the subject in this way. The best use of this book would be as essential reading for students who are completing their degree or equivalent course rather than students new to the subject. I would add one qualification to this generalization—the bright, intelligent and enthusiastic student will be immensely stimulated by this book. He will see new dimensions and will be encouraged to search deeper into the basic principles of the subject with the aid of the useful list of further reading at the end of each chapter. The majority, however, could easily fall into the trap of collecting a mass of incoherent facts because they lack the discipline and ability to search out and understand each topic as it is presented.

This book forms a comprehensive survey of metallurgical knowledge covering all aspects of metallurgical science and technology. There is little which has escaped mention and with the aid of a good index it is possible to read up a description of any topic very easily.

DEREK HULL

## SYMMETRY AND FIELDS

### Brandeis University Summer Institute in Theoretical Physics, 1965

Edited by M. Chretien and S. Deser. Vol. 1: *Axiomatic Field Theory*. Pp. xi+516. \$32.50. Vol. 2: *Particle Symmetries*. Pp. xi+691. \$35. (New York: Gordon and Breach, Science Publishers, Inc.; London: Blackie and Son, Ltd., 1966.)

THE Brandeis University Summer Institute in Theoretical Physics is by now an established annual event, and the 1965 session on particle symmetry and axiomatic field theory maintains the high standard we have come to expect from this school.

The first volume covers axiomatic field theory, comprising major expositions, running to 100–150 pages each, on analytic properties of scattering amplitudes in quantum field theory, the connexion between Wightman and LSZ quantum field theory, algebraic aspects of relativistic quantum field theory and relativistic invariance and internal symmetries by, respectively, H. Epstein, K. Hepp, D. W. Robinson and L. Michel. Finally, there is a rather short contribution by R. G. Newton on the three body scattering problem, which does not pretend to mathematical rigour and only just about gets to the Fadeev equations.



All these authors are great experts in their respective and overlapping fields which require considerable familiarity with some highly non-trivial tools in functional analysis and generalized function theory. The lectures of Epstein, Hepp and Robinson are reasonably self-contained, whereas that of Michel requires knowledge of his older Istanbul course on group extension. Hepp's course has the strongest physical basis and can be strongly recommended as an exposition of the Haag-Ruelle scattering theory; by way of introduction non-relativistic scattering is treated in detail. There is good reason to think that the mathematics of these lecture courses will play an increasingly important part in all future physics and that it constitutes the best possible training for the younger theoreticians.

The second volume is much less coherent. There are two highly skilled introductory expositions, "Weak Interactions" and "Symmetries and Elementary Particles", by N. Cabibbo and F. Low respectively. A longish contribution by R. Cutkosky examines "Boot Strapping Models". This is an extremely readable account of a rather obscure subject, obscure because of the widely differing assumptions current in this work. A few of the other contributions have lost some topical interest, such as the review of SU(6) by B. W. Lee and the lectures by T. D. Lee on "Possible C-non-invariance in the Electromagnetic Interactions". Finally, there are lectures with strongly phenomenological flavour, by A. H. Rosenfeld, on "Mesons". This subject is in rapid flux and is constantly being reviewed again. It is of course unavoidable that some of the work presented at summer schools should be overtaken by events before the publication of the lectures. There remains enough first class material in these lectures to justify the issue in book form—that some of the proof reading is sloppy is regrettable.

There are paperback editions of each volume available at \$6.50. S. ZIENAU

## SPECTRA AND SURFACES

### Infrared Spectra of Adsorbed Species

By L. H. Little, with supplementary chapters by A. V. Kiselev and V. I. Lygin. Pp. xii + 428. (London: Academic Press, Inc. (London), Ltd.; New York: Academic Press, Inc., 1966.) 100s.; \$16.50.

THE study of the adsorption of molecules on surfaces is of technological importance in fields such as chemical catalysis, lubrication and adhesion. It is also a subject where scientific knowledge and understanding are still remarkably limited in relation to what is interesting import.

In many areas of chemistry much progress has been made during the past fifty years by the application of the diffraction (particularly X-ray diffraction) and spectroscopic methods of structure determination. The X-ray method depends mainly on the presence of large three-dimensional ordered arrays of molecules, and these are not to be found in monomolecular surface layers. Electron beams can usefully interact with layers a few atoms thick. The method of low energy electron diffraction (LEED) has thus recently made some useful contributions to surface studies; but there is still uncertainty about the interpretation of the results obtained by this technique, and its application appears to be limited to the relatively few cases where very high vacua can be achieved. Applications of spectroscopic methods, which can provide valuable information about the structures and environment of molecules but do not require ordered systems, are therefore of even greater importance in this field than they are elsewhere in chemistry. Most of the spectroscopic methods have made contributions to the study of adsorption. The versatility of infra-red spectro-

scopy, and in particular its ability to obtain information from small samples in all the states of matter, has led to a particularly extensive use of this method on adsorbed molecules.

This book is the first to be published specifically on the spectroscopy of adsorbed molecules. It surveys the results of infra-red studies up to the time of its completion (late 1965).

The interpretation of infra-red spectra of chemisorbed molecules is not an easy matter, partly because some surface species may not find ready analogues among isolated chemical compounds, and partly because there is no guarantee that a particular spectrum does not contain contributions from more than one species. This means that neither the literature itself, nor this comprehensive review of it, makes easy reading for the non-spectroscopist. The author has therefore wisely commenced with an introductory chapter on general principles in which he has also cited some of the typical successes of the method; this should tempt the non-spectroscopic surface chemist to explore the remainder of the book for the significant results in his field of interest. The second chapter provides a clear account of the experimental techniques used in this type of work. The next six chapters are concerned with the spectra obtained from small molecules (carbon monoxide, nitric oxide, carbon dioxide, hydrogen and nitrogen) chemisorbed on a variety of surfaces; and from hydrocarbons and other organic molecules similarly adsorbed on metals or on oxides. The final brief chapter in this section discusses a number of general spectroscopic topics concerned with chemisorption and catalysis.

The second part of the book is concerned largely with infra-red spectra from physically adsorbed molecules on oxide surfaces (particularly with respect to interactions with surface hydroxyl groups) and on related adsorbents such as clay minerals and zeolites. For physical adsorption the interpretation of the spectra is more straightforward, for this leads only to perturbations of spectra familiar in other phases. Infra-red spectroscopy has contributed greatly and directly to an understanding of the nature of physical adsorption on such surfaces. A valuable feature of this part of the book consists of contributions from two Russian authors, Professor Kiselev and Dr Lygin, in the form of separate chapters or supplements to those written by Dr Little. Much of the pioneering spectroscopic work, particularly on physical adsorption, was carried out by the late Academician Terenin and his colleagues in Leningrad and in a number of cases this work is only available in Russian language journals. It is therefore particularly valuable that these and later substantial contributions have been authoritatively reviewed by the Russian co-authors.

In summary, this book provides a welcome, comprehensive and fairly critical review of the whole field of infra-red spectroscopic investigations of adsorbed molecules; the only notable omission noticed concerned a number of publications from the Italian school under Bonino and Fabbri. It is a very valuable reference book for specialists in this field, and should be of interest and use to many other surface chemists.

N. SHEPPARD

## TOPOLOGICAL TEXT

### Elementary Topology

A Combinatorial and Algebraic Approach. By Donald W. Blackett. (Academic Press Textbooks in Mathematics.) Pp. ix + 224. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1967.) 76s.

THIS is one of a number of attractive textbooks beginning to appear on the market which are designed to foster the undergraduate student's interest in algebraic (that is non-point-set) topology without making heavy demands on

his technique. Its presentation, which is informal and incorporates many beautiful pictures, gives one the feeling "this isn't going to hurt". Unfortunately, many readers will suffer agony before reading the last chapter. Much of this is because of the sheer difficulty of the subject—each problem has to be tackled with bare hands and technical details have to be glossed over, with consequent loss of clarity. There does seem to be, however, a general lack of motivation; for example, in the chapter on network theory, the reader is led through pages of difficult calculations involving the boundary operator, without being previously told what they are for. There are seven chapters; in the first two, compact surfaces are classified by triangulation and in the third there are applications to Riemann surfaces. Winding number, the degree of a map, the Brower fixed-point theorem and the Borsuk-Ulam theorem for surfaces, with applications to complex analysis, occupy chapter four. The fifth treats vector fields and their singularities. The sixth and seventh, respectively, cover network theory and some topology of 3-manifolds. There are about three pages of exercises at the end of each chapter. The book would certainly be of value to anyone teaching topology at this level.

C. B. RAYNER

## TEXT FOR GEODESISTS

### Physical Geodesy

By Weikko A. Heiskanen and Helmut Moritz. (A Series of Books in Geology, edited by Gilluly and Woodford.) Pp. vi + 364. (San Francisco and London: W. H. Freeman and Company, 1967.) 110s.

NINE years ago one of the authors of this book, Professor Heiskanen, and the late Professor Vening Meinesz wrote *The Earth and its Gravity Field* which was widely acclaimed. Until then the application of measurements of the intensity of gravity to the study of geodesy, and the determination of the Figure of the Earth, was largely based on the well known theorem of Stokes, first published in 1849. The great disadvantage of this approach is that gravity anomalies have to be reduced to a co-geoid which is a bounding equipotential surface before the theorem can be used, necessitating assumptions as to the density of the Earth's topography which has to be computationally removed. In 1945 Molodensky proposed a new approach which used ground level gravity anomalies without further reduction; in the last ten years or so many scientists have followed and further developed such methods. Consequently Heiskanen and Moritz have written a book which is completely new, rather than a revision of *The Earth and its Gravity Field*. The major difference between the new book and the old is that the new one describes this modern theory and, as a consequence, there is no space for any of the chapters on the geophysical aspects of gravity which formed a large part of the old book.

The first five chapters cover the material for a basic course in physical geodesy. The first chapter is entitled "Fundamentals of Potential Theory". It is complete and well explained although many long derivations of formulae are omitted and the reader is referred to standard textbooks. The second chapter is entitled "The Gravity Field of the Earth"; this again is well presented and includes full discussions of the generalized Stokes formula and the determination of the physical constants of the Earth. The third chapter, "Gravimetric Methods", describes the various conventional forms of reduction and the practical determination of the geoid, including the theory and use of isostasy.

The fourth and fifth chapters, "Heights above Sea Level" and "Astro-geodetic Methods", formulate the reductions necessary to height and astronomical measurements to bring them to the geoid or ellipsoid. Although there are paragraphs on accuracy and the effect of gravity and

density errors on height, there appears to be nowhere in the fourth chapter a reasoned statement as to when normal or theoretical gravity may be used instead of measured gravity without significant loss of accuracy. The authors, however, state in their preface that the book "is intended to be theoretical in the sense in which the word is used in the term 'theoretical physics'", so they are being consistent. A corollary of this is that there is no chapter on instruments or the measurement of gravity.

The remaining four chapters present more specialized and advanced topics. The sixth chapter gives formulae for the "Gravity Field outside the Earth". The seventh chapter gives a short discussion on "Statistical Methods in Physical Geodesy" which are in wide use today, while the eighth chapter is entitled "Modern Methods for Determining the Figure of the Earth". If criticism has to be made, it is that these last two chapters are too short; for instance de Graaf-Hunter's "Model Earth" is merely referred to in one line. The ninth chapter devotes twenty-six pages to "Celestial Methods", including the use of the Moon as well as artificial Earth satellites; it thus necessarily gives but an outline of the subject matter.

Attention must be drawn to one important feature: the concept of the geoid is fully maintained, not rejected as obsolete, and geometrical interpretations are given of all modern methods, adding much to their understanding and appreciation. The authors thus show that the supposed dichotomy between the new thinking and the old is not nearly as great as some would imply, and a full reconciliation of the two approaches is made.

As is inevitably the case today, the most up to date information on all the topics discussed in the book can only be found in many and widely different journals or university publications, not all of which are readily available to the average geodesist. Almost every geodetic measurement depends in a fundamental way on the Earth's gravity field, and so the authors have performed a signal service to geodesy in collating such information in one volume, and presenting it in a logical form and sequence which is readily understandable. They are to be congratulated not only on producing a much-needed book which every geodesist should buy, but also on writing in such impeccable English, which is the native tongue of neither.

It remains to add that each chapter has a full list of references at the end, and that there is a detailed list of contents and a good index. The book is well produced and the numerous diagrams are very clear; this and the apparent lack of misprints show a happy co-operation between the authors and the publisher. A. R. ROBBINS

## POLYMERS RE-ISSUED

### High-Temperature Polymers

Edited by Charles L. Segal. Pp. viii + 197. (London: Edward Arnold (Publishers), Ltd.; New York: Marcel Dekker, Inc., 1967.) 70s. net.

IN 1966 Marcel Dekker, Inc., launched the *Journal of Macromolecular Chemistry*. By the time the second or third issues of the first volume appeared plans must have been advanced to replace this journal in 1967 by the *Journal of Macromolecular Science* published in four sections. In Britain the total subscription to this journal is \$154 and many libraries may await an assessment of its quality before ordering, for this is more than four times the price of the pilot journal of 1966. Part A of the *Journal of Macromolecular Science*, devoted to chemistry, is the direct successor of the 1966 journal. The first two issues consist of papers presented at symposia, one on high temperature polymers and one on polyaldehydes. Subsequent issues of Part A, Volume 1, contain contributed research papers.

The two symposium issues have now been re-issued separately as hard-backed volumes. The first is being reviewed here. Because all these publishing activities are of recent origin I have felt it advisable to draw the attention of readers to them. Those who receive or intend to order the new journal may not wish to duplicate material and expenditure by purchasing some issues again in hard-backed form.

This symposium on high temperature polymers was held by the American Chemical Society at a Western Regional Meeting in November 1965. It contains eight papers, an editor's introduction and one summary paper. About half of the papers take a general approach to their subjects, particularly those of Marvel and Van Wazer, so that the lapse of time since they were given does not detract from their interest. They show that, despite much work and progress, a great deal more is urgently needed in this challenging field. Other papers are reports on work in progress to which fresh results have since been added.

It is doubtful whether such a regional one-day symposium justifies this double publication, but if it is to be done it should be done in such a way that the book version has, in isolation, a lasting value. In his preface the editor speaks of the extensive discussion which followed the papers, but none of this is recorded in the book. A number of serious misprints occur which should have been corrected in such a re-issue; examples are the omission of scale numbers from thermograms and an error in equation (1) of Van Wazer's article. As a collection, the papers do not represent a deliberately systematic coverage of high temperature polymers. The addition of several more specially commissioned general articles and an index would have greatly enhanced the scientific value of this publication as a separate book. PATRICK MEARES

## HOW PROTEINS ARE MADE

### Techniques in Protein Biosynthesis

Vol. 1. Edited by P. N. Campbell and J. R. Sargent. Pp. xii+336. (London: Academic Press, Inc. (London), Ltd.; New York: Academic Press, Inc., 1967.) 80s.; \$15.

### Regulation of Nucleic Acid and Protein Biosynthesis

Edited by V. V. Koningsberger and L. Bosch. (B.B.A. Library, Vol. 10.) Pp 412. (Amsterdam, London and New York: Elsevier Publishing Company, 1967.) 140s.

THE first of these two books does not, as the title might suggest, contain precise details of techniques used to investigate protein biosynthesis, but contains a series of reviews with more or less emphasis on practical procedures. The first chapter, written by the editors, is an excellent summary of the experiments that have led to our present knowledge of the structure and biosynthesis of proteins. The second and third chapters, by A. von der Decken and J. R. Sargent, respectively, review experiments on protein biosynthesis in mammals. The fourth chapter, by M. S. Bretscher and O. W. Jones, reviews experiments that have elucidated the genetic code. Practical procedures are emphasized in the second, third and fourth chapters. K. S. Kirby, in the next chapter, after paying due attention to the importance of ribonucleic acids in protein biosynthesis, proceeds to present a valuable summary of methods of isolating RNA. The volume ends with thirteen pages of odd techniques with practical details. The exact purpose of this book is difficult to grasp, for no biochemist wants to make a routine procedure of solving the genetic code, or elucidating the mechanism of protein biosynthesis; and even if he did, the practical details presented would be inadequate. Nevertheless, articles such as these are essential for teaching advanced biochemistry, and it is lucky that leading research workers are willing to write them. I can

think of no better article on protein biosynthesis for a student than the first chapter of this book.

The second book contains thirty-three papers presented at a symposium in the Netherlands in June 1966, together with a summary of the meeting by H. Chantrenne. The authors include many stars from outside the Netherlands such as A. Kornberg, S. Ochoa, S. Spiegelman, F. Jacob, F. Lipmann, J. Bonner, J. Brachet and C. E. Sekeris. The book contains what were in mid-1966 the latest experimental results and ideas of most of the principal workers in nucleic acid and protein biosynthesis and their regulation. Progress in this area is not so rapid as it was a few years ago, and the volume will be invaluable reading for research workers for a year or so. The first of four sections is entitled "DNA Synthesis and its Regulation". It contains papers on the ultrastructure of the animal chromosome, and the biosynthesis and repair of DNA. The second section, "RNA Synthesis", is largely concerned with the mechanism of action of enzymes that synthesize RNA. The third section, "Protein Synthesis", contains papers on the mechanism of incorporation of amino-acids into polypeptides. The fourth section, "Regulation of RNA and Protein Synthesis", is the largest. Its topics include histones and other possible gene regulators; the regulation of protein synthesis in bacteria, yeast, liver and enucleate cells; and the effect of ecdysone on RNA and protein metabolism. J. M. BARRY

## NUTRITION AFTER BIRTH

### Utilization of Nutrients during Postnatal Development

By P. Hahn and O. Koldovsky. (International Series of Monographs in Pure and Applied Biology. Division: Zoology, Vol. 33.) Pp. xi+177. (Oxford, London and New York: Pergamon Press, Ltd., 1966.) 50s. net.

NEONATAL physiology and medicine must be reckoned among the growing points of knowledge at the moment, and some time ago the Czechoslovakian Academy of Sciences had the foresight to recognize this and to establish a special department of developmental physiology, which has now justly earned a reputation in many parts of the world. Had it not been for language difficulties, moreover, and the all too common parochial attitude to international literature, its fame might have been even greater.

The authors of this book have done the English speaking world a service by summarizing certain aspects of the work on developmental physiology, based particularly on studies made on the rat and to a lesser extent on man. They have, naturally, drawn largely, but by no means entirely, on their own experiences, and this is probably the most valuable thing they could have done, for they have freer access to the literature of Eastern Europe and Russia than most of the rest of us.

The authors have been concerned mainly with the nutritional aspects of development. This has involved a review of the way the nutrients are obtained, the amounts that have to be obtained and the way they are expended. In connexion with this last topic the authors rightly felt it necessary to consider thermal regulation in the newborn and its cost in terms of food. This was a service in itself, for it led them to review much of the earlier fundamental Czech and Hungarian literature on this subject. The authors' own experiments have largely been concerned with weaning rats earlier or later than the normal practice, and the part played by carbohydrate and fat in the normal metabolic sequences of development. This is the main theme also in the chapter on the human foetus and newborn which has been contributed by V. Melichar and M. Novak. Protein foods and protein metabolism have been relatively neglected, and the role of the gut in absorbing unchanged proteins and antibodies has scarcely been mentioned. This is a pity, for this is one of the most

fascinating aspects of developmental physiology and a very important one for survival in some species. There are 600 references, which, in a book of this length, gives cause for reflexion.

The rat has been a very productive animal in this field, for it is normally born in an immature state. Thus, many developmental changes, which cease long before birth in a sheep or a guinea-pig, take place after birth in the rat, and are consequently open to similar experimental investigation. The authors therefore did well to choose it and, short of marsupials, which have so far scarcely been studied from this point of view, they could hardly have chosen better. Perhaps the authors, with all their experience and access to a literature which is difficult for the rest of us to enjoy, could have made the book a little less factual and objective, and given us more of their own judgments and criticism. This, however, is becoming a more and more difficult thing to do, and the more literature one sets out to quote the more difficult it tends to become.

The ability to write books of this kind and to sponsor small conferences on subjects of international interest are two of the privileges of Czechoslovakian scientists, and they are making the most of them.

R. A. McCANCE

## CEREBROSPINAL TREATISE

### Physiology of the Cerebrospinal Fluid

By Hugh Davson. Pp. vii + 445. (London: J. and A. Churchill, Ltd., 1967.) 84s.

It is rare to find a book which can be said to be faultless, but it is almost true of Dr Davson's book, which has been eagerly awaited and now is found to fulfil all expectations. The only fault I could find was a transposition of figures 46 and 47, on pages 76 and 77.

Although the present work is based on Dr Davson's original book *The Physiology of the Ocular and Cerebrospinal Fluids* published in 1956, it shows so few traces of the old that it can justly be regarded as a new book devoted to the cerebrospinal fluid. If this should seem too narrow a field, a few minutes looking through the pages will disprove it. Pharmacology, electron microscopy, brain metabolism, renal and respiratory physiology are all included, so that the cerebrospinal fluid becomes integrated with other body fluids and processes in a concise and sparkling style.

Although designated as an introductory account of the physiology of the cerebrospinal fluid, it is very much more and must surely be regarded as the authoritative text. The author is world renowned for his experimental work on the cerebrospinal fluid and this experience is evident in his lucid explanations and critical analyses of the vast mass of experimental work on this subject. The production, composition, pressure and absorption of cerebrospinal fluid naturally occupy a good deal of the text, most of it referring to experimental work. There are, however, intriguing references to clinical material, largely gathered into the last three chapters, concerned with blood brain barrier, acid base characteristics of respiration and the cerebrospinal fluid pressure. The illustrations are clear and well placed with regard to the text, while there are more than 1,000 references from a wide range of sources.

Dr Davson's book arrives opportunely at a time when a great deal of interest is being focused on the cerebrospinal fluid, especially among clinicians, who will find in this work the answers to many of their queries and, perhaps even more important, will find that a large number of questions are raised. Dr Davson is to be congratulated on a work of scholarship and this book can be recommended unreservedly.

EDWARD HITCHCOCK

## OBITUARIES

### Professor J. A. Chalmers

JOHN ALAN CHALMERS, who died on March 14, was born in 1904 within the sound of Bow Bells and was a Foundation Scholar of Highgate School, from which he won an open scholarship in natural sciences to Queen's College, Cambridge. He went up to Cambridge in 1923 and in the course of a distinguished undergraduate career gained first class honours in parts one and two of the natural sciences tripos. He then did research in radioactivity under Rutherford, but left in 1928 to take up a lectureship in physics at the University of Durham, where he spent the remaining thirty-eight years of his academic life. In 1932 Chalmers took up the study of atmospheric electricity, a subject which became his dominant research interest and to which he and his research students made many contributions. Those who knew Chalmers best can see how perfectly his chosen research fitted his love of the open air and his intense interest in natural phenomena. Chalmers' lively mind ranged over a wide field—point discharge phenomena, precipitation electricity, atmospheric ionization, and the Earth's electric field. Although at first he enjoyed only limited resources of money and equipment, by his persistence and enthusiasm he gradually built up a large research group with well equipped field laboratories at the department of physics in Durham and at the university observatory. With Pasquill he measured the electrical charges carried by raindrops, and with Whipple he worked out a theory of the capture of ions by falling drops, a theory later extended to ice crystals. With Hutchinson he investigated the mechanism of the charging of drops and ice crystals. He worked extensively on natural objects and, for example, demonstrated the part played by living trees in transferring electrical charge from clouds to the Earth. He also investigated the electrical field conditions of artificial points on masts and tethered balloons in various meteorological conditions. Some of the problems chosen were not perhaps the most fruitful because of their inherent complexity and the vagaries of the weather, but Chalmers made them challenging and stimulating, and as his resources increased he developed more powerful instrumentation. His more recent work was concerned with a theoretical analysis of the electrode effect at the Earth's surface and the identification of an unexpected negative potential gradient in conditions of mist and fog as the secondary product of corona discharges from distant electrical power transmission lines. His researches were not confined to atmospheric electricity; early in his career he wrote a series of papers on contact potential, and throughout his life he worked on problems in classical electricity and magnetism. He also gave vigorous support to the M.K.S. system of units. His output of papers was prolific, and he published about a hundred papers and two books on atmospheric electricity, the second of which is probably the best treatment of the subject in English.

Chalmers served Durham well in teaching and administration. He was well known to generations of students and his feats of memory were legendary. His detailed knowledge of degree regulations and his work on the modernization of first degree courses made him an almost automatic choice for dean of the faculty of science in 1965, an office which he held until his death. The university recognized his many contributions to its work by electing him to a personal chair.

In the wider life of the city and county, Chalmers' name was synonymous with scouting. He devoted all his spare time to this work, and at weekends and in vacations could be seen leaving Durham for a camping or climbing holiday with his touring car, or in later years his van, bulging with boys.

G. D. ROCHESTER  
W. C. A. HUTCHINSON



## University News :

### Birmingham

PROFESSOR R. B. HUNTER, at present professor of materia medica in the University of St. Andrews, has been appointed vice-chancellor and principal of the university.

### Bradford

MR D. P. HOWSON, at present senior lecturer in the Department of Electronic and Electrical Engineering, University of Birmingham, has been appointed to the additional chair of electrical engineering, and Dr J. B. Helliwell, at present reader in gas dynamics in the University of Strathclyde, has been appointed to the chair of mathematics.

### Cambridge

DR H. LEHMANN, university biochemist to the Cambridge United Hospitals, has been appointed to the chair of clinical biochemistry.

### Leeds

A NEW Division of Virology, under the headship of Dr Jaroslaw Czekalowski and within the Department of Bacteriology, has been created in the university.

### London

THE following appointments have been made: Dr R. F. L. Logan, at present reader in social medicine in the University of Manchester, to the chair of organization of medical care at the London School of Hygiene and Tropical Medicine; Professor W. R. Niblett, at present dean of the Institute of Education, to the newly established chair of higher education tenable at the institute; Dr. A. J. Rutter, reader in ecology in Imperial College, to the chair of botany tenable at that college. The title of professor of chemistry has been conferred on Dr C. A. Vernon, in respect of his post at University College.

### Sussex

PROFESSOR J. C. WEST, at present dean of the School of Applied Sciences, has been appointed as pro-vice-chancellor of the university for a period of two years from August 1; Professor T. B. Bottomore, at present head of the Department of Political Science, Sociology and Anthropology in the Simon Fraser University, British Columbia, has been appointed to the chair of sociology, and Mr. A. Kirk, at present director of the Computer Laboratory in the University of Liverpool, has been appointed director of the Computing Centre.

## Appointments

THE following appointments have been made by the Medical Research Council to its three advisory research boards: *Biological Research Board*, Professor A. S. V. Burgen of the Department of Pharmacology, University of Cambridge; Professor K. Burton of the Department of Biochemistry, University of Newcastle upon Tyne; Professor P. M. B. Walker of the Department of Zoology, University of Edinburgh. Professor J. L. Gowans of the Sir William Dunn School of Pathology, Oxford, already a member of the board, will succeed Professor W. D. M. Paton as chairman. *Clinical Research Board*, Professor A. W. Kay, regius professor of surgery in the University of Glasgow. *Tropical Medicine Research Board*, Professor W. Melville Arnott of the Department of Medicine, University of Birmingham; Dr D. J. Lewis of the British Museum (Natural History).

PROFESSOR H. R. HEWER, professor of zoology in Imperial College, has been appointed chairman of the new Farm Animal Welfare Advisory Committee, which has been set up to advise the Ministry of Agriculture, Fisheries and Food and the Scottish Office on matters involving the welfare of farm animals kept under intensive husbandry systems.

MR J. A. H. PAFFETT, at present holder of the chair of naval architecture at the Royal Naval College, Greenwich, has been appointed superintendent of the Ship Division of the Ministry of Technology's National Physical Laboratory. He is succeeding Mr A. Silverleaf, who is now deputy director (C) at the National Physical Laboratory.

## Announcements

THE *Royal Society Leverhulme Visiting Professorships* have been awarded as follows: Professor S. Devons, professor of physics and chairman of the Department of Physics at Columbia University, to visit the University of Andhra, Waltair; Professor D. Whitteridge, professor of physiology and head of the Department of Physiology at the University of Edinburgh, to visit the Vallabhbhai Patel Chest Institute, University of Delhi; Professor W. Williams, professor of plant science and head of the Department of Plant Science in the University of Newcastle upon Tyne, to visit the Agricultural College of Prague, the Faculty of Natural Science of the Charles University and the Institute of Experimental Botany of the Czechoslovak Academy of Sciences. The *Royal Society Visiting Professorships* have been awarded to Professor F. C. Champion, professor of physics at King's College in the University of London, to visit the University of Malaya, Kuala Lumpur, and Professor N. Tinbergen, professor of animal behaviour at the University of Oxford, to visit the University of East Africa.

DR S. H. SHAW, adviser to the Ministry of Overseas Development and in charge of the Overseas Division of the Institute of Geological Sciences, has been elected president of the Institution of Mining and Metallurgy for the session 1968-69.

THE Institution of Civil Engineers has awarded the *Telford Gold Medal* jointly to J. K. Anderson, J. A. K. Hamilton, W. Henderson, J. S. McNeil, Sir Gilbert Roberts and H. Shirley-Smith for their paper, "The Forth Road Bridge". The *James Watt Medal* has been awarded to A. R. Wilkinson; and the *George Stephenson Medal* to P. A. Scott.

THE Colworth Medal, which has been donated by the Unilever Research Laboratory, is awarded annually by the Biochemical Society to the most promising young British biochemist. Nominations are invited for the award of this medal for the year 1967, and further information can be obtained from the Honorary Secretary, The Biochemical Society, 7 Warwick Court, London, WC1.

# CORRESPONDENCE

## Projects for Schools

SIR,—Nigel Hawkes reports (*Nature*, 215, 122; 1967) that a boy at a certain British school carried out on mice experiments which, so far as a reader can judge, ought not to be done without a Home Office licence and Certificate A. Whether little or much discomfort was caused by the changes of temperature mentioned by Mr Hawkes, the matter is serious because it could be the thin end of the wedge. In America children are encouraged to repeat even severe experiments on animals, and British publishers have, to my knowledge, refused the British rights of at least three American books telling amateurs how to amuse themselves in this way. British scientists have a highly responsible tradition in such matters but there is strong American pressure on European education, and vigilance seems to be called for.

Yours faithfully,

C. W. HUME

The Athenaeum,  
Pall Mall, S.W.1.

## APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

**PRINCIPAL SCIENTIFIC OFFICER** (with a good honours degree, preferably in physics or in mathematics or engineering, and experienced in the administration of scientific work) to take charge of the SCIENTIFIC ADMINISTRATION GROUP at the Rutherford Laboratory which is the leading research establishment in elementary particle physics in the U.K.—Mr. G. N. Pickles, The Rutherford Laboratory, Chilton, Didcot, Berkshire, quoting Ref. 409/34 (August 17).

**RESEARCH ASSISTANT** to Professor D. J. Carr, Head of the DEPARTMENT OF BOTANY—The Secretary, Department of Botany, Queen's University, David Keir Building, Belfast 9, Northern Ireland (August 19).

**RESEARCH FELLOW** (with a Ph.D. degree and experience of analytical geochemistry) in the DEPARTMENT OF GEOLOGY to carry out analyses of carboniferous sedimentary rocks—The Registrar, University College of Swansea, Singleton Park, Swansea (August 19).

**TUTOR LIBRARIAN** (graduate with library qualifications and experience) in the Institute of Education—The Registrar, The University, Keele, Staffordshire (August 19).

**PRINCIPAL** (national of the United Kingdom or the Republic of Ireland, science graduate with wide industrial and technical college experience preferably including responsibility for laboratory technician courses, and preferably a knowledge of Portuguese) of the SCHOOL OF MEDICAL LABORATORY TECHNOLOGY, Federal University of Minas Gerais, Brazil—The Council for Technical Education and Training for Overseas Countries, Eland House, Stag Place, London, S.W.1, quoting Ref. TET/BRZ/12 (August 21).

**PRINCIPAL** (national of the United Kingdom or the Republic of Ireland, with a good degree in science or engineering and wide experience in technical education) of the NORTHERN TECHNICAL COLLEGE, Zambia—The Council for Technical Education and Training for Overseas Countries, Eland House, Stag Place, London, S.W.1, quoting Ref. TET/NDZ/12 (6) (August 21).

**COX CHAIR OF ANATOMY**—The Secretary, The University, Dundee, Scotland (August 26).

**RADIATION OFFICER** (preferably with a knowledge of radiation protection and electronics) in the DEPARTMENT OF PHYSICS—The Registrar, The University of Manchester, Manchester, 13, quoting Ref. 120/67/Na (August 26).

**RESEARCH FELLOW** (with computer programming experience, an interest in information retrieval, and preferably some knowledge of statistical methods) in MEDICAL DATA PROCESSING—The Registrar, The University, Keele, Staffordshire (August 26).

**LECTURER or ASSISTANT LECTURER in PSYCHOLOGY**—The Secretary, The University, Dundee, Scotland (August 31).

**LECTURER** (preferably with previous experience in model building for computer control) in CONTROL ENGINEERING—The Registrar, University of Bradford, Bradford, quoting Ref. L/COE/5/X (August 31).

**LECTURER** (with wide experience of general medicine, but previous experience in rheumatology not essential) in RHEUMATOLOGY—The Registrar, The University, Manchester 13, quoting Ref. 128/67 (August 31).

**LECTURERS/SENIOR LECTURERS in PURE MATHEMATICS** at the University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 31).

**ASSISTANT LECTURER** (with the M.B. or Ph.D. degree in the physiological sciences and preferably experience in teaching and research) in PHYSIOLOGY—Head of the Department of Physiology, University College, Merrion Street, Dublin 2, Republic of Ireland (September 1).

**ENTOMOLOGIST, SCIENTIFIC OFFICER** (with a first- or upper second-class honours degree and preferably postgraduate experience in entomology) to study resistance to aphids in sugar beet and other crop plants—The Secretary, Plant Breeding Institute, Maris Lane, Trumpington, Cambridge (September 1).

**LECTURER** (with basic training in bacteriology and pathology) in the DEPARTMENT OF BACTERIOLOGY—The Secretary, The London Hospital Medical College (University of London), Turner Street, London, E.1 (September 1).

**SCIENTIFIC OFFICER** (with a good degree and an interest in the developmental physiology of plants and in genetics) in the CYTOGENETICS SECTION to work on the developmental aspects of the present programme for the cytogenetic analysis of wheat—The Secretary, Plant Breeding Institute, Trumpington, Cambridge (September 1).

**LECTURER or ASSISTANT LECTURER in the COMPUTING LABORATORY** to give lectures on programming languages and techniques to undergraduate and graduate students and to advise computer users on the preparation of programmes—The Secretary, The University, Dundee, Scotland (September 2).

**LECTURER or ASSISTANT LECTURER in PHYSIOLOGY**—The Registrar, The University, Sheffield (September 2).

**LECTURER** (registered medical practitioner and preferably with some experience in hospital laboratory work, particularly in a biochemical department) in the DEPARTMENT OF CLINICAL CHEMISTRY—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh (September 2).

**RESEARCH FELLOW** (with a Ph.D. or equivalent) in PHYTOCHEMISTRY in the Postgraduate School of Studies in Pharmacy—The Registrar, University of Bradford, Bradford 7, quoting Ref. P/R/F (S)/1/E (September 4).

**SENIOR LECTURERS (2), and a LECTURER in the DEPARTMENT OF PHYSICS**, University of Ife, Nigeria—The Inter-University Council, 33 Bedford Place, London, W.C.1 (September 4).

**LECTURER or ASSISTANT LECTURER in BIOLOGY (Senior College); and LECTURERS (2) in BIOLOGY (Junior College)** at the Royal University of Malta—The Inter-University Council, 33 Bedford Place, London, W.C.1 (September 6).

**ASSOCIATE PROFESSOR in the DEPARTMENT OF PATHOLOGY; and a LECTURER/SENIOR LECTURER in PATHOLOGY**, University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 8).

**LECTURER in the DEPARTMENT OF SURGICAL SCIENCE**—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh (September 9).

**CHAIR OF SURGERY at Monash University**—The Secretary-General, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1; or the Academic Registrar, Monash University, Clayton, Victoria, Australia (Australia and London, September 11).

**CHAIR OF GEOGRAPHY at the proposed UNIVERSITY OF DUNDEE**—The Secretary, Queen's College, Dundee, Scotland (September 15).

**LECTURER** (with experience in industry or chemical engineering) in APPLIED CHEMISTRY at the University of Otago, Dunedin, New Zealand—The

Secretary-General, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, September 15).

**RESEARCH FELLOW** (with some experience in the application of computers to structural analysis and preferably completed work for a higher degree) in STRUCTURAL COMPUTING to work on the application of digital computer methods to the optimum design of multi-storey frames—The Registrar, The University, Manchester 13 (September 15).

**LECTURER in MICROBIOLOGY (non-clinical)**—The Secretary, The Queen's University, Belfast, Northern Ireland (September 30).

**LECTURER** (with medical qualifications and training in pharmacology) in PHARMACOLOGY in the DEPARTMENT OF MATERIA MEDICA AND THERAPEUTICS—The Secretary, The University, Aberdeen (September 30).

**READER** (preferably with interests in the fields of numerical mathematics or statistics) in MATHEMATICS; and a **SENIOR LECTURER or LECTURER** (with a higher degree in chemical engineering or a related discipline coupled with academic research or professional engineering experience; or a good honours degree and some years industrial experience, and preferably an interest in fluid mechanics or heat and mass transfer) in CHEMICAL ENGINEERING at the University of Canterbury, Christchurch, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (September 30).

**TEMPORARY RESEARCH ASSISTANT** (with a good honours degree in botany and some postgraduate training or research experience) in the DEPARTMENT of CELL BIOLOGY to work on plant cell hybridization—The Secretary, John Innes Institute, Colney Lane, Norwich, NOR 70F (September 30).

**TAXONOMIC MYCOLOGISTS** (with a good honours degree with postgraduate experience of the systematics of micro-fungi) in the Commonwealth Mycological Institute's identification service—The Secretary, Commonwealth Agricultural Bureaux, Farnham House, Farnham Royal, Bucks (October 15).

**HEAD OF THE STATISTICS DEPARTMENT**—The Secretary, Rothamsted Experimental Station, Harpenden, Herts (October 21).

**LECTURER in the SCHOOL OF CHEMICAL ENGINEERING**, University of New South Wales—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 30).

**ASSISTANT EXPERIMENTAL OFFICER** (with a degree or equivalent in horticulture, agricultural botany or a biological subject) for field and laboratory work with pesticides and soil organisms at the Field Station—Dr N. G. Hague, Imperial College Field Station, Ashurst Lodge, Sunninghill, Ascot, Berks.

**DIRECTOR (M.D. or Ph.D. with current knowledge and experience in biomedical engineering)** of the BIOMEDICAL ENGINEERING DEPARTMENT—The Executive Director, Ottawa Civic Hospital, 1053 Carling Avenue, Ottawa 3, Ontario, Canada.

**GRADUATE RESEARCH ASSISTANT in the DEPARTMENT OF CHEMICAL PATHOLOGY** to work on protein metabolism in brain by tracer techniques—The Secretary, Institute of Neurology (University of London), The National Hospital, Queen Square, London, W.C.1.

**RESEARCH ASSISTANT (biochemist or veterinary pathologist)** (preferably with postgraduate research experience and/or a higher degree, experience in immunological techniques, protein and enzyme chemistry and/or tumour transplantation in pure strain animals) in the IMMUNOLOGY CANCER RESEARCH UNIT—The Registrar, The University, Liverpool, 3, quoting Ref. RV/100/N.

**RESEARCH FELLOW or RESEARCH ASSISTANT** (physical chemist or biophysicist, preferably with postgraduate research experience and/or a higher degree) in the IMMUNOLOGY CANCER RESEARCH UNIT—The Registrar, The University, Liverpool, 3, quoting Ref. RV/99/N.

**RESEARCH SCIENTIST** (with a Ph.D. degree in science or postgraduate research experience of an equivalent standard and duration, preferably in the field of ecology, supported by satisfactory evidence of research ability, and preferably experience with animal ectoparasites or disease vectors) in the DIVISION OF WILDLIFE RESEARCH, Commonwealth Scientific and Industrial Research Organization, Canberra, to undertake a study of the epidemiology of myxomatosis, with a view to assessing the present status of the disease in various geographic regions in Eastern Australia—Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, quoting Appointment No. 560/226.

**SENIOR LECTURER/LECTURER** (with a higher degree in philosophy, or both a degree in philosophy and a degree in a subject related to their special interest, for example, mathematics, physics, anthropology) in PHILOSOPHY at the University of Queensland, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1.

**S.R.C. RESEARCH STUDENT in the DEPARTMENT OF CHEMISTRY** for work on the inhibition of the combustion of polymers—Professor C. F. Cullis, Department of Chemistry, The City University, St. John Street, London, E.C.1, quoting Ref. 42 CH.

**TECHNICIAN** (with previous experience of assembling electronic equipment) in the DEPARTMENT OF MACHINE INTELLIGENCE AND PERCEPTION for duties which will include assisting with the design, construction and maintenance of experimental devices for use with the Department's Elliott 4100 computer, and the maintenance of off-line electro-mechanical equipment—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh, 8.

## REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

### Great Britain and Ireland

**Safety in Numbers.** By Prof. H. L. Price. (Inaugural Lecture delivered before the University of Leeds on 14 November 1966.) Pp. 28. (Leeds: Leeds University Press, 1967.) 2s. 6d. [156]

**Lawes Agricultural Trust.** Rothamsted Experimental Station—Report for 1966. Pp. 411. (Harpenden, Herts: Rothamsted Experimental Station, 1967.) 20s. [156]

**Mechanical and Physical Properties of the Austenitic Chromium-Nickel Stainless Steels at Elevated Temperatures.** Pp. 25. (London: International Nickel, Ltd., 1967.) [166]

**Seventh Special Report from the Select Committee on Science and Technology, Session 1966-67.** Pp. 4. (London: H.M. Stationery Office, 1967.) 8d. net. [166]

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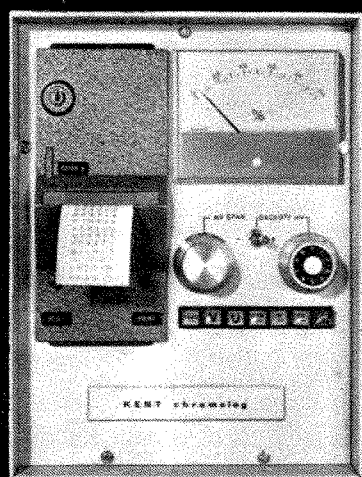
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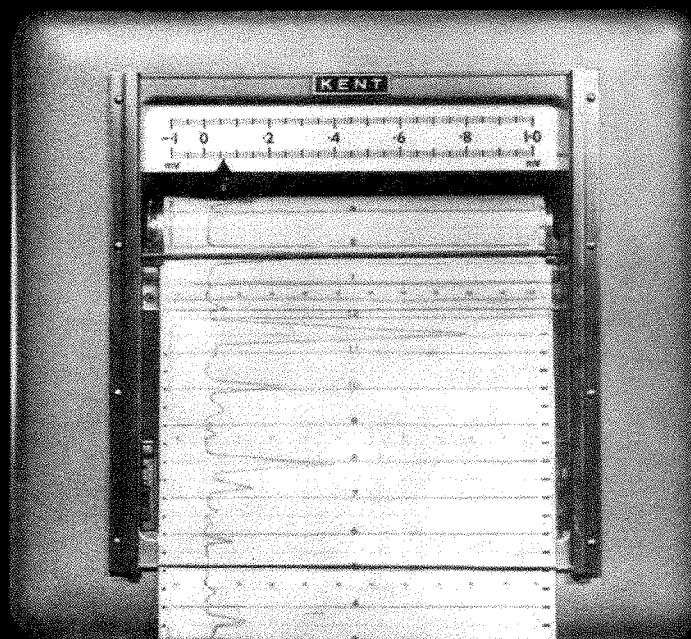
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MELBOURNE, AUSTRALIA  
LECTURER IN PHYSICS**

Applications are invited for appointment to this position in the Physics Department. The Department of Physics has active research interests in theoretical and experimental solid state physics, for which a range of facilities is available. Current research projects are concerned with low temperature physics (<sup>3</sup>He and <sup>4</sup>He), superconductivity, magnetism, optical properties of solids, laser physics, solid state plasmas, electron microscopy, E.S.R. and N.M.R. spectroscopy, nuclear orientation, Mössbauer effect. Further information on the Department is obtainable from the Chairman. The salary scale at present is \$A4,800 to \$A6,300 per annum, but is under review and substantial increases are expected effective from July 1, 1967. Superannuation on the F.S.S.U. basis is provided.

Benefits: Travelling expenses for appointee and family; removal allowance; repatriation after three years if desired; subsidised housing initially; STUDY LEAVE is granted at the rate of one term's leave for six terms' service, with provision for financial assistance. Loans for home purchase are available.

General information is available from the Academic Registrar, Monash University, Clayton, Victoria, Australia, or the Secretary-General, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London S.W.1.

Closing Date: September 29, 1967.

The University reserves the right to make no appointment or to appoint by invitation at any time. (556)

**UNIVERSITY COLLEGE OF  
NORTH WALES  
BANGOR****SCHOOL OF PLANT BIOLOGY  
RECLAMATION OF METAL  
CONTAMINATED SOILS**

Applications are invited for the post of Research Assistant to investigate the use of plant populations tolerant to toxic metals for the reclamation of toxic mine waste. The work will include the investigation of ecological conditions on metalliferous mine workings and the development of genetic reservoirs of adapted material. A full-time laboratory technician will be available, and there will be support for travel outside the British Isles. Applicants should have a good honours degree and interests in plant ecology, genetics, or mineral nutrition. Salary, £775 to £825 for a period of three years, with superannuation.

Further details can be obtained from the Registrar, to whom applications should be sent, together with the names of two referees, before August 31, 1967. (574)

**WHARFEDALE HOSPITAL  
MANAGEMENT COMMITTEE  
WHARFEDALE GENERAL HOSPITAL  
MIDDLETON, NR. ILKLEY, YORKS  
ALLERGY RESEARCH UNIT**

Applications are invited from graduates for the post of IMMUNOLOGIST in the Allergy Research Unit. Candidates should have a degree in a biological science, together with appropriate postgraduate training or practical experience. The successful candidate will be required to undertake work in the immunological aspects of Respiratory Disease.

Further particulars from the Group Secretary, Wharfedale General Hospital, Otley, to whom applications, stating age, qualifications and experience should be sent immediately. Closing date August 19, 1967. (573)

**UNIVERSITY OF IFE  
NIGERIA**

Applications are invited for (a) Senior Lectureships (2) and (b) Lectureship in Department of Physics. Preference given to candidates with qualifications and experience in the following fields: (i) Applied Physics, especially Electronics and related subjects. Candidates with qualifications in Electrical Engineering considered; (ii) Geophysics, Pure and Applied; (iii) Theoretical Subjects, especially Classical and Quantum Mechanics, Mathematical Methods. Salary Scales (a) £2,275 to £2,575 per annum; (b) £1,200 to £2,175 per annum. 20 per cent supplementation basic salaries (generally free of income tax) payable to U.K. staff; Superannuation Scheme; return family passages; children's and car allowances; annual U.K. leave, where applicable.

Detailed applications (six copies), naming three referees by September 4, 1967, to Inter-University Council, 33 Bedford Place, London W.C.1, from whom particulars are obtainable. (523)

# NATURAL ENVIRONMENT RESEARCH COUNCIL

## NATURE CONSERVANCY

### 3 Posts : SCIENTIFIC OFFICER CLASS

#### 1 Post : EXPERIMENTAL OFFICER CLASS

The Natural Environment Research Council has vacancies at the following Nature Conservancy stations.

(1) **ATTINGHAM PARK, SHREWSBURY.** A SENIOR SCIENTIFIC OFFICER/SCIENTIFIC OFFICER AND AN EXPERIMENTAL OFFICER/ASSISTANT EXPERIMENTAL OFFICER to work in the newly established conservation and land use section.

**Duties:** The section, which will operate on a Great Britain basis, will deal with selected projects involving large-scale changes in land and water use.

**Qualifications S.S.O./S.O.:** A good honours degree in a relevant subject, with knowledge of ecology and practical experience in conservation. Preference will be given to candidates with a special knowledge of (a) geography and plant ecology or (b) vertebrate zoology and agriculture of forestry.

**Experimental Officer/A.E.O.:** General Certificate of Education (or equivalent) in five subjects, two of which should be mathematical or scientific subjects at Advanced level. At age 22 entrants are expected to have a pass degree (or equivalent).

(2) **BANGOR HEADQUARTERS.** A SENIOR SCIENTIFIC OFFICER/SCIENTIFIC OFFICER to work in the Pedology Section.

**Duties:** To provide advice on pedological problems relevant to conservation and research. The work includes mineralogy of soils and their parent material, morphology, clarification, distribution, and chemical and physical characteristics in relation to ecological problems with particular reference to upland soils in Wales.

**Qualifications:** A good honours degree in preferably chemistry, geology or soil science with post-graduate experience.

(3) **MONKS WOOD EXPERIMENTAL STATION, HUNTINGDON.** A SCIENTIFIC OFFICER/SENIOR SCIENTIFIC OFFICER to work in the Toxic Chemicals and Wildlife Division.

The person selected will take charge of the laboratory working on pesticide residues using gas-liquid chromatography. He will have an Assistant Experimental Officer and two Scientific Assistants as his staff.

**Qualifications:** a good honours degree in Chemistry, Agricultural Chemistry or Biochemistry with post-graduate experience.

**Salaries:** Senior Scientific Officer Grade (normally at least 26 years and under 32 years at December 31, 1967), £1,744 to £2,155.

Scientific Officer Grade (under 29 years at December 31, 1967), £926 to £1,574.

Experimental Officer Grade (at least 26 and under 31 years at December 31, 1967), £1,365 to £1,743. Assistant Experimental Officer Grade (18 and under 28 years at December 31, 1967), £568 (age 18) to £1,017 (age 26) to £1,243.

Initial appointment will be on unestablished terms, but there are prospects of a permanent and pensionable appointments.

Application forms and further particulars available from The Natural Environment Research Council (E), State House, Holborn, W.C.1.

Closing date: August 29, 1967. (522)

## UNIVERSITY OF CAMBRIDGE CHAIR OF MATHEMATICAL PHYSICS

Applications are invited for the newly established Professorship of Mathematical Physics. Preference will be given to candidates whose work is in the field of Theoretical Quantum Physics. Pensionable stipend £4,360.

Applications (ten copies) marked "Confidential" should be sent by October 21, 1967, to the Registry, The Old Schools, Cambridge, from whom further particulars may be obtained. (569)

# assistant librarian

The Nicholas Research Institute require a librarian to be responsible for the day to day running of its Technical Library.

Applications are invited from either librarians with a scientific background or from young science graduates interested in information work.

The Company has a non-contributory Pension and Life Assurance Scheme and also a Profit-Sharing Plan.

Please write, giving full details to:

D. J. Goddard, Esq., Personnel Manager (R/G/9),

**NICHOLAS RESEARCH INSTITUTE LTD,**  
225 Bath Road, Slough, Bucks.

A MEMBER OF THE ASPRO-NICHOLAS GROUP

(561)

## Leading Swiss Chemical Firm requires

# AGRONOMIST FOR INDONESIA

### Qualifications :

Degree in entomology

Experience with insecticides and in experimental techniques.

### Responsibilities :

Evaluating insecticides in Sumatra.

Supervision of trials of demonstrations in rice.

Promotional activities for insecticides.

Applicants should send their handwritten *curriculum vitae* together with references to

Box No. 931, *Nature*, T. G. Scott & Son Ltd., 1 Clements Inn, London, W.C.2.

Employees of the firm are aware that this position is being advertised.

(X585)

## UNIVERSITY OF WESTERN AUSTRALIA TEMPORARY LECTURER IN CLINICAL PSYCHOLOGY

Applications are invited for the above-mentioned appointment on the staff of the Department of Psychology (Professor A. J. Yates) for a period of one year from September, 1967. The appointee will be required to teach in the course for the M.Psych. Degree and to assist with course administration and organization. The current salary range for the post is \$A4,800 to \$A6,340 but is likely to become \$A5,400 to \$A7,300 per annum in the near future. An allowance will be made towards fares.

Intending applicants are requested to obtain details of the procedure to be followed in applying for the post and a copy of the conditions of appointment before submitting their applications. This information is available from the Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London S.W.1. Applications close in Australia and London on September 10, 1967. (552)

## EWELL COUNTY TECHNICAL COLLEGE DEPARTMENT OF BIOLOGICAL SCIENCES PHARMACOLOGY

Senior Technician (T3) required for September 1. To be in charge of the technical services of this Section of the Department.

### PHYSICS

Technician required for September 1, or as soon as possible thereafter. To maintain and develop electronic and optometric equipment used in all branches of applied biology, and to assist in the preparation of biophysics classes.

The appointment may be made on any of the grades T3, T2 or T1, according to the qualifications and experience of the successful applicant. Salary scales, T3, £820 to £1,020; T2, £735 to £860; T1, £315 to £735. A London weighting allowance of £30, £50 or £75 according to age is payable in addition.

Application forms from the Registrar, Ewell County Technical College, Reigate Road, Ewell, Surrey. (578)



# MEDICAL RESEARCH COUNCIL

## SENIOR ADMINISTRATIVE APPOINTMENT

THE MEDICAL RESEARCH COUNCIL will have a vacancy shortly for a University graduate, preferably aged 35-45, for work in the Administrative Division of their Headquarters Office. The work will be concerned with all administrative aspects of the formulation of Council policy and its execution. The duties fall mainly under the four following heads (1) the administration of the Parliamentary grant-in-aid and the Council's endowment funds (2) the management of the Council's building programme and other arrangements for accommodation for the Council's staff (3) the personnel work arising from the Council's staff of over 3,300 in many different grades at home and abroad, and (4) the administration of the Council's programme of research grants, scholarships and training awards and the control of the budgets for laboratory supplies, equipment and recurrent expenses.

Salary scale—the Civil Service Assistant Secretary scale (starting pay at present £3,500 a year rising to £4,500 by six annual increments, with a London allowance of £85 a year).

The appointment will be a permanent one subject to the satisfactory completion of a trial period, the length of which will depend on age and experience but should not normally exceed one year.

*Superannuation provision: Federated Superannuation System for Universities. Apply, giving full curriculum vitae and names of two professional or business referees, to:*

**Establishment Officer, Medical Research Council,  
20 Park Crescent, London, W.1 by 8th September, 1967.  
(quoting E2/337/N)**

(568)



## INFORMATION SCIENTIST

Expansion of the existing library and information service is taking place at the Ranks Hovis McDougall Research Centre at High Wycombe. This is concurrent with the opening of a new major laboratory unit which will result in greatly enlarged research facilities for the Group.

A young man or woman under 25 with a good degree in chemistry, biochemistry or other discipline relevant to work in the field of food science and technology, is required for the post of Assistant Information Officer.

Previous experience of information work is desirable, but applicants without it will be considered if they can evince a strong inclination for this kind of work and a willingness to provide a really useful service for their colleagues on the bench.

This appointment offers scope and good financial reward. Some help might be given with studying for a further qualification.

*Please write with details of qualifications and career to the Research Manager,*

**RANKS HOVIS McDOUGALL (RESEARCH) LTD.,**

**Cressex Laboratories,  
Lincoln Road, High Wycombe, Bucks.**

(517)

## Immunologist/ Zoologist

A graduate is required for an active research programme in the A.R.C. Cattle Blood Typing Service. One of the important lines of investigation is the study of white cell and tissue antigens in cattle and other species. Excellent laboratory facilities are available. A person with some immunological experience is preferred. It will be possible to study for a Ph.D.

Appointment in the range of S.O. £926 to £1,574 or S.S.O. £1,744 to £2,155 with F.S.S.U. benefits.

Applications to and further details from the Secretary, A.R.C. Animal Breeding Research Organisation, West Mains Road, Edinburgh, 9. (525)

## UNIVERSITY OF DUNDEE

### COMPUTING LABORATORY

A vacancy occurs for a LECTURER or ASSISTANT LECTURER who will be required to give lectures on programming languages and techniques to undergraduate and graduate students and to advise computer users on the preparation of programmes. The Laboratory is installing an Elliott 4130 Computing System which will include magnetic tape, disc files, and remote access consoles. Salary scales: Lecturer, £1,470 to £2,630; Assistant Lecturer, £1,105 to £1,340, with placing according to qualifications and experience. F.S.S.U.; grant towards removal of household effects.

Applications (6 copies), containing the names of three referees, to be lodged by September 2, 1967, with the Secretary, The University, Dundee, from whom further particulars may be obtained. (518)

## BROOKE BOND RESEARCH LABORATORIES

BLOUNTS COURT, SONNING  
COMMON, READING, BERKS

Owing to the expansion of research effort several vacancies will occur in the near future for Graduates in Chemistry, Biochemistry, Botany, or Food Technology.

One of the posts will be for an **Information Scientist**, the remainder being concerned with research and development. It is anticipated that one of the successful candidates will be stationed in Kenya for at least one tour of duty whilst the others will be located in new research laboratories near Reading. The starting salary will be commensurate with age and experience. The Company operates a contributory pension scheme.

Applications (in duplicate, please) to Head of Research at the above address. (544)

## UNIVERSITY OF OXFORD DEPARTMENT OF ZOOLOGY

Principal Technician required September 1967 to be in charge of Chemistry teaching laboratories and to be responsible for supervision of all first-year teaching laboratories in a new building to be ready for occupation in 1969. Salary according to age, qualifications and experience, on University scale.

Applications, with full details and names of two referees to be sent to the Administrator, Department of Zoology, Parks Road, Oxford. (576)

# WOOLWICH POLYTECHNIC LONDON, S.E.18

## DEPARTMENT OF BIOLOGY AND CELL SCIENCE

### SENIOR LECTURER OR LECTURER IN BIOCHEMISTRY

Applications are invited for this post to commence September 1, 1967, or as soon as possible thereafter. In addition to teaching undergraduate and postgraduate students the successful applicant will be required to collaborate with Dr. P. B. Gahan in studies on lipid-protein metabolism or enzyme pathways in ageing, for which full facilities are available.

Salary scales: Senior Lecturer, £2,140 to £2,380 plus £70 London allowance; Lecturer, £1,875 to £2,140 plus £70 London allowance.

Closing date: September 8, 1967.

Assistance may be given towards household removal expenses.

Particulars and application form from Clerk to the Governors, Woolwich Polytechnic, Wellington Street, London, S.E.18. (575)

## PRINCIPAL SCIENTIFIC OFFICER AND SENIOR SCIENTIFIC OFFICER

### EAST AFRICAN COMMON SERVICES ORGANISATION

A Principal Scientific Officer and a Senior Scientific Officer are required at the East African Institute for Medical Research, Mwanza, Tanzania, to plan and carry out research on Schistosomiasis and methods of control and also to train newly appointed science graduates in research methods.

Candidates must be citizens of and permanently resident in the United Kingdom or the Republic of Ireland. They must possess a degree in Zoology preferably "Honours" or a Medical degree, and postgraduate research experience in tropical parasitology. Postgraduate degrees (e.g., Ph.D., M.Sc., etc.) desirable but not essential.

Salary according to qualifications and experience in scale £1,818 to £3,159 per annum for Scientific Officers and in scale £2,448 to £3,000 per annum for medical research officers. Terminal gratuity. Free family passages. Education allowances. Accommodation available at low rental. Two-year contract. Generous paid leave on completion.

Please apply for further particulars and application forms stating full name to:

THE MINISTRY OF OVERSEAS  
DEVELOPMENT,  
Room 403,  
Eland House,  
Stag Place,  
London, S.W.1. (571)

## UNIVERSITY OF LIVERPOOL

### THE HARTLEY BOTANICAL LABORATORIES

Applications are invited for the post of TEMPORARY DEMONSTRATOR/ ASSISTANT LECTURER or LECTURER in BOTANY for the period October 1, 1967 to September 30, 1968. Preference will be given to candidates with an interest in Plant Physiology or Plant Biochemistry. The initial salary will be in the range £895 to £1,285 per annum for a Demonstrator; £1,105 to £1,340 per annum for an Assistant Lecturer; or £1,470 to £1,740 per annum for a Lecturer.

Applications, including the names of three referees, should be submitted by August 26, 1967, to the Registrar, The University of Liverpool, Liverpool 3, from whom further details may be obtained.

Please quote Ref.: RV/120/N. (566)

# analytical chemist

A well-qualified analytical chemist is required, for an expanding programme of analysis for pesticide residues in crops, soil and animal tissues. We are looking for a man aged between 25 and 35 with either a Ph.D. in Chemistry or a good honours degree plus experience in this or a related field. The successful applicant will be expected to provide and apply creative ideas in a well-equipped active department, working in parallel with chemistry, biology and toxicology teams, in a large Research Station, 12 miles south of Cambridge, concentrating on pesticides. An attractive salary is envisaged. *If you are interested please write for an application form (Quoting Ref. No. 122/10) to:*



# FISONS

Personnel Officer,  
Chesterford Park Research Station,  
FISONS PEST CONTROL LTD.,  
Nr. Saffron Walden, Essex. (536)



# PHYSICIST

## UNITED KINGDOM ATOMIC ENERGY AUTHORITY, WANTAGE RESEARCH LABORATORY, BERKSHIRE.

The Wantage Research Laboratory, which is generally concerned with the application of radioactive materials to scientific and technological problems, has a vacancy for a physicist to develop methods of elemental analysis based on the interaction of radiations with matter. X-ray, beta-particle and neutron attenuation and scatter, and X-ray fluorescence techniques now form the basis of instruments for on-line analysis and of portable instruments for use in factory, laboratory and field conditions. There has been a rapid development and extension of instruments of this type during recent years and many new and important applications have been established.

A vacancy exists in the team exploring new techniques for automatic process control and geological prospecting. Current problems include studies of inter-element effects and the effects of grain size in X-ray fluorescence analysis of mineral ores. It should appeal to a physicist with combined theoretical and experimental interest in radiation interactions who wishes to extend his experience to problems of immediate economic importance.

Although previous experience in radiation measurement is desirable, ability, enthusiasm and drive are more important and recent graduates with these qualities are encouraged to apply.

Candidates should have a 1st or good 2nd Class Honours degree. The appointment will probably be made to the grade of Scientific Officer, with a starting salary in the range £995 to £1,685, but an exceptional candidate might be appointed as a Senior Scientific Officer, with a salary range £1,870-£2,310.

Authority housing will be available for married officers living beyond daily travelling distance.

For further details and application forms please write to:

Personnel Department A (A.5198/34),  
Atomic Energy Research Establishment,  
Harwell, Didcot, Berks. (539)

## UNIVERSITY OF NOTTINGHAM DEPARTMENT OF PHYSIOLOGY AND ENVIRONMENTAL STUDIES

Applications are invited for the post of LECTURER in Plant Physiology. Candidates should preferably have a first degree and postgraduate training to Ph.D. level in Plant Physiology, Biochemistry or Biophysics. Salary will be within the range £1,470 to £2,630 per annum.

Forms of application and further particulars, returnable not later than September 1, 1967, from the Registrar. (533)

## THE UNIVERSITY OF MANCHESTER MANCHESTER, 13

### RADIATION OFFICER IN THE

### DEPARTMENT OF PHYSICS

Applications invited from graduates for this post. Knowledge of radiation protection and electronics additional qualifications though not essential. Salary range per annum: either £920 to £1,525; or £1,450 to £2,200, according to qualifications and experience. F.S.S.U.

Particulars and application forms (returnable by August 26), from the Registrar. Quote ref. 120/67/Na. (441)

## BIOCHEMISTRY

### UNIVERSITY OF ALBERTA

### EDMONTON, ALBERTA, CANADA

Applications are invited from potential Post-Doctoral Fellows in Biochemistry for research into physicochemical aspects of lipid-protein interactions. Stipend \$6,000 and over (tax free).

Apply to Dr. P. G. Baron, Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada, enclosing details of academic attainments and names of two or three referees. (547)

SENIOR TECHNICIAN OR TECHNICIAN required for Microbiology at Queen Mary College (University of London), Mile End Road, E.1. Previous experience is essential and the possession of the I.S.T. or City and Guilds Science Laboratory Technician's Certificate, preferably advanced, or O.N.C. or similar qualification is desirable though not essential. Salary according to age and ability on the scale £912 to £1,150 or £653 to £938 per annum plus London weighting £60 and possible £30 or £80 special qualification award. Five-day week. Four/five weeks annual leave. Pension scheme.—Letters only to Registrar (B/ST), stating full details of age, experience and present work. (558)

## CSIRO AUSTRALIA

### RESEARCH SCIENTIST DIVISION OF PLANT INDUSTRY F. C. PYE FIELD ENVIRONMENT LABORATORY

**GENERAL.** The F.C. Pye Field Environment Laboratory located at Canberra, A.C.T., conducts experimental and theoretical research into the physics of plant environments and plant growth processes. Its facilities include laboratories for studies in soil physics, gas physics, solar radiation, fluid mechanics, micrometeorology, and biology, as well as mobile laboratories for studies of the field environment of plants. It has access to the CSIRO CDC 3600 computer and to CERES, a controlled environment research facility. The laboratory's staff includes mathematicians, physicists and biologists.

**DUTIES.** The appointee will have the opportunity to undertake research in one of the following fields: (a) Mathematical Ecology; (b) Transfer processes within plants; (c) The mechanics of plant growth.

(a) **Mathematical Ecology.** One of the Laboratory's aims is to contribute to quantitative aspects of ecological methodology and theory. The appointee would be expected to develop a research programme in this area. Typical topics could be the mathematical-statistical description of the spatial distribution of plants and foliage, and the dynamics of population growth and dispersal.

(b) **Transfer processes within plants.** The appointee would be expected to conduct research into the mechanisms of movement of water and solutes within the plant, and the effects of water stress on these processes. Some integration of this work with current studies of the transfers of water vapour and carbon dioxide in the field and the water relations of plants would be expected. Liaison with the Division's Plant Physiology Section which is concerned with problems of translocation within the plant would also be encouraged.

(c) **The mechanics of plant growth.** This appointment is for investigations of mechanical aspects of the growth and function of plant tissues. Typical topics could be: mechanical aspects of cell enlargement, particularly in relation to the uptake and movement of water; the propagation of turgor through cells; root penetration and elongation and the uptake of water and nutrients; momentum transfer and the oscillation of foliage elements.

**QUALIFICATIONS.** Applicants should have a Ph.D. degree, or have had appropriate postgraduate research experience of equivalent standard and duration, supported by satisfactory evidence of research ability. For (a), training in Mathematics or Statistics is required, while for (b) and (c) training in Physics, Biophysics or Physiology is required.

**SALARY.** Depending upon qualifications and experience, the appointment will be made within the salary ranges of Research Scientist, \$A5,250–\$A6,622 p.a. or Senior Research Scientist, \$A6,892–\$A7,974 p.a. An applicant with an outstanding record of research achievement would be considered for appointment as Principal Research Scientist, \$A8,242–\$A9,490 p.a. Salary rates for women are \$A428 p.a. less than the corresponding rates for men. Promotion within CSIRO to a higher classification is determined by merit.

**CONDITIONS.** The duration of the appointment will be determined in consultation with the successful candidate; some applicants may prefer a fixed term appointment for three years. Fares paid for the appointee and his dependent family. Further details supplied on application.

Applications, quoting reference number 130/870, and stating full name, place, date and year of birth, nationality, marital status, present employment, details of qualifications and experience, together with the names of not more than four persons acquainted with the applicant's academic and professional standing should reach—

The Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, by the 8th September, 1967. (577)

### UNIVERSITY OF NATAL DEPARTMENT OF GEOLOGY AND GEOGRAPHY

Applications are invited from suitably qualified persons for appointment to the post of LECTURER IN GEOLOGY AND GEOGRAPHY, Pietermaritzburg. The salary scale attached to the post is: R.3,000 by R.150 to R.4,800 (£1,500 by £75 to £2,400) per annum. The commencing salary notch on this scale will be determined by the qualifications and/or experience of the successful applicant.

Further particulars of the post and of the concomitant amenities such as travelling expenses on first appointment; pension, medical aid, staff bursary and housing loan schemes; long leave conditions, etc., are obtainable from the Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1. Applications, on the prescribed form, close in South Africa and London on September 15, 1967. (555)

### UNIVERSITY OF ALBERTA EDMONTON, ALBERTA, CANADA DEPARTMENT OF PHARMACOLOGY

Applications are invited for the post of Assistant Professor or Associate Professor in the Department of Pharmacology from persons interested in Cardiovascular Pharmacology or Biochemical Pharmacology. The salary scale attached to this position is \$9,000 to \$12,450 for an Assistant Professor or \$12,500 to \$16,550 for an Associate Professor. The commencing salary will depend on the qualifications and experience of the successful applicant.

Further particulars of the position and of the concomitant amenities such as travelling expenses on first appointment and pension scheme may be obtained from Professor G. B. Frank, Department of Pharmacology. (513)

### UNIVERSITY OF SHEFFIELD DEPARTMENT OF GEOLOGY

Applications are invited for a post of ASSISTANT LECTURER or LECTURER IN APPLIED GEOLOGY with particular interests in Economic Geology or in fields related to Engineering Geology. The Department is equipped with Geochemical, X-ray Fluorescence and Electron Microscope Laboratories for research in these fields. Initial salary according to qualifications and experience in the range: Assistant Lecturer, £1,105 to £1,340; Lecturer, £1,470 to £2,630, with F.S.S.U. provision.

Further particulars from the Registrar, to whom applications (four copies), should be sent by September 4, 1967. (541)

### MACAULAY INSTITUTE FOR SOIL RESEARCH SPECTROCHEMISTRY

Experimental or Assistant Experimental Officers required to fill three vacancies in the Department of Spectrochemistry, to take part in the general analytical work of the department and in the application of direct reading techniques to soil and plant analysis. Applicants should have interests in physico-chemical or electronic techniques, general analytical chemistry, geochemistry or soil science and should possess an ordinary degree, H.N.C., or equivalent qualification in a relevant scientific subject. Salary on the A.E.O. scale (£744 at age 21 years, £1,017 at age 26 or over, rising to £1,243) or E.O. scale (£1,365 to £1,734) according to age and experience. Contributory superannuation scheme.

Application forms obtainable from the Secretary, The Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen, AB9 2QJ, to whom they should be returned by September 11, 1967. (572)

### SIR JOHN CASS COLLEGE RESEARCH ASSISTANTSHIPS IN PHYSICS

A number of Research Assistantships in Physics will be available from September 1, 1967 for graduates with First- or Second-class (Upper or Lower) Honours degrees to undertake postgraduate research leading to M.Phil. or Ph.D. Internal degrees of the University of London.

The research interests are Magnetic thin films; Surface physics in u.h.v.; Scintillation processes (u.v. and X-ray); Dielectric properties of solids; Flow anisotropy in liquids.

Salary £810 by £30 to £870 per annum (to include six hours weekly demonstrating). Applications to the Head of the Department of Physics, Sir John Cass College, Jewry Street, London, E.C.3. (548)

### UNIVERSITY OF LANCASTER SCIENTIFIC OFFICER

Applications are invited for a post of Scientific Officer in the Department of Chemistry. The person appointed is expected to have postgraduate (or equivalent) experience in one or more of the following techniques—G.L.C., I.R. spectroscopy, N.M.R. spectroscopy, mass spectrometry; he will be responsible for the day-to-day running of instruments and will be required to give assistance to research workers in the department. The starting salary is likely to be about £1,200 per annum, and the appointment will be for three years in the first instance.

Further particulars may be obtained from Professor J. C. Bevington, Department of Chemistry, St. Leonardgate, Lancaster, to whom applications should be sent before September 1, 1967. (542)

### UNIVERSITY OF LEEDS DEPARTMENT OF INORGANIC AND STRUCTURAL CHEMISTRY

Applications are invited for a POST DOCTORAL FELLOWSHIP in THE DEPARTMENT OF INORGANIC AND STRUCTURAL CHEMISTRY at a salary of £1,105 a year. Preference will be given to candidates with some experience in magnetochemistry.

Applications, stating age, qualifications and experience and the names of two referees, should reach Professor H. Irving, Department of Inorganic and Structural Chemistry, The University, Leeds, 2, as soon as possible. (540)

### UNIVERSITY OF SYDNEY CHAIR OF PHYSIOLOGY

Applications are invited for the Chair of Physiology which became vacant on the resignation of Professor P. O. Bishop. Candidates should be qualified in the field of Neurophysiology. Salary will be at the rate of \$A10,400 per annum. Academic salaries are at present under review. There is retirement provision under either the Sydney University Professorial Superannuation Scheme or the New South Wales State Superannuation Scheme. Under the Staff Members' Housing Scheme, in cases approved by the University and its Bankers, married men may be assisted by loans to purchase a house. The Senate reserves the right to fill the Chair by invitation.

A statement of conditions of appointment and information for candidates may be obtained from the Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1. Applications close in Australia and London on September 29, 1967. (553)

## EMPTY WORDS ON SPACE

THOSE who hope that the British House of Commons will eventually grow to be an effective critic of Government policy in science and technology will not have been much encouraged by the publication a week ago of the report of the sub-committee of the Estimates Committee on the conduct of space research and development in Britain (see *Nature*, **215**, 685; 1967). With all its brash espousal of popular but thoughtless causes, the report is unlikely to have much influence in the ways in which its authors intended. Indirectly, it will probably reduce the credibility of the Estimates Committee, which is unfortunate at a time when it is clear that a conscious attempt is being made to give the committee a sharper cutting edge on policy, and when other sub-committees of the same body are trying to win support for daring but imaginative proposals for penal reform. In the circumstances, it might be kinder to let the report sink quietly into the wastepaper basket where it belongs, yet many of the questions raised by its appearance and its failure are of lasting interest. To know just why many of the conclusions to which the sub-committee was driven are faulty is in itself important. And how did the committee go so badly adrift? And what kinds of conclusions should it have reached? These questions also deserve an answer.

The first complaint against the committee's handiwork is that it has somehow failed to appreciate the subtleties of the problem with which it has been wrestling since February 1966. In the event it is not surprising that some among the many recommendations in the report should be entirely sensible. If you keep on tossing pennies in the air, some of them are bound to come down in the way which is predicted. Unfortunately, however, the committee's argument is so thin that very little of what it has to say will carry conviction. It is sound enough, of course, to resist the claims of the National Industrial Space Committee that extra rockets on the ELDO pattern should be ordered quickly even though there are no customers for them in prospect, although it cannot have taken particular cleverness for the members of the committee to have recognized this echo of the familiar cry that it is often more important to order equipment than to use it. Politicians should be familiar enough with that by now.

The committee is also right in saying that there should be no further increase in the rate at which the ELDO organization is empowered by money to launch its clever apogee-perigee rockets, but it has inexplicably shrunk from what would have been the useful task of asking whether and in what form ELDO should continue after current programmes have come to an end. The truth is that ELDO in its present form owes too much to the mixed motives which attended its forma-

tion at the beginning of the decade. Although the committee collected plenty of evidence to show that the work of ELDO is badly co-ordinated, and that participants in the programme do not easily acquire a sense of familiarity with those developments not directly in their charge, much of this trouble can be traced to the haste and the muddle in which the organization was set up. At the same time, however, if European countries are ever to produce large launching rockets—and it is not obvious that they should—it will obviously be easiest to do this within the framework of European collaboration. In other words, if ELDO did not exist, it would be necessary to think of inventing it. The committee would have done a public service if it had tried to decide whether reform is feasible.

Instead, the committee has settled for some woolly statements about the need that "within the total space budget the greater share should be spent on the national programme and the lesser share on the international programme". As it happens, in the current financial year, the British contribution to the budget of ELDO will be £9.69 million out of a total of £30.55 million, with the European Space Research Organization (ESRO) and the International Telecommunications Satellite (INTELSAT) taking £600,000. One thing that rankles with the committee is that international obligations are a smaller proportion of what is spent in France, but this is of course beside the point. There can be no simple rule to determine how a balance should be struck between domestic and international expenditure.

These, however, are almost minor transgressions. It is much more serious, and much more important, that the committee seems to have emerged from its long sequence of meetings entirely innocent of a clear view of what space research and development is for. Only this can account for the strange repetition of the cry that there should be what the committee calls "a national space programme with a budget of its own" in the charge of a single minister—the Minister of Technology. The truth is that there are at least three quite different sets of reasons why countries such as the United Kingdom should put money into space research and development, which means that there must be at least three different sets of yardsticks for deciding what to spend. In other words, where the objective is to provide opportunities for people from British universities and laboratories to carry out experiments with rockets or satellites, it is entirely right and proper that decisions about how much to spend should be taken within the framework of the Science Research Council, for only then is it possible to match the benefits of such expenditure against those likely to be obtained by spending money on other



kinds of scientific research. In the same way, it is proper that the Ministry of Defence should take decisions about the usefulness of certain military applications of Earth satellites, chiefly in telecommunications, by matching the potential advantages against the costs of these and alternative developments. Without knowing much more than it troubled to find out about the criteria which are being used in making these comparisons, it is, of course, nonsense for the Estimates Committee to claim that an expenditure of between £30 million and £35 million a year is more or less what Solomon would have settled for.

The committee's sense of muddle extends to what it has to say about the commercial benefits which may eventually stem from space research and development. Prompted by the National Industrial Space Committee, the Estimates Committee sheds a great many tears about the way in which British manufacturers have not won enough contracts from organizations like ESRO to balance out what the British Government contributes to them. Considerations like these, however, have nothing at all to do with the question of how much money should go into the commercial development of devices such as communications satellites, and how governments like the British should seek to organize and to exploit them. If there is a case at all for the development in Europe of means of building, launching and operating communications satellites, it must rest on a hard-headed estimate that the economic benefit to be derived by means like this is measured in hundreds of millions of pounds a year. British companies could obviously do better in winning contracts from ESRO, but carrying out jobs for a research organization can never bring in more than chicken feed. It is foolish of the committee to suggest that there should be what it calls a national space programme in order that the companies can be better equipped to acquire a bigger share of this marginal business. There is rather more sense in what the committee has to say about the need of a tighter relationship between the Ministry of Technology, blessed for better or worse with involvement in ELDO, and the General Post Office, traditionally responsible for negotiations on the operation of the international communications network. It is also sensible of the committee to draw attention to the opportunity that will present itself, in 1970, for a revision of the rules which at present govern the operation of INTELSTAT—the consortium which owns and operates the system of communications satellites launched from the United States. These—with the splendid index—are the few virtues in a bad report.

What has gone wrong? Why has the committee followed so many false scents? Nobody who reads the minutes of evidence can fail to be impressed with the amount of time spent on explanation. Witnesses were more often tutors than defendants, and it is clear that many of the lessons they attempted to impart have failed to sink home. Although it is unreasonable to expect that members of the estimates committee should be knowledgeable about the technicalities of

space research and development, it is entirely proper to ask that they should provide themselves—or be provided—with professional help when they embark on highly technical enquiries. Congress in the United States, which has a long experience of legislative committee work, learned this lesson long ago. Those who would increase the influence of the British Parliament by strengthening the committees will undermine their own efforts if they do not quickly follow suit.

The estimates committee was also exceedingly unwise in its choice of witnesses. It is entirely understandable that the civil servants responsible for the administration of money now spent on space research should have been fully represented, but why should the only outside body represented in the evidence have been the National Industrial Space Committee—a lobby if there ever was one? Certainly the chairmen of the scientific committees to which the British Government looks for advice could have given a much needed account of how policies on space research are determined. The Post Office, which has still to live down its recent brash advocacy of a system of low-level satellites for communications, is not the only authority on whether, and when, independent launchers for communications satellites would be a European asset.

What, then, should be done? Scepticism about the report may at least have the virtue of suggesting the kinds of studies which need to be carried out if countries such as the United Kingdom are to form rational policies. Clearly there is a need for a more deliberate appraisal of the potential value of ESRO as a means of sustaining scientific research. It would also be splendid if Europe as a whole could decide what needs to be done about communications satellites.

Where ESRO is concerned, the example of CERN is instructive. Both institutions have been established so as to provide facilities for research which are beyond the resources of any single member. Both of them, to some extent at least, involve expensive capital installations. It is true that CERN has from the beginning had the advantage of a much more easily recognizable centre for its operations—the accelerator at Geneva—but this does not entirely explain why ESRO has won many fewer friends. A part of the trouble seems to have been a somewhat muddled organization, which is why there are now hopes that Professor Bondi will be able to do much to invigorate the organization when he arrives there in the autumn. It is also true, however, that some of the uncertainty which surrounds ELDO has afflicted ESRO. It is even suggested that the two organizations should be merged, which makes very little sense unless on the principle that if all your eggs are rotten, they may as well be in the same basket. The ideal would be to keep ESRO going as an independent unit and to see whether it can become a vehicle for executing a substantial part of the research in this field which European scientists wish to undertake and their governments are prepared to underwrite. It is entirely possible that the constitution of ESRO might be modified in

such a way as to make the organization a more effective focus for space research in Europe. One of the strengths of CERN is that countries which have paid their subscriptions are tempted to get the best value for their investment by carrying out as much work as possible at the central establishment (which has not, of course, brought strictly national activity in high energy physics to a halt). If there were an incentive like this for ESRO, that organization would be enormously strengthened.

It is also important that there should now be a cool appraisal of the commercial benefits of building large launching rockets in Europe. As things are, the ELDO launcher casts a long shadow over everything. People are naturally reluctant to acknowledge that the usefulness of this machine is limited after so much money has been spent on it. The result is that plans for satellite development in ESRO, and plans for launching communications satellites in Europe, are being made to hang on this one rocket. Even at this late stage, however, it is entirely possible that it would be better to start again from scratch on the development of another rocket. That is something which ought to be considered. It is also possible that a suitable organization in Europe could make a sensible (and lasting) agreement with the United States on the use of even bigger rockets. Certainly this is a possibility which ought to be explored. Much will depend on the form of the revised INTELSTAT agreement, which is certain by the seventies to seem on both sides of the Atlantic to be too much dominated by the United States. But in this sense, some hard work now on the diplomacy of international communications could help enormously to simplify the problem of deciding what kinds of rockets, if any, should be developed and built independently in Europe. To begin with, however, it is essential that these discussions should be undertaken on a European basis. The common interest of the non-rocket countries is too powerful for any other course to be acceptable.

## HOW BIG IS CHINA?

How soon will China be a nuclear power to be reckoned with? The prophecies which the Joint Committee on Atomic Energy has been making about the development of nuclear weapons and missiles in mainland China (see page 805) are sobering but not much of a surprise. For a decade now, Chinese statesmen have made no secret of their intention to develop a strategic striking force. Indeed, on several occasions since the official opening in 1958 by Mr Khrushchev of the Russian research reactor in Peking, commentators elsewhere have been inveigled into the assumption that Chinese ambitions had already been translated into fact. Although it is now clear that preparations for the manufacture of nuclear explosives were well under way even before 1958, the course of events since then has not in any way been unexpected. The interval

of time between the first fission explosion and the first fusion explosion in China may have been shorter than elsewhere, but that is a simple consequence of what must have been an explicit decision in China that the only weapons worth having are thermonuclear weapons. Certainly the obvious difference between the speed of development in China and in France seems to stem directly from Chinese confrontation on the building of diffusion plants for the manufacture of fissile uranium. The joint committee is understandably puzzled that the French are making what seems to be heavy weather of the production of a thermonuclear weapon, but it is probably prudent—even fair—to remember that the origin of the French decision to manufacture nuclear weapons was clouded, to say the best of it. To begin with, the French government talked chiefly of tactical uses for nuclear weapons. Given Chinese single-mindedness, it is not surprising that China has forged ahead with the manufacture of nuclear explosives of all kinds.

The development and manufacture of missiles are another matter. The joint committee seems to have convinced itself that the Chinese are already possessed of medium range ballistic missiles and that rockets able to travel greater distances will be in service in the early seventies. No doubt the committee has access to unpublished information about developments in China, and there is no question that the news it has to give of the development of delivery systems for nuclear weapons is potentially important. Because the Chinese aircraft industry is as yet comparatively unsophisticated, it has always been evident that China could not become an effective nuclear power without some machinery for the delivery of nuclear explosives. It would be entirely consistent with the policy of seeking to become a nuclear power as soon as possible if the Chinese had decided not to bother with the development of aircraft but to go straight for long range missiles. Nevertheless, it would be surprising if China were able by the early seventies not merely to design and prove a long range missile, but to manufacture it in large numbers and then integrate it into a strategic striking force. Experience elsewhere shows that there is often a lag of several years between the first appearance of a new sophisticated weapon and its effective deployment in the field. This is why it is probably safe to think that it will be nearer the middle of the next decade than the beginning when the Chinese are able to level a credible threat against other strategic nuclear powers, to the west or to the east of China. This no doubt is why Mr Robert McNamara in the United States has been able to insist that an anti-ballistic missile system will not be strictly necessary before the mid-seventies.

A lot can happen before then. One possibility is, of course, that domestic troubles in China may prevent the Chinese government from maintaining a coherent and consistent defence policy but it would be rash for people outside China to rely on that. It is, however, more than likely that long before the Chinese government would wish directly to pose a missile threat

against—say—the United States, it will find itself preoccupied with more immediate and more local problems. In particular, the Chinese government is likely to find itself increasingly made militarily impotent in the immediate neighbourhood of mainland China by the lack of advanced conventional weapons. For one thing, this deficiency is likely to limit the extent to which the Chinese are able to think of using their new nuclear explosives even in the affairs of south-east Asia.

In other words, one of the immediate and somewhat paradoxical consequences of the speed with which nuclear explosives have been developed in China may well turn out to be a recognition that single-mindedness has created a lopsided military apparatus. Obviously it would be folly to hope that the Chinese will noticeably slacken their efforts on nuclear weapons for the sake of a more balanced programme, but the next few years are likely to bring some sharp reminders that the mere possession of nuclear weapons does not make up for a lack of conventional strength. China, like the other nations which have developed nuclear weapons for themselves, will soon begin to learn that nuclear explosives do not bring instant prowess.

What will happen after that? If it is reasonable to assume that Chinese nuclear weapons will be substantial by the middle seventies, it is natural to expect that the existing nuclear powers will be forced in some way to respond. The development and deployment of anti-ballistic missiles in the United States is one obvious possibility, although it is not immediately obvious why the kind of strategic balance which has developed between the Soviet Union and the United States should not also help to nullify the Chinese threat. Is it so entirely outrageous to look for some kind of dialogue between China and the other members of the nuclear club? In practice it is hard to think that the isolation of China from the rest of the world can persist unchanged for yet another decade. In the long run, and possibly in the immediate future as well, it may be more profitable for the rest of the world to respond to what is happening in China by talk rather than by the development of nullifying hardware.

## MOLECULAR MODELS

A REALIZATION of the importance of molecular model building was a major factor in Pauling's determination of the  $\alpha$ -helix of proteins and Watson and Crick's discovery of the structure of DNA. But model building—apart from providing a way of refining molecular structures which are being determined by X-ray crystallography and giving tangible representations of the final molecular structure—can by itself, in the right hands, provide a method for predicting the probable atomic structures of macromolecules which, for one reason or another, cannot be directly determined by X-ray diffraction. Such predictive model building is, however, fraught with pitfalls and only when it is

treated as a strict discipline can any reliance be placed on the results it yields. Of necessity, assumptions must replace data, and the validity of a model structure entirely depends on the validity of the principal assumptions; but a model must in addition bring together every scrap of relevant chemical and stereochemical data that is available, no matter how obtained, and synthesize it into a coherent whole.

The elucidation of a unique structure of a small but very interesting part of the transfer-RNA molecule, the anticodon loop, by Fuller and Hodgkin, which is reported on page 817, is a case in point. Crystallographic analyses of *t*RNA are impossible simply because, despite numerous efforts and some red herrings, it has so far proved impossible to crystallize the material. However, since Holley and his collaborators determined the nucleotide sequence of alanyl *t*RNA, the sequences of three other species of *t*RNA have revealed certain features common to the four molecules. Moreover, the structure of double helical RNA, that of reovirus, has been determined by X-ray diffraction, and the importance of the phenomena of base stacking in non-helical regions of nucleic acid molecules has been recognized. Fuller and Hodgkin have used these data and the accumulated information on bond lengths and angles in ribonucleotides and nucleotides to construct their model of the anticodon loop. By restricting their effort to this small region of the *t*RNA molecule, Fuller and Hodgkin have provided themselves with fixed points—the two free ends of the loop and a region of base-pairing. When the seven unpaired nucleotides that form the anticodon loop are arranged to maximize base stacking, taking into account all the known stereochemical constraints, there appears to be a unique structural solution for the four species of *t*RNA: five bases are stacked and two are in a less ordered configuration.

The model engenders confidence because of its stereochemical neatness and because it is based on two eminently reasonable assumptions—that there should be maximum base pairing and maximum base stacking. Moreover, any model must of course be reconcilable with the known biological functions of a molecule and the proposed structure of the anticodon loop is. It accommodates the genetic data on which Crick based his wobble hypothesis and it allows two *t*RNA molecules simultaneously to recognize adjacent codons of a messenger RNA molecule.

Obviously the structure of a molecule can never be proved by model building alone. Sometimes it may only show what structures are impossible, but may leave several alternative probabilities. When, however, a model accounts uniquely for all the known structural and functional properties of a molecule, it is probably substantially correct. It remains to be seen whether or not the model now proposed will stand when the nucleotide sequences of more species of *t*RNA have been determined. Meanwhile, model building may well be used to find the probable structure of another vital part of the *t*RNA molecule—the amino-acid recognition site.

## NEWS AND VIEWS

### Unfinished Business

THE disappearance of the British Parliament on vacation has left several important issues in abeyance. By the time the political season begins again, and certainly by the time that Parliament reassembles in October, a great many tasks will need urgently to be attended to. In science and technology, perhaps the most glaring need is for a decision on the future of the Atomic Energy Authority; although it seems to be unlikely that a radical change in the structure of the authority will be made in the months immediately ahead, the fact that a successor to Lord Penney (who moves to Imperial College in October) has not yet been appointed is one sign that there are decisions to be made. (The annual report of the authority, which usually appears in the summer, is being held up until the autumn, but this is probably more a sign that Lord Penney wishes to make its appearance his final public comment on the authority's business than a symptom of uncertainty.)

The report of the Advisory Council on Scientific Policy, expected about May this year, is also late, although it is known that the final draft has now gone to the Secretary of State for Education and Science. In the weeks and months immediately ahead, the council is likely to be preoccupied with the fixing within the government of targets for spending by the research councils into the early seventies. A year ago it seems to have been agreed that government expenditure on civil science should continue to increase, but at a declining pace. In particular, it has been laid down that in three consecutive years increases should amount to 10, 9 and 8 per cent respectively. Having established the point that plans for spending on research should be settled in broad outline three years in advance, the advisory council is now faced with the need to combat the Treasury's instinct to continue the progression 10, 9, 8 in such a way as to entail a continuing reduction in the pace of growth. The appearance of the council's report on the cost of what is called "sophistication", also due soon, is expected to make an important contribution to this question.

Another matter that will be looked at closely in the autumn is the operation of the Science Research Council, then to be blessed with a new chairman in the person of Professor Brian Flowers. The Science Research Council is, of course, the largest of the four research councils concerned with the natural sciences.

### Women not Wanted

FOR a nation suffering from a shortage of doctors, Britain seems to be remarkably prodigal of willing helpers. Last week a twenty-two year old girl left Britain to take up a place in a medical school in Prague, after she had failed even to be interviewed for a place at a British school. The girl, Miss Diana Pearce, who had three A levels and ten O levels, had been applying to London and provincial hospitals for

the past four years. She will now, presumably, be learning Czech in order to qualify as a doctor. Miss Pearce is unusual only in her determination; at various London hospitals last year the number of women applicants for each place varied between twenty and forty. Only about twenty per cent of the places at medical schools are for women, and competition for them is significantly higher than for men.

When Miss Pearce returns from Czechoslovakia, fully qualified, she will still not be able to practise in Britain. This is a predicament she will share with doctors holding qualifications from every country outside the Commonwealth, South Africa, Eire and Burma. If she only intends to remain in Britain for a short time, she may be registered temporarily with the General Medical Council. If, however, she decided to stay, she would have to acquire one of a number of British qualifications, a process that could possibly take as much as two or three years.

At the same time, two foreign doctors working in Britain have had their temporary registrations cancelled. One of these, a Polish woman doctor, had married an Englishman, and by signifying her intention to remain in Britain had disqualified herself from temporary registration. The other, whose case is still under review by the General Medical Council, and whose name has not been revealed, apparently had his registration cancelled while he was in process of taking examinations for British qualifications. He had already passed one examination. If the Council refuses to rescind its decision he has said that he will go abroad.

The reason why, under Act of Parliament, the General Medical Council will not allow licentiates of foreign medical colleges, including those of the United States and Europe, to practise in Britain without these preliminaries is that reciprocal agreements have not been made with its counterparts in these countries. Before the Second World War agreements did exist with Belgium, Italy and Japan, but these lapsed during the war.

### Cheap Digs for Students

THE University of Lancaster is going ahead with a scheme for cheap student accommodation that may prove a model for universities in the future. In its new building, to be completed in 1969, there will be no large communal rooms, but only self-contained flatlets. Each of these will house ten or eleven students, in single or double rooms. While the flats will have dining-room kitchens, students will be able to eat in their colleges, with which the new building will be integrated. Because of the lack of expensive communal facilities in the new building, and because it will stand in the university's own grounds, the cost will be only £700 per student, compared with £1,200 at other institutions. This in turn will mean low rents—three pounds for a single room, ten shillings less for a double room. Even so, the university hopes to make a profit, and so has been able to finance the scheme through an insurance company, instead of the University Grants Committee. £500,000 will be borrowed, and repaid over thirty years at an annual rate of £43,000. The cost of heating, light and maintenance will bring this sum to £66,500, while the revenue from 605 students will be £68,500, if all goes according to plan.



Conventional university discipline, communal meals, and times for visitors, will be impracticable in the new building. And it will be interesting to see how students take to living together in such large groups. The social and financial implications of the scheme will be discussed by a sub-committee of the Committee of Vice-Chancellors, in order that other universities may be informed of Lancaster's experiment.

## Post Office Computers

AFTER the haste with which the Post Office (Data Processing Service) Bill went through Parliament, the service seems to be taking some time to get down to work. The service is being planned by the General Post Office Computer Development Department, whose director is Mr C. R. Smith. Although computer time will be offered for sale almost immediately, the data processing service is not expected to become fully operational for about two years.

At present the Post Office has computer centres in London, Derby, Portsmouth, Edinburgh and Lytham St Annes, with computer equipment worth £4 million already installed and working. It plans to set up further centres at Bootle, Leeds, Glasgow, Bristol and Manchester. The cost of setting up this service is expected to be about £12 million. By 1971 the Post Office hopes to have twenty large computers in operation up and down the country, all linked together.

The computers will be primarily for the use of the Post Office—the Bootle centre, for example, will work with the Giro banking system, but as part of the service the Post Office will sell off surplus time. Twelve of the GPO computers are English Electric-Elliott and one is made by ICT. There are further English Electric-Elliott computers on order.

## Computers for Columbia

A SCHEME for permitting access to the computers being used at Columbia University from the offices of individuals making use of them is to be carried out by 1969. Altogether, the university is planning to spend \$3 million on the provision of 200 access points scattered around the Columbia campuses. The intention is to allow individual researchers to communicate directly with the whole of the computer system at Columbia. It has been calculated by those responsible for the development of the new system that the provision of several access points will increase five-fold the present power of the computers already installed at Columbia.

Apparently the planners are thinking simply of the extension of the present uses of the computer centre at Columbia, which has in the past four years grown so that it handles an average of between 6 and 7 million jobs each day. One advantage of providing multiple access to the computing network is that it will be easier for demands for small blocks of computer time to be met. In practice, it appears that the computer centre at Columbia tends to accumulate throughout each week a backlog of work which is cleared at the weekend. The network of access points now being provided is intended simply to help with the management of the computers' time. The system differs, of course, from true multiple access systems in which several jobs are processed more or less simultaneously.

## Air Fares Go Up

FROM September, air fares in Britain are to rise between 11 and 15 per cent. The Air Transport Licensing Board last approved an increase of fares in April 1966 so that this second fare rise within eighteen months has caused a certain amount of comment.

Comparisons are often made between the costs of travelling between London and Glasgow, and between San Francisco and Los Angeles: the British trip costs 7.49 cents per mile, the American only 3.96 cents. But San Francisco and Los Angeles are two poles of population density in a way in which London and Glasgow are not. A better comparison could be made with the New York-Pittsburgh route, on which passengers are travelling between a huge metropolis and one of a number of large industrial cities in a densely populated region. The cost of this journey, of length similar to the other two, is 6.87 cents per mile; and it is interesting to note that the sector is served by two carriers, one of which, Allegheny Airlines, is subsidized by the Federal Government. Pilot salaries are, of course, higher in the United States, but airport charges are heavy in Britain—the first thirty passengers on a Vanguard flight between Manchester and London pay for the airport charges, according to BEA. Air fares in Europe are often more expensive than in Britain, so that the new increases may well have been unavoidable.

Yet even if fares are relatively low, it is impossible to say how far the size of the increase is justified. This is because, although the Licensing Board has access to the accounts of the various airlines, it is not permitted to make them public. Private individuals have no right to object to the raising of fares, but the board sometimes allows them to make statements, especially if they represent groups of people likely to be affected by changes in fares. These individuals are then allowed to "cross-examine" the airlines; the airlines do not, however, have to reply.

Clearly the Licensing Board is unhappy with the situation. In its report on its decision to raise fares, the board says: "It is unfortunate that the powers under which it (the information on which the decision rests) was obtained do not allow us to make this information public. . . . We think that a general disclosure of such information would be beneficial to British aviation".

## More Scientists as Astronauts

AMONG the new NASA intake of 11 scientist-astronauts there is a Welsh chemist and an Australian physicist. The inclusion of these two naturalized American citizens breaks new ground for the American programme—indeed the Australian, Dr Philip Chapman, only gained his US citizenship this May though he has been working at the Experimental Astronomy Laboratory of MIT since 1961. The Welshness of Dr John Llewellyn—despite 9 years' work in North America—is not to be concealed; besides his own good Welsh name his wife was born Valerie Davies-Jones and his 3 children rejoice in the names Gareth, Sian and Ceri Elum-med. Both naturalized astronauts have gravitated to their present appointments by way of Canada. The acceptance by NASA of naturalized citizens for their sensitive manned space programme reflects the new emphasis on appropriate scientific background for the second generation of manned space operations

(post-Apollo). This group is clearly intended for the manning of the Apollo Applications Programme where the premium is put on manned supervision of astronomy from orbit. Four of the 11 have research experience in astrophysics.

As a group, the 16 scientist-astronauts now under active training are less stereotyped than the previous 49 jet pilot recruits. They do not average 2.3 children each as before, and their age spread is much wider—the geophysicist of the new group is only 25 and one of the astronomers is already 40. Dr Garriott, an ionospheric physicist selected in 1965 as one of the first group of scientific astronauts, was in London recently attending the IQSY-COSPAR meeting and renewing acquaintance with the Radio and Space Research Station, Slough, where he had worked for a year before becoming an astronaut candidate. He made it clear that scientists selected were not led to believe that their expertise had been earmarked in advance for a particular mission—quite the contrary. Training, which is a continuous process up to the moment of lift-off, is aimed at broadening scientific experience as well as technical skills—such as piloting jet aircraft. In fact Dr Garriott had come to England from a geological field party in Iceland where some of the volcanic regions are thought to resemble lunar terrains more closely than most terrestrial landscapes.

Of the total tally of 66 astronauts, 56 are on the active list today. An Air Force doctor in the 1965 intake of scientist-astronauts resigned; one of last year's pilot officer recruits was killed in a motor accident in June; three of the regular astronaut groups II and III were killed in aircraft crashes during training; three, including space-walker White, died in this year's tragic Apollo ground-test accident. US Navy Commander Scott Carpenter has just returned to the Navy to pursue his work in "inner space" in the "Man-in-the-Sea" experimental programme, and John Glenn has been seconded to other duties.

## Doctorate Production

THE National Science Foundation of the United States has issued its estimates of scientific manpower for the next decade. On the assumption that the student population will continue to increase at its present rate, the United States will need three hundred and seventy thousand science lecturers and research workers in universities by 1970. It is expected that the percentage of those university workers with PhD degrees will fall over the next few years, but will return to the present level in the mid seventies. The estimates of those taking the bachelors' and doctors' degrees are as follows:

	Bachelors	Doctors
1965	238,000	12,100
1970	348,000	20,000
1975	428,000	30,500

Of the newly qualified PhDs of recent years, about 15 per cent were foreign, and the estimates assume that there will be no change in this proportion.

Though the United States will be doubling what the foundation calls its "baccalaureate and doctorate production" in the ten year period, its population is only expected to increase by 18 per cent, to two hundred and thirty million. Alternatively, the progression of the United States towards a nation in mortar boards can be shown by calculating the proportion of babies

born in any year who are destined to become science graduates at the age of twenty-one. Of those born in 1944, one in twelve received a B.Sc. in 1965. Of those born ten years later, one in nine are expected to graduate in science, and more than one in twenty-nine will acquire a higher degree.

The report gives no indication of how far into the future we may expect the expansion of American education to continue. There are more ways to produce doctorates than by training first class minds; the report says nothing of the maintenance of standards. And if baccalaureate production is to double every decade, as it has done, while population output lags behind, can quality control be stringent?

## Missiles for Mao

THE Joint Committee on Atomic Energy of the United States Congress now believes that China will have inter-continental ballistic missiles with nuclear warheads in the early 1970s. Its report states that there is evidence that the sixth and latest Chinese test device was dropped from an aircraft. The fall-out from the fifth test, details of which were not released by the American Atomic Energy Commission at the time, indicated that uranium-238 was being used as fissionable material, and that the fusion material, tritium, was produced by fission-induced decay from lithium-6. The Chinese therefore seemed to have solved most of the problems of designing fusion bombs by last December, and the committee believes that the yield of the fifth bomb may have been deliberately limited, to keep the fall-out over China to a low level. The explosion of a megaton bomb was fully expected. The first four tests conducted in China took place in October 1964, May 1965, and May and October 1966. The third of these was the largest, the size of the weapon being between two hundred and a thousand kilotons. There is evidence that lithium-6 was involved in this third bomb, as well as the fifth. The other three were smaller, the first being twenty kilotons, while the other two were slightly larger, in the low intermediate range of between twenty and two hundred kilotons.

Reviewing the French atomic programme in the same report—a strange juxtaposition, since the report is supposed to deal with the effect on United States security of the Chinese weapons—the Committee concluded that France is a long way behind China. It even doubts whether the French will explode a hydrogen bomb in 1968. On the other hand, the French do have a strategic force of Mirage IVs; the Chinese are not known to have a means of delivering their more powerful weapons. China has already developed an intermediate range missile, according to the report, but there are no signs that this is in service. The Chinese are also supposed to be "interested in the development of submarines" armed with long range rockets, though the committee has no knowledge of actual developments.

The report will, no doubt, influence the argument about the development of anti-missile defences in the United States. The committee mentions that the United States will lack defences against a Chinese attack, but does not reveal its views on the establishment of an anti-missile system. Nor is there any discussion in the report of the possible effects of the cultural revolution on Chinese activities. It is difficult

to believe that scientists can long remain undisturbed, or that the Chinese economy can continue to bear the huge burden of the nuclear programme.

## No Diagnosis, No Cure

DURING its twenty year life, the British National Health Service has often been criticized. Usually criticism has been directed at the service, rather than the doctors and nurses who make it up. Recently this unwritten rule has been broken by a book, *Sans Everything*, compiled by an organization called AEGIS (Aid for the Elderly in Government Institutions) and written by Mrs Barbara Robb, chairman of the organization. The book alleges bad nursing practices, mismanagement and even physical ill-treatment of elderly patients in some geriatric and psychiatric hospitals. The book does not name the hospitals, nor its sources of information, explaining that this is done to protect the informers from becoming scapegoats.

After some weeks of negotiation, Mr Kenneth Robinson, the Minister of Health, has persuaded Mrs Robb to tell him confidentially the names of the hospitals involved. This, he said, would enable the truth to be established. But he has now set in motion enquiries which seem calculated to reassure only those who never believed the allegations in the first place.

First, the enquiries are to be held by the hospital boards. In effect the boards are being asked to set up enquiries into their own management of their own hospitals. True, the ill-effects of this will be mitigated by the fact that the committees of enquiry (each with a Queen's Counsel as chairman) will be selected from people from outside the region, but the hospital boards will be responsible for selecting them. (The Lord Chancellor has put forward a list of names of QCs to the minister, who has forwarded them to the boards for selection. Other members of the committees will be a doctor, a nurse, and a member of the public, man or woman.)

Second, proceedings will be held in private, which is never the best way of convincing critics outside. Staff and patients, the ministry says, will be able to give evidence about hospitals in complete confidence. But if they want to make allegations against named individuals, the ministry adds, they must be prepared to do so in the presence of the individuals accused. It is hard to think that nurses, who are best placed to know what is going on, will come forward to give evidence against matrons.

## Merger for Engineers?

THE Institution of Mechanical Engineers and the Institution of Heating and Ventilating Engineers are considering the possibility of a merger. The stimulus for the talks seems to have been the desire of the IHVE to join the Council of Engineering Institutions—for this a Royal Charter is necessary. The IHVE has twice applied for a Royal Charter, a form of official recognition which entitles the institution to represent its members, award qualifications and give evidence or comment on legislation to government departments or working parties.

When the IHVE first applied for a charter, in 1948, its request was turned down. In 1962 it applied again, but the decision was held in abeyance. The institution

also applied to join the CEI, and during discussions it was suggested that the position might be easier if the IHVE were to consider the possibility of a merger with the mechanical engineers, already members of the CEI. This is the possibility which is now being considered, although both institutions point out that the discussions are still at an early stage.

## Astronomy by Computer

THE Institute of Theoretical Astronomy at Cambridge is now in business. Armed with the tools of their trade—paper, sharp pencils, and an IBM 360/44—the astronomers will have been at work for several months by the time the institute is officially opened at the end of the year.

The institute, of which Professor Fred Hoyle is the director, has been made possible by a grant of £250,000 from the Nuffield Foundation to cover staff costs, and a matching grant from the SRC to buy the computer. The Wolfson Foundation gave a grant to pay for the special building, and the University of Cambridge is also contributing towards running costs. The common factor behind these generous gestures of support seems to have been the fear that Professor Hoyle would turn his thinly veiled hints of emigration into reality. That, at least, seems to have been averted, but Professor Hoyle will continue to be something of a trans-Atlantic commuter. One of the features of the institute will be the provision of facilities for visiting workers, many of them no doubt from America, and joint studies in collaboration with the California Institute of Technology are proposed.

When complete, the institute will have a staff of about 20, all of them post-doctoral. No teaching in the conventional sense will be expected of them, and the institute is not part of any of the Cambridge faculties. There will, however, be some lectures suitable for graduate students. The research work will cover subjects such as stellar structure, relativity, cosmology and celestial mechanics. The joint studies with Caltech, which will give the Cambridge workers access to the outstanding facilities for observational work in California, will include work on the synthesis of elements in stars.

## Immunochemistry Moves to Oxford

A new Medical Research Council Unit for research into immunochemistry is to be set up at the University of Oxford, under the directorship of Professor R. R. Porter, FRS, who succeeds Sir Hans Krebs, FRS, as Whitley Professor of Biochemistry in the university in the coming academic year. The unit will be concerned with investigating the structure of antibodies, and its relation with their function. The problem is to discover how antibodies can combine specifically with an unlimited number of different antigens. It is hoped that research into this will throw light on the formation of antibodies, and allow a greater control of immune reactions. Some reactions, such as tissue graft rejection, are, however, caused by cells, not antibodies, and the unit will begin studies of the relationship of the two types of reaction.

Professor Porter, who has been Professor of Immunology at St Mary's Hospital Medical School since 1960, established the MRC Research Unit in Immuno-

chemistry there two years ago. In 1966 he was given the Gairdner Award of Merit for his work in this field.

## More Defenders for Aldabra

THE US National Academy of Sciences has allied itself with the Royal Society in the society's war with the British Ministry of Defence over the proposed airfield on the island of Aldabra in the Indian Ocean. The Academy has been having informal conversations with Federal agencies in the hope that another site might be found.

The Royal Society also announced this week details of an expedition to the island to gather as much information as possible before any development might start. The expedition will consist of three phases: a short-term dry season phase, during this month and next, to complete reconnaissance observations started in 1964, to investigate the lagoon area, to study land molluscs, and to begin long term studies on the giant tortoises, birds and marine life; a long term dry season phase from September to December, to continue the studies on tortoises, birds and marine life; and a wet season phase, from December to March 1968, to continue this study and begin studies on insects and land plants. The overall leader of the expedition is Dr David R. Stoddart, a lecturer in the Geography Department at the University of Cambridge. He helped carry out the reconnaissance observations in 1964.

A decision is expected from the Ministry of Defence on the future of Aldabra by May 1968, but scientists may be able to take heart from the fact that in some circles the strategic value of a base on the island is in doubt. Mr Alastair Buchan, director of the Institute for Strategic Studies, in a letter to *The Times*, said that he had great difficulty in understanding what function a base tucked away in that corner of the Indian Ocean would serve, unless it was for British or American military intervention in Southern and East Africa—a role that had dubious credibility. He suggests that Gan, Diego Garcia and the Cocos Islands provide natural staging bases for south-east Asia unless the ministry is contemplating the bizarre alternative of trooping from Britain to Asia via Ascension and Aldabra, which are 4,500 miles apart, the extreme load range of the most powerful aircraft under development.

## Where to put Reactors

THERE is every prospect that the US Atomic Energy Commission will persist with its cautious policy on the siting of reactors and that it will in the process win the approval of most interested parties. So much can be inferred from the report of the hearings on the licensing and regulation of nuclear reactors held in April and May this year by the Joint Committee on Atomic Energy, the report of which has now appeared. The AEC itself, in the person of the chairman, Dr Glen Seaborg, takes the line that more experience of the operation of reactors with the capacity of those now being installed at electricity generating stations will be necessary before it will be possible to lay down criteria for deciding when reactors can be placed, as many of the utility companies would wish, in or near urban areas. In his statement to the joint committee, Dr Seaborg pointed out that although there were in

April 323 nuclear reactors of all types operating in the United States, and although these have accumulated 1,870 reactor-years of operating time, there were only 14 power reactors licensed for civilian operation with an accumulated operating time of 60 reactor-years.

The incentives which tempt utility companies to site reactors close to cities are easy enough to identify, of course. It also seems that in its operation of the licensing procedures, the AEC has been prepared to relax a little the stringency of its rules for deciding how close to centres of urban population reactors can be sited. In granting permission for the operation of a reactor, the AEC specifies a region called an exclusion zone around the reactor which must be entirely within the control of the reactor operator, and then a more extensive area in which the population must be low. The sizes of both areas are determined by the size of the reactor and by some of its design characteristics. In the consent for the building of the San Onofre plant in California, the AEC reduced the exclusion distance from 0.8 miles to 0.5 miles, while the radius of the low population zone was reduced from 12.5 miles to 4 miles. In some respects this site is exceptional, chiefly because it is surrounded by a military reservation, but it appears that similar relaxations of the strictest rules have since been applied to power reactors being constructed in New England.

## Making Medicine Scientific

A FRESH look at the scientific and technical services in British hospitals is to be taken by a committee set up jointly by the Minister of Health and the Secretary of State for Scotland. As the ministry points out, there has been a rapid increase in the use of science and technology in medicine, but so far the expansion has been disjointed and has not followed a coherent policy. The committee will be considering the future organization of the scientific and technical services, and the broad pattern of staffing required. It will be making recommendations to the ministers.

The scope of the survey will be wide. It is to cover the use of physicists, biochemists and physiologists, with their supporting technicians, which will presumably include all the laboratories within the health service. It is also likely to include the newer services such as the cervical cancer smear tests. Since the intention here is to make smear tests available to all women at risk, large laboratory facilities are likely to be needed. The problems of staffing renal dialysis units—the reason given by the ministry several months ago for the slow expansion of the units—might also be worth considering. There are also enormous possibilities of mechanization in intensive care units, with the possibility of continuously monitoring the patient's condition automatically. So far the health service has done little in this direction.

The new committee is to be chaired by Sir Solly Zuckerman, Chief Scientific Adviser at the Cabinet Office and chairman of the Advisory Council on Science and Technology. The other members will be Professor A. R. Currie of the University of Aberdeen, Dr R. Gaddie from the General Hospital at Birmingham, Professor J. E. Roberts from the Middlesex Hospital and the University of London, Mr. A. B. Scott from the United Manchester Hospitals Board of Governors, Professor J. P. Shillingford from the Hammersmith Hospital,



and Dr S. Shone from Sheffield Regional Hospital Board.

## Rabbits by Radio

SOME Australian rabbits are being fitted with miniature radio transmitters to reveal their movements. This and other wildlife research—aimed at the control or conservation of a variety of mammals and birds—is described in the latest annual report of the CSIRO Division of Wildlife Research. A recurrent problem in the control of rabbits is re-infestation, and the animal's movements are being studied to find out how far they can travel. Detailed recording of movements at night—previously difficult in large enclosures or field conditions—will be much easier now that transmitters small enough, and an effective means of harnessing them to the rabbit, have been developed. Transmitted signals are picked up by receivers with directional aerials.

The division's largest single research programme—on the biology of rabbits—also includes investigations into the activity and use of the odoriferous glands which have shown that the sub-mandibular and anal glands are primarily used for territorial marking, while the inguinal gland is associated with sexual attraction. While a rabbit is engaged in social activities, such as examining an object which carries the scent of another rabbit, it drops pellets with the strong odour of the anal gland. This odour is much fainter, or absent, when the rabbit is engaged in non-social activities. This knowledge may help to explain the significance of the "dung-hills" which are found in rabbit territories.

As part of a long term study of fluctuations in numbers of nematode parasites in rabbits in Queensland and New South Wales the CSIRO are looking for the reason for a striking increase in nematodes in females during the breeding season, while at the same time there is a decrease in the parasites in males. Experimental results suggest that the state of susceptibility of a rabbit—particularly a female—to the nematode *Trichostrongylus retortaeformis* is closely associated with its hormonal status. Luteinizing hormones given to does at eighteen day intervals to cause ovulation decreased their susceptibility to the nematodes, while cortisone increased the susceptibility of bucks. The CSIRO feel that this work could lead to the use of hormones in controlling parasites in domestic stock.

## Dissemination of Tumours

from a Correspondent

THE greatest problem in the treatment of cancer is the ability of cancers to invade surrounding tissues and form metastases. Despite this, little attention has so far been paid to the development of screening tests which will detect compounds which affect tumour dissemination or the growth of newly formed metastases. The Committee on Experimental Chemotherapy of the International Union against Cancer recently organized a one day meeting to discuss the possibility of designing such tests.

The meeting opened with a description of the mechanism of cell adhesion and how the altered surface charge of tumour cells decreases their adhesiveness by affecting the properties of the cellular membrane. The importance of this loss of adhesiveness in the shedding of

individual cells from a tumour was discussed. This first stage of tumour dissemination from the point of view of a physical chemist was then supplemented by accounts from biologists of the various steps in the formation of secondary deposits. Tumours penetrate surrounding tissues by invasion or infiltration or may be intravasated by mechanical pressure. Then follows dissemination through the circulation followed by lodgement and growth. The ability of various organs to trap disseminated tumour cells varies greatly. Intracerebral inoculation of tumour cells to rats or mice is followed by the appearance of these cells in the lung and liver and only later in the kidney. The presence of malignant cells trapped by tissues can be demonstrated by a number of techniques including tissue bioassay, isolated organ perfusion and plating techniques. But even if an organ is efficient in trapping disseminated tumour cells, this is no indication that a secondary tumour will eventually appear. Injection of rodent tumour cells into chick embryos has shown that particular tumours are trapped most efficiently by liver, spleen and kidney, with brain the least efficient organ, but it is only in the brain that tumours appeared. The site of tumour growth in the chick embryo depends on whether or not the injected tumour cells have lost their strain specificity or on the degree of malignancy of the tumour. Time did not allow the presentation of the majority of papers on the factors affecting cancer dissemination and metastases, although evidence was presented that known chemotherapeutic agents could influence the behaviour of tumours, probably by affecting immunological mechanisms.

The actual test systems available for studying the effect of compounds in inhibiting cancer dissemination or the growth of metastases are few. Three dimensional growth of tumours or embryonic tissue in a matrix consisting of cellulose sponge coated with collagen enables the study of tissue architecture and of the interaction between tumours and embryonic tissue. Such a system would enable an investigation of the effects of chemical agents in preventing tumour invasion or infiltration which is usually the first stage of dissemination. The technique of intracerebral tumour implantation is already in use as a system to detect agents which might prevent the systemic spread of the tumour. A subcutaneously transplanted tumour which leads to the formation of metastases in the lung is also used for the same purpose.

The conclusion of the meeting was that this important aspect of experimental cancer chemotherapy should be extended. Although it was agreed that the tests available at present were very few, there was every hope that new tests could be designed. In order to stimulate further interest in the subject the Experimental Chemotherapy Committee of the UICC plan to hold a symposium in 1969.

## Hormone Insecticides

from a Correspondent in Cell Biology

WILLIAMS and his collaborators at Harvard are doing some very exciting work which shows that insect juvenile hormone and naturally occurring and synthetic analogues can be used as a potent insecticide for the control of some very noxious insects. The group's latest finding (Vinson and Williams, *Proc. US Nat. Acad. Sci.*, **58**, 294; 1967) is that synthetic juvenile hormone

effectively blocks the hatching, metamorphosis and sexual maturation of the human body louse *Pediculus humanis*—a vector of epidemic typhus, trench fever and epidemic relapsing fever—which in many areas of the world has become resistant to virtually all the currently available insecticides.

Williams (1956) first recognized the potential value of juvenile hormone as an insecticide when he found that hormonally active extracts of certain moths cause lethal derangements of metamorphosis when applied to silkworm pupae. This was followed by the intriguing discovery (Sláma and Williams, 1965) that American, but not European, paper contained a natural analogue of juvenile hormone. The principal source of this material was eventually tracked down to pulp from the balsam fir *Abies balsamiae*, an indigenous American pulp tree. Law, Yuan and Williams (1966) then made a synthetic juvenile hormone of high activity which blocks the metamorphosis of the yellow fever mosquito *Aedes aegypti* (Riddiford and Williams, *Proc. US Nat. Acad. Sci.*, **57**, 595; 1967) as well as the human body louse.

In the current experiment, Vinson and Williams induced body lice to lay eggs on woollen or nylon pads impregnated either with synthetic juvenile hormone or, as a control, peanut oil. Only 24 per cent of the eggs on the hormone treated woollen pads hatched; none of those on the nylon pads but 82 and 88 per cent of the controls hatched. Moreover, if the female lice were exposed to hormone for more than 24 h before laying, none of the eggs laid on hormone impregnated pads hatched, whereas 85 per cent of the controls hatched.

Only 46 per cent of the eggs laid on unimpregnated pads by females given a 24 hour exposure before laying hatched, compared with 96 per cent hatching of eggs from females previously exposed to peanut oil. This effect, however, is shortlived. Eggs laid on unimpregnated pads more than 24 hours after the end of exposure of the females hatched successfully.

Synthetic hormone also prevents the normal development of nymphs. Up to the third instar, nymphs exposed to hormone behave like controls exposed to peanut oil. At this stage the controls undergo metamorphosis, become sexually mature and produce a new generation, but in striking contrast none of the nymphs exposed to hormone reaches sexual maturity.

Under laboratory conditions, synthetic juvenile hormone is obviously a very effective insecticide and ovicide. It will be of great interest to see whether these experiments can be scaled up to provide a commercially feasible method for the control of disease carrying insects. Significantly, the United States Army is a co-supporter of the work. Perhaps the greatest obstacle apparent from the experiments is that the effect of the hormone is so short-lived that the insects must be more or less continuously exposed to it.

## Pattern Vision after Lesions

from a Correspondent in Neurophysiology

Galambos, Norton and Frommer have now published results which show that even after extensive lesions to the optic tracts—severing up to 98.5 per cent of the fibres present—the cat is able to perform a visual form discrimination (*Exptl. Neurol.*, **18**, 8 and

26; 1967). Lesions were made electrolytically under stereotactic control; the illustrations show that they were well placed and that they destroyed at least as much tissue as was claimed. On the whole, there is little apparent damage to surrounding areas. Five cats with lesions destroying continuity of between 80 and 96 per cent of optic tract fibres on both sides were observed for visual deficits after the operation. Four were quite normal while one showed slight difficulty in manoeuvres involving vision.

Histological results obtained from these animals were used to guide lesion placements in a further three cats which were examined more thoroughly for visual defects in both flux discrimination and pattern discrimination. Animals were tested in a discrimination box for their ability to distinguish 6 from 9. One cat which had nearly all of both optic tracts destroyed between the chiasm and lateral geniculates learned by the fifth day after operation to make 40 out of 50 correct responses (29 of the last 30 were correct). The second, with lesions sparing only 3 per cent of fibres on one side, had reached the criterion of 90 per cent success within 350 trials before the operation, but afterwards could not at first see the choice panels. Eight days later, however, it began to show continual improvement above chance and gradually reached the 90 per cent criterion. The third cat, with no pre-operative training, reached criterion for a flux discrimination in two days, taking sixteen days for pattern discrimination. In a further group of six cats with lesions interrupting more than 99 per cent of the optic tracts, three with total lesions were blind, while three with no more than 1 per cent of optic tract fibres intact appeared blind in gross behavioural tests but none the less performed a flux discrimination 35 days after the operation. They were unable to re-learn a pattern discrimination.

The authors consider that these results are at variance with the apparently orderly structure of connexions within the visual system of the cat. But this is not particularly surprising. First, the capacity of the optic tract for transmitting information far exceeds the capacity of the brain for receiving it, if subsequent behaviour is considered to be determined only by the information processed. Many experiments on both human subjects and other mammals have clearly shown the limited capacity of the central nervous system for accepting information. Barlow, in the NPL symposium "The Mechanization of Thought Processes" (HMSO, 1958), gives a good discussion of redundancy in sensory systems. Secondly, we know that to establish recognition of an object it is not necessary for the same set of photoreceptors to be stimulated at each presentation. There is considerable size and orientation independence in pattern recognition: it is clear that the process of interest involves the central abstraction of features from a stimulus which may be conveyed from the periphery by a great number of paths. Within limits, it is not therefore essential that every pathway should maintain its integrity, and Galambos *et al.* give an estimate of these limits under the circumstances of their experiment. For the initial phase of an animal's life in which pattern recognition is learnt it is much more likely that the integrity of the visual pathway is necessary, but just as this learning involves recognition that many different stimuli can give rise to the same percept, so are there many ways of presenting the

necessary information for the performance of a visual discrimination. Lesions to the visual pathway must limit the number of ways in which a stimulus can be represented, but unless they are very extensive they need not effectively limit the rate of information transmission.

## Problems in Associating Systems

from a Correspondent in Molecular Biology

THE study of protein systems in which sub-units exist in association-dissociation equilibrium has come to have a fundamental interest in protein chemistry and enzymology. Many examples have recently come to light of situations in which the state of aggregation of the protein controls its function, and increasing attention has therefore been focused on the theoretical problems of analysing such systems by transport and equilibrium methods, notably sedimentation velocity and equilibrium, free boundary electrophoresis and partition chromatography. In all these processes the act of fractionation leads to the displacement of the equilibrium in a manner which is determined by the properties of the aggregated and disaggregated forms, the total concentration and the equilibrium constant(s) for the association. In principle it is possible to derive information about the stoichiometry, the equilibrium and the value of the equilibrium constant; the theory for such systems has been developed in general terms by Gilbert and others.

An article by Kegeles, Rhodes and Bethune (*Proc. US Nat. Acad. Sci.*, **58**, 45; 1967) now draws attention to a serious experimental source of error, hitherto completely overlooked, which may invalidate many of the results obtained on associating systems studied in the ultracentrifuge by sedimentation velocity. In high-speed sedimentation experiments a substantial hydrostatic pressure is generated, which can amount to hundreds of atmospheres towards the bottom of the cell. In aqueous systems, at least, this is insufficient to have more than a trivial effect on sedimentation velocities, but it can have a drastic influence on an equilibrium process. There is ample evidence that association processes are commonly accompanied

by an increase in molar volume, which can be observed by dilatometry. The application of pressure consequently leads to a displacement of the equilibrium towards the dissociated form. For realistic values of the molar volume of association, it turns out that the pressures generated in the ultracentrifuge at high speeds are sufficient to produce a substantial change in the equilibrium constant, which would therefore vary through the liquid column, and make analysis intractable. Kegeles *et al.* show that concentration distributions of quite misleading and unexpected shapes could be obtained, and evaluate some of the consequences of this situation.

In any event it appears clear at this stage that low-speed sedimentation equilibrium and partition chromatography ('Sephadex' gel filtration, for example) offer safer methods of studying such equilibria. The applicability of gel filtration has been discussed by Gilbert, Ackers and others, and a new analysis by Ackers (*J. Biol. Chem.*, **242**, 3026; 1967) has now appeared. Gel filtration involves a simple partition process of the molecules between the insides of the gel globules and the free solvent outside. The method has several experimental advantages, and Ackers has shown that dissociation constants may be determined even when the partition coefficients of the molecules are concentration-dependent. Although gel filtration provides probably the most advantageous approach to these equilibria, it cannot give molecular weights, except of an approximate nature, and sedimentation equilibrium is probably best used for this purpose. It is probable, however, that the increasingly firm theoretical foundation of the gel filtration technique, allied with its experimental precision and simplicity, will encourage widespread application.

An interesting example of the application of sedimentation equilibrium is the work of van Holde and Rossetti (*Biochemistry*, **6**, 2189; 1967) on the association of purine in aqueous solution (which presumably involves the stacking mechanism operative in polynucleotides). The results compare well with earlier studies by other methods, and support the conclusion that the aggregation process can be described by a single association constant. Thermodynamic parameters are derived.

## Steady State Crustal Spreading

by

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Evidence which bears on the processes of continental drift, ocean floor spreading and the appearance of mid-oceanic rises is rapidly accumulating. The assumption that the mid-oceanic rises represent a steady state<sup>1,2</sup> leads to estimates of the rate of ocean floor spreading and of subsidence which in turn suggest geophysical tests of the theory.

### Crust

RECENT identification of the relationship between the observed symmetric magnetic anomaly patterns across mid-ocean ridges<sup>4</sup> and magnetic field reversals during the

past  $3.4 \times 10^6$  yr B.P.<sup>5</sup> has provided a unique method of determining ocean floor spreading rates. Vine<sup>6</sup> determined rates of 0.95 cm/yr for the Mid-Atlantic Ridge south of Iceland, 1.9 cm/yr at 38° S., 4.4 cm/yr for the East

Pacific Rise at  $51^\circ$  S., and 2.9 cm/yr for the Juan de Fuca Ridge, while Pitman and Heirtzler<sup>7</sup> determined a spreading rate of 4.5 cm/yr for the Pacific-Antarctic Ridge. Thus it appears that crustal spreading rates vary along the length of the mid-ocean ridge system by at least a factor of four. This longitudinal variation in velocity may distort the usual parallelism of the anomaly pattern associated with mid-ocean ridges<sup>7</sup>. The magnetically determined spreading rates determined for the Atlantic Ocean are plotted as a function of latitude in Fig. 1.

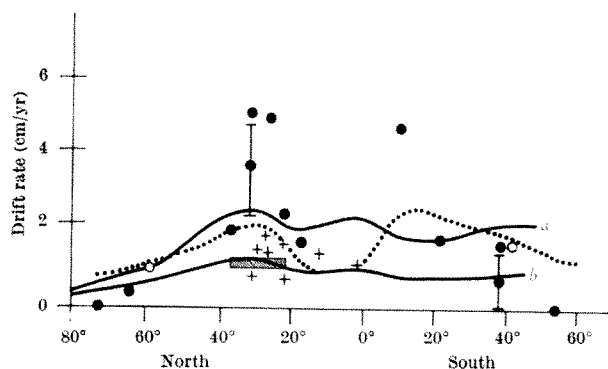


Fig. 1. Variation of drift rate with latitude in the Atlantic Ocean. Closed circles are computed from island ages<sup>11,12</sup>, and latitude of island is plotted. Vertical bars at  $33^\circ$  N. and  $38^\circ$  S. are due to uncertainty in age of Madeira, and distance of Tristan da Cunha from axis, respectively. Open circles are computed from magnetic anomalies<sup>6</sup>. Horizontal bar indicates upper limit of drift rate for North American Basin<sup>13</sup>. Crosses are drift rates and latitudes of oldest cores<sup>13</sup>. Solid curves are expected variation of drift rate if rifting began at  $120 \times 10^6$  yr B.P.<sup>9</sup> (a) or  $260 \times 10^6$  yr B.P.<sup>8</sup> (b), and is proportional to east-west width of Atlantic Ocean. Dotted curve is variation if rate is proportional to east-west width of mid-Atlantic ridge, and edges of ridge are  $50 \times 10^6$  yr B.P. isochrons.

Besides these magnetic data there is other evidence for longitudinal variations in crustal spreading rates. Such evidence includes the ages of sediment cores from the ocean floor; geological evidence of continent separation; variation in width of mid-ocean ridges; the age of islands as a function of distance from the axis of the Mid-Atlantic Ridge; and the occurrence of numerous lateral offsets along oceanic ridges.

From the ages of sediment core J. Ewing *et al.*<sup>8</sup> have concluded that rifting of the Atlantic floor began at least  $260 \times 10^6$  yr in the region of the North American Basin. If spreading has progressed at a constant rate since initial rifting, the region  $20^\circ$ – $40^\circ$  N. is moving at 1.2 cm/yr. This value is plotted as a horizontal bar in Fig. 1. In contrast, King<sup>9</sup> has used geological evidence to argue that the separation of South America and Africa was initiated about  $120 \times 10^6$  yr ago. Again assuming no temporal variations, the average spreading rate for the South Atlantic would then be 2.5 cm/yr.

If, on the other hand, simultaneous east-west separation of the American-Eurafrican rift is taken as a constraint, then the variation in width of the Atlantic Ocean should directly reflect longitudinal variations in crustal spreading rates. Using the above two starting dates of 120 and  $260 \times 10^6$  yr B.P. as illustrative examples of synchronous rifting, variations in spreading rate of the Atlantic as a function of latitude are as plotted in Fig. 1.

The width of a segment of the mid-ocean ridge may also be a measure of its rate of spreading. This follows if crestral and abyssal depths are relatively constant, mid-ocean ridges are steady-state features for periods of  $10$ – $50 \times 10^6$  yr and subsidence of the flanks is to first approximation only a function of time after extrusion at the axis.

The variation of ridge width in the Atlantic Ocean is plotted as a function of latitude in Fig. 1. The datum for this curve was arbitrarily taken to be the magnetically

determined drift rate of 0.95 cm/yr (ref. 6) south of Iceland. The curve is seen to pass through the other magnetically determined drift rate of 1.9 cm/yr at  $38^\circ$  S.<sup>6</sup> and roughly reflects the variation of spreading rates computed from island ages<sup>10</sup> discussed below. Available data<sup>6</sup> indicate that the generally wider East Pacific Rise is also spreading more rapidly.

The contention that the width of a mid-ocean ridge is related to the spreading rate is readily tested by detailed magnetic surveys, for the width of anomalies should then vary inversely with the regional slope. Pitman and Heirtzler<sup>7</sup> have analysed magnetic anomalies over the Pacific-Antarctic Ridge and the Reykjanes Ridge to get crustal spreading rates. From their data it follows that (a) for at least the last  $3.4 \times 10^6$  yr the spreading rate has been 1 cm/yr for the Reykjanes Ridge and 4.5 cm/yr for the Pacific-Antarctic Ridge. (b) Spreading has been linear with time, and thus with distance, at least out to the  $3.4 \times 10^6$  yr isochron on both ridges. This corresponds to a distance of 34 km from the axis of the Reykjanes Ridge and 153 km from the axis of the Pacific-Antarctic Ridge. Because the magnetic signature of the two ridges can be matched by linear adjustments of horizontal scale, it appears that spreading is linear out to 100 km on the flanks of the Reykjanes Ridge and 500 km on the Pacific-Antarctic Ridge flanks<sup>7</sup>. This supports the suggestion that the spreading rate increases gradually with distance from the rift axis<sup>14,15</sup> only if the rate of increase is proportionately equal at the areas compared. (c) Estimates from Pitman and Heirtzler's<sup>7</sup> Figs. 2, 3 and 4 give an average subsidence rate to the  $10 \times 10^6$  yr isochron of about 10 cm/ $10^3$  yr for both the Reykjanes and Pacific-Antarctic Ridges, if these ridges are assumed to have been steady-state for the last  $10 \times 10^6$  yr. Comparison of these two ridges thus suggests that ridge width is related to spreading rate.

Wilson<sup>10</sup> first plotted radiometrically determined ages of islands in the Atlantic Ocean against distance from the axis of the ridge. The data<sup>11</sup> (Fig. 2) suggest a general increase of age with distance, thus supporting the hypothesis that islands form near the crest and drift away at the rate of a few cm/yr. Because the oldest rocks may not have been sampled the indicated drift rates represent upper limits (unless some of the volcanic islands originated on the ridge flanks rather than at the axis of rifting<sup>2</sup>).

If the dated rocks originated at the axis of rifting and the drift rate has been constant with time, from Fig. 1 it seems that spreading rates are low at the northern and southern extremities of the Mid-Atlantic Ridge but exceed 2 cm/yr in the regions  $20^\circ$ – $40^\circ$  N. and  $10^\circ$ – $40^\circ$  S. The two drift rates calculated from magnetic anomalies<sup>6</sup> are in rough agreement with the drift rates determined from island ages.

Some of the scatter in Fig. 2 may be due to the assumption that spreading has been east-west from the rift axis. Suppose instead that the islands were carried away from the axis of the Mid-Atlantic Ridge along flow lines suggested by Carey<sup>12</sup>. These flow lines are the trajectories along which continents have supposedly separated. In Fig. 3 the oldest core and island dates<sup>10</sup> have been replotted as a function of distance from the rift axis along the flow lines. These distances were scaled to an estimated accuracy of about  $\pm 5$  per cent.

If rifting began simultaneously throughout the Atlantic, then the drift rate of a unit of the ocean floor should be proportional to the total length of the flow line passing through it and extending from the rift axis to the edge of the continent. Fig. 4 shows the same data as Fig. 3 but plotted against a dimensionless number which is the ratio of distance from the axis to total length of the flow line. These attempts to reduce the scatter on Fig. 2 do not conclusively demonstrate either that drifting began simultaneously or that the spreading rate has been constant in time.

The paleontologically determined ages of some pre-



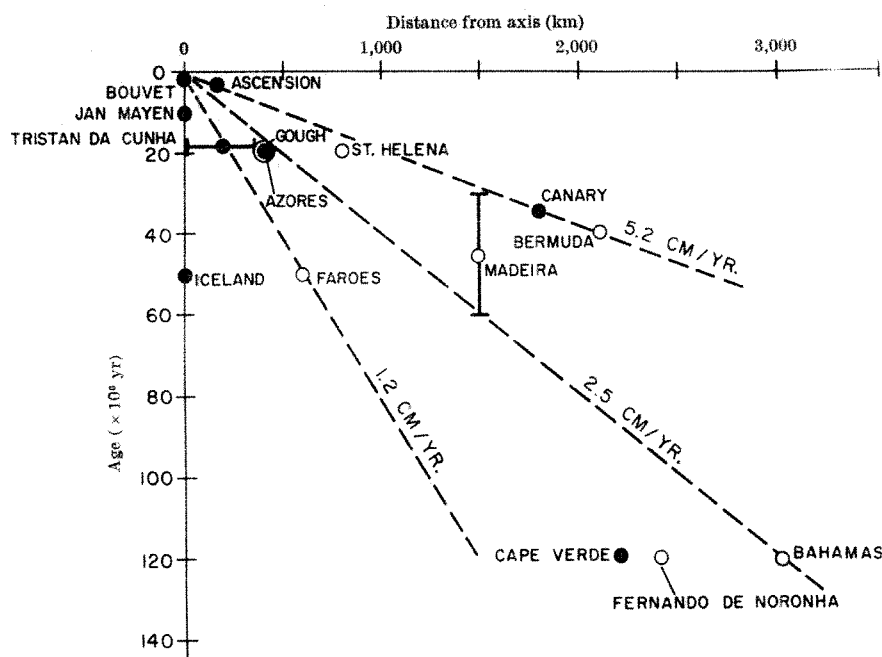


Fig. 2. Age of oldest rock from various islands in the Atlantic Ocean, plotted against distance from axis of mid-Atlantic ridge<sup>11,22</sup>. Solid circles indicate active volcanoes; bars indicate uncertainty in age or distance from axis of ridge.

Pleistocene sediment cores from the Atlantic have been plotted against distance from the mid-Atlantic Ridge (Fig. 5)<sup>13</sup>. By dividing these ages into their respective distances from the ridge axis the average drift rates can be estimated. Data for some of the oldest cores (with the lowest drift rates) are plotted in Fig. 1. The average drift rates range from 1 to 2 cm/yr and, despite the sparse data, there is a faint suggestion of a maximum at 34° N. Because the sediments sampled at each locality are not necessarily the oldest, the calculated drift rates are upper limits. The drift rates implied by sediment ages appear significantly lower than those computed from island ages. As the islands are generally further from the axis, there are at least two interpretations also consistent with magnetic data<sup>6,7</sup> which show an increase of anomaly wavelength several hundred km from the axis (about  $10 \times 10^6$  yr at spreading rates of a few cm/yr): (a) spreading is steady state, with velocity increasing gradually away from the axis<sup>14,15</sup>; or (b) spreading rates were higher in the past and diminished about  $10 \times 10^6$  yr.

If the width of a mid-ocean ridge is a measure of the rate of spreading, then changes in ridge width ought to be marked by transverse faulting. Such changes in ridge width are observed, for example, in the equatorial Atlantic and north of Iceland. Transverse fractures occur in the same regions. Inspection of the physiography of the Atlantic Ocean might lead to the supposition that transverse fracture zones are somehow required by changes in direction of ridges. The mid-ocean ridge curves around the southern end of Africa without significant offsets, however.

If rifting began roughly synchronously in the Atlantic Ocean, the present variation in ocean width implies that, at least in the past, spreading rates have varied along the ridge. This variation in spreading rate is not, however, valid if the ocean crust has been moving at a greater rate than continents, that is, if down welling convection cells have existed along the margins of the Atlantic Ocean. If this were the case (assuming synchronous rifting) the spreading rate could have been constant along the length of the Atlantic, with most intense down welling occurring where the ocean is at present narrow, for example, in the Greenland Sea. There is no geological evidence, however, to suggest active Tertiary trenches in that area.

There is evidence that rifting may not have been synchronous in the Atlantic. Non-synchronous rifting rules out the possibility that the continents have been displaced and rotated as rigid wholes, and requires some tectonic deformation on land, regardless whether the oceanic crust is moving faster than the continents or not. The limiting case of a longitudinally constant spreading rate would mean a direct correlation between width of ocean and age. This would mean that the north equatorial Atlantic was of mid-Mesozoic age, the South Atlantic of late Mesozoic, and the Arctic Atlantic of Tertiary age. Such a rifting model implies tectonic deformation on the adjacent continents, especially in regions where the ocean narrows abruptly such as between 40° N. and 50° N. and between 70° N. and 80° N. in the Atlantic. A correlation between terrestrial tectonics and ocean floor spreading history, as

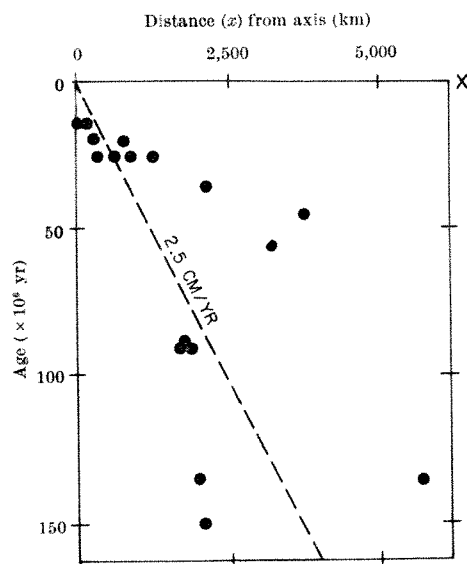


Fig. 3. Age of seventeen oldest sediment cores and islands in the Atlantic Ocean<sup>10</sup> plotted against distance ( $x$ ) from axis of mid-Atlantic ridge as measured along flow displacement lines<sup>12</sup>. A dashed line which indicates drift rate of 2.5 cm/yr has been added for reference.

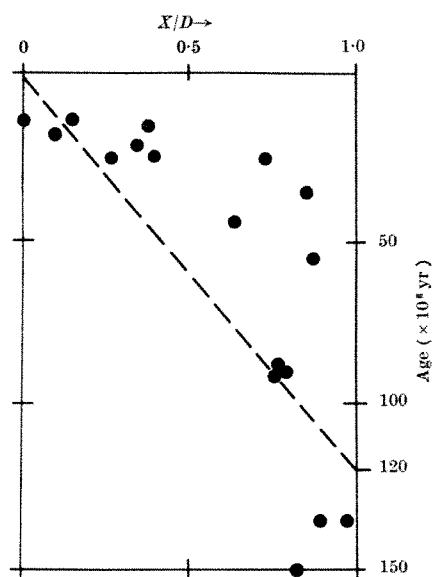


Fig. 4. Same ages as above, plotted against  $X/D$ , where  $D$  is distance along flow displacement line<sup>12</sup> from mid-Atlantic ridge to edge of continent, and passing through dated points. If rifting began simultaneously at  $120 \times 10^6$  yr, and proceeded along flow lines, all points would lie above dashed line.  $D$  varies from less than 500 km (Greenland Sea) to over 5,000 km (Florida).

unravelling by magnetic anomalies, may discriminate among these hypotheses. Until then a longitudinal variation of spreading rate, at least in the past, appears most reasonable.

It has also been suggested<sup>14,15</sup> that spreading velocity may increase gradually away from the axis. This possibility places another perturbation on ocean floor displacement which will be considered later.

### Steady-state Model

If the flank profile of a mid-ocean ridge is constant with time the ridge can be considered a steady-state feature. Suppose that material wells up in the central region of a ridge and spreads laterally, sinking at a rate which does not vary with time for a given distance from the axis.

Let  $y$  denote distance measured along the length of the ridge, and  $x$  the distance from the axis measured along the trajectory of spreading. If spreading proceeds essentially at right angles to the crest, the regional slope at a point  $P(x,y)$  on a steady-state ridge will be  $\tan^{-1} S(x,y)/f(x,y)$ , where  $S$  denotes the spreading velocity and  $f$  denotes the rate of subsidence. According to the linear spreading hypothesis of Dietz<sup>1</sup> and Hess<sup>2</sup>,  $S(x,y)$  would be constant with respect to  $x$ . In this case the topographic slope at a point would uniquely determine the subsidence rate  $f(x,y)$ . If, however,  $S(x,y)$  increases gradually from zero at the crest to a maximum value somewhere on the flanks<sup>11,13</sup>, the spreading rates plotted in Fig. 1 are average values, always less than the actual spreading rate at the point  $P(x,y)$  considered. Therefore, division of the average spreading rate by the local slope will yield a lower limit for local subsidence rate. If the elevation of mid-ocean ridge crests above abyssal levels is taken as nearly a constant the width of ridges is then inversely proportional to the average regional slope of the flanks. Therefore, a wide ridge could mean either rapid spreading or slow sinking. In the preceding discussion evidence was given that  $S(x,y)$  varies at least by a factor of four in the  $y$  direction. Consider now whether or not  $f$  is constant with time, that is, does it vary with  $x$ ? Sinking of a unit of ocean floor with time could be caused by a number of processes including chemical changes involving change in volume, isostatic settling or thermal contraction caused by progressive cooling of mantle material spreading away from the axis of upwelling.

Langseth *et al.*<sup>3</sup> computed ridge profiles on the assumption that hot material ascends at the axis, spreads laterally at a uniform rate, and contracts as it loses its heat through the Earth's surface. Their data shows that the average subsidence rate ( $f$ ), independent of  $S$  if  $S > 0.5$  cm/yr, is about 2.5 cm/1,000 yr for the first  $50 \times 10^6$  yr. This agrees closely with the subsidence rate determined from various atolls and guyots (Fig. 6). Nevertheless Langseth *et al.*<sup>3</sup> think that crustal spreading has not been operative on the Mid-Atlantic Ridge since the beginning of Tertiary time, except possibly during the last few million years. This conclusion is based on the absence of a broad heat flow maximum which their model predicts. However, they admit that if mantle material ascends over a broad

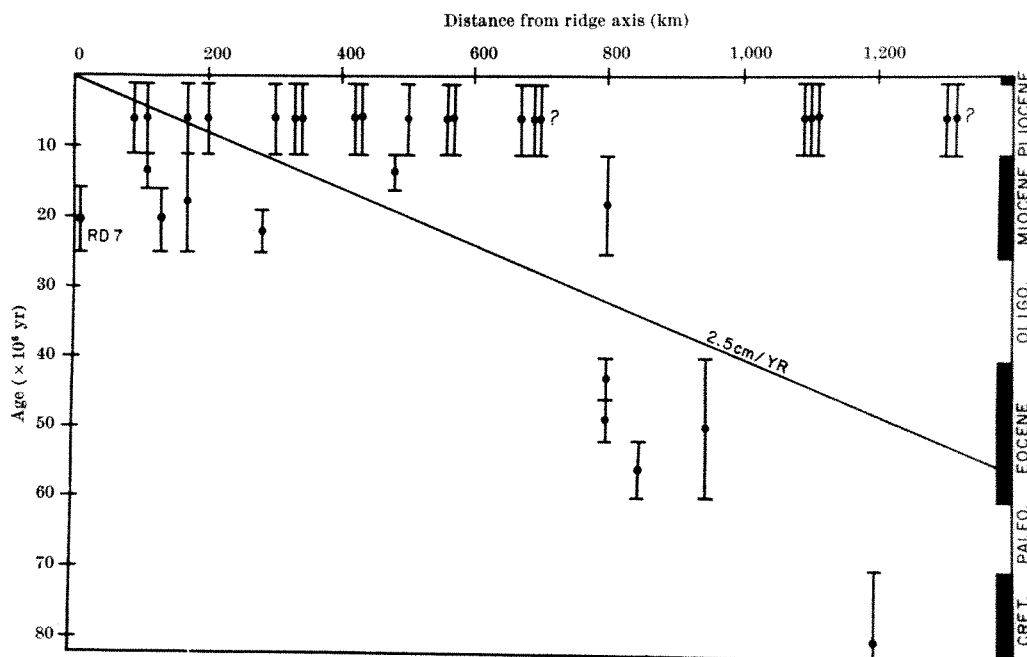


Fig. 5. The ages of sediment cores<sup>13</sup> as a function of distance from the axis of the mid-Atlantic ridge. Vertical bars indicate uncertainty of the absolute dates associated with stratigraphic dates of cores. Straight line indicates uniform drift rate of 2.5 cm/yr.

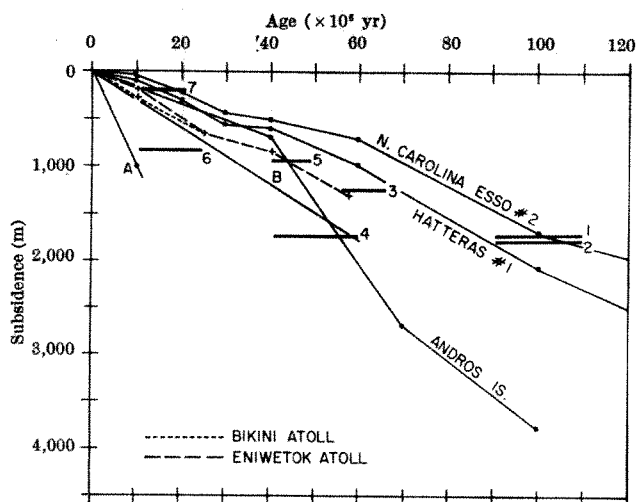


Fig. 6. Subsidence plotted against time<sup>3,7,10-12</sup>. Curve B is the average subsidence rate due to the thermal contraction model<sup>3</sup>. Curve A represents average actual subsidence rate on upper flanks of Reykjanes and Pacific-Antarctic ridges, if the ridges are steady-state features. Note that curves A and B represent subsidence as a function of time after extrusion, whereas the other data are a record of subsidence looking backwards in time.

region and then spreads at a rate which gradually increases with  $x^{14,15}$ , or if there is an insulating surface layer which is not perfectly coupled to the flow, then the expected heat flow maximum would be much reduced. Further, they acknowledge that available data permit spreading of the East Pacific Rise as an admissible hypothesis.

To examine the thermal expansion model in greater detail an example of a smoothed profile from part of the mid-ocean ridge is shown in Fig. 7, in which the crestal elevation for the theoretical profile has been selected to give the best fit to the observed profiles. The dashed horizontal line indicates the theoretical equilibrium datum, that is, the abyssal plain at great distances from the axis, and after sediments have been removed.

Although Langseth *et al.*<sup>3</sup> reject this model on the basis of observed heat flow across the Mid-Atlantic Ridge there are three points of similarity between the theoretical and actual mid-ocean ridges: the calculated subsidence rates are 2.5 cm/10<sup>3</sup> yr (curve B, Fig. 6) which compares with rates of 10 cm/10<sup>3</sup> yr for the Reykjanes and Pacific-Antarctic Ridges (curve A, Fig. 6) and 2 cm/10<sup>3</sup> yr for the average subsidence of atolls and guyots during the last 50–100 × 10<sup>6</sup> yr; the calculated elevation of the crest above the abyssal plain is about 2 km (independent of  $S$  for  $>0.5$  cm/yr), which compares favourably with actual ridges; the calculated regional slopes (out to at least 550 km from the crest) compare favourably with actual slopes on mid-ocean ridges, with the best fit corresponding to an  $S$  of 1 or 1.5 cm/yr.

If thermal expansion contributes to the elevation of mid-ocean ridges, then at least spreading rates should be reflected in the width of heat flow anomalies. Unfortunately, data are not yet adequate to confirm this relationship along the Mid-Atlantic Ridge. It appears, however, that the heat flow anomaly is wider over the East Pacific Rise where spreading is faster<sup>6,7</sup>. For instance the width of isothermal belts enclosing values of heat flow exceeding 3  $\mu\text{cal}/\text{cm}^2/\text{sec}$  is 600 km over the East Pacific Rise<sup>16</sup> and no more than 200 km over the Mid-Atlantic Ridge<sup>17</sup>. Thus the assumption that the elevation of ridge crests is caused by thermal expansion, or conversely that the subsidence of flank slopes is caused by cooling contraction seems valid.

Langseth *et al.* took the surface conductivity as representative of the upper 100 km of the Earth. To reconcile the observed  $f$  of 10 cm/10<sup>3</sup> yr across the Reykjanes and Pacific-Antarctic Ridges to their calculated  $f$  of 2.5 cm/10<sup>3</sup> yr requires that conductivity increases with depth.

Rapid subsidence would imply rapid cooling and thus higher conductivity. For a given spreading rate higher conductivity would narrow the predicted heat flow maximum, but the ridge profile would be narrowed as well. We have found that the 1 cm/yr spreading model best fits the profiles of the average mid-ocean ridge. Therefore, if this fit is to be retained while the conductivity is increased in such a way as to multiply  $f$  by 4, then  $S$  must also be multiplied by 4. The required spreading rate is then about 4 cm/yr which is the order of observed  $S$  for the East Pacific Rise. These changes in  $S$  which are required by the observed  $f$  would presumably leave the dimensions of the heat flow anomaly essentially unchanged. Thus, a mere change in conductivity cannot reconcile the model of Langseth *et al.* with the fact that the Mid-Atlantic Ridge and the East Pacific Rise have similar profiles but dissimilar heat flow anomalies. A thermal model must be sought which incorporates the known difference in crustal structure as well.

### Subsidence of Oceanic Islands

Independent estimates of the subsidence rate  $f$  may possibly be derived from the sinking of atolls and guyots in the Pacific. Islands may form at the axis of a mid-ocean ridge and ride down the continuously foundering "conveyor belt" toward the abyssal plains<sup>2</sup>. As soon as vulcanism ceases, wave erosion begins to truncate the seamount, and further sinking will produce a guyot. Maximum subsidence possible on this hypothesis will be for an island formed and truncated on the crest, which is on the average 2–3 km deep and 2–3 km above the abyssal plains. For a guyot on the abyssal plain, the subsidence can then be no greater than 2–3 km. Actually the equilibrium level will be somewhat deeper after the sediments have been removed. Total subsidence might be as great as 4–5 km if basement depths under continental rises of the Atlantic Ocean are interpreted at least partly in terms of subsidence from the crests of mid-ocean ridges. Lesser depths to the tops of guyots which now rise from abyssal depths could be a result of islands retaining their

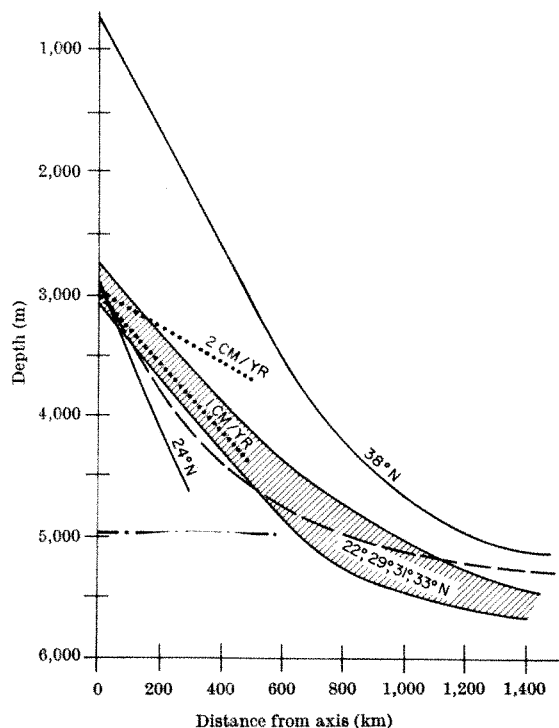


Fig. 7. Smoothed profiles<sup>14,15</sup> across the mid-Atlantic ridge, between 22° N. and 38° N., compared to the thermal contraction models<sup>3</sup>. Dotted curves are model profiles for 1 cm/yr (steeper profile) and 2 cm/yr drift rates<sup>8</sup>. Dashed curve is a pure exponential, shown for comparison.

volcanic activity for some distance as they were carried down the flanks, or of islands formed somewhere on the flanks<sup>2</sup>. The present existence and even volcanic activity of Atlantic islands now hundreds or thousands of km from the axis of the mid-ocean ridge suggests that "late truncation" is possible (see Fig. 2).

If sinking of the flanks is caused by thermal contraction of ascended mantle material spreading from the axis of a ridge, the sinking rate of a parcel of oceanic crust may be only a function of time after extrusion. In that case, should convection cease, the mid-ocean ridge will subside as it cools, and a parcel of crust containing the guyot will continue sinking at the same rate as when the ridge was still "active". The total subsidence after convection ceases will then depend on the distance  $x$  of the guyot from the axis, and can be no greater than the height of the crest of an active ridge above the abyss, namely, 2 or 3 km. Menard<sup>18</sup> has argued that many of the guyots in the southwestern Pacific were associated with a Late Cretaceous rise, now totally subsided (Darwin Rise). There are many other guyots, however, not thought to be related to extinct rises. We suggest that the subsidence rates should be the same whether the island rode down the flanks or subsided as part of a dying ridge. According to the present hypothesis the time for a seamount to sink 2–3 km should be comparable to the time required for material from the crest to reach the outer flanks of an active ridge. If the sinking rate of islands should prove to be greater than the rate of subsiding of flanks, or if guyots with tops much deeper than 3 km can be found, regional subsidence does not suffice, and it would be necessary to suppose that sea level has risen, or that subsidence relative to the crust has taken place. It should be noted that subsidence rates estimated from islands, unlike those determined from the flanks of mid-ocean ridges and discussed previously, are subject to the additional uncertainty of sea level changes. If there is no discrepancy in these two estimates of subsidence, large changes in sea level can be ruled out.

Hamilton<sup>19</sup> has studied the sinking seamount problem in detail and concludes that sinking, not a gradual rise in sea level, is the dominant process involved. A plot of dated depths from guyots and atoll bore holes<sup>20</sup> is shown in Fig. 6. The average subsidence rate has been about 2 cm/10<sup>3</sup> yr for at least the last 60 × 10<sup>6</sup> yr. Two guyots (1 and 2 in Fig. 6) suggest a levelling off in the subsidence rate from 60 to 100 × 10<sup>6</sup> yr after sinking began. Because of the uncertainty in age (the depth being precisely known), the range in average subsidence rates may be as great as 1.5–5 cm/10<sup>3</sup> yr.

Guyots and atolls are rare in the Atlantic. Shallow-water carbonates were encountered in a deep borehole on Andros Island<sup>21</sup>, however, and these are plotted in Fig. 6. The subsidence rate of Andros Island is the same (2 cm/10<sup>3</sup> yr) obtained for Pacific atolls and guyots. The oldest horizons on Andros Island may have suffered additional depression from thick sedimentary overburden, but since at least 60 × 10<sup>6</sup> yr ago there appears to have been no significant difference between the sinking rates of the guyots, which are free of sediment, and the atolls.

The sinking rate of seamounts has therefore been of the same order (1–5 cm/10<sup>3</sup> yr) but lower than the subsidence of the upper slopes of the Pacific–Antarctic Ridge and the Reykjanes Ridge. Subsidence on the latter ridges has been 10 cm/10<sup>3</sup> yr for the last 3–4, and probably the last 10 × 10<sup>6</sup> yr, if those ridges are steady-state features. This subsidence rate has been added to Fig. 6 (line A). Let us try to account for the difference between ridge and island rates of sinking. First, it is reasonable that the initial subsidence rate should be higher than the average over many tens of millions of years because  $f(x)$  must be a decreasing function. Second, it is possible that cessation of volcanic activity and truncation of an island often did not occur until the island had been carried some distance away from the crest, that is, to a region where the sinking

rate was less. Some of the guyots and atolls may have long since ceased their subsidence so that the average rate is much lower than the actual rate. Less likely explanations for the difference in subsidence rates are that the flanks of the Pacific–Antarctic and Reykjanes Ridges are sinking unusually rapidly, or that the time scale of magnetic reversals is too short by a factor of about four, or that sea level has been falling at about 8 cm/10<sup>3</sup> yr, or that the ridges are not steady-state features.

Let us apply the proposed model to two of the few dated guyots presently near an active ridge. First, Erben Guyot rises from abyssal depths west of southern California, about 1,500 km from the supposed axis of the East Pacific Rise. A truncated top submerged 750 m has been dated as Miocene (10–25 × 10<sup>6</sup> yr)<sup>20</sup>. These data are combined with an average ridge profile in Fig. 8. The average subsidence rate must lie between 3 and 7 cm/10<sup>3</sup> yr, which is, and should be according to the present model, less than the initial rate of 10 cm/1,000 yr discussed above. If the seamount drifted down the flanks of a typical ridge, it must have been truncated at about half the present distance to the axis of the ridge (Fig. 8). Let us assume a value of 3 cm/yr based on a magnetically determined drift rate of 2.9 cm/yr for the Juan de Fuca Ridge<sup>6</sup>. Then Erben Guyot was truncated at 25 × 10<sup>6</sup> yr, which is consistent with the Miocene age determined for its top. If this former island originated at the crest of the ridge, it must have been active between about 25 and 50 × 10<sup>6</sup> yr ago. This consequence of the hypothesis could be checked with isotope dating of dredge basalt from the guyot.

Guyots among the Tuamotu Islands have been dated at 40–45 × 10<sup>6</sup> yr (Fig. 8). Their tops are submerged 900 m and are about 2,700 km from the axis of the East Pacific Rise, or about 2,000 km from their possible location on the flanks when they were truncated. Taking the drift rate of about 4.5 cm/yr determined recently<sup>6,7</sup> for the Pacific–Antarctic Ridge, the time since truncation of the Tuamotu guyots should be 2,000 km/4.5 cm/yr, or about 44 × 10<sup>6</sup> yr. This compares perhaps too well with the Upper Eocene data obtained for their tops. Menard<sup>18</sup> would ascribe the sinking of the Tuamotu guyots to the subsidence of an ancient ridge, the Darwin Rise. Hess<sup>2</sup> has suggested that the crest of the entire Pacific Ocean was generated at the axis of the Darwin Rise. According to those hypotheses the above agreement in ages of truncation would have to be fortuitous.

If regional subsidence down the flanks of the East Pacific Rise correctly explains the submergence of the as yet undated Pratt–Walker group in the Gulf of Alaska, the ages of truncation should increase from east to west because the tops are concordant (700–800 m) and the slope of the sea floor slight. If sinking has been local, or a result of a rise in sea level, there would be no reason to expect such an increase of age from east to west, and a sinking rate of 2 cm/10<sup>3</sup> yr would suggest a common age of 40 × 10<sup>6</sup> yr for the group of guyots. If the guyots came from the Darwin Rise<sup>2</sup> they must be still older. But Pratt guyot, for example, is only about 500 km from the axis of the ridge, so that according to the present hypothesis its top must be younger than 16 × 10<sup>6</sup> yr. Drift rates inferred from magnetic anomalies, and dating of the guyot tops will decide the question.

The interpretation of the subsidence of guyots in the Mid-Pacific Mountains and the Bikini–Eniwetok group is more complicated, as they might be related to the Melanesian Rise, the East Pacific Rise, the extinct Darwin Rise, or to several of these features.

The uniformity of sinking rates for widely separated atolls and guyots of different sizes suggests that the foundering is regional, not local. According to the present hypothesis, the total subsidence cannot exceed the height of a mid-ocean ridge above the abyssal plain (after sediments have been removed), which generally does not



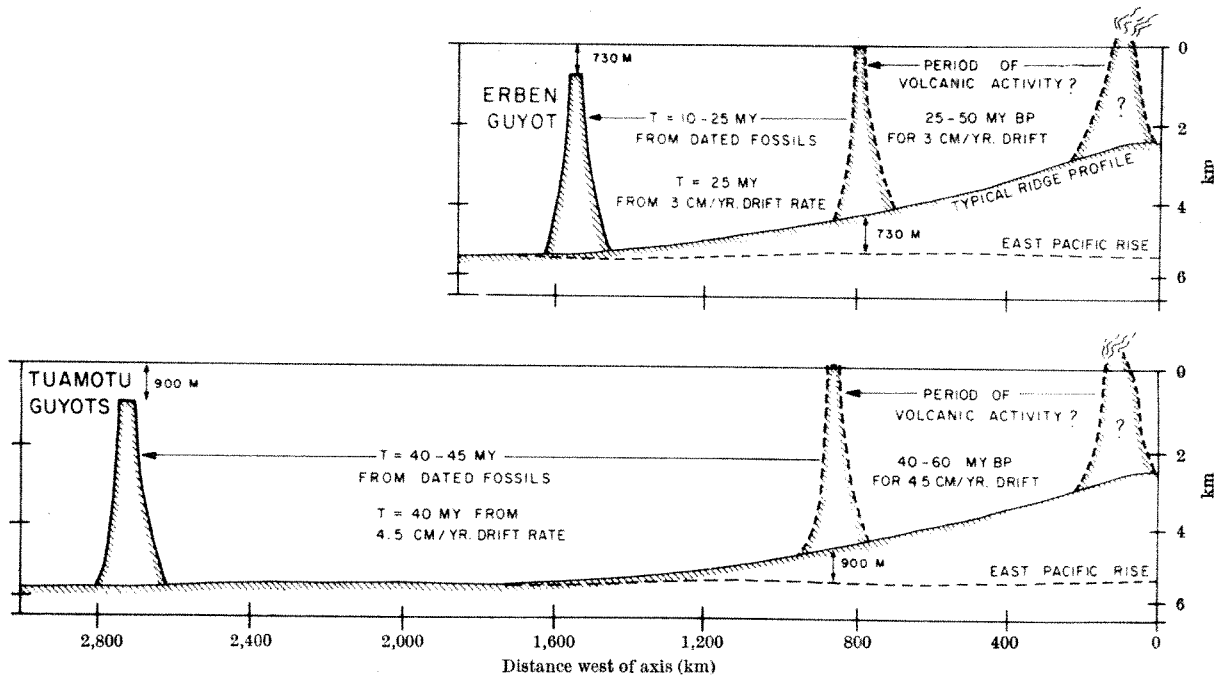


Fig. 8. Origin and subsidence of Erben and Tuamotu guyots<sup>20</sup> according to hypothesis<sup>2</sup> that subsidence is result of drifting down the flanks of average mid-ocean ridge. All elements except guyot flanks are drawn to scale. Note that fossil ages from guyot tops agree closely with ages computed from distance travelled after truncation, and crustal spreading rates determined from magnetic profiles in the vicinity of the seamounts. Erben Guyot is at 34° N., while a 2.9 cm/yr drift rate was established at 49° N.<sup>6</sup>. The Tuamotu Guyots are at 15° S., while a 4.5 cm/yr drift rate was established between 40° S. and 55° S.<sup>7</sup>.

exceed about 3 km. Fig. 6 shows that this is the case for the limited number of features so far dated. Unfortunately there could be another possible explanation for the absence of subsidence much greater than about 3 km. The oldest dates are about  $100-200 \times 10^6$  yr, which is the order of time required to sweep the ocean basins clear of seamounts at spreading rates of a few cm/yr. Thus depths greatly exceeding about 3 km are not likely to occur, regardless of how the seamounts are sinking.

### Continental Shelves

Pre-Cretaceous erosional surfaces dip seaward under several shelves bordering the Atlantic Ocean. Two boreholes drilled through the wedge of younger sediments east of Cape Hatteras yield a record of subsidence at about the same rate (a few cm/ $10^3$  yr) as that of Andros Island, Eniwetok and Bikini atolls, and a number of guyots in the Pacific. In Fig. 6, the subsidence curves for the North Carolina Esso No. 2 and the Hatteras No. 1 boreholes have been constructed from the data of Heezen<sup>22</sup>. It is tempting to suggest that the same mechanism of regional contraction is involved in the subsidence of guyots, atolls, banks, and mid-ocean ridges themselves.

The subsidence of shelves on rifted coast lines (where there are no trenches) may be incorporated into the hypothesis of mid-ocean ridge formation if we make two suppositions: the erosional surface which dips seaward under the wedge of Mesozoic and younger sediments was formed when Eurafica and the Americas were at the stage of rifting represented by the East African rift belt of today; erosional lowering of this pre-Atlantic uplift was rapid enough to remove several km of material in the highest, steepest region along the axis of the uplift before significant lateral spreading had occurred. It then follows that, as the severed continental edges withdrew from the axis of uplift and subsided, an initially nearly level surface could acquire a seaward dip. In areas where the erosion had been sufficiently active, subsidence could lower the land below sea level, where it began to receive sediment in Cretaceous and later times. Isostatic loading and compaction could then account for still further depression of the shelves.

If this view is correct, then the material eroded from the pre-Cretaceous uplift must have been carried eastward into Eurafica and westward into the Americas, although some of the sediment would have been trapped in axial graben valleys. Erosion must have been in rough proportion to the elevation, so that the axial zone of greatest uplift was most deeply eroded. Therefore, a series of boreholes penetrating the pre-Cretaceous basement would be expected to encounter generally older rocks under the edge of the shelf than nearer to the continent. The Cretaceous and younger sediments should thicken seaward, and the deeper units should dip more steeply. This is known from boreholes on the Atlantic coastal plain and continental shelf<sup>22</sup>. The slope of the pre-Cretaceous erosional surface should be comparable to or less than the regional slope of mid-ocean ridges (if equilibrium has been reached). The basement slopes seaward at about 20 : 1,000 east of Cape Hatteras<sup>22</sup>. This compares with 2 to 10 : 1,000 for typical mid-ocean ridges (Fig. 7). Therefore, either the initial uplift was steeper, or isostatic depression and compaction have steepened the dips. The widths of subsiding shelves should be comparable to or less than half the width of mid-ocean ridges. Inspection of any chart of the Atlantic Ocean reveals this to be the case. If the edges of rifted continents have no shelves at all, the implication is that erosion was inadequate compared to the initial uplift and the rate of rifting. In any case there should be evidence for some subsidence since rifting began, and, conversely, coast lines that have not been rifted should be free of shelf-wedges. A maximum subsidence of at least 3 km and possibly 4-5 km could be accounted for in this manner. Greater subsidence is indicated by seismic refraction, but has not been proven by boreholes, for the basement under the edge of the shelf off eastern North America<sup>21</sup>. In some areas the basement has been located as much as 8 km below sea level, but it is not proven that the seismic interface can be identified as the same pre-Cretaceous erosional surface penetrated by boreholes nearer the present shoreline, such as the two boreholes plotted in Fig. 6; and Heezen *et al.*<sup>22</sup> have linearly extrapolated the borehole data seaward and found that benches in the

continental slope can be related to specific Tertiary rock formations. This suggests that the seismic discontinuity which forms depressions underneath the outer parts of the shelves and the continental slope is an older surface. Is this downbuckling an isostatic effect, a buried trench, or even a buried rift valley that did not widen into an ocean?

By analogy to present East Africa it would appear that several branches of the rift may have existed before formation of the Atlantic Ocean. Perhaps the offshore depressions in the basement are, like the Triassic lowlands (Newark Series), grabens filled with non-marine clastics and eruptive rocks. Whatever the final answer to this problem, it appears that a substantial part of the subsidence of shelves may be related to the same mechanism (thermal contraction by cooling?) that causes the flanks of mid-ocean ridges to subside. Alternatively, the depression of continental margins has been linked to descending limbs of convection cells, and the buried grabens identified as marginal trenches which once resembled the present Puerto Rico Trench<sup>21,22</sup>.

Fig. 6 suggests that subsidence has continued into geologically recent times. It may be objected that subsidence of the basement under Andros Island and the continental shelf should have ceased long ago, as these features are presently over 3,000 km from the ridge axis. The abyssal plains slope toward the continents after sediments have been removed, however, suggesting that equilibrium has not been reached. Further, if thermal contraction is the mechanism, it follows that the mantle under the oceanic crust seaward of the shelf has come closer to thermal equilibrium than adjacent shelves. This follows because the shelves both insulate the mantle and supply some radiogenic heat of their own. Thus the continental edges may continue to subside after the adjacent abyss has reached equilibrium. For some unknown interval of time after rifting the abyss contracts more rapidly than the immediately adjacent shelf. This process may contribute to keep the shelf edge abrupt.

Theoretical calculations such as ref. 3 can explore this possibility.

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## Conformation of the Anticodon Loop in tRNA

by

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A molecular model for the anticodon arm is proposed which is compatible with chemical, X-ray and genetic evidence. It provides a stereochemical basis for Crick's "wobble" hypothesis.

NUCLEOTIDE sequences determined for a number of amino acid specific tRNA molecules<sup>1-4</sup> have led to the suggestion that these molecules have a "clover leaf" structure (Fig. 1). This was because, despite their different nucleotide sequences, there are striking structural homologies when the tRNA molecules are folded so that the number of intramolecular Watson-Crick base-pairs is a maximum (Fig. 1). Diagrams like Fig. 1, however, indicate little of the three-dimensional appearance of such structures and their implications. Therefore we have constructed three-dimensional models, and here describe a molecular model-building study of the anticodon arm.

Using chemical information about nucleotide sequence and X-ray evidence on the conformation of base-paired regions in the tRNA, the maintenance of reasonable stereochemical constraints leads to a model for the anticodon arm. This model accounts for the observed

degeneracy in the reading of the third position of the codon and also makes a prediction about the site of the distortion required to accommodate this degeneracy.

### Model Building Technique

We used Corey, Pauling and Koltun<sup>5</sup> spacefilling models and also skeletal models with a scale of 4 cm to 1 Å (ref. 6). The former ensure that short van der Waals contacts are avoided during preliminary investigations. Because, however, the atomic centres in them are inaccessible, we used skeletal models when preliminary study suggested that a particular conformation merited detailed analysis. Lengths and angles of covalent bonds and short van der Waals contacts were calculated from atomic co-ordinates measured on skeletal models and the co-ordinates were adjusted until acceptable stereochemistry was obtained,

that is lengths of covalent bonds within 0.05 Å of accepted values, covalent angles within 6° and no non-covalently bonded contacts more than 0.4 Å short of the sum of the atomic van der Waals radii. We do not necessarily believe that our models describe the actual molecular conformations to an accuracy of a few hundredths of an angstrom, but the analysis shows that a model with the general characteristics we propose can be built with acceptable stereochemistry. Only if model building is treated as a rigid discipline with strict attention paid to detailed stereochemistry can the results of a study such as this be considered reliable and meaningful.

### Conformation of the Anticodon Arm

X-ray diffraction suggests that the molecules of tRNA (ref. 7), in common with all RNA molecules so far studied by this method, contain helical regions with a conformation similar to that determined for two-stranded reovirus RNA (ref. 8). We have assumed that the Watson-Crick base-paired regions in the clover leaf structure have a conformation like the eleven-fold double-helical structure of reovirus RNA (rather than the less favoured ten-fold possibility). In the anticodon arm there is a loop of seven nucleotides at the end of the helical region. From considerations of biological function, the structural homologies in the different tRNA species might be expected to extend to the conformation of this loop.

The characteristic features of polynucleotide secondary structure are provided by interbase hydrogen bonding and base-stacking. The tRNA nucleotide sequences so far determined do not suggest an intramolecular base-pairing scheme which would give a similar structure for all the anticodon loops (Fig. 2). Therefore we searched for conformations of this loop which maximized single-

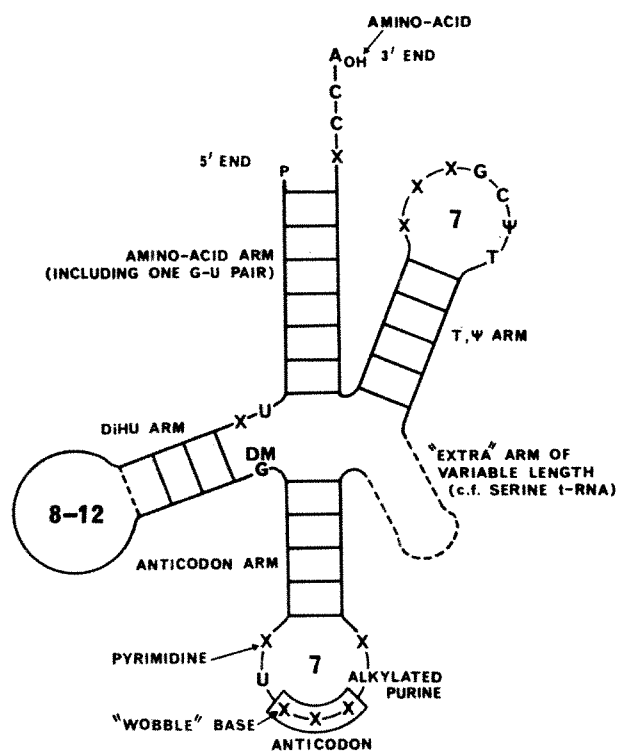


Fig. 1. Generalized clover-leaf structure for yeast tRNA based on sequences determined for tRNAs specific for alanine<sup>1</sup>, serine<sup>2</sup>, tyrosine<sup>3</sup> and phenylalanine<sup>4</sup>. A base-pair is indicated by a line linking two parts of the RNA chain (a dashed line indicates a base-pair occurring in some tRNAs but not in others); X indicates a nucleotide which varies with the tRNA species; nucleotides which occur at an equivalent position in all sequences are denoted as follows—uracil (U), dimethylguanine (DMG), adenine (A), cytosine (C), thymine (T), pseudouracil (ψ). The number at the centre of each loop indicates the number of nucleotides in the loop.

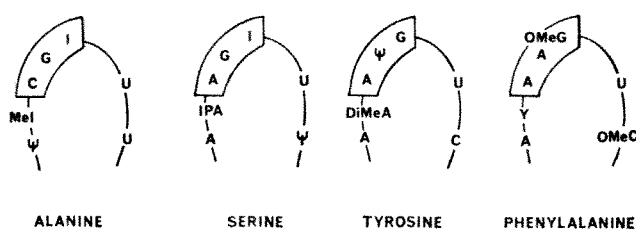


Fig. 2. The nucleotide sequences in yeast tRNA anticodon loops. The anticodon is shown boxed. The symbols ψ, MeI, C, G, I, U, A, IPA, DiMeA, Y, OMeG, OMeC stand for respectively: pseudouracil, 1-methyl-inosine, cytosine, guanine, inosine, uracil, adenine, isopentenyl-adenine, dimethyl-adenine, a so far unidentified purine, O-methyl guanine, O-methyl cytosine. (The references from which the sequences were taken are in the caption to Fig. 1.)

stranded base-stacking. In doing this we also attempted to: (a) avoid negatively charged phosphate groups coming closer to each other than in accurately determined crystalline fibrous structures; (b) ensure that hydrogen bond donor groups on unpaired bases and ribose sugars were not buried in the structure, so they were unavailable for hydrogen bonding; (c) maintain single bond orientations in the polynucleotide chain (for example, the conformation at the glycosidic link) within the limits of values observed in model compounds and other polynucleotides.

When these stereochemical constraints are maintained, model-building studies suggest that the polynucleotide chain has surprisingly little conformational freedom. Furthermore, orientation of the single bonds in the only two polyribonucleotides whose structures have been determined in detail by X-ray analysis (two-stranded helical RNA (ref. 8), and two-stranded polyadenylic acid<sup>9</sup>) are rather similar. Therefore if stacking is to be maintained, the polynucleotide chain might be expected to have a conformation similar to that in one of the structures described for ribopolynucleotides. Stacking as much as possible of the anticodon loop on top of the double helical region of the anticodon arm might be expected to "nucleate" the structure of the single-stranded region so that its nucleotide conformation is similar to that in the double helical region, that is that of the eleven-fold model for reovirus RNA. (We refer to this conformation as standard.) There is some support from physical studies on solutions of polynucleotides and dinucleotides for postulating that the conformation of a single-stranded polynucleotide with base-stacking is similar to the conformation it would have as one of the strands in a two-stranded structure<sup>10,11</sup>.

Figs. 3 and 4 illustrate the structure which stacks the greatest number of the nucleotides in the anticodon loop. Five nucleotides are stacked in the standard conformation so that they lie on the same helix as that chain in the anticodon arm double-helix nearer the tRNA 3' end. This structure represents a unique solution to the problem of maximizing base-stacking in the anticodon loop. Conformations with slightly different base tilt and rotation and translation of each nucleotide (for example if the standard nucleotide conformation was that of the ten-fold rather than eleven-fold RNA model) could of course give a similar degree of base-stacking. Stacking combinations of nucleotides other than those stacked in this structure, however, result in less than five nucleotides being stacked. In particular five nucleotides cannot be arranged so that they lie on the same helix as that chain of the anticodon arm double-helix nearer the tRNA 5' end. This is shown in Fig. 4 where A and B are closer together than they would be for a structure with bases perpendicular to the helix axis. If, however, the five bases were stacked on the chain of the two-stranded helix nearer the tRNA 5' end, the base tilt would make the distance to be spanned by the two non-standard nucleotides greater than for a structure with bases perpendicular to the helix axis. In addition to this increased distance, the two nucleotides would have to span the RNA groove containing the 2-keto

groups rather than that containing the 6-keto groups which is spanned in the model illustrated in Figs. 3 and 4. The RNA conformation is such that spanning the groove containing the 2-keto groups requires a much longer polynucleotide chain.

In the structure illustrated in Figs. 3 and 4 the two nucleotides of the anticodon loop not in the standard conformation have the planes of their bases approximately parallel, with some overlap of their hydrophobic surfaces. There is, however, some flexibility in this region of the structure and this conformation should be regarded as typical of a number of related possibilities. All the tRNA nucleotide sequences so far determined are compatible with the poorer stacking of these two nucleotides as compared with that in the standard helix because these bases are always pyrimidines (of which at least one is uracil). These bases are generally thought to stack least well. In all these sequences, the second of the five nucleotides in the single stranded helix (that is 7 in Fig. 4) has a chemically modified hydrogen bonding donor group on the base. In addition to inhibiting base-pairing which might favour alternative structures to that in Figs. 3 and 4, this group may increase hydrophobic stabilization of this stacked conformation.

The structure illustrated in Figs. 3 and 4 could describe the conformation of the loop of 7 unpaired bases in the T $\psi$  loop (Fig. 1). The occurrence of uracil and cytosine, however, at what would be positions 6 and 7 in the single-stranded helix (Fig. 4) make it a rather less attractive solution than it is for the anticodon loop.

A loop at the end of an RNA double helix need be no longer than three nucleotides<sup>12</sup>. The base-stacking in such a structure, however, is probably much less perfect than that in the standard conformation and such a loop would only be expected to occur if it contained nucleotides with poor stacking interactions, for example the loop with UUU and UCU at the end of the extra arm in the two serine tRNAs (Fig. 1).

### Codon-Anticodon Interactions

From consideration of the likely anticodon in a number of tRNAs and a knowledge of the different codons which will

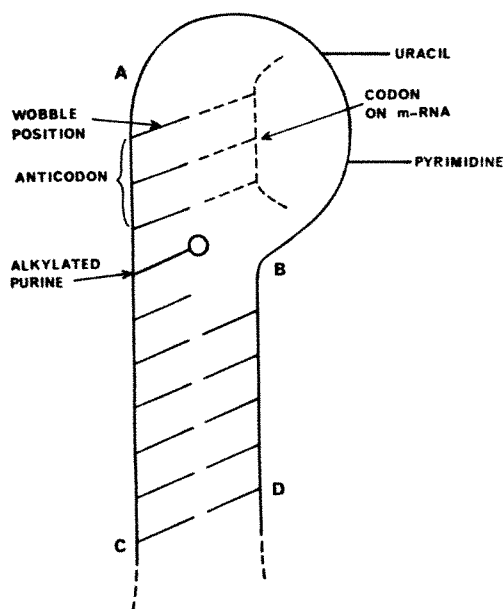


Fig. 3. Schematic diagram of the model for the tRNA anticodon arm illustrating its relationship to the codon. The helical regions are shown as straight in this diagram. CD is the first base-pair in the double helical region of the anticodon arm and all the bases between A and C are stacked on one another and follow a regular helix. The companion set between B and D and the set of three bases in the codon follow the complementary helix. In space (see Fig. 4) A and B are quite close together because five nucleotide pairs is about half a turn of the helix.

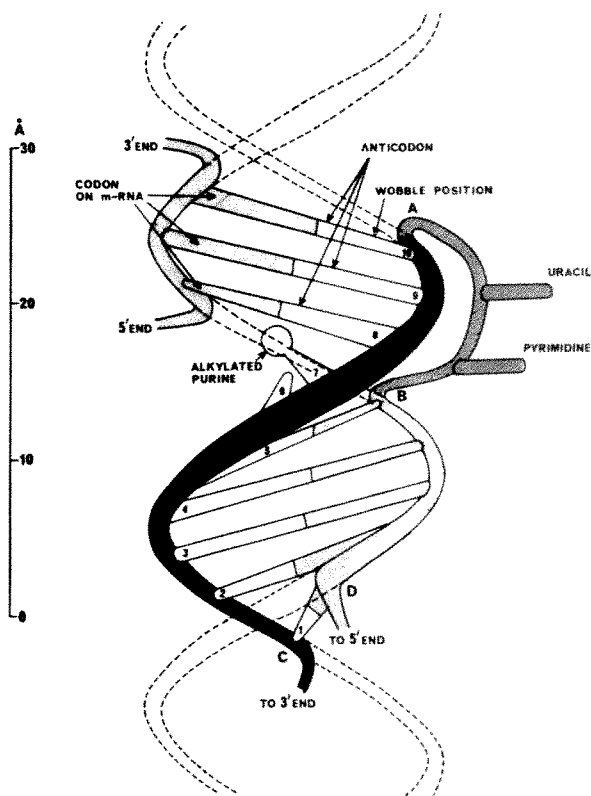


Fig. 4. Schematic diagram of the tRNA anticodon loop illustrating its relationship to the codon and the helical character of the structure. The letters A, B, C and D identify the same points on the structure as in Fig. 3. The bases in nucleotides 1 to 10 are stacked on one another and follow the regular helix which is shown black. The chain of the anticodon double helix between D and B is shaded like the codon to indicate that they follow the same helix. This helix is complementary to the black one. The two nucleotides not in the standard conformation are represented by dark line shading. The representation of their conformation is very schematic because they lie behind nucleotides 8, 9 and 10 in the black chain. The dotted lines indicate the generic helix from which the structure can be imagined to be derived.

recognize a particular tRNA species, Crick<sup>13</sup> has proposed a hypothesis for codon-anticodon recognition. This involves standard Watson-Crick base-pairing between the bases in the codon and anticodon triplets while allowing the possibility of "wobble" or limited alternative pairing in the third position. When codon and anticodon are paired in this way, the atomic sequence in one chain is the reverse of that in the other and the two triplets can be arranged as the two strands in a regular RNA double helix. In our model the anticodon triplet occupies positions 8, 9 and 10 of the anticodon helix (Figs. 3 and 4) and a messenger RNA (mRNA) codon can be base-paired to it without steric hindrance between the rest of the anticodon arm and adjacent mRNA codons. In fact simultaneous recognition of two anticodon arms by adjacent codons is stereochemically possible (Fig. 5). There is not much flexibility in the relative position and orientation of two anticodon arms when they are interacting simultaneously with adjacent mRNA codons. They can interact, however, with each other in a way similar to neighbouring helices in crystalline fibres of reovirus RNA (ref. 8) (see caption to Fig. 5). The possible biological significance of intermolecular hydrogen bonds between the sugar hydroxyl of one helix and the phosphate oxygen of another has been noted<sup>8</sup>. One such hydrogen bond is formed between the two anticodon arms when arranged as in Fig. 5. Preliminary studies suggest that it is possible to arrange the clover leaf arms of the generalized tRNA molecule (Fig. 1) so that there is no steric hindrance between two tRNA molecules whose anticodon arms are interacting in this way.

It might be asked if mistakes in translation could occur by codon "recognition" of bases at positions 7, 8 and 9



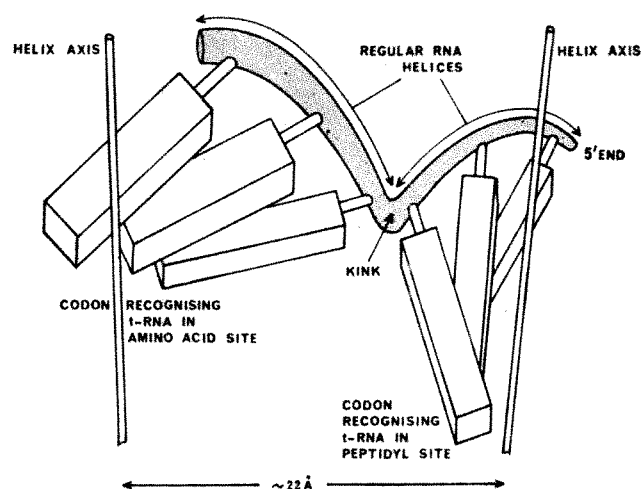


Fig. 5. Schematic diagram of successive codons in mRNA simultaneously recognizing anticodon arms. Each codon of the mRNA has the conformation illustrated in Figs. 3 and 4. The operation required to move the anticodon arm from the amino-acid site to the peptidyl site involves a rotation  $\theta$  about and a translation  $t$  along the anticodon helix axis and a translation  $d$  perpendicular to the helix axis along a line joining the two helix axes illustrated in this figure. If the anticodon helices are linked by a hydrogen bond and have a stereochemical relationship like two reovirus RNA helices in the crystalline fibre then the symmetry operation can be defined precisely as follows (otherwise it is an approximate description).  $\theta = 87.3^\circ$  (that is  $120-32.7$ ),  $t = -2.73$  Å,  $d = 22$  Å. Values of  $\theta$  and  $t$  which would move nucleotide 9 into the position occupied by nucleotide 10 (Fig. 4) are taken as positive.

(or even 6, 7 and 8) rather than 8, 9 and 10 of the anticodon helix. Studies with spacefilling models suggest that recognition of both 7, 8 and 9 and 6, 7 and 8 could not be excluded even if it is insisted that adjacent codons recognize anticodon arms simultaneously. Recognition of 6, 7 and 8 is the less plausible stereochemically. If the geometry of the tRNA-mRNA-ribosome interaction is inadequate to prevent mis-reading of this kind, it may be that it is prevented by the chemical modification which occurs at nucleotide 7 (Fig. 4) in all sequences determined so far<sup>1-4</sup>.

### Stereochemical Aspects of the "Wobble" Hypothesis

We have considered possible distortions of our model for codon-anticodon interaction which would accommodate the alternative base-pairings described by Crick in his "wobble" hypothesis<sup>13</sup> (Table 1). The separation and relative orientation of the glycosidic links in these alternative base-pairs differ from that in the standard Watson-Crick pairs. The position of the wobble base-pair in our model is illustrated in Figs. 3 and 4. Accommodation of adenine-inosine in this position requires extension of the sugar-phosphate chain linking the second and third bases of the anticodon (at positions 9 and 10) or compression of the chain joining the second and third bases of the codon. The chain in the RNA helix is already rather compressed (about 5.6 Å between successive phosphates as compared to about 7 Å in a completely extended chain) and further compression results in steric hindrance between the 2' hydroxyl (and the sugar carbon to which it is attached) and the base of the previous nucleotide. Therefore the principal distortion involved in accommodating adenine-inosine (and any other alternative pairs with an interglycosidic link separation larger than the standard pair) must occur at the anticodon.

In contrast accommodation of an alternative pair with an interglycosidic link separation smaller than the standard pair would require either extension of the sugar-phosphate chain between the second and third bases of the codon or compression of the chain between the second and third bases of the anticodon. Significant compression of the chain can be excluded, and so it appears that the principal distortion involved in accommodating pairs with

an interglycosidic link separation significantly shorter than standard must occur at the codon.

### Site of Distortion in "Wobble" Pairing

Using skeletal models we have found that all the alternative pairing required to account for the genetic evidence on degeneracy in the third position of the codon-anticodon interaction can be accommodated in our model by distortion of the anticodon alone (Table 1). Distortion of the codon conformation is not required. Further, our model building studies indicate that a uracil-uracil pairing can be accommodated if distortion is allowed at the codon. Therefore, because the genetic evidence excludes such pairing in this position, we can conclude that it does not occur because the codon conformation cannot be significantly distorted. It should be noted, however, that our criteria for an acceptable pairing relate to the geometry of the interbase hydrogen bonds and the stereochemistry of the sugar-phosphate chain. While these are clearly necessary requirements, other considerations may also be relevant to the occurrence of a particular alternative pair, for example interbase dipole-dipole interactions. (It may be that non-occurrence of the uracil-uracil pair is the result of such effects rather than of the codon being rigidly held.)

The assignment of "wobble" distortion to the anticodon rather than the codon seems reasonable from general considerations because one might expect each codon to be held on the 30S ribosome in a way which is independent of its position in the mRNA and therefore through bonds involving groups near to or part of the sugar-phosphate chain of the codon currently being read. Such bonds would be expected to limit the conformational flexibility of the codon as compared with the anticodon (which is a relatively small part of the tRNA molecule and not necessarily close to the ribosomal binding site on the tRNA) in a way which is compatible with the above assignment of the distortion

Table 1. THE ACCOMMODATION OF ALTERNATIVE BASE-PAIRS AT THE "WOBBLE" POSITION IN THE CODON-ANTICODON COMPLEX AS A FUNCTION OF WHETHER THE DISTORTION IS ALLOWED IN THE CODON OR ANTICODON CONFORMATION

Alternative codon-anticodon pairs (groups involved in interbase hydrogen bonding)	Genetic evidence for its occurrence (— denotes occurrence) (X denotes non-occurrence)	Stereochemistry of the polynucleotide chain according to the site of distortion (— denotes acceptable stereochemistry) (X denotes unacceptable stereochemistry)	
		Distortion at anticodon only	Distortion at codon only
Adenine-inosine (6-amino to 6-keto and N1 to N1)	—	(The torsion angle of the inosine glycosidic link is about $5^\circ$ outside the acceptable range)	X
Guanine-uracil (6-keto to N1 and N1 to 2-keto)	—	(There is a hydrogen-oxygen non-bonded contact of about 2.2 Å, i.e., about 0.3 Å less than the sum of the van der Waals radii of these atoms)	X
Uracil-guanine (N1 to 6-keto and 2-keto to N1)	—	(There is a hydrogen-oxygen non-bonded contact of about 2.2 Å, i.e., about 0.3 Å less than the sum of the van der Waals radii of these atoms)	X
Uracil-uracil (6-keto to N1 and N1 to 2-keto)	X	X	X
Uracil-uracil (N1 to 6-keto and 2-keto to N1)	X	X	—
Uracil-cytosine (6-keto to 6-amino and N1 to N1)	X	X	X

For none of the pairings denoted as "stereochemically acceptable" is the stereochemistry quite as satisfactory as that in the undistorted standard conformation. The departures from acceptable stereochemistry are noted and are small enough for it to be concluded that the codon-anticodon complex could be distorted to accommodate these pairs. In contrast the stereochemistry of the pairs denoted "stereochemically unacceptable" is quite unacceptable with non-covalently bonded contacts 1 or 2 Å less than normal values and with torsion angles 40 to  $50^\circ$  outside the range of observed values.

associated with "wobble" pairing to the anticodon. Further, the occurrence of the "wobble" base at the top of the single strand anticodon helix allows distortion in the part of the sugar-phosphate chain to which it is attached to be absorbed in the conformational flexibility of the two unstacked pyrimidines next to it.

The model we propose for the anticodon arm of tRNA allows codon-anticodon interaction through Watson-Crick base-pairing. The codon and anticodon nucleotide triplets have the conformation of the two strands in a regular RNA double helix. The alternative or "wobble" pairings suggested for the third base of the anticodon can be accommodated in this model by distortion of the anticodon conformation. It is not necessary to postulate distortion of the anticodon conformation. The observation that uracil-uracil is not a wobble pairing suggests that codon conformation distortion does not take place. This model of the anticodon arm allows adjacent mRNA codons to simultaneously recognize anticodon arms.

In a study such as this it is important to identify clearly the principal assumptions on which the model building is based. Essentially the only assumption we make is that the number of stacked bases in the anticodon loop should be a maximum; this leads to a unique solution for the conformation of the loop. The model receives support from the base sequences which have been determined for the anticodon loop in a number of tRNAs (Fig. 2): the pyrimidines (mainly uracil) are in the irregular part of the loop, the wobble base is at that position in the stacked part of the structure which has the most conformational

flexibility, and the modified purine is at a position which could prevent a wrong set of three nucleotides in the anticodon being recognized by the codon. There is no structural or genetic evidence in conflict with this model and, while the model building study does not prove it to be correct, its stereochemical neatness and the manner in which it accounts for what is known about the anticodon region of tRNA suggest that it is essentially correct.

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## Aetiology of Multiple Sclerosis

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Are dietary defects a cause of multiple sclerosis? There is some evidence that a lack of certain fatty acids during the maturation of the brain results in demyelination.

ONE hypothesis of the aetiology of multiple sclerosis is that the disease is a result of a dietary deficiency of polyunsaturated fatty acids (PUFA). Sinclair<sup>1</sup> concluded that the diets of civilized countries were becoming deficient in these fatty acids and thought that one sequela of such a deficiency could be demyelination of the nervous system. Swank<sup>2</sup> has suggested that multiple sclerosis is the result of the ingestion of a diet rich in animal fats deficient in polyunsaturated fatty acids. These two concepts may not be incompatible, because a low saturated fat intake may cause a sparing of these fatty acids<sup>3</sup>. In areas of the world where there is a high incidence of multiple sclerosis (Northern United States, Central and Northern Europe), a dietary deficiency of polyunsaturated fatty acids is unlikely to occur, and this makes it unlikely that such a deficiency is a cause of the disease.

At least two of the polyunsaturated fatty acids, namely, linoleic and linolenic acid, have different biological functions<sup>4</sup>. In the animal organism, the biosynthesis of polyunsaturated fatty acids proceeds along two independent metabolic pathways<sup>5,6</sup>. Longer chain  $\omega$ 6 series acids are synthesized from  $\Delta$ 9,12 linoleic acid (18 : 2  $\omega$ 6) as a precursor. (The  $\omega$ -number is designated in reference to the

position of the double bond starting from the methyl end of the molecule.)  $\alpha$ -Linolenic acid (18 : 3  $\omega$ 3) is required for the biosynthesis of the longer chain  $\omega$ 3 fatty acids. The interconversion of  $\omega$ 6 and  $\omega$ 3 series acid does not appear to occur in the animal organism<sup>7</sup> or in developing chick brain<sup>8</sup>.

The two principal polyunsaturated fatty acids in mammalian<sup>9,10</sup> and chick brain<sup>11</sup> are arachidonic (20 : 4), an  $\omega$ 6 acid, and docosahexaenoic (22 : 6), an  $\omega$ 3 series acid. These acids are found primarily in the glycerophosphatides and more specifically in the phosphatidylethanolamine (PEA) and phosphatidylserine (PS) fractions, with lesser amounts in phosphatidylcholine (PC). Rathbone<sup>12</sup> found 8.6 per cent and 11.4 per cent of the total fatty acids of rat brain consisted of 20 : 4  $\omega$ 6 and 22 : 6  $\omega$ 3, respectively. In the fully developed chick embryonic brain, 20 : 4 constituted 13.5 per cent, 4.1 per cent and 3.8 per cent of the total fatty acids of the PEA, PS and PC fractions, respectively. The comparable values for 22 : 6 were 19.8 per cent, 26.2 per cent and 1.1 per cent<sup>13</sup>. O'Brien and Sampson<sup>14</sup> found that 20 : 4  $\omega$ 6, 22 : 5  $\omega$ 6 and 22 : 6  $\omega$ 3 were present at a relatively high level in PEA and PS in human brain. It is apparent that the

brain cephalin fraction is relatively rich in  $\omega 6$  and  $\omega 3$  series polyunsaturated fatty acids.

The composition of brain fatty acids may be influenced by the fatty acid composition of the diet. This relationship has been demonstrated in chickens<sup>15</sup>, young rats<sup>16</sup> and older rats<sup>12</sup>. Feeding 18:2 leads to an increase in longer chain  $\omega 6$  acids and feeding 18:3 to an increase in  $\omega 3$  series acid, and so Mohrhauer and Holman concluded that at least one member of each family of polyunsaturated fatty acids must be able to pass through the blood-brain barrier (this term is used in a functional sense in further discussion). Lipid-soluble substances are known to penetrate the blood-brain barrier more readily than most aqueous-soluble compounds<sup>17</sup>, and Sperry *et al.*<sup>18</sup> have determined that labelled octanoic acid can penetrate the blood-brain barrier.

Usually 18:2 constitutes about 1 per cent of the fatty acids in the brain<sup>19</sup>, and this fatty acid pool may be utilized for the biosynthesis of longer chain  $\omega 6$  polyunsaturated fatty acids. There is a slow turnover of phospholipids in adult brain<sup>20</sup> and so the brain may be able to survive any 18:2 deficiency in the diet for extended time periods. In our work with chicks, however, as well as those already cited with mammalian species, virtually no brain phospholipid 18:3 could be detected. In the absence of any pool of 18:3, any requirements for this  $\omega 3$  fatty acid would seem to depend on an adequate exogenous source. If the intake of polyunsaturated fatty acids is less than marginal during development, subsequent requirements for  $\omega 3$  acids as a result of normal turnover cannot be met. Alternatively, the structure of developing nervous tissue formed during periods when an adequate exogenous source of  $\omega 3$  acids is unavailable may be defective and break down after an extended time period.

The known stability of myelin and the low turnover of myelin lipids<sup>21-23</sup> may seem inconsistent with the foregoing concept. The same studies, however, demonstrated that the turnover of the lecithin and cephalin fractions is greater than that of spingomyelin, cerebrosides or cholesterol. It should be borne in mind that there may be a relative deficiency of  $\omega 3$  acids early in life, and perhaps even *in utero*, on a diet deficient in  $\omega 3$  fatty acid. The margin of safety involved here is unknown. In addition, the primary metabolic lesion may not occur in myelin, but demyelination may be a secondary effect of abnormal metabolism in other brain structures; for example, neurones, glia or neuronal processes. Thus Terry and Harkin<sup>24</sup> indicated in Wallerian degeneration that the periodic layers of myelin were intact at a time when the axoplasm and mitochondria are damaged. Another factor to be considered is the relatively late time of peak onset of the disease (about 30 yr) (ref. 25), a fact which may be compatible with the slow myelin lipid turnover.

Experimental data which tend to support the concept of  $\omega 3$ -deficiency have been reported. Weil<sup>26</sup> reported a decrease of phospholipids in the brain in multiple sclerosis. Gerstl *et al.*<sup>27,28</sup> found deficits in phospholipids and fatty acids in intact white matter from brains of patients with multiple sclerosis. They also reported that the lipid content of myelin from such brains was less than that of the control. Kishimoto *et al.*<sup>29</sup> analysed the fatty acids of glycerophospholipids from apparently normal white matter derived from patients with multiple sclerosis, and reported that the 22:6  $\omega 3$  content of the ester-linked fatty acids was less than half of that found in normal white matter. The number of cases thus far reported is too small to permit generalizations to be made. Lecithin from the white matter of patients with multiple sclerosis contains a larger percentage of saturated fatty acids than that of control subjects<sup>30</sup>. Analysis of the cerebrospinal fluid phospholipids in multiple sclerosis<sup>31,32</sup> has shown that a relative increase in the cephalin fraction occurs and the severity of the clinical picture has shown a good correlation with the increase in cerebrospinal fluid cephalin. A group of patients with multiple sclerosis studied

by Tourtelotte *et al.*<sup>33</sup> gave results which can be recalculated in units used by McArdle and Zilkha to show a similar trend. No determinations for polyunsaturated fatty acids were attempted in these studies. Other phospholipid fractions were normal.

The two principal dietary sources of the  $\omega 3$  fatty acids are a variety of seed and marine oils<sup>34</sup>, the latter rich in polyunsaturated  $C_{20}$ - and  $C_{22}$ -acids. Differences in species and environmental conditions influence the  $\omega 3$  fatty acid content but the following generalizations may be made<sup>34</sup>. Edible seed oils with a significant 18:3 content are found in soybeans, whole wheat, millet, a number of nut and fruit oils, and some non-edible seeds yield oils which are used for cooking in many parts of the world; for example, linseed and rapeseed. In addition, a wide variety of vegetable and fruit oils indigenous to tropical and sub-tropical climates contain 18:3 as a minor component. Marine oils have a high content of  $C_{20}$ - and  $C_{22}$ -unsaturated fatty acids, while oils from fresh water fish contain  $C_{18}$ -unsaturates<sup>35</sup>. Cod-liver oil is reported<sup>36</sup> to contain 0.7 per cent 18:3 and 8.7 per cent 22:6. The fish oils of the sardine, herring, menhaden and mackerel are rich in  $C_{20}$ - and  $C_{22}$ -unsaturates. The principal site of lipid storage in fish is the liver<sup>37</sup> and the amount present in extra-liver sources depends on the nutritive condition and the species. It appears that where the whole fish is consumed, an increased supply of  $\omega 3$ -type acids would be available. The ready anti-oxidizability of these highly polyunsaturated fatty acids is relevant to fish oils. Drying of the fish, lengthy exposure to air, and excessive cooking would cause peroxidation or polymerization of the essential fatty acids and make the amount of protective factor available uncertain.

The difficulties of studying the epidemiology of a low-incidence disorder like multiple sclerosis has already been detailed by McAlpine, Lumsden and Acheson<sup>38</sup>, and the citations that follow refer to the page number in their treatise. If the known epidemiological data are correlated with the distribution of foods containing fatty acids of the linolenic acid type, the following points can be made.

(a) In North America, Europe, the Near East and probably Australia and Asia (except for Japan and China) there appears to exist a north-south gradient, with the incidence of the disease lower in warmer latitudes (p. 43). This low incidence occurs in areas where the consumption of animal protein is generally low and where there is an availability of a large variety of seed and fruit oils, in many cases dietary staples, which contain small to moderate amounts of 18:3. Thus the use of palm oil in the tropics, millet in parts of Asia, safflower, cottonseed and rapeseed oils, as well as berries and fruits containing 18:3 in warm latitudes, could be preventive of multiple sclerosis.

(b) The low incidence of multiple sclerosis in Japan (p. 5) and China (p. 27) may be attributed to the widespread use of soybeans in the diet as well as the availability of marine foods in many cases eaten uncooked, which would tend to preserve the content of polyunsaturated fatty acids.

(c) There are variations in the patterns of distribution of multiple sclerosis in the high incidence area of Central and Northern Europe. Where statistics are available (Scotland and Norway) they indicate that high incidence areas involve "... remote, circumscribed or mountainous tracts of country where there may be special problems such as ... lack of variety in the diet" (p. 32). Thus in Norway (p. 48) the disease is uncommon in coastal fishing areas and common in interior agricultural communities. Sallstrom<sup>39</sup> found the incidence of the disease high in agricultural workers in Sweden.

(d) Whether the incidence of the disease differs in urban versus rural areas is not well documented (p. 32). Miller *et al.* (p. 38) have suggested that in England and Wales the risk of the unskilled worker developing multiple sclerosis was half that of someone in the professional or

managerial class. This may be a reflexion of economic status and the tendency to substitute animal protein for fish and vegetable proteins by the more affluent. In a study of Army inductees, Beebe *et al.*<sup>40</sup> concluded that the incidence of multiple sclerosis was higher in metropolitan or urban areas when compared with rural areas.

(e) Migration from a high risk zone to a low risk zone carries with it in part the risk of the country of origin (p. 39). Migration from a low risk to a high risk area may increase risk (p. 40). Other variables, however, such as age at the time of migration, obscure any definitive assessment. In a study of immigrants to Israel originating from Europe and Afro-Asia, Alter<sup>41</sup> found no change in the low incidence of the disease in the latter group. In Europeans under 15 yr old at immigration, the incidence of the disease approached that of the Afro-Asians, indicative of a protective effect of migration. In Europeans migrating at 15–29 yr the incidence was 8.9 times the Afro-Asian, and at 30–44 yr this figure was 5.4. The authors remark that their data indicate that multiple sclerosis is acquired many years before the onset of clinical symptoms, and the early life history of the patient might be examined for an aetiological factor. Older people, however, may be habituated to dietary preferences and the statistics may reflect a resistance to changes in food selection with age.

(f) The tendency for the disease to be familial (p. 61) is explicable on the dietary deficiency hypothesis. Mackay and Myrianthopoulos<sup>42</sup>, in an investigation of monozygotic and dizygotic twins, concluded that if genetic effects are involved in the aetiology of multiple sclerosis they are subjected to powerful environmental influences.

The problems that present themselves in advancing this hypothesis are numerous.

(a) The inability to produce demyelination of the nervous system in experimental animals on an  $\omega 3$  fatty acid-deficient diet may be the result of either differences in specie sensitivity or the inability of animals to survive such a diet for the period of time required to produce lesions. Much of the experimental work reported on a linolenic acid deficiency has involved a deficiency of polyunsaturated fatty acids.

(b) The data on the composition of polyunsaturated fatty acids in the brain in multiple sclerosis is limited. It will require more detailed analysis of various lipids and lipid fractions before any assessment of the effect of deficiency of these acids can be made.

(c) Some of the epidemiological data cited indicating partial protection in the young in moving from a high risk to a low risk area suggest some exogenous requirement before the brain matures.

(d) There may be other unknown metabolic pathways where a requirement exists in the absence of any precursor pool. Thus the aetiology of multiple sclerosis may reside in more than one dietary deficiency. Alternatively, the dietary intake of  $\omega 3$  fatty acids may be adequate but the pathway from ingestion to the final deposition of 22:6 in the brain may be impaired. Thus absorption from the gastro-intestinal tract, transport mechanisms in the blood, passage across the blood-brain barrier, or the  $\omega 3$  elongation system, may be defective in patients who develop multiple sclerosis. We have shown in chick brain<sup>43</sup> that the elongation of 18:3  $\omega 3$  to 22:6  $\omega 3$  may proceed with a regulatory mechanism different from that of the  $\omega 6$  acids. Multiple sclerosis may thus be a result of some aberration in the control mechanism of 18:3 elongation to 22:6 resulting in an apparent deficiency in brain concentrations of the latter. Competition has been demonstrated between 18:2 and 18:3 and Rahm and Holman<sup>44</sup> showed in rats that an increase in the intake of 18:2 depressed the concentrations of 22:6 in liver fatty acids. We have found that the incorporation of <sup>14</sup>C-linolenic acid into 22:6 in brain slices was markedly inhibited by the presence of  $\Delta 6,9,12$  6 isomer of linolenic acid (unpublished results of Stephanides and Bernsohn).

(e) It has been pointed out<sup>45</sup> that monomolecular surface films of fatty acids are influenced by unsaturation. Unsaturated acids produce films of the expanded type, and as the double bond moves toward the methyl end of the molecule, the film expands more readily. Thus a deficiency of  $\omega 3$  fatty acids in the membrane may produce a more rigid structure which may not be consistent with normal functioning in the central nervous system.

Many basic data need to be obtained before the theoretical aspects of this problem can be fully elucidated. Such data and the development of an animal model would be a desideratum but the nature of the clinical problem and its seriousness for the patient and family has prompted this presentation despite the apparent gaps in knowledge.

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# Cotton Effects in Plant Ferredoxin and Xanthine Oxidase

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The position and magnitude of the optically active absorption bands in the non-haem iron proteins xanthine oxidase and spinach ferredoxin are found to be very similar. A study of the Cotton effects in these proteins suggests that xanthine oxidase contains four identical pairs of iron atoms while spinach ferredoxin contains only one pair.

SPINACH ferredoxin (photosynthetic pyridine nucleotide reductase), isolated from the chloroplasts of spinach leaves, acts as an electron carrier in photosynthesis<sup>1,2</sup>. It contains two atoms of iron per mole<sup>3,4</sup>, undergoes reversible oxidation and reduction and only one electron is transferred per mole in the process<sup>3,4</sup>. By contrast, bacterial ferredoxin contains about six atoms of iron per mole and two electrons are transferred per mole in the oxidation-reduction process<sup>5,6</sup>. These two proteins can, however, be interchanged in the reduction of triphosphopyridine nucleotide using chloroplast extracts free of ferredoxin<sup>7</sup>.

Xanthine oxidase from mammalian milk has a function and size different from those of the ferredoxins. It contains about eight atoms of iron, two atoms of molybdenum and two moles of flavin adenosine dinucleotide (FAD) per mole<sup>8</sup>; up to ten electrons are transferred per mole in the oxidation-reduction process<sup>9</sup>. It has, however, an electronic spectrum rather similar to that of spinach ferredoxin.

The optical properties of bacterial ferredoxin have been previously studied as have those of rubredoxin (a red non-haem iron protein isolated during the purification of bacterial ferredoxin and containing one iron atom per mole)<sup>10,11</sup>. We now present a similar study of spinach ferredoxin, parsley ferredoxin and xanthine oxidase.

Spinach ferredoxin was kindly provided by Professor F. R. Whately, and parsley ferredoxin by Dr H. E. Davenport. Measurements on these proteins were carried out in 0.05 molar *tris*-hydrochloric acid buffer pH 7.4. Xanthine oxidase was purified according to a procedure developed by Drs L. I. Hart and R. C. Bray (unpublished), based on the observation<sup>8</sup> that salicylate treatment selectively denatures enzymes free of molybdenum. The specific activity/ $E_{450}$  of the enzyme used was 153 and it had iron : FAD : molybdenum : enzyme ratios of 7.2 : 1.7 : 1.7 : 1. (Dr L. I. Hart kindly prepared and analysed the enzyme for us.) The enzyme was stored at 4°C in molar phosphate buffer (pH 6) containing 3 mmolar sodium salicylate and 1 mmolar EDTA. Measurements were carried out in 0.055 molar pyrophosphate buffer (pH 8.2) containing 1 mmolar EDTA. Methanol-inactivated xanthine oxidase was prepared by the dialysis method using salicylaldehyde as substrate<sup>12</sup>. The reduced spectra

were obtained by adding crystalline sodium dithionite to the samples of spinach and parsley ferredoxins; aeration restored the original oxidized spectra. Xanthine oxidase was reduced by adding a calculated amount of xanthine dissolved in 0.02 molar sodium hydroxide to give approximately a 10 molar excess of substrate or by adding a solution of sodium dithionite in 0.001 molar sodium hydroxide again to give a 10 molar excess. Special anaerobic heads for both the circular dichroism and optical rotatory dispersion cells were used. The procedure for anaerobic addition has been described previously<sup>13</sup>. In other experiments, reduction of xanthine oxidase was accomplished by adding solid dithionite; this procedure gave identical results to those where the addition of sodium dithionite was controlled. Samples of xanthine oxidase were transferred anaerobically from the circular dichroism cells to electron spin resonance tubes and the state of oxidation of the flavin, molybdenum and iron studied<sup>14</sup>.

The Cotton effects of spinach ferredoxin are shown in Fig. 1 and those of xanthine oxidase in Fig. 2. Measurements from these spectra are given in Tables 1 and 2 together with those of other non-haem iron proteins. The results are presented in molecular form using a molecular weight of 274,000 (ref. 14) and  $E_{1\text{cm}}^{1\%}$  of 2.4 (ref. 8) at 450 mμ for xanthine oxidase and a molecular weight of 13,000 (ref. 3) for spinach ferredoxin. The present results are in essential agreement with the previous studies of the rotatory dispersion of these proteins<sup>15-18</sup> over a limited wavelength range and also with the more informative property, circular dichroism, of oxidized xanthine oxidase (Keleti, T., unpublished results).

Despite the widely differing composition and molecular weights of the two proteins, it is evident that the spectra of the oxidized forms are very similar in the region 350–800 mμ with the exception of a positive circular dichroism maximum at about 600–640 mμ in spinach ferredoxin. Thus it is clear that the spectra are produced by similar chromophores in similar asymmetric environments. In spinach ferredoxin this chromophore can only arise from the two iron atoms, and similar iron atoms must also constitute the visible chromophores in xanthine oxidase. The molybdenum atoms therefore do not contribute appreciably to the observed spectra in the visible region

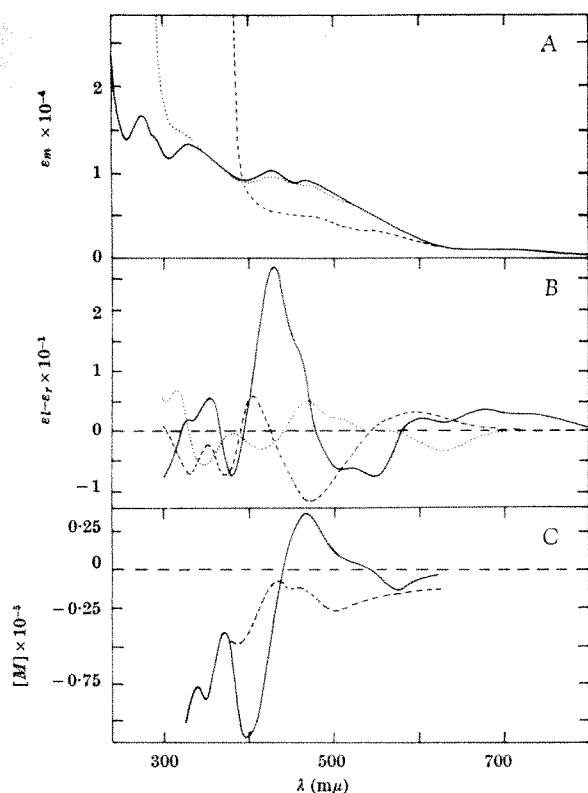


Fig. 1. The optical properties of spinach ferredoxin. *A*, Absorption; *B*, circular dichroism; *C*, optical rotatory dispersion; —, oxidized; . . . , oxidized (8 molar urea); ---, reduced.

except perhaps at about 600 mμ. Similarly the flavins, which have an absorption band at 450 mμ, give rise to little if any optical activity. These flavin molecules must therefore be in a site of low asymmetry.

A comparison of the intensities of the circular dichroism spectra from oxidized xanthine oxidase and spinach ferredoxin (Table 1) reveals that over the wavelength range 400–800 mμ the values for xanthine oxidase are about four times the corresponding values for spinach

ferredoxin. The slight differences in the positions of the absorption maxima are reflexions of differences in the primary, secondary, or tertiary structure of the protein bound to the chromophore.

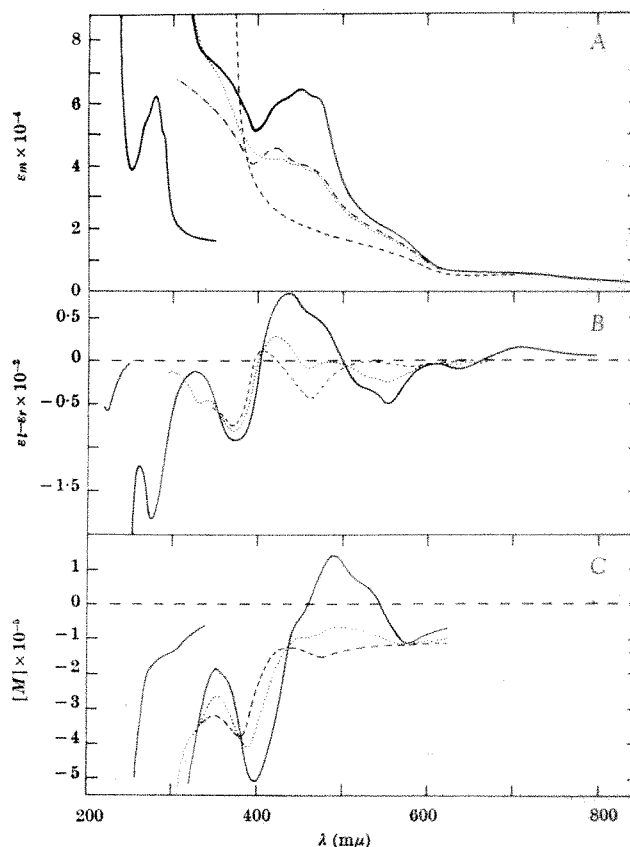


Fig. 2. The optical properties of xanthine oxidase. *A*, Absorption (at wavelengths below 300 mμ the scale is five times reduced); *B*, circular dichroism (at wavelengths below 250 mμ the scale is one hundred times reduced); *C*, optical rotatory dispersion (at wavelengths below 300 mμ the scale is five times reduced); —, oxidized; . . . , reduced with xanthine; ---, reduced with dithionite; — · — · —, oxidized "Flavin-Mo" free enzyme (replotted from the data in ref. 19).

Table 1. CIRCULAR DICHROISM AND ABSORPTION MAXIMA OF NON-HAEM IRON PROTEINS (OXIDIZED FORM)

Xanthine oxidase (a)		Spinach ferredoxin (buffer) (a)		Spinach ferredoxin (8 molar urea) (a)		<i>P. elsdonii</i> rubredoxin (b)		<i>P. elsdonii</i> ferredoxin (b)		<i>C. pasteurianum</i> ferredoxin (c)	
λ (mμ)	ε <sub>l</sub> -ε <sub>r</sub>	λ (mμ)	ε <sub>l</sub> -ε <sub>r</sub>	λ (mμ)	ε <sub>l</sub> -ε <sub>r</sub>	λ (mμ)	ε <sub>l</sub> -ε <sub>r</sub>	λ (mμ)	ε <sub>l</sub> -ε <sub>r</sub>	λ (mμ)	ε <sub>l</sub> -ε <sub>r</sub>
705	+14.3	710	+2.87								
635	+8.4	678	+3.16	635	-3.6	632	-5.0	679	-1.7	683	-1.9
553	-50.8	593	+2.06								
530	-33.6	548	-7.8			564	+13.2	565	+2.3	567	+3.3
		508	-6.33	505	+2.28	502	-11.3				
475	+10.4	460	+10.6	468	+4.71			473	+1.9		
432	+82.1	426	+27.2	420	-2.87	441	+8.7	425	+1.4	410	+4.15
375	-97.2	380	-7.6			400	+4.5				
		356	+5.23	346	-5.45	345	-3.4	c. 330	c. +1.7	c. 325	c. +2.6
275	-194	325	+2.05	317	+3.53	328	-4.2			c. 295	c. +1.5
218	-6,540					275	-5.3	c. 290	c. +1.6	c. 275	c. -0.8
						224	-89.5				

(a) This work. (b) Taken from ref. 11. The values given here are corrected by a factor of ten owing to a calculation error. (c) This work. The sample was obtained from the Worthington Biochemical Corporation, New Jersey, USA. ε<sub>m</sub> is taken as 2 × 10<sup>4</sup> cm<sup>2</sup> M<sup>-1</sup> at 390 (refs. 5 and 15).

Table 2. CIRCULAR DICHROISM AND ABSORPTION MAXIMA OF NON-HAEM IRON PROTEINS (REDUCED FORM)\*

Xanthine oxidase (xanthine reduced 60 per cent)		Xanthine oxidase (dithionite reduced)		Spinach ferredoxin		<i>P. elsdonii</i> rubredoxin		<i>P. elsdonii</i> ferredoxin		<i>C. pasteurianum</i> ferredoxin	
λ (mμ)	ε <sub>l</sub> -ε <sub>r</sub>	λ (mμ)	ε <sub>l</sub> -ε <sub>r</sub>	λ (mμ)	ε <sub>l</sub> -ε <sub>r</sub>	λ (mμ)	ε <sub>l</sub> -ε <sub>r</sub>	λ (mμ)	ε <sub>l</sub> -ε <sub>r</sub>	λ (mμ)	ε <sub>l</sub> -ε <sub>r</sub>
550	-25.3	580	-8.4	596	+3.24						
525	-18.8										
461	-8.4	460	-46.4	470	-11.95			465	+1.6	410	+1.1
417	+29.5	407	+11.0	406	+5.08					365	+4.25
370	-86.4	367	-82.5	374	-7.52						
333	-51.6			333	-7.15						
						336	+11.1				
						317	-20.0				
						295	+10.0	395	-2.3		
						287	+9.8	348	+2.5		
						258	+9.0	295	+4.0	285	+7.15
						224	-89.5				

\* Sources and comments as in Table 1.

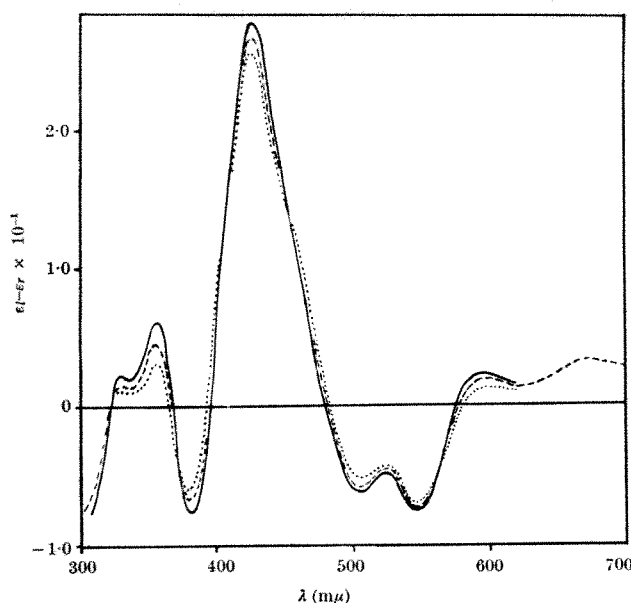


Fig. 3. The variation of the circular dichroism of spinach ferredoxin with temperature: —, 1° C; ---, 22.5° C; ···, 32.2° C. This sample contained a polyphenolic impurity giving rise to the large negative effect at 300 mμ.

It has been found that in FAD and molybdenum-free xanthine oxidase, the extinction coefficient (at 450 mμ) is 5,000 cm<sup>2</sup> moles<sup>-1</sup> (ref. 19) per iron; this agrees well with that calculated by Bray<sup>20</sup>. Using this value, the contribution of FAD and molybdenum to the molar extinction at 450 mμ was estimated in oxidized and reduced xanthine oxidase. This figure correlates with the contribution from the FAD<sup>21</sup>; it is known that free FAD and bound FAD have essentially the same absorbancy at 450 mμ (ref. 22). The suggestion<sup>23</sup> that the visible absorption spectrum of xanthine oxidase is due to molybdenum appears to be incorrect. We have confirmed the findings of Rajagopalan and Handler<sup>19</sup> using our more highly purified xanthine oxidase preparation and have compared the absorbances with those from spinach ferredoxin (Table 3). It can be seen (comparing columns 2 and 5) that the values for xanthine oxidase are four times those for spinach ferredoxin. The same result is obtained when the molar optical rotatory dispersions of these two materials are compared (Table 3).

It is possible that the iron atoms in the plant ferredoxin chromophore are in a mixed spin state under physiological conditions. If this were so, small changes in temperature would radically alter the observed spectral properties. We have therefore measured the circular dichroism of spinach ferredoxin over the temperature range 0°–30° C (Fig. 3). As was found previously in rubredoxin<sup>11</sup> the overall shape of the spectrum remains unchanged and it would seem likely that the slight reduction in intensity on raising the temperature resulted from thermal broadening of the vibronic absorption bands. Thus it can be concluded that mixed spin states are not involved over the physiological range of temperature though the possibility remains open whether they occur at lower temperatures<sup>24</sup>.

Further insight into the environment of the iron chromophore may be obtained by altering the conformation of the protein. We have investigated this possibility by measuring the spectra of spinach ferredoxin in 8 molar urea (Fig. 1) and as a function of time at pH 11 in sodium hydroxide-sodium carbonate buffer (Fig. 4). Similar experiments were carried out with xanthine oxidase solutions in buffer containing 9 molar urea and also in solutions saturated with urea at room temperature.

Although dissolving the ferredoxin in 8 molar urea has only a slight effect on the absorption spectrum, the circular dichroism is much changed. The original spectrum is replaced by one with very different and much weaker absorption bands. The effect of urea is presumably to alter the helical nature of the protein around the non-haem iron chromophore. The results indicate that urea treatment leads to a chromophore which is in a centre of lower asymmetry due to loss of helical structure. The circular dichroism at 635 mμ in the protein treated with urea probably results from an iron-sulphur band<sup>25</sup>.

At pH 11 the protein is attacked by hydroxyl ions hydrolysing the amide linkages; this treatment will, of course, affect the more exposed portions of the molecule initially. Again it is evident that the optical activity observed for the iron chromophores arises largely from the conformation dictated by the protein structure. Thus

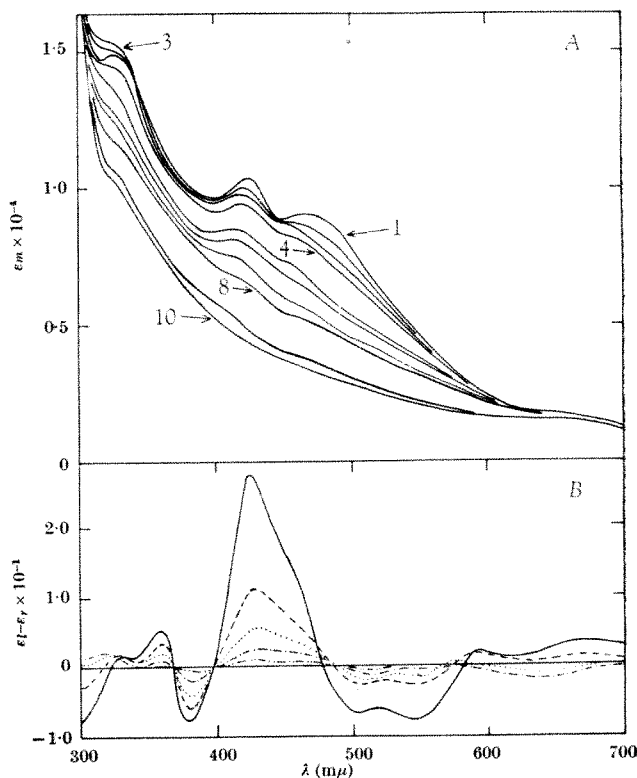


Fig. 4. The variation of absorption and circular dichroism of spinach ferredoxin at pH 11 with time. A. Absorption: 1, 3 min; 2, 11 min; 3, 23 min; 4, 74 min; 5, 110 min; 6, 160 min; 7, 178 min; 8, 235 min; 9, 507 min; 10, 730 min. B. Circular dichroism (scan speed 1 mμ/8 sec commencing at 700 mμ): —, untreated; ···, 28 min; ---, 115 min; - · - ·, 188 min; - - - -, 690 min. This sample contained some polyphenolic impurity.

Table 3. COMPARISON OF ABSORPTION AND OPTICAL ROTATORY DISPERSION PROPERTIES OF XANTHINE OXIDASE WITH ITS COMPONENTS AT 450 Mμ

	Xanthine oxidase $\epsilon_m$ (cm <sup>2</sup> moles <sup>-1</sup> l.)	Iron only xanthine oxidase* $\epsilon_m$ (cm <sup>2</sup> moles <sup>-1</sup> l.)	Difference = FAD + Mo contribution $\epsilon_m$ (cm <sup>2</sup> moles <sup>-1</sup> l.)	Spinach + ferredoxin $\epsilon_m$ (cm <sup>2</sup> moles <sup>-1</sup> l.)	Free FAD† $\epsilon_m$ (cm <sup>2</sup> moles <sup>-1</sup> l.)
Oxidized	66,000	40,000	26,000	8,980	11,300
Reduced (dithionite)	22,500	(45,400)‡	2,500	(10,320)§	—
[M] (amplitude between 480 and 395 mμ)¶ oxidized form	67,800	20,000	—	4,550	980
				159,000	—

\* Estimated from the data given in ref. 19. † Taken from the data in ref. 3. ‡ Taken from ref. 21. § Values at 420 mμ. ¶ This work.

after about 45 min the circular dichroism has fallen by more than half, but the effect on the absorption spectrum is only slight. Further reaction causes a steady loss of absorption between 300 and 600 m $\mu$  together with loss of optical activity. The circular dichroism curves do not, however, pass through common points (isodichroic points) on the base line, indicating that, although the original optically active chromophore is disappearing, a new one is being formed. In particular, a new Cotton effect appears at about 640 m $\mu$ , associated with the shoulder at 650 m $\mu$  in the absorption spectrum (this region of the absorption spectrum is only slightly affected by the treatment with alkali). This band is at the same wavelength as the band obtained with 8 molar urea. The effect of treatment at pH 11 is probably to produce the same effect as urea, that is to destroy the helicity of the protein in the neighbourhood of the iron chromophore leaving small Cotton effects as a result of isolated iron-sulphur chromophore.

With xanthine oxidase there was no change in the absorption, circular dichroism or optical rotatory dispersion spectra when these were measured immediately in 9 molar urea, but after 30 h at 0° C the circular dichroism had decreased by 21 per cent while there was a 24 per cent loss in absorption at 450 m $\mu$ . These findings probably indicate that iron and flavin have been lost from the enzyme as reported previously from studies in 4 molar urea<sup>19</sup>. Using a room temperature saturated urea solution (20 molar) denaturation occurred rapidly; all characteristic optical activity above 300 m $\mu$  was lost, and the fully denatured protein showed a 74 per cent loss in absorption at 450 m $\mu$  after about 6 h. In no case was a change comparable with that found with spinach ferredoxin observed. It has been previously noted<sup>26</sup> that at low concentrations urea tends to act as a competitive inhibitor for xanthine rather than to cause conformational changes in the protein. The differences in the effect of urea on xanthine oxidase and spinach ferredoxin are presumably a reflexion of the great difference in the protein structures and sizes of these proteins.

We have also measured the spectra of parsley ferredoxin. These are shown in Fig. 5. The results have been calculated using a molecular weight of 13,000 as with spinach ferredoxin (Bendall, Gregory and Hill<sup>27</sup> give the molecular weight as 12,200 from ultracentrifuge measurement and 14,000–19,000 as the minimum molecular weight from amino-acid analysis. We have used here the same value for the molecular weight as in spinach ferredoxin (13,000)). The spectra are very similar both in intensity and shape

to those of spinach ferredoxin. The visible and near ultra-violet spectra in these proteins arise therefore from almost identical chromophores.

A previously reported optical rotatory dispersion curve<sup>10</sup> referred to a sample of parsley ferredoxin which contained some colourless (probably poly-phenolic) impurity. A similar impurity is often present in spinach ferredoxin, which has a strong absorption band at 265 m $\mu$ , a strong positive circular dichroism at 265 m $\mu$  and a much weaker negative circular dichroism at 295 m $\mu$ . The presence of this impurity does not affect the results of circular dichroism above 320 m $\mu$  but introduces a plain positive optical rotatory dispersion curve into the visible region.

The optical properties of the reduced proteins are given for spinach ferredoxin (Fig. 1) and xanthine oxidase (Fig. 2). Measurements from these spectra are given in Table 2 together with those for other non-haem iron proteins. The spectra of dithionite-reduced spinach ferredoxin and xanthine oxidase are very similar above 300 m $\mu$ . The only difference is the change in sign of the long wavelength band, a similar effect to that seen in the oxidized spectra. The negative band at 580 m $\mu$  in reduced xanthine oxidase cannot arise from a reduced "ferredoxin-like" chromophore and the differences at long wavelength for both oxidized and reduced xanthine oxidase may arise from an electronic transition involving molybdenum. This conclusion is supported by the parallel electron spin resonance study which failed to reveal any flavin semiquinone—a component which might have been expected to contribute to the absorption spectrum of the reduced xanthine oxidase in this region. The electron spin resonance signals found for molybdenum and non-haem iron in the present samples of xanthine oxidase were identical to those reported previously (ref. 12 and Bray, R. C., and Knowles, P. F., unpublished) under each of the reduction conditions examined.

The absorption spectra of xanthine- and dithionite-reduced xanthine oxidase were compared after both the circular dichroism and optical rotatory dispersion experiments. Several workers<sup>28–30</sup> have reported that the extent of rapid reduction of the enzyme with xanthine does not exceed 50–60 per cent of the value obtained with dithionite. No attempt was made in the present investigation to follow the rapid phase of reduction; the results obtained refer to reactions lasting about 45 min. It was found that the extent of reduction produced by xanthine was 60–65 per cent of that produced by dithionite. The extent of reduction calculated from the circular dichroism and optical rotatory dispersion spectra agreed well with that obtained from the absorption spectra of the same solution. There was no indication of further slow changes in the spectra as reported by Bray *et al.*<sup>13</sup>, possibly because the present studies were made with a substantially more highly purified enzyme.

These results suggest that xanthine only partially reduces the iron chromophores. This is confirmed by the observation that the circular dichroism and optical rotatory dispersion curves for the oxidized and reduced species all pass through the same points, isodichroic points being seen at 502 m $\mu$ , 405 m $\mu$  and 352 m $\mu$  and isorotatory points at 432 m $\mu$ , 377 m $\mu$  and 333 m $\mu$ . In addition, on re-admitting air to the cells, spectra were obtained which passed through the same isospectral points. The observation of these points (analogous to isosbestic points) can only be interpreted as indicating the presence of just two species of iron, the oxidized and the reduced. It should, however, be pointed out that from electron spin resonance studies<sup>12</sup> it is known that the molybdenum species present in xanthine- and dithionite-reduced xanthine oxidase are quite different.

The molybdenum of methanol-inactivated xanthine oxidase has been shown<sup>12,17</sup> by electron spin resonance to be in the pentavalent state with a less than axially symmetrical environment. The valency of molybdenum in the oxidized enzyme is probably six<sup>20</sup>. A difference might

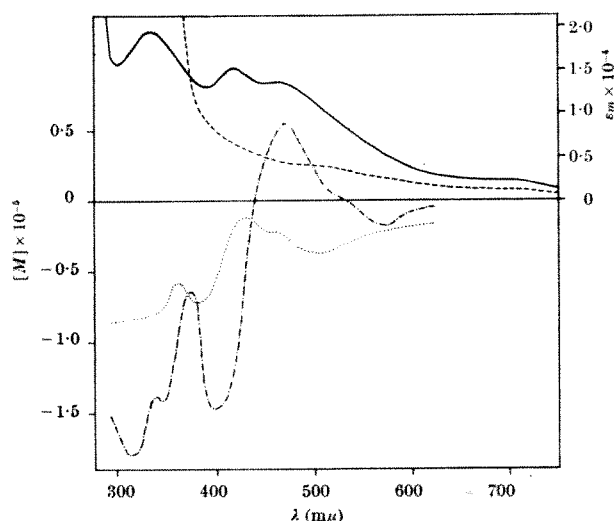


Fig. 5. The optical properties of parsley ferredoxin. Absorption (—), and optical rotatory dispersion (— · — · —), of the oxidized protein. Also absorption (with dithionite) (---) and optical rotatory dispersion (·····) of the reduced protein.



be expected between the optical properties of active and of methanol-inactivated xanthine oxidase due to the change from  $\text{Mo}^{\text{VI}}$  to  $\text{Mo}^{\text{V}}$ . The absorption, circular dichroism and optical rotatory dispersion spectra obtained for oxidized- and dithionite-reduced methanol-inactivated xanthine oxidase were found to be identical with those of the active enzyme. This again indicates that the contribution to the spectra from molybdenum is small and is masked by the "ferredoxin-like" chromophores. Until more evidence is available, the assignment of the band at 580 m $\mu$  to molybdenum must remain tentative.

The study of the position and magnitude of optically active absorption bands gives a very delicate indication of molecular environment. Those observed for plant ferredoxin and xanthine oxidase are very similar not only in position but also in the relative magnitude per iron atom and must therefore arise from virtually identical non-haem iron chromophores. A comparison of the magnitudes per protein molecule indicates that xanthine oxidase probably contains four identical pairs of non-haem iron atoms while spinach ferredoxin contains one such pair. This confirms an earlier report based only on optical rotatory dispersion measurements<sup>31</sup>. Our results also suggest that xanthine only effects partial reduction of the iron relative to dithionite.

These findings correlate with the findings of electron spin resonance studies (Gibson, J. F., and Bray, R. C., unpublished) and with Mössbauer studies<sup>32</sup>. The latter indicate that at pH 6.0, dithionite reduction affects all the iron atoms while reduction with salicylaldehyde affects only up to 20 per cent of the iron present. A model to explain all these experimental observations, which involves spin-paired iron atoms, has been proposed by Gibson *et al.*<sup>33</sup>. This is a refinement of an earlier model by Brintzinger, Palmer and Sands<sup>34</sup>. A low spin ferrous atom interacting with a radical<sup>35</sup>, however, cannot be ruled out as a possibility from the available data, though the transfer of only one electron during the catalytic cycle of spinach ferredoxin would favour the two iron atom model.

The presence in xanthine oxidase of four pairs of iron atoms, two atoms of molybdenum and two molecules of FAD, would require ten electrons to be fully reduced which agrees well with the figure estimated by Ehrenberg and Bray<sup>9</sup>. This two iron-two sulphur chromophore has been found in material of plant and mammalian origin; it may therefore be expected as a common unit in nature and to occur in many enzymes with redox function. There is already good evidence for the chemical similarity of non-haem iron proteins from mammalian, plant and bacterial sources<sup>2,19,36-43</sup>. There must be large differences in protein structure, however, to account for the facts that although the optical rotatory dispersion spectra of spinach ferredoxin and adrenodoxin<sup>36</sup> are virtually identical, the electrode potentials differ widely ( $-432$  and  $+164$  mV respectively).

The bacterial ferredoxins<sup>2,5-7,15,44</sup> have been found to substitute for spinach ferredoxin in cell extracts and have a similar function, namely electron transport. It should be emphasized that this interchangeability is probably due to their similar redox potentials and not to similarity in structure<sup>45</sup>. They do in fact differ greatly in their spectroscopic properties. Not only do the wavelengths of the optically active absorption bands differ, but the whole character of the Cotton effects changes. Bacterial ferredoxin has a relatively simple circular dichroism spectrum<sup>10,11</sup> with only about one-tenth of the intensity of plant ferredoxin although it contains about six atoms of iron. These results can be compared with those of spinach ferredoxin and xanthine oxidase in Tables 1 and 2. Furthermore, treatment with 8 molar urea causes only a slight change in the optical activity<sup>19</sup> of bacterial ferredoxin, showing that protein conformational changes do not affect the chromophore. The small intensity of the circular dichroism also indicates that the environment of the chromophore here is in a site of low helicity; it is

significant that the circular dichroism of spinach ferredoxin in 8 molar urea is of the same order of magnitude as that of untreated bacterial ferredoxin. The reactivities of the ferredoxins (plant or bacterial) with the chelating agents 2,2'-dipyridyl and 1:10-*ortho*-phenanthroline are also different. In bacterial ferredoxin loss of iron from the protein is more rapid from the oxidized species than from the reduced species<sup>46</sup>, the reverse being true with spinach ferredoxin (Garbett, K., and Stangroom, J. E., unpublished). The iron chromophores in bacterial ferredoxin are evidently of a totally different nature from those reported in this work.

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# Absence of an Oxygen Effect and the Genetically Determined Repair Processes

by

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Certain mutants are more sensitive to  $\gamma$ -irradiation in the absence of oxygen than in its presence. This unusual effect is controlled by one gene.

It is generally observed that cells exposed to sparsely ionizing radiations in the presence of oxygen suffer more damage than if exposed in its absence. The extent of the enhancement varies according to the organism and strain tested, environmental conditions and the criterion of damage<sup>1</sup>. Two systems which do not behave in this way are bacteriophage and transforming principle, which exhibit similar radiosensitivity in the presence and absence of oxygen<sup>2,3</sup> when irradiated extracellularly. Another aspect of the oxygen effect was recently reported by Alper<sup>4</sup>, who observed that not only were ultra-violet sensitive bacterial mutants frequently more sensitive to ionizing radiations, but also that they had a smaller oxygen enhancement ratio (OER) than their parental strains. We have recently isolated several mutant strains of the unicellular green alga, *Chlamydomonas reinhardtii*, which are highly sensitive to ultra-violet light and the enhanced sensitivity of which is probably caused by a defective capacity for repairing damage. Some of these mutants have an unchanged response to ionizing radiation, others are more sensitive, whereas still others are more sensitive than the parental form only when irradiated in the absence of oxygen. In the present communication the response to  $\gamma$ -radiation of one of this last class of mutants is described.

Synchronous populations of haploid cells of the parental wild type (WT) and of one of the ultra-violet sensitive (UVS 1) forms (both of mating type +) were grown in liquid cultures using a standard medium<sup>5</sup>. To ensure absolute uniformity of developmental stage in all experiments, the cells were denitrified to convert them into gametes<sup>6</sup>, although all available evidence indicates that the vegetative and gametic stages have similar sensitivities. Diploid stages were used for some of the experiments and these were produced by mating gametes of + and - mating type, using techniques described elsewhere<sup>7</sup>. In all instances assays of viability after irradiation were made by plating cells at suitable dilutions on a yeast acetate supplemented medium in 1.5 per cent agar, and the criterion of survival was the production of a colony of 100 cells from a single cell. Cultures were maintained in an incubator at 25°C and 500 ft.-candles light intensity. A 4,000 c. cobalt-60 'Hotspot', which gave a dose rate of 14,900 rads/min, was the source of  $\gamma$ -radiation. Samples were exposed in liquid minimal medium and vigorously bubbled with oxygen or nitrogen (<10 p.p.m. oxygen) during irradiation and for 15 min before. Hersch cell measurements showed that the level of oxygen in the samples bubbled with nitrogen for this period of time was less than 130 p.p.m. It is necessary to irradiate a photosynthesizing system such as *Chlamydomonas* in the dark to ensure that there is no intracellular generation of oxygen.

The response of haploid WT and UVS 1 cells irradiated in oxygen or nitrogen is shown in Fig. 1. The WT cells have an OER of 2.8. UVS 1 cells irradiated at the same time and under identical conditions have a similar response to that of WT when irradiated in the presence of oxygen, but are more sensitive than WT when irradiated under

anoxic conditions. In nitrogen there is an apparently exponential decrease in survival of UVS 1 cells with increasing dose, at least for survival down to  $10^{-3}$ , and superficially oxygen appears not to be dose modifying—a feature noted also by Alper<sup>4</sup> in one of the bacterial mutants sensitive to ultra-violet light. Closer examination, however, indicates that the dose response curve in nitrogen could be a composite one, one part—representing the majority of the population (about 90 per cent)—which is more sensitive in nitrogen than in oxygen, and the remaining 10 per cent which has a normal OER. The response of this latter 10 per cent in nitrogen, if normalized to a 100 per cent survival value, is identical with that of the WT. Proof of the heterogeneous nature of this UVS 1 haploid population has been obtained by the isolation of two distinct types of cell population. One is identical to UVS 1 in having the composite response in nitrogen as shown in Fig. 1. The other, termed sub-population UVS 1/B, has a response to  $\gamma$ -radiation in nitrogen similar to that of the WT (as in Fig. 1), that is, it has a normal OER. There must, of course, then be another sub-population UVS 1/A, representing 90 per cent of the UVS 1 population, which is more sensitive in nitrogen than in oxygen, but we have been unable to isolate this UVS 1/A form uncontaminated from the B sub-population. The nature and origin of these sub-populations will be discussed later, and the reason for this inability to isolate pure UVS 1/A will then become apparent.

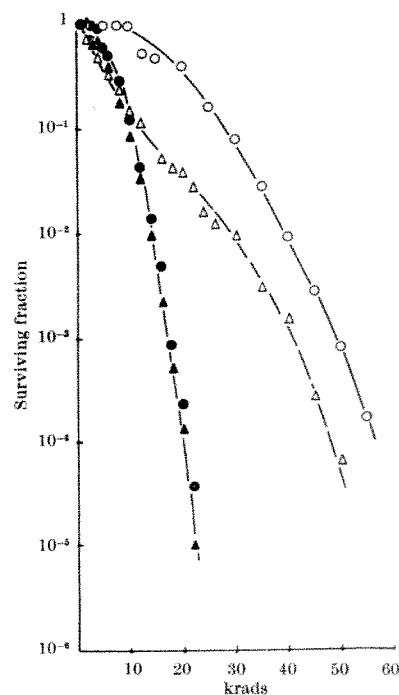


Fig. 1. The response of haploid cells of WT (○) and UVS 1 (△) to  $\gamma$ -radiation. Closed symbols, irradiation in oxygen; open symbols, in nitrogen.

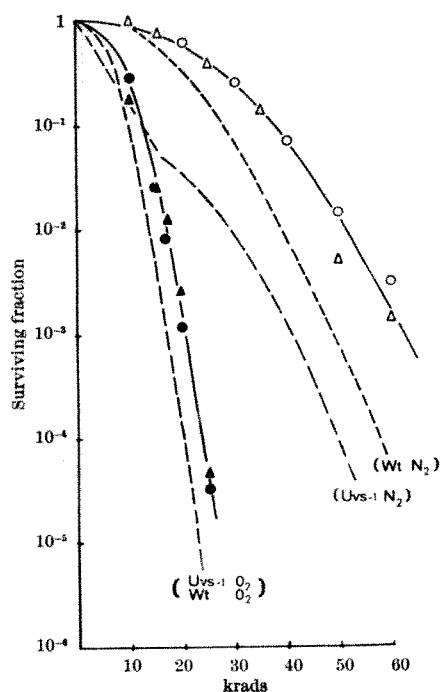


Fig. 2. The response of haploid cells of WT ( $\circ$ ) and UVS 1 ( $\Delta$ ) to  $\gamma$ -radiation, in the presence of  $10^{-2}$  molar cysteamine. Closed symbols, irradiation in oxygen; open symbols, in nitrogen. Broken lines indicate the responses obtained in the absence of cysteamine (see Fig. 1).

Though the *B* sub-population has a normal OER like the WT, it is not WT in its response to UV, but remains as sensitive as UVS 1. Furthermore, *B* cells are distinguishable from WT by their less vigorous growth after irradiation with high doses in nitrogen; in other words, the *B* change may not involve a full restoration of the wild type phenotype for resistance to  $\gamma$ -radiation in nitrogen.

An apparent reversal of the oxygen effect has been shown to be caused by the production of toxic cuprous ions under anoxic conditions<sup>8</sup>. Because the minimal medium used in the present experiments had trace quantities of copper salts, a test was made of their role in determining the response of UVS 1 to  $\gamma$ -radiation. Haploid cells were thoroughly washed, and then irradiated in buffer free of copper. The results obtained were similar to those shown in Fig. 1, indicating that copper toxicity was not responsible for the enhanced sensitivity of UVS 1 when irradiated in nitrogen<sup>8</sup>.

To further characterize the difference in the response of UVS 1 and WT cells, haploid strains of both genotypes were exposed to  $\gamma$ -radiation under oxic and anoxic conditions in the presence of  $10^{-2}$  molar cysteamine. Freshly prepared solutions of cysteamine were used and the pre-treatment with nitrogen was as previously described. In the case of the oxic irradiations, solutions were bubbled with oxygen for only 3 min before irradiation to reduce oxidation of the cysteamine. As the data in Fig. 2 show, this protective compound was effective for both genotypes under both gas conditions, but particularly so for UVS 1 under anoxic conditions. This contrasts with the greater protection usually observed in cellular systems following irradiation in oxygen, but agrees with the results obtained for bacteriophages<sup>9</sup>. A particularly significant feature of these results was that cysteamine completely eliminated the characteristic heterogeneity of the haploid UVS 1 population following irradiation in nitrogen and raised the level of survival of both sub-populations to that observed in the WT. Cysteamine thus corrected the genetic defect in UVS 1 and allowed a better survival after irradiation in nitrogen than in oxygen.

When homozygous diploid spores of UVS 1 and of WT were irradiated at the "2 h stage"<sup>10</sup> in either oxygen or

\* I am grateful to Miss T. Alper for suggesting this experiment.

nitrogen the survival curves shown in Fig. 3 were obtained. In contrast to the haploid cells, diploid UVS 1 cells were more sensitive than WT in the presence of oxygen. Whereas the WT cells had an OER of 2.4, anoxia conferred no protection on the UVS 1 cells—in fact the latter were consistently more sensitive in nitrogen than in oxygen. Furthermore, in contrast to the haploids, the whole of the UVS 1 population, when in the diploid state, was homogeneous in this respect—that is, *A* and *B* could not be distinguished. To confirm the observation that the two sub-populations, *A* and *B*, of UVS 1 did not differ in their response when in the diploid state, diploid homo-

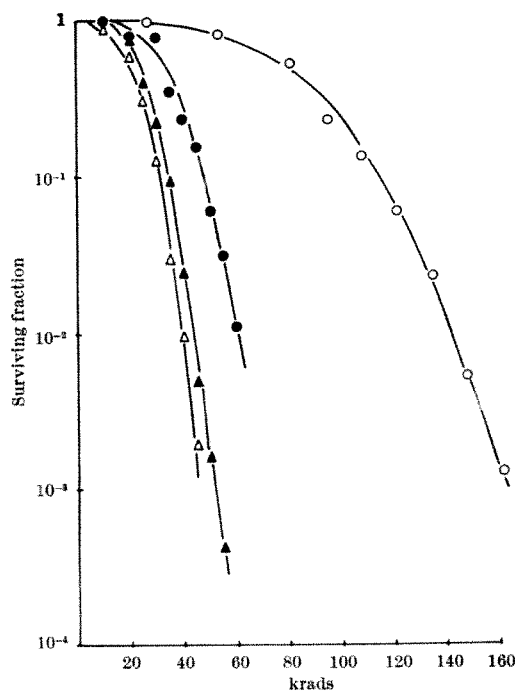


Fig. 3. The response of diploid cells of WT ( $\circ$ ) and UVS 1 ( $\Delta$ ) to  $\gamma$ -radiation. Closed symbols, irradiation in oxygen; open symbols, in nitrogen.

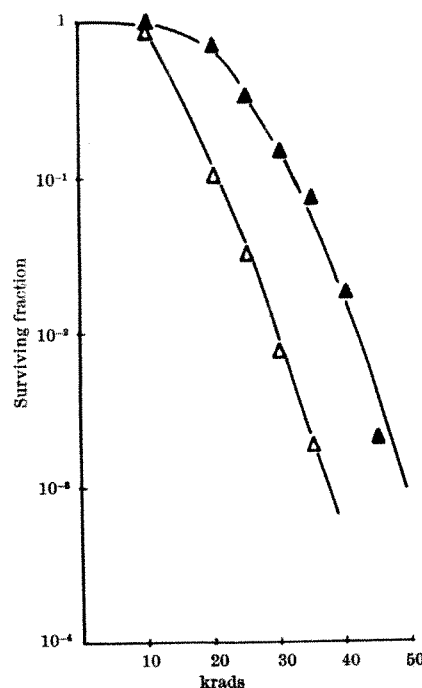


Fig. 4. The response of diploid cells of UVS 1/*B* to  $\gamma$ -irradiation in the presence of oxygen ( $\blacktriangle$ ) and of nitrogen ( $\triangle$ ).

zygous UVS 1/B cells were irradiated. When these cells were irradiated in oxygen or nitrogen those in nitrogen were more sensitive to radiation (Fig. 4). Thus whereas haploid UVS 1/B cells show a normal OER, diploid UVS 1/B cells like haploid and diploid UVS 1 cells are more sensitive in nitrogen than in oxygen. There is, of course, no *a priori* reason why haploid and diploid cells should behave in a similar manner. The initial lesions induced by radiation in the two forms may well be identical but subsequent steps in the development of the lesion and the critical lethal event may be different.

### Mutational Changes

The two changes we are concerned with are the nature of the mutational change involved in the induction of the UVS 1 strain from the WT, and that involved in the origin, during vegetative reproduction, of the UVS 1/B population.

The isolation of the UVS 1 mutant after nitrosoguanidine treatment will be described elsewhere. The genetic nature of the change involved was analysed by crossing it with the WT parental strain. Diploid spores were WT in their response to  $\gamma$ -radiation, and the characteristic UVS 1 sensitivity to  $\gamma$ -radiation in nitrogen thus behaves as a recessive character. Tetrad analysis (twenty-three tetrads) of the spores gave a 1:1 segregation among the haploid progeny for the UVS 1 versus WT response. Furthermore, all the former were sensitive to ultra-violet light and the latter resistant. Tests of another fifty single cell isolates from this cross, which showed the UVS 1 response to  $\gamma$ -radiation, showed that all were also sensitive to ultra-violet light. Thus the absence of an oxygen effect in UVS 1 cells is due to a nuclear factor which is controlled by a single gene, and the response to ultra-violet light is controlled by the same or by a closely linked gene.

The problem of the origin of the UVS 1/B population is a much more difficult one. If single cells of UVS 1 are isolated, and each allowed to form a new population, approximately 90 per cent of these populations are heterogeneous, and two sub-populations UVS 1 and UVS 1/B can be isolated from each one (see Fig. 5). Thus during vegetative reproduction UVS 1/A cells are continually giving rise to UVS 1/B cells. To determine whether the reverse change occurs from B to A one hundred single cells were isolated from a B population and allowed to multiply; the hundred populations were then tested for their response to  $\gamma$ -radiation in nitrogen. All had a normal OER, that is, no type A had been produced. B populations have been maintained by vegetative propagation for several months and the change from A to B is therefore heritable and stable.

A more detailed analysis of the UVS 1/A to UVS 1/B change was precluded by the fact that when UVS 1/B strains were crossed with any strain other than UVS 1/B, a high proportion of the diploid zygospores gave rise to aberrant haploid progeny. Meiosis appeared to be completed normally, but later growth and division of the haploid products was extremely slow and aberrant. In

the limited number of normal tetrads (thirty) obtained when UVS 1/B (as + or - parent) was crossed with UVS 1, all progeny had the UVS 1/B response to  $\gamma$ -radiation. This suggests an extrachromosomal control—possibly UVS 1/B has a cytoplasmic suppressor<sup>13</sup> for part of the UVS 1 gene, though some form of chromosomally based instability cannot be ruled out.

The mutant form UVS 1, selected on the basis of its high sensitivity to ultra-violet light, represents the first known example of an actively metabolizing cellular system which is more sensitive to a sparsely ionizing radiation when irradiated in the absence than in the presence of oxygen. Whatever interpretation is put on this observation it must be reconciled with the fact that the parental strain from which UVS 1 was derived, when irradiated at the same time and under identical conditions, showed a normal oxygen effect. It seems unlikely that there is an inherent difference in sensitivity between UVS 1 and WT; if this were the case differences in the response to oxygen would also be expected, and this did not occur in the haploids. Alper<sup>4</sup> has pointed out that in some bacteria the repair system which deals with ultra-violet photo-products also deals with a certain part of the damage induced by ionizing radiation. She observed that forms of bacteria sensitive to ultra-violet light had a lower OER when exposed to sparsely ionizing radiation than did the more ultra-violet resistant parental forms, and has interpreted these results on the basis of the induction and differential repair of two types of damage—N and O. The former has a low, and the latter a high, OER. Because the increased sensitivity of bacterial mutants is caused by an inability to repair N type damage, the end result of the greater importance of this class of damage is the lower OER. This interpretation of the situation, however, cannot apply to the *Chlamydomonas* data, for if there were two similar classes of lesions induced in this species, then the contribution of N type to the total damage in UVS 1 would have to be large to be capable of swamping the O type. The presence of these extra N type lesions in UVS 1 would necessarily give rise to an enhanced sensitivity of cells irradiated in oxygen, unless an increase in N (caused by a lack of repair) was accompanied by a concomitant decrease in O, which is unlikely. Although such an enhanced sensitivity in oxygen did occur in the diploid, it did not occur in the haploid.

A possible interpretation of our data is as follows: after the initial absorption of energy, different reactions occur with the radicals in the target molecule, or possibly in its environment, in the presence and in the absence of oxygen. Although many of the lesions induced as a result of the reactions in these two gas conditions will be identical in kind, there will be quantitative as well as qualitative differences. In UVS 1 a system concerned with the recognition and repair of some of the particular lesions induced under anoxic conditions is defective, and the survival level, as a consequence, remains low. This concept can be accommodated within a framework such as that developed by Alexander<sup>11</sup> and Howard-Flanders<sup>9</sup>. This proposes that a radical induced in the target molecule

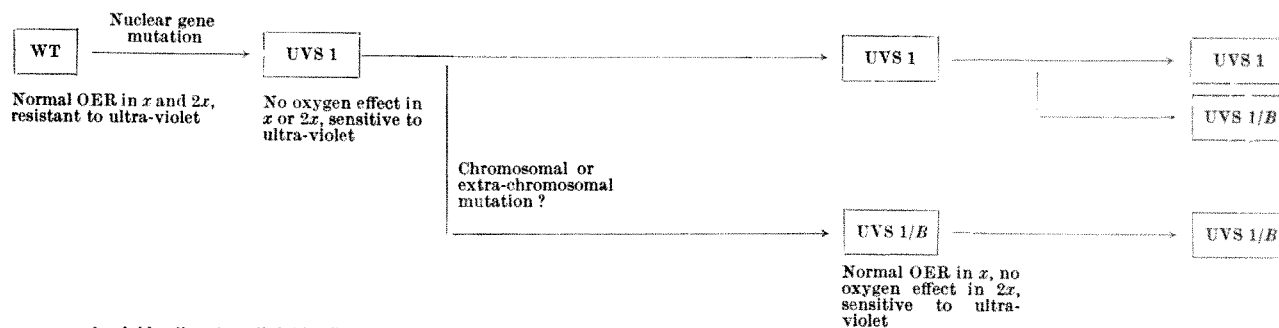


Fig. 5.



(presumably DNA) can undergo one of three reactions—interaction with oxygen, interaction with another radical, or hydrogen donation to restore the original configuration; in the presence of oxygen the first predominates. In the present interpretation it is assumed that the lesions resulting from the peroxy-radicals formed in oxygen as well as some of those resulting from the formation of other radicals in nitrogen may be repairable by one system. In the absence of oxygen, other reactions, possibly radical-radical interaction leading to cross-linking, may occur, and these lesions are repairable by a second system. This repair system, which also deals with certain ultra-violet photoproducts, could be defective in UVS 1. The third reaction—hydrogen donation—may be important in interpreting the results obtained with cysteamine. Several theories have been proposed to account for the protective activity of this compound, but any interpretation of its action in the present system has to be reconciled with the observation of a greater protection in UVS 1 than in WT and in anoxic rather than oxic conditions. If the "radical scavenger" hypothesis is accepted it is difficult to understand why UVS 1 and WT should differ, whereas the "induced anoxia" theory is clearly invalid; the "mixed disulphide" hypothesis could in theory be reconciled with a protection of a radiosensitive repair enzyme in UVS 1. The last model of cysteamine action involves its activity as a hydrogen donor, and there is good evidence to support this model for some systems in which the target molecule is clearly DNA<sup>12</sup>. If this is the mechanism involved in the *Chlamydomonas* experiments then the absence in UVS 1 of that particular repair system which copes with some part of the anoxic damage can be masked by the restoration of damage which follows hydrogen donation from cysteamine in the absence of oxygen.

The nature of the relationship demonstrated genetically in the present experiments, between the repair of certain

ultra-violet photoproducts and of lesions induced by ionizing radiations in anoxic conditions, has yet to be elucidated at the biochemical level.

If the interpretation of differential repair systems acting on some part of the damage induced under oxic and under anoxic conditions is shown to be correct, then this concept will be significant in a re-evaluation of certain radiobiological observations and also possibly ultimately in radiotherapy. It is a general problem in radiotherapy that tumour foci can be relatively anoxic and thus more resistant to sparsely ionizing radiations than the better oxygenated surrounding tissues. Compounds which inhibit the repair of ultra-violet photoproducts are well known; if similar compounds can be found which block the systems responsible for the repair of anoxic damage, that is, which simulate the genetic block in UVS 1, then this could be a means of enhancing damage in the tumour cells while leaving the response of surrounding tissues unchanged.

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## Plasmodial Fusion and Lethal Interaction between Strains in a Myxomycete

by  
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After plasmodial fusion between certain strains of *Physarum polycephalum* there is a lethal reaction which may end in the total destruction of one or both plasmodia.

THIS is a preliminary account of a study of fusion between plasmodia of the Myxomycete *Physarum polycephalum* Schweinitz and of a lethal reaction which follows some fusions. The plasmodium of *P. polycephalum* is a motile mass of protoplasm of variable form without a rigid wall and not sub-divided into cells (that is, coenocytic). Vigorous protoplasmic streaming takes place continuously through a network of channels ("veins") at rates as great as 1 mm/sec with reversals of flow approximately once/min<sup>1,2</sup>. The nuclei divide synchronously even in a large plasmodium which may cover an area of many square centimetres<sup>3</sup>.

The plasmodial phase, which alternates in the life cycle with a haploid amoeboid phase, is initiated by a sexual process, the fusion of two amoebae (myxamoebae). One of us (J. D.) has shown that the two amoebae must differ in mating type, and that the mating types, of which four have been identified, are determined by a series of alleles at a single locus<sup>4</sup>. In appropriate conditions the plasmodium produces uninucleate spores, from which haploid uninucleate amoebae emerge. Nuclear fusion certainly takes

place before sporulation, because spore formation is accompanied by meiosis<sup>5</sup> and recombination of genetic markers<sup>6</sup>.

The strains of *P. polycephalum* used in our investigations were all derived from a plasmodium supplied to one of us (J. D.) by Professor H. P. Rusch of Wisconsin University in 1957. Clones of amoebae were maintained in two-membered culture with *Escherichia coli*. Plasmodia were obtained by mixing clones of amoebae of differing mating type. Details of the method by which clones were obtained, cultured and mated have been published previously<sup>4,6</sup>. Plasmodia were freed from *E. coli* by migration on acidified plain agar, pH 4.6, sometimes with the addition of streptomycin. They were then maintained in pure culture on a semi-defined agar medium containing glucose, peptone, mineral salts, vitamins and haematin, closely resembling that advocated by Daniel and Baldwin<sup>7</sup>. The interaction between two plasmodial strains was examined by inoculating the strains on opposite sides of a Petri dish containing semi-defined agar medium and observing the response when, after 2–3 days of growth, the plasmodia met at the centre of the dish.



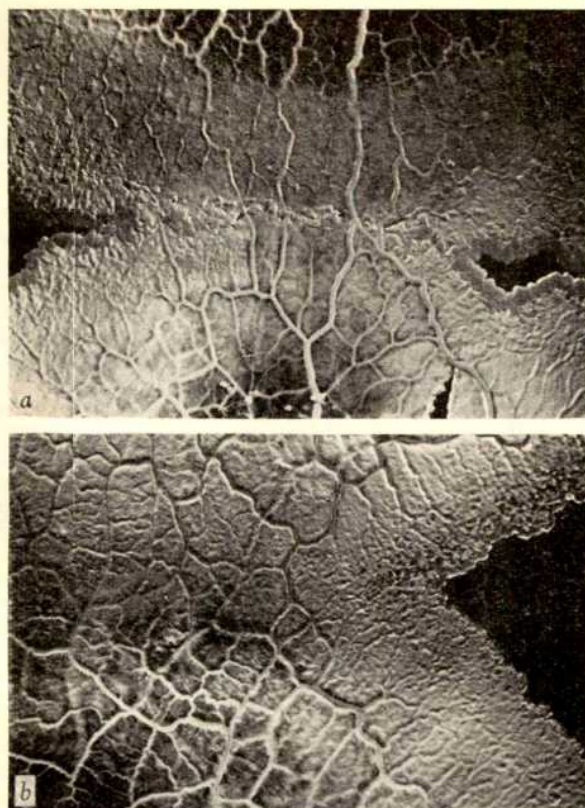


Fig. 1. Fusion between plasmodia of the same strain showing appearance (a) about one hour and (b) several hours after contact. ( $\times 2.8$ .)

### Plasmodial Fusion

When the advancing margins of two plasmodia of the same strain meet they rapidly fuse. Within a few minutes of contact movement of protoplasm between the plasmodia can be detected, and within about 1 h prominent "veins", visible to the naked eye, link the two plasmodia (Fig. 1a). By this time the plasmodia usually will have merged completely over an extensive front, and within a few hours it is no longer evident that the resulting plasmodium originated from two separate plasmodia (Fig. 1b). When plasmodia of different strains are tested for fusion with each other in various combinations of two at a time, some pairs of plasmodia consistently fuse with each other and other pairs consistently fail to fuse (Fig. 2a). These latter plasmodia may remain in contact for days without fusing, although after a few hours one plasmodium may grow over the other making observation difficult (Fig. 2b). Thus whether plasmodial fusion occurs depends in the first instance on which strains are paired rather than on their physiological state, a conclusion reached by Gray on the basis of less extensive data<sup>8</sup>.

In order to investigate the factors involved in determining plasmodial fusion, we have carried out a series of experiments in which groups of plasmodia derived from closely related clones of amoebae have been tested for fusion with each other in all combinations of two at a time. Because of the known relationships among the plasmodia, we were able to make definite predictions of the results expected on the basis of various inherited compatibility systems. For one related set of plasmodia, all descended from the two clones of amoebae previously designated<sup>6</sup> *a* and *i*, we found that a scheme based on one pair of gene alleles ( $f^1$  and  $f^2$ ) was sufficient to account for all the results. On this scheme, fusion occurs only between plasmodia with identical genotype at the *f* locus. The plasmodia are diploid (or dikaryotic) and so each carries two alleles at each gene locus (Table 1). In similar tests with other groups of plasmodia derived from more dis-

Table 1. INTERACTION OF PAIRED PLASMODIA OF VARIOUS FUSION LOCUS GENOTYPES

	Genotype		
	$f^1f^1$	$f^1f^2$	$f^2f^2$
Genotype $f^1f^1$	+	-	-
$f^1f^2$	+	+	-
$f^2f^2$			+

+, Denotes fusion; -, denotes failure to fuse.

tantly related clones of amoebae, the results are more complex and suggest the presence of other loci influencing plasmodial fusion. We are still analysing these, and results will be published in detail elsewhere. We have so far found no evidence that the mating type locus (*mt*) influences plasmodial fusion. In this respect, our results differ from those of Collins<sup>9,10</sup>, who has studied plasmodial fusion in the Myxomycete *Didymium iridis* and has tentatively concluded that it is controlled in this species by four loci, including the mating type locus.

### The Lethal Interaction

As indicated here, pairing plasmodia which are of different strains but of identical genotype with respect to the fusion locus (*f*) results in apparently normal fusion. Within about 1 h prominent "veins" connect the two plasmodia. With some combinations of strains the final result of fusion is a single plasmodium of which the origin from separate plasmodia is no longer evident, as with the fusion of plasmodia of the same strain. With other combinations of strains, however, fusion may be followed by a destructive reaction which varies considerably in severity. The first indication of the reaction, occurring after about an hour, is a cessation of streaming in "veins" at and near the region where fusion occurred. Gelling of protoplasm probably takes place, as Brownian movement of particles in the protoplasm ceases, and changes in colour and appearance make the affected area obvious

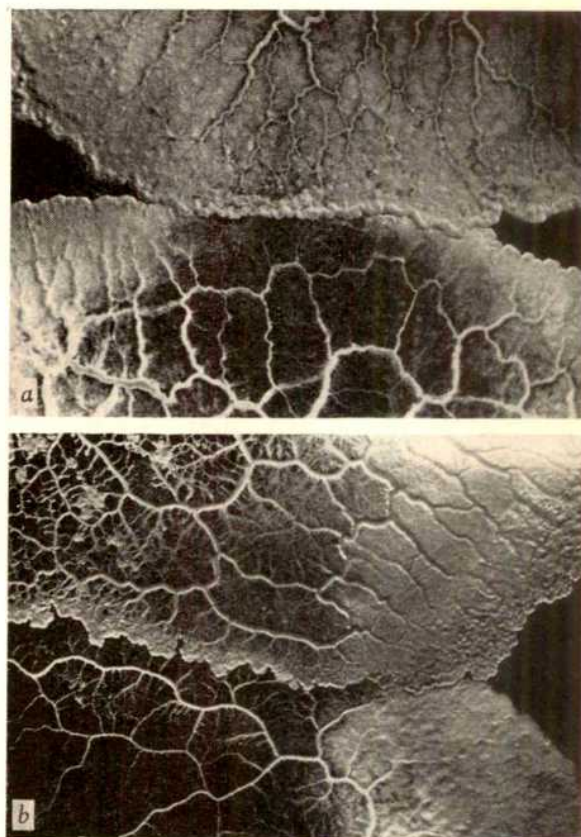


Fig. 2. Failure of fusion between plasmodia of different strains showing appearance (a) about one hour and (b) several hours after contact. ( $\times 3.5$ .)



to the naked eye. The area affected may remain restricted, or may spread to occupy much of one or both interacting plasmodia. In extreme instances total destruction of one or both of the plasmodia occurs, no further growth takes place, and attempts to sub-culture demonstrate the absence of viable material. When, as is usual, some of the plasmodium survives, the affected area becomes sharply delimited and is subsequently invaded and digested by plasmodia from the surviving regions. Lethal interaction between strains has not been seen in the absence of plasmodial fusion. Although the phenomenon clearly has a genetic basis, in that some combinations of strains have never been observed to result in a lethal interaction, whereas some consistently do, genetic analysis of the phenomenon has not yet been attempted. It is also unknown whether the surviving regions are genetically identical with either of the interacting plasmodia.

We are unaware of any previous report of a lethal reaction after plasmodial fusion in Myxomycetes. A lethal interaction after vegetative fusion has, however, been reported in the filamentous fungus, *Neurospora crassa*, where heterocaryons are successfully established only between strains identical at certain loci. When hyphae from strains not identical at the appropriate loci meet, normal fusion occurs, but death of the cells immediately adjacent to the fusion follows<sup>11</sup>. The sequence of events described and figured is similar to what we have observed in *Physarum*. The reaction in the Myxomycete is, however, far more spectacular, because many square centimetres instead of two adjacent cells are involved. The greater extent of the reaction in the Myxomycete is probably the result of efficient mixing of protoplasm from the interacting plasmodia resulting from the very rapid protoplasmic streaming. In *Neurospora*, studies involving micro-injection strongly suggested that the material that brought about death through transfer between the genetically unlike cells was a soluble cytoplasmic protein<sup>12</sup>.

In *P. polycephalum*, the process of cell fusion is apparently controlled by one gene locus (*mt*) when it occurs in amoebae and by another gene locus (*f*) when it occurs in plasmodia, the *mt* locus being without effect on plasmodial fusion and the *f* locus without effect on the fusion of amoebae. Thus each of these two genes seems to have expression in only one of the two phases of the life cycle. It is of interest that another gene (*act*), which confers actidione resistance on the amoebae, also appears to influence only one phase of the life cycle and to lack similar expression in plasmodia<sup>6</sup>. As well as acting at

different stages in the life cycle, the *mt* and *f* genes differ in their mode of action; amoeboid (sexual) fusion occurs only between amoebae differing in *mt* genotype, whereas plasmodial (vegetative) fusion occurs only between plasmodia identical at their *f* loci. The discovery of lethal interactions following plasmodial fusion indicates that for plasmodial fusion to proceed to completion, even closer genetical identity is required.

The mating system controlling sexual fusion in *P. polycephalum* is similar to systems promoting outbreeding and leading to genetic recombination in a wide variety of other organisms. There are few studies in other organisms on the genetic control of vegetative fusions, that is fusions regarded as not directly related to sexual reproduction. In the Fungi<sup>13</sup> vegetative fusion resulting in the establishment of a heterocaryon seems often to be limited to material that is genetically closely similar, either through a genetic system which prevents fusion or through a lethal interaction following protoplasmic mixing. In mammalian cells in tissue culture, however, fusion between cells of widely different genotype (for example, those of mouse and man<sup>14</sup>) can occur and result in viable heterocaryons, a situation wholly different from that found in the Fungi and in *P. polycephalum*. Our present results suggest that the latter organism constitutes particularly favourable materials for the study of such genetically restricted vegetative fusions.

We thank Professor E. B. Chain for helpful discussion and Mr G. D. Gilder for technical assistance. This work is being continued as part of a broad programme of research on myxomycetes now being undertaken at the Department of Biochemistry, Imperial College, and at the Department of Genetics, University of Leicester.

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## Relationship between Thymus and Hypophysis

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Young adult mice injected with anti-hypophysis serum develop wasting disease such as after neonatal thymectomy and atrophy of the thymus. This and the finding of changes in the hypophysis after neonatal thymectomy suggest a relationship between the function of the hypophysis and that of the thymus.

THE thymus plays a dominant part in the development of immunological function<sup>1-3</sup>. Impaired antibody synthesis, failure to develop cell mediated immunity and wasting disease result from thymectomy shortly after birth<sup>4,5</sup>. The wasting syndrome, which is characterized by a rapid weight loss and death of the animals, begins to manifest itself only several weeks after neonatal thymectomy and at a stage of development when the animals

seem to be normally healthy. Contradictory views exist as to the mechanism responsible for the wasting disease<sup>6-12</sup>. It has also been suggested that the thymus plays a part in body growth and development (see, for example, ref. 13).

There is an important relationship between the thymus and several endocrine glands<sup>14-18</sup>. The anterior pituitary gland deserves special mention because growth hormone causes a marked enlargement of the thymus<sup>18</sup>. In an

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attempt to analyse the relationship between the thymus and the pituitary two main types of experiments were performed. In the first experiments, young adult mice were injected with rabbit anti-mouse hypophysis serum. In the second experiments, newborn mice were thymectomized and their hypophysis examined histologically at various times after thymectomy. It was noted that anti-hypophysis serum produced loss of weight, wasting disease and thymus involution or even complete disappearance of the thymus. Neonatal thymectomy, on the other hand, resulted in definite morphological changes in the hypophysis.

**Mice treated with anti-hypophysis serum.** Inbred C57/BL and outbred NMRI male mice, 25–35 days old, ranging in weight from 14 to 22 g, were used for the experiments with anti-hypophysis serum. Rabbit antisera were prepared by giving two subcutaneous injections of mouse hypophyseal cells incorporated into Freund's complete adjuvant and subsequent intravenous and intramuscular injections of mouse hypophyseal cells. Sera from bleedings on day 35, 42 and 49 after the onset of the injection schedule were prepared, inactivated at 56° C for 30 min and stored at –20° C. A single intraperitoneal injection of 0.15 ml. immune serum/5 g body weight was given. Control groups included mice injected with normal rabbit serum, and anti-thymus serum prepared in a manner similar to the anti-hypophysis serum.

Table 1 summarizes the results. Fifteen out of sixty-seven NMRI mice injected with anti-hypophysis serum died within 8–30 days after a single injection. Post-mortem examination showed that the thymus of these animals was completely atrophic. Before death they exhibited the typical clinical symptoms of wasting disease, such as pro-

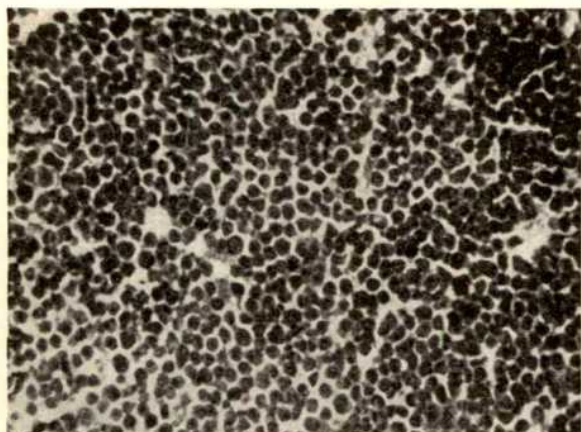


Fig. 1. Thymus cortex of a normal NMRI male mouse, 30 days old, stained with haematoxylin and eosin. ( $\times 360$ .)

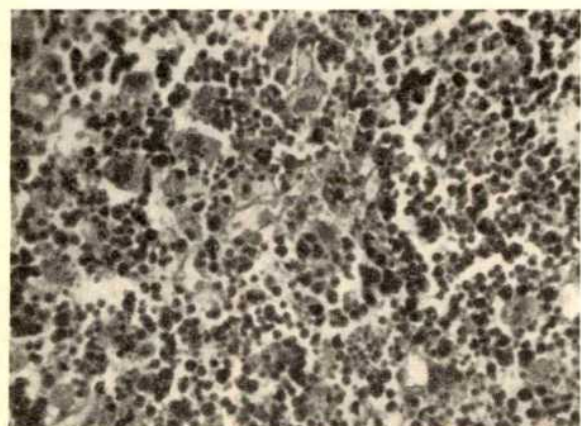


Fig. 2. Thymus cortex of a wasting NMRI male mouse, killed 10 days after a single intraperitoneal injection of rabbit anti-mouse hypophysis serum. Massive lysis and pycnosis of the cortex thymocytes. Stained with haematoxylin and eosin. ( $\times 360$ .)

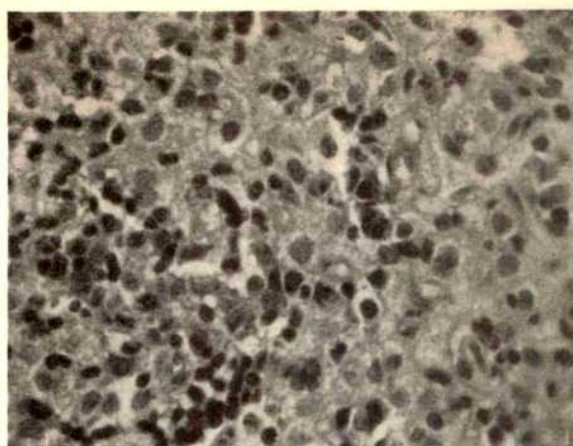


Fig. 3. Thymus cortex of a wasted NMRI male mouse which died 12 days after a single intraperitoneal injection of rabbit anti-mouse hypophysis serum. The cell picture in the cortex is exclusively dominated by epithelial and reticular cells. Stained with haematoxylin and eosin. ( $\times 360$ .)

gressive loss of weight (for example, from 19–20 g to 13–14 g), ruffled hair, hunched posture, diarrhoea, oedema and inactivity. Animals injected with normal rabbit serum or with rabbit anti-mouse thymus serum showed a normal growth pattern.

Histological examination revealed a progressive loss of thymic cells after treatment with anti-hypophysis serum (Figs. 1–3). Fig. 2 shows one stage of the progressive disappearance of thymocytes, and Fig. 3 the final stage of a wasting animal in which thymocytes can no longer be observed in the thymus.

Fig. 4 shows the average growth curves of various groups of NMRI mice. The following experimental groups were used: (1) untreated controls; (2) mice wasting after treatment with anti-hypophysis serum; (3) mice treated with anti-thymus serum; (4) mice wasting after neonatal thymectomy. Untreated control animals and those treated with a single injection of anti-thymus serum behaved similarly except for the passing initial reduction in weight after the injection of the immune serum. Wasting animals injected with anti-hypophysis serum showed a growth pattern similar to the neonatally thymectomized mice. It must be emphasized that the mice were injected with the anti-hypophysis serum at approximately the age when they would develop the wasting syndrome if neonatally thymectomized. The results in Fig. 4 suggest that the age of the mice chosen for effecting the wasting by anti-hypophysis serum may be critical. Experiments are being performed to test this point.

It is interesting to note that in no experiment did all the animals treated with anti-hypophysis serum show the same pattern of wasting and thymus involution and a high percentage survived and grew in a normal way. This was at first attributed to the mice being of outbred NMRI strain. Similar results, however, were obtained with inbred mice. Fig. 5 gives a typical picture of individual growth curves and the time of death in C57/BL mice. Three out of eight animals wasted and died without thymus on day 8 or 9. One wasted but recovered and at the time of killing (day 31) had a normal thymus. One animal was wasting and when it was killed 21 days after the injection of antiserum it exhibited only very small

Table 1. WASTING SYNDROME AND THYMUS ATROPHY IN YOUNG ADULT NMRI MALE MICE AFTER A SINGLE INTRAPERITONEAL INJECTION OF RABBIT ANTI-MOUSE HYPOPHYSIS SERUM

Normal rabbit serum	Anti-hypophysis serum Wasting no thymus	Wasting and recovering	Anti-thymus serum
0/27	15/67	4/67	0/21

Dose of serum injected: 0.15 ml./5 g body weight. Weight of injected mice: 15–20 g.



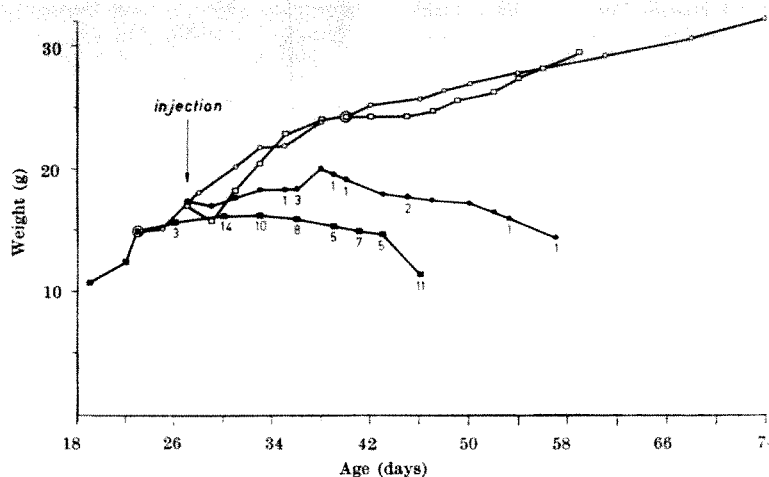


Fig. 4. Comparison of growth of *NMRI* male mice after a single intraperitoneal injection of rabbit anti-mouse hypophysis serum, anti-thymus serum or after neonatal thymectomy. The numbers in the curves indicate the wasted mice dying on the various days after thymectomy or anti-hypophysis serum treatment. ○, Untreated (10); ●, anti-hypophysis serum (10); □, anti-thymus serum (10); ■, neonatally thymectomized (63).

remnants of thymus. Three animals had a normal growth pattern and when they were killed on day 31 they all had a normal thymus.

The observed differences in the growth pattern after treatment with anti-hypophysis serum seemed at first surprising. Although the injected male mice were of similar weight (14–17 g) and age (28–32 days), they did not belong to the same litter and therefore the differences may have resulted from the different stages of physical, and possibly sexual, maturity of the animals used. This suggests a hypophyseal mechanism regulating thymus growth and cell turnover, which is dependent on the age or maturity of the animal.

The animals wasting and dying with complete thymus atrophy after treatment with anti-hypophysis serum showed no macroscopic reduction or microscopic modifications of the lymph nodes and spleen as compared with mice injected with normal rabbit serum. No particular changes within sections of the pituitary of such mice were observed. The possibility that an alteration occurred in the adrenals, thyroid and gonads is at present being investigated.

*Modification of the hypophysis after neonatal thymectomy.* *NMRI* mice of the same litter were thymectomized or

sham-operated within 12 h of birth. The animals were killed at various times thereafter and serial transverse sections were prepared. (The techniques involved and detailed observations will be described elsewhere.) A marked degranulation of acidophilic cells in the anterior lobe of the pituitary was observed. This process seemed to be very pronounced about 20 days after thymectomy when the animals were still growing normally without outward signs of wasting disease (Figs. 6 and 7). These results lend additional support to the existence of a relationship between the thymus and the hypophysis. In agreement with earlier findings by Law *et al.*<sup>18</sup>, we did not find any signs of alteration in the hypophyses of thymectomized wasting mice.

These findings show that a single injection of rabbit anti-hypophysis serum given to young male mice may result in wasting disease, thymus atrophy or disappearance and death. The effect of anti-hypophysis serum seems to have a certain degree of specificity because anti-thymus serum and normal serum have no such effect on the thymus. In several experiments not reported here, repeated injections of anti-hypophysis serum did not result in toxic effects and there was no increase in the incidence of wasting. These findings indicate that a non-

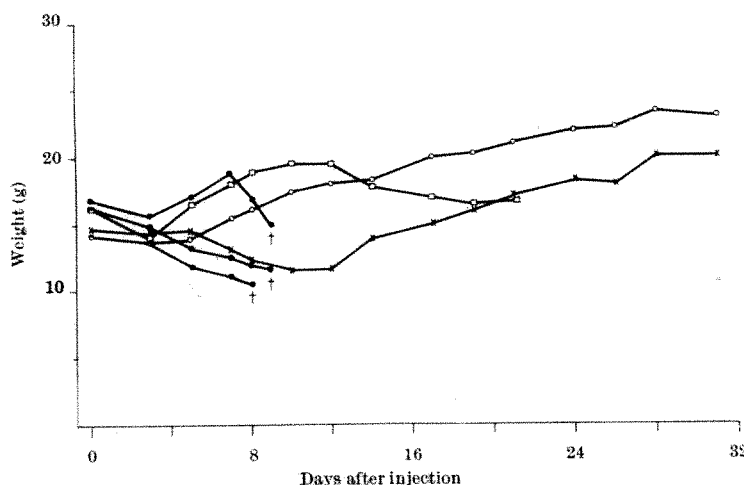


Fig. 5. Growth of *C57/BL* mice after a single intraperitoneal injection of rabbit anti-mouse hypophysis serum. Dose of serum injected: 0.15 ml./5 g body weight. ○, No wasting, normal thymus (3); ●, wasting, no thymus (3); ×, wasting, recovering, normal thymus (1); □, wasting, killed, small thymus (1).

specific toxic effect or stress mechanism is not involved. The observed effects, however, are not seen in all animals of similar weight. Some animals waste but eventually recover and finally possess a normal thymus and most grow quite normally.

A striking observation was the similarity in the behaviour of neonatally thymectomized mice and young adult mice treated with a single injection of anti-hypophysis serum. Both groups of animals showed typical wasting disease which resulted in death. A likely explanation seems that there exists a direct relationship between the thymus and the hypophysis; namely, neonatal thymectomy results in changes in the hypophysis (Figs. 6 and 7) and wasting, while anti-hypophysis serum produces thymus atrophy and also wasting. The fact that after neonatal thymectomy mice start to waste at the time when injection of anti-hypophysis serum into young adults (28-32 days) produces wasting disease and thymus atrophy suggests that a close relationship may exist between hypophyseal and thymic factors which is dependent on age. Thymectomy or inhibition of the hypophyseal factors at a certain stage in the development of the animals results in wasting and death, thus indicating the possible significance of the thymus-hypophysis axis for growth, maturation and possibly differentiation. It is not known what factor(s) and/or cells are inhibited by anti-hypophysis serum. Based on the known growth

promoting effect of somatotrophic hormone on the lymphoid tissue and the thymus<sup>15</sup>, we would predict that this hormone should be capable of preventing the wasting disease in mice treated with anti-hypophysis serum. It seems likely that the thymocytes or one or more thymus factors are under the control of the somatotrophic hormone. Thymotropic hormone does not seem to be the essential factor because thyroxine promotes peripheral lymphoid tissue but not thymus growth<sup>16</sup>. The possibility that other unknown hypophyseal factors are acting directly on the thymocytes or on the epithelial cells of the thymus medulla cannot be excluded<sup>20,21</sup>.

According to Metcalf<sup>20</sup> and Everett *et al.*<sup>22</sup>, 95 per cent or more of the cells produced in the thymus are rapidly destroyed there, and thus it is likely that hypophyseal factors exist at a certain stage in the development which control the turnover of thymocytes through action on thymus target hormones or factors. Blockade of cells producing these factors by anti-hypophysis serum would result in only the destructive pathway being active.

The mode of action of anti-hypophysis serum is unknown. The effect on the hypophysis could be cytolytic, cytotoxic or else it could neutralize one or several hypophyseal hormones or factors. Recent experiments have shown that its activity can be absorbed out by hypophysis but not by thymus or spleen cells. A specific direct effect on the hypophysis is therefore likely. While no gross abnormality in the lymph nodes and spleen was observed, the absolute number of circulating leucocytes decreased sharply in the C57/BL wasting animals.

Thus, summing up, our experiments show that a single injection of anti-hypophysis serum given to young adult mice can result in wasting disease, thymus atrophy and death of the animals. Normal serum and anti-thymus serum have no such effects. Neonatal thymectomy results in morphological changes in the acidophilic cells of the anterior pituitary gland. These experiments complement each other and support the concept that there is a relation between the thymus and the hypophysis. Whether hormones in the hypophysis or some unknown factors are responsible for the reported findings has yet to be determined.

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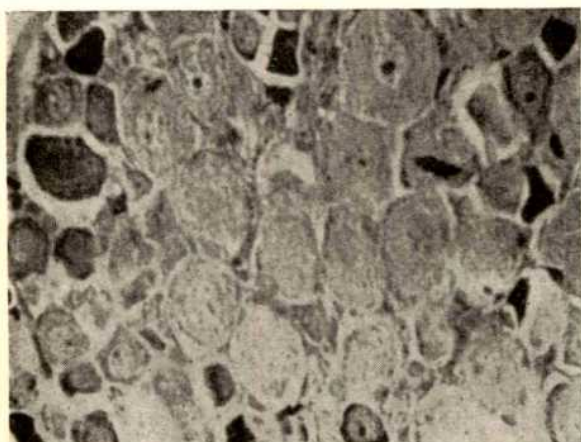


Fig. 6. Section of the anterior pituitary gland of a neonatally thymectomized NMRI mouse, killed 20 days after the operation. Typical nests of sharply degranulated acidophilic, growth-hormone-producing cells. Stained with azan. ( $\times 300$ .)

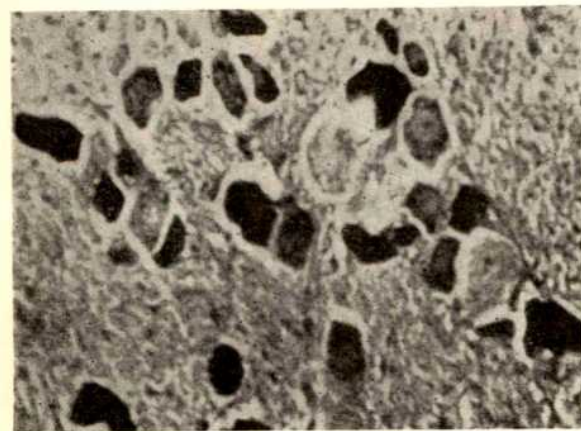


Fig. 7. Section of the anterior pituitary gland of a neonatally sham-operated littermate, killed when 20 days old (compare with Fig. 6). Typical nests of normally granulated acidophilic, growth-hormone-producing cells. Stained with azan. ( $\times 300$ .)



## LETTERS TO THE EDITOR

## ASTRONOMY

## Origin of Planetary Nebulae

STATISTICAL arguments have been advanced to demonstrate that all or a large fraction of stars with masses comparable with that of the Sun become planetary nebulae during the course of their evolution<sup>1,2</sup>. It has further been argued that these stars are the evolutionary products of horizontal branch stars, although the mechanism for the ejection of the expanding shell has remained a mystery.

Details of the structure of post horizontal branch stars, however, are now available from the work of Hayashi, Hoshi and Sugimoto<sup>3,4</sup>. They find that as the carbon-oxygen core grows in the centre of the star the luminosity increases up to  $8 \times 10^4$  L. This is considerably brighter than the luminosity at the time of the helium flash. As the star gets brighter the fraction of the star in the region of low temperatures increases, and if a sufficient fraction of the star is at temperatures below  $5 \times 10^4$  °K, with a consequent ratio of specific heats  $\gamma$  less than  $4/3$  due to hydrogen and helium ionization, then the possibility arises that the star will be dynamically unstable.

The stability of a star is determined by the equations for small perturbations about equilibrium. If the perturbations have a time dependence proportional to  $\exp(i\sigma t)$  then when  $\sigma^2$  becomes negative dynamical instability ensues. The eigenvalue equation that determines  $\sigma^2$  admits a variational principle, which gives<sup>5</sup>

$$\sigma^2 \leq \frac{\int_0^R \gamma p r^4 (d\xi/dr)^2 dr - \int_0^R r^3 \xi^2 [d(3\gamma - 4p)/dr] dr}{\int_0^R \rho r^4 \xi^2 dr}$$

where  $p$  is the pressure,  $r$  the distance from the centre of the star,  $\rho$  the density and  $\xi(r)$  any trial function for the proper eigenfunction that is finite at the centre and surface. If  $\gamma$  is constant and less than  $4/3$  for  $r \geq r_1$ , then a trial function  $\xi = (r - r_1)$   $r \geq r_1$ ,  $\xi = 0$   $r \leq r_1$ , gives  $\sigma^2 = 0$  when

$$\int_{r_1}^R p \left\{ \left( 5 + \frac{\gamma}{3\gamma - 4} \right) r^4 - 8r^3 r_1 + 3r^2 r_1^2 \right\} dr = 0$$

The critical value of  $r_1$  depends both on the value of  $\gamma$  and the variation of pressure with radius from  $r = r_1$  to  $R$ . The equation has been solved for  $p \propto 1/r^2$ ,  $1/r^4$ ,  $1/r^6$  for  $\gamma = 1, 1.1, 8/7, 1.2$ . The results are given in Table 1. If  $r_1/R$  is less than the value shown then the star is dynamically unstable.

Table 1. VALUES FOR  $r_1/R$  FOR DIFFERENT  $\gamma$  AND  $p$

$p/\gamma$	1.0	1.1	8/7	1.2
$\frac{1}{r^2}$	0.52	0.44	0.33	0.20
$\frac{1}{r^4}$	0.47	0.34	0.28	0.13
$\frac{1}{r^6}$	0.38	0.15	—	—

The detailed value of  $r_1/R$  as a function of luminosity and radius of a star could be calculated by solving the equilibrium and stability equations, but because of the uncertainty attached to the theory of convective energy transport, which is vitally important for giant stars, the above values given in Table 1 should be representative. From the models available we tend to prefer a value at the smaller end of this range.

It is instructive to compare these values with the fractional radius at which  $T = 5 \times 10^4$  as computed from

Table 2. DETAILS OF POST HORIZONTAL BRANCH STARS  
 $X = 0.900$   $Y = 0.099$   $Z = 0.001$

$M_2/M_\odot$	0	0	0.13	0.46	0.47	0.50	0.60
$M_1/M_\odot$	0.53	0.53	0.53	0.53	0.53	0.60	0.70
$\log(L/L_\odot)$	1.77	1.93	1.99	2.90	3.19	3.61	3.90
$r_1/R$	1	1	1	0.56	0.39	0.18	0.10

stellar models of the post horizontal branch stage of stellar evolution. Hayashi *et al.*<sup>3,4</sup> have only considered the core of the star whereas  $T = 5 \times 10^4$  occurs in the envelope. Fortunately we may use the relation  $T \propto 1/r$  outside the shell source with reasonable confidence, and from the known temperature and radius at the shell source calculate the radius at  $5 \times 10^4$  °K. Moreover, the total radius of the star is determined by its luminosity and scarcely affected by anything else so that the radius of the star can be calculated. The resulting values of  $r_1/R$  are given in Table 2.  $M_2$  is the mass of the carbon-oxygen core and  $M_1$  the mass interior to the hydrogen envelope.

As the luminosity increases up the tip of the giant branch—the last model is at the third helium flash—the depth of the unstable layer increases. By comparison with the value in Table 1 we see that we would expect the star to be dynamically unstable as it moves up the giant branch. This then is the mechanism proposed for the origin of planetary nebulae.

Because the structure of the giant is insensitive to the mass in the envelope unless this mass is less than about 4 per cent of the mass of the star<sup>6</sup>, we can see that almost all the envelope will be ejected, and we should expect a star of about  $0.7 M_\odot$  as the central star of planetary nebulae. The star will contract very quickly at first, and then cool down to become a white dwarf. The radius when the instability occurs will be about  $300 R_\odot$  giving the escape velocity comparable with the observed expansion velocities of the nebulae. If the total mass of the star was originally 1 solar mass there would be 0.3 solar masses in the envelope.

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## Bouncing Spheres in General Relativity

I REPORT here an analysis recently completed of the radial adiabatic motion of spherically symmetric models of uniform density and isotropic pressure in general relativity. An appropriate form of the metric is

$$ds^2 = 4F(t) \dot{R}^2 R^{-2} dt^2 - R^2 (r^2 dr^2 + d\theta^2 + \sin^2 \theta d\varphi^2)$$

with

$$R(t, r) = r \alpha(t) / [1 - r^2 \beta(t)]$$

$r$  and  $t$  being radial and time-like labels, respectively, and dots denoting differentiation with respect to  $t$ . At this stage  $\alpha(t)$  and  $\beta(t)$  are arbitrary functions of  $t$ , and

$$\frac{8\pi\rho}{3} = \frac{1}{4F(t)} - \frac{4\beta}{\alpha^2} = \frac{2m_s}{r_s^3} \left( \frac{1 - r_s^2 \beta}{\alpha} \right)^3$$

$$\frac{p}{\rho} = -1 + \frac{\dot{R}_s}{R_s} \frac{R}{\dot{R}}$$

suffix  $s$  referring to the surface of the model and  $m_s$  being the mass of the model according to the external Schwarzschild metric. It can readily be shown from this that

$$\frac{1}{2} \left( \frac{dR_s}{ds} \right)^2 = z - 2 \frac{x-1}{x^2}, \quad \left( x = 1 - \beta r_s^2, z = \frac{m_s}{R_s} \right)$$

where  $ds$  is the proper time of a co-moving observer on the surface of the model. Furthermore (with suffix  $c$  denoting the centre)

$$\rho = \frac{3z^3}{4\pi m_s^2}, \quad \frac{\rho}{\rho_c} = \frac{x}{z} \frac{dz}{dx} - 1.$$

Accordingly, imposition of a relation between  $\rho$  and  $p_c$  will give a track for the model in the  $(x, z)$  plane, real motion being confined to the part of the plane above and to the left of the curve (to be called  $Q$ )  $z = 2(x-1)/x^2$ . In particular, intersection of the model track and  $Q$  implies a bounce, that is, a reversal of motion. Attention has been concentrated on finding, for a given  $(p_c, \rho)$  relation, the ranges of initial values of  $z$  ("potential"),  $v = dR_s/ds$  ("velocity") and  $f = d^2R_s/ds^2$  ("acceleration") leading to a bounce after the initial inward motion. The initial acceleration is required if the  $(p_c, \rho)$  relation involves an otherwise unknown constant. The pressure density relations considered were of the form  $p_c \rho^{-\gamma} = \text{a constant}$  for  $\gamma = 1, 4/3, 5/3$ , together with the truncated form of the last two laws if the condition  $p_c \leq \rho/3$  is imposed. The corresponding curves in the  $(x, z)$  plane are power law type, straight lines and hyperbolae respectively.

A sample of the results obtained is the following set of conditions for the occurrence of a bounce.

If  $\gamma = 4/3$ , the minimum initial outward acceleration  $f$  leading to a bounce is found in terms of a quantity  $F = R_s f / z (\frac{1}{2} v^2)^{\frac{1}{2}}$ , where  $F = 2$  for small  $z$  and increases with  $z$ , more rapidly for large than for small  $v$ . For the largest permissible  $z$  (that is, giving finite  $p_c$ )  $F$  varies between 5.20 for  $v = 0$  ( $z = 0.4444$ ) and 10.56 for  $\frac{1}{2} v^2 = z = 0.2963$ . If the further restriction  $p_c \leq \rho/3$  is imposed a more complicated situation results. If initially  $\frac{1}{2} v^2 = z$ , no bounces are possible for  $z > 0.13396$  and bounces can occur without attaining  $p_c = \rho/3$  only if the initial  $z < 0.10993$ , the exact behaviour always depending on the initial acceleration.

If  $\gamma = 5/3$ , stable equilibrium configurations can occur for  $z < 0.23449$ , and oscillating models exist, but their largest  $z$  cannot exceed the value 0.34234. For general initial conditions, the most convenient quantity to consider is now the minimum initial value of  $w = (R_s f + z)/z^2$  as function of the initial  $z$  and  $v$ . For small  $z$ , the least value of  $w$  leading to a bounce is 10.93, increasing with  $z$  for large  $v$ , but decreasing with  $z$  for small  $v$ . The largest critical  $w$  value is 22.78 ( $\frac{1}{2} v^2 = z = 0.2963$ ); the smallest is 2.25 ( $v = 0, z = 0.4444$ ). Note that these positive  $w$  values imply negative  $f$  values (inward accelerations!) for reasonably small  $z$  ( $< 0.23449$  for  $v = 0$ ,  $< 0.1$  for  $\frac{1}{2} v^2 = \frac{1}{2} z$ ,  $< 0.084$  for  $\frac{1}{2} v^2 = z$ ). Again the imposition of  $p_c \leq \rho/3$  leads to greater complication. Bounces not involving  $p_c = \rho/3$  are now possible only from starting positions below the nearly straight curve (given in terms of the parameter  $q$ )

$$x = \frac{1}{2} q \left[ 3 \frac{2-q}{3-2q} \right]^{\frac{1}{2}}, \quad z = 2 \frac{q-1}{q^2} \left[ 3 \frac{q-1}{3-2q} \right]^{\frac{1}{2}}$$

which passes through (1, 0.0868) and (1.2, 0.2778), the latter being on  $Q$ .

For  $\gamma = 1$ ,  $R_s f / z = u$  is best considered as function of  $z$  and  $y = \frac{1}{2} v^2 / z$ . For small  $z$  the condition for a bounce is the same as in Newtonian theory, so that  $y$  and the minimum  $u$  are related by

$$y = (1+u) \log(1+u) - u$$

but for larger  $z$  the necessary  $u$  values become somewhat larger. For the largest  $z$  possible with the restriction  $p_c \leq \rho/3$  we still have  $u = 0$  for  $y = 0$ , but for  $y = 0.0328$ ,  $z = 0.2344$ ,  $u = 0.373$ ; and with  $y = 1$ ,  $z = 0.13396$ ,  $u = 2.732$ .

Full details of this work (which is related to two recent papers<sup>1,2</sup>) will be published elsewhere.

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<sup>1</sup> Thompson, I. H., and Whitrow, G. J., *Mon. Not. Roy. Astro. Soc.*, **207**, 136 (1967).

<sup>2</sup> Bonnor, W. B., and Foulkes, M. C., *Mon. Not. Roy. Astro. Soc.* (in the press). In this paper an oscillating model is obtained.

## PLANETARY SCIENCE

### Devitrification of Tektite Glass

THEORIES of the origin of tektites have usually been based on the assumption that tektite glass is stable. In view of the critical nature of this assumption, the tendency of various natural tektite glasses to crystallize has been studied by subjecting them to heat treatments for specified lengths of time. Two types of experiments were performed, one involving the heat treatment of supercooled glass above the strain point, and the other, crystallization of tektite liquid on slow cooling from the melt. The tektite samples used in these experiments were lent by R. S. Clarke, jun., of the Smithsonian Institution, and E. C. T. Chao of the US Geological Survey.

In order to study the devitrification of tektite glass, samples several cubic centimetres in volume were subjected to various heat treatments well below the liquidus temperature in an air atmosphere in an electric furnace. Only the outer skin of the glasses were subjected to air oxidation, and the oxidation state of the iron did not change in the interior, where crystallization was studied. Some identical samples were also treated in a vacuum furnace. Crystal morphology was studied microscopically and by X-ray diffraction.

Experiments designed to study the crystallization of cooling tektite liquids were performed by melting tektite glass and placing the molten material in a temperature gradient in the vicinity of the liquidus temperature for a period of about 24 h. These experiments were performed in platinum wound electric furnaces; the glass was contained in platinum boats. Obvious oxidation effects were again confined to the periphery of the sample.

Tektite samples listed in Table 1 were exposed to the heat treatment listed. Detailed descriptions will be summarized for the Vietnam, Pugad Babuy and Texas tektites. Fig. 1 shows that the tektites crystallized to two or three phases, denoted by  $A$ ,  $B$  and  $C$ . The Bohemian and Texas tektites did not crystallize to hypersthene, phase  $A$ ; phases  $B$ , cristobalite, and  $C$ , unidentified rod and shapes, were noted in these specimens.

Table 1. HEAT TREATMENTS AND CRYSTALLIZATION DATA OF SELECTED TEKTITES

Temperature (° C)	Heat treatment °C		Viscosity (poises)
	Time (h)		
950	12		10 <sup>10</sup>
1,000	10		10 <sup>9</sup>
1,050	10		10 <sup>8</sup>
Sample	Phases observed optically		
<i>USNM</i> 2005 Pugad Babuy	3		
<i>USNM</i> 2010 Pugad Babuy	3		
<i>USNM</i> 2175 Thailand	3		
<i>USNM</i> 2141 Vietnam	3		
<i>USNM</i> 2057 Bohemia	2		
<i>USNM</i> 1975 Santa Mesa	3		
<i>USNM</i> 1910 Santiago	3		
<i>USNM</i> 1012 Santiago	3		
<i>USNM</i> 1880 Lee County, Texas	2		
Western Australia East of Kilgoorie	3		
Ortigas	3		

Samples were loaned by R. S. Clarke, USNM, and E. C. T. Chao, US Geological Survey.



Table 2. AVERAGE TEKTITE COMPOSITIONS FROM SEVERAL LOCALITIES

Locality/type	No. of samples	SiO <sub>2</sub>	Al <sub>2</sub> O <sub>3</sub>	Fe <sub>2</sub> O <sub>3</sub>	FeO	MgO	CaO	NaO	K <sub>2</sub> O	TiO <sub>2</sub>	P <sub>2</sub> O <sub>5</sub>	MnO	H <sub>2</sub> O
Martha's Vineyard <sup>4</sup>	1	80.6	11.3	0.4	2.2	0.7	0.7	1.1	2.4	0.5	—	0.05	0.1
Texas bediasites <sup>5</sup> , Lee County	2	76.37	13.78	0.19	3.81	0.63	0.65	1.54	2.08	0.76	0.04	0.04	—
Pugad Babuy, P. I. <sup>6</sup>	3	70.8	14.02	0.71	4.25	2.54	3.17	1.39	2.44	0.79	—	—	—
Santa Mesa, P. I. <sup>7</sup>	3	70.7	13.88	0.61	4.35	2.61	3.17	1.40	2.40	0.80	—	—	—
Australite <sup>8</sup>	17	73.45	11.53	0.58	4.05	2.05	3.50	1.28	2.28	0.69	0.03	—	—
Moldavite <sup>9</sup> , Bohemia	2	78.27	10.12	0.42	1.56	2.18	3.20	0.47	3.38	0.33	—	—	—
Indochinites <sup>7</sup> , Thailand and Vietnam	12	73.0	12.83	0.64	4.37	2.48	1.91	1.45	2.40	0.73	—	0.09	—

Phase *A* crystallized in the form of transparent laths and rods. The index of refraction was greater than the surrounding glass, the cleavage traces were length slow (positive elongation) biaxial negative and the average size was 90–100 $\mu$ . Petrographic and X-ray diffraction identified phase *A* as hypersthene (Mg,Fe)<sub>2</sub>(SiO<sub>3</sub>)<sub>2</sub>, with diffraction lines at 3.18, 2.87, 2.53 and 2.45 Å (ref. 1).

Phase *B* crystallized in the form of 30° dendrites on the lath shapes of phase *A* (Fig. 2) and as isolated spherulites (Fig. 3). X-ray diffraction identified this phase as cristobalite, with lines at 4.09, 3.15, 2.85 and 2.50 Å. The cristobalite phase is apparently a modification of the more pure silica form, because it appears here with an index of refraction higher than that of the glass ( $N=1.51$ ). Substitution of alumina modified by alkaline earth ions in the cristobalite structure are suspected to account for the higher index.

Phase *C* was interspersed throughout the body of all the glass samples investigated. It appeared in the shape of small rods, T-shapes and Y-shapes. The 3–4 $\mu$  size and its high degree of dispersion in the glass made it difficult to identify either optically or by X-ray diffraction. Fig. 4 depicts phase *C*.

In addition to the tektites which were heat treated for 32 h, two samples from Vietnam were heated at 950° C for shorter periods of 2 and 10 h. These also crystallized to phases *A*, *B* and *C*.

Gradient furnace liquidus studies performed on both heating and cooling of Philippine Island tektites and artificial tektite glasses show the liquidus to be in the range 1,330°–1,350° C with tridymite and cristobalite as the phases at the liquidus. The test lasted for 24 h but similar results were obtained in 17 h.

The liquidus<sup>2</sup> temperature sets the upper limit of crystallization for the tektite compositions investigated.

It also shows that these glasses cannot be slowly cooled through the range 1,350°–950° C without substantial internal devitrification.

The fine grained internal crystallization of hypersthene which takes place rapidly on heating of the tektite glasses, particularly those from south-east Asia, is unknown among other high silica natural glasses. Such fine grained devitrification is frequently associated in artificial glasses with thermally induced micro-immiscibility or the formation of a fine emulsion of two glasses.

It is known that aluminosilicate glasses which contain appreciable amounts of ferrous ion tend to separate into two components, iron-silicate and aluminosilicate. The system leucite (KAlSi<sub>3</sub>O<sub>8</sub>)–fayalite (Fe<sub>2</sub>SiO<sub>4</sub>)–silica (SiO<sub>2</sub>) is an example of this. The stable liquid immiscibility occurs on the liquidus where an iron-silicate liquid coexists with a potassium–aluminosilicate liquid<sup>3</sup>. In the system Na<sub>2</sub>O–FeO–Al<sub>2</sub>O<sub>3</sub>–SiO<sub>2</sub> and FeO–Al<sub>2</sub>O<sub>3</sub>–SiO<sub>2</sub>, similar metastable immiscibility occurs. We propose that tektite glasses, which contain considerable FeO, separate on heating into a component rich in iron silicate and one rich in aluminosilicate. The iron silicate portion is unstable and devitrifies to ferro-hypersthene. The fine grain size is explained by the fine nature of the emulsion.

Cristobalite, or other phases, may form with increased heat treatment on the hypersthene nuclei. This results in a material of considerable crystallinity, which is very fine grained and resembles chert in appearance.

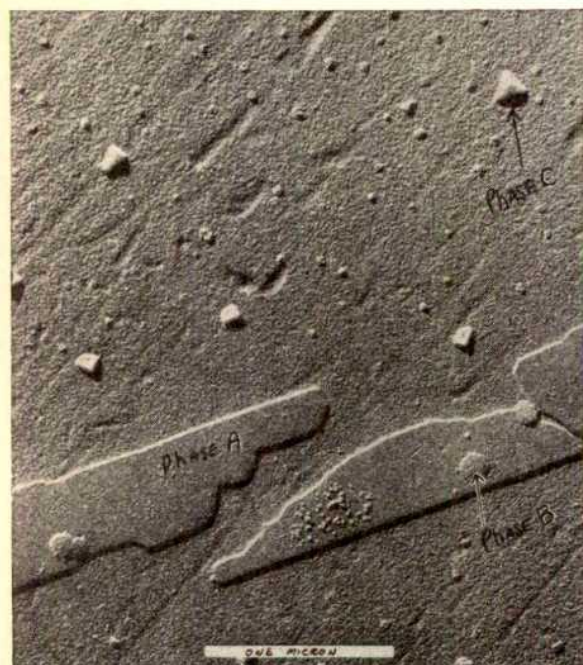


Fig. 1. Electron photomicrograph of a Pugad Babuy sample, USNM 2010, showing the three phases *A*, *B* and *C*. ( $\times c. 24,000$ .)

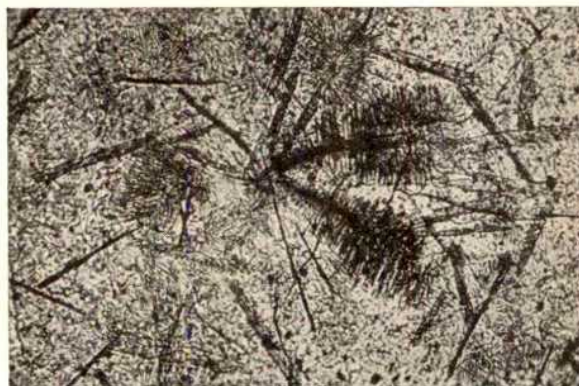


Fig. 2. Phase *A*, laths; phase *B*, dendrites. ( $\times c. 70$ .)

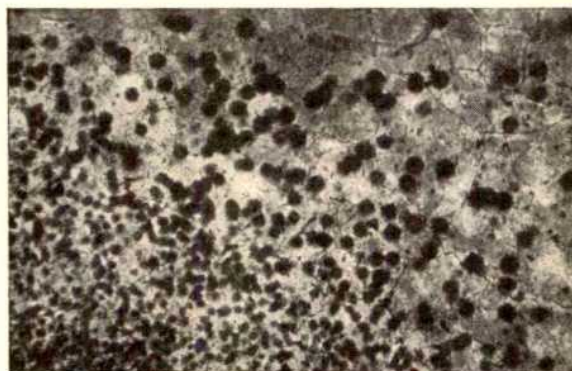
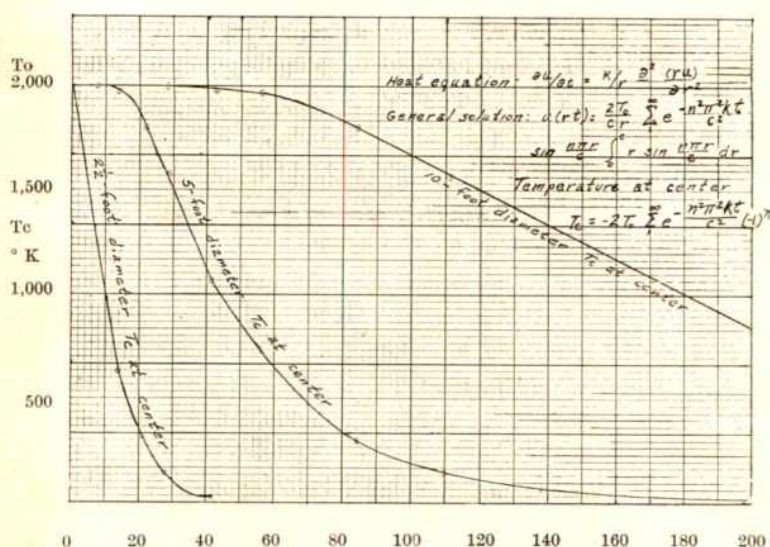


Fig. 3. Cristobalite spherulites.



Fig. 4. Phase C rods, Y-shapes. ( $\times c. 700$ .)Fig. 5. Cooling curves for tektite spheres. Thermal diffusivity ( $k$ )  $= 5 \times 10^{-3} \text{ cm}^2/\text{sec}$ .

The rate of devitrification of tektite material on cooling from a melt depends on the relative stability of liquid and crystal; the rate on heating from cold glass depends on the stability of the glass towards both immiscibility and crystal formation. Artificial high silica and aluminosilicate glasses which contain soda and potash are known to be very resistant to devitrification, while iron and magnesium aluminosilicates are usually unstable and devitrify more readily.

It has been noted from the heating experiments described that the tendency for the hypersthene devitrification on heating is greatest in the philippinites and indochinites, and least among the moldavites and North American tektites. This tendency seems to be related to composition (Table 2). Tektites, poor in silica, particularly those richest in FeO, MgO and  $\text{Al}_2\text{O}_3$ , would be expected to devitrify most readily, while tektites with more silica, particularly those richest in the alkalis, would be expected to be more stable glasses.

The great instability of the indochinites low in silica, which transform to cherty hypersthene crystal bodies when heated for 2–10 h above  $900^\circ \text{C}$ , suggests that tektites with even less silica may exist, but if they do they are in a devitrified form difficult to distinguish from many common rocks. It is possible that diligent searching in fields strewn with tektites will uncover crystalline materials which are tektitic in origin.

The susceptibility of tektite glass to internal devitrification sets rigid limits on the maximum size of a molten tektite body which can cool to a rigid condition and still

remain a glass. The key parameter in such a calculation is  $k$ , the thermal diffusivity, which has been measured at Corning Glass Works for a typical indochinite composition glass. This parameter has a value of about  $5 \times 10^{-3} \text{ cm}^2/\text{sec}$  in the general temperature range of interest. From the heat equation with spherical co-ordinates, as shown in Fig. 5, time-temperature curves can be drawn which can be used to determine the critical size of a tektite. According to this equation, a sphere 5 ft. in diameter of tektite liquid at  $2,000^\circ \text{K}$ , the surface of which is quenched and held at  $0^\circ \text{K}$ , would cool so slowly that its centre would remain at a temperature of  $950^\circ\text{--}1,350^\circ \text{C}$  for more than 10 h. This is sufficient time for a typical indochinite-philippinite tektite to devitrify. Radiative cooling from the surface would involve even slower cooling rates than quenching to absolute zero; a 5-ft. sphere can therefore be considered a maximum size for any cold entirely glassy tektite body of the indochinite type which has cooled from a temperature in excess of its liquidus temperature.

The tendency for hypersthene to devitrify as fine microlaths on short heating of many south-east Asian tektite glasses above  $900^\circ \text{C}$  limits the thermal history of susceptible glasses which do not display devitrification. Such glasses must never have been reheated above  $900^\circ \text{C}$  for more than a few hours subsequent to their last period of fusion.

The ease of internal crystallization of south-east Asian tektite glass on slow cooling severely limits the size of the parent mass, unless this mass was either not formed by conventional melting above the liquidus, was substantially crystalline, or was never cooled below the liquidus before breaking up into smaller fragments.

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### Ionospheric No-echo Occurrences

THE use of ionogram data to identify periods of high absorption has recently been criticized by Schaeffer<sup>1</sup>. This criticism is justified at a number of stations, including Mawson, but does not apply to stations where adequate precautions are taken in the ionogram analysis. At Halley Bay, in particular, it is relatively easy to obtain high sensitivity of the ionosonde down to 0.7 Mc/s by using

large aeriels, so that changes in the frequency minimum,  $f_{\min}$ , detect only large changes in absorption.

Every no-echo event at Halley Bay during the IGY and IQSY was scrutinized, taking into account background noise level, the value of critical frequency immediately before and after the "blackout" and any evidence for tilts which would be expected if the critical frequency had fallen during the "blackout" record. Because more than half the "blackouts" seen at Halley Bay last for half an hour or less and ionograms are available for every quarter hour, these controls are very effective in preventing misinterpretations and it is not expected that more than 1 per cent of the total "blackouts" recorded could be challenged on the grounds raised by Schaeffer.

The diurnal variation of the  $F_2$  layer critical frequency was examined on the days which showed no-echo conditions and compared with the corresponding variation for other days. These showed no significant differences of the type suggested by Schaeffer; in fact, as would be expected from disturbed to quiet day ratios of the critical frequency of the  $F_2$  layer,  $f_oF_2$ , at Halley Bay, the critical frequencies were somewhat higher than normal on the days with no-echo conditions. As an additional check, a high sensitivity absorption equipment (A.1) was used during many of the events. This clearly showed increased absorption with normal heights of reflexion whenever echoes were received during the recovery stage.

Despite the widely different critical frequencies in the IGY and IQSY, the "blackout" phenomena reported by Bellchambers and Piggott<sup>2</sup> have recurred, at a lower rate of occurrence but at essentially the same times as in the IGY, and have shown the same relation to magnetic activity. In both epochs auroral types of high absorption are often present at times when the magnetic field is undisturbed, particularly near 1900 L.M.T. Most of these periods are seen within 12 h of classical events of high planetary magnetic disturbance index.

Piggott and Shapley<sup>3</sup> and others have pointed out that riometer absorption events often differ greatly from those found using ionosonde or by field strength measurement at lower frequencies. This is because the riometer is relatively more sensitive to absorption processes where the collisional frequency is high, typically below 75 km, whereas the other techniques are relatively more sensitive to absorption at greater heights. One would expect different spatial and temporal distributions for particles capable of ionizing the atmosphere in these two regions.

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<sup>1</sup> Schaeffer, R. C., *Nature*, **214**, 376 (1967).

<sup>2</sup> Bellchambers, W. H., and Piggott, W. R., *Proc. Roy. Soc., A*, **256**, 200 (1960).

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## MOLECULAR STRUCTURE

### Charge Transfer Complexes of Lipids with Iodine

THERE is much interest in the role of charge transfer interactions in biology<sup>1</sup>. In a study of the semiconductive properties of lipids in the solid state, Rosenberg and Jendrsiak (ref. 2 and unpublished work) have shown that when lipid molecules, such as egg lecithin and synthetic lecithin, are exposed to iodine vapour, they exhibit a large increase in electrical conductivity with a concomitant decrease in the activation energy from the dry state value. They suggest that a charge transfer mechanism may be involved. Because they also found that iodine added to the aqueous media surrounding

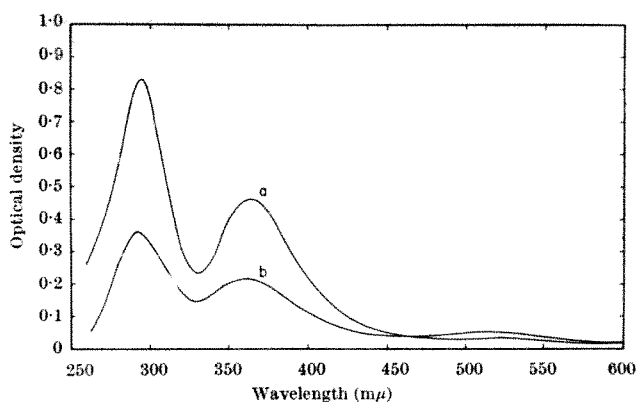
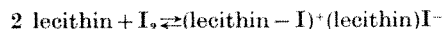


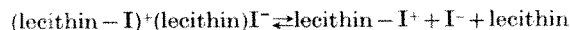
Fig. 1. Absorption spectra of lecithin and iodine in carbon tetrachloride. a,  $1.18 \times 10^{-4}$  molar egg lecithin; b,  $6.21 \times 10^{-3}$  molar synthetic lecithin;  $5.63 \times 10^{-2}$  molar iodine.

lipid bilayers (egg lecithin or oxidized cholesterol) increased the bilayer conductivity also by a factor of more than  $10^4$ , it was suggested that a charge transfer mechanism might also be involved in this case. It was with the intention of investigating these possible charge transfer mechanisms that we undertook the work reported here.

When iodine in carbon tetrachloride was added to a solution of lipids in carbon tetrachloride, the original violet colour of iodine changed. In the case of lecithin (synthetic and egg) two new absorption bands appeared in the region of 366  $\mu$  and 294  $\mu$ . By varying the concentrations of iodine and lecithin, both dissolved in carbon tetrachloride, it was found that the new bands arose from a 2 : 1 complex between lecithin and iodine. One mechanism of the reaction might be represented by



According to Mulliken<sup>3</sup> this would be the inner complex, whereas the outer complex ( $\text{lecithin} - \text{I}_2$ ) would be a molecular complex of the usual type. This inner complex is an essentially ionic structure and it slowly dissociates as



As soon as  $\text{I}^-$  forms it combines with free  $\text{I}_2$  molecules to give  $\text{I}_3^-$ , which is responsible for the bands at 366  $\mu$  and 294  $\mu$ . The spectra of lecithin (egg and synthetic) with iodine are shown in Fig. 1. The optical density of these solutions increased with time and thus all the spectra were recorded within 30 min of mixing the solutions. Of the two lecithins, egg lecithin is more than a hundred times stronger than synthetic lecithin in forming the complexes as observed from the change in optical density of the same concentration of iodine with different concentrations of lecithin.

Iodine, on the other hand, was found to form an outer complex with cholesterol. The visible iodine band at 518  $\mu$  ( $\epsilon = 935$ ) shifted to 509  $\mu$  ( $\epsilon = 800$ ) and at the same time a new band appeared at 332  $\mu$  where both donor and acceptor have no absorption. This band of iodine and cholesterol at 332  $\mu$  was considered to be a charge transfer band. By varying the concentrations of cholesterol and iodine in carbon tetrachloride, it was found that the new band came from a 1 : 1 complex formation. Applying the Ketelaar equation<sup>4</sup> at the  $\lambda_{\max}$  of the complex, the equilibrium constant was estimated, using a donor concentration range of  $9.68 - 48.40 \times 10^{-3}$  moles/l. with a fixed concentration of acceptor of  $11.26 \times 10^{-4}$  moles/l. The results are  $K = 6.26$  l./mole and  $\epsilon_{\max} = 795.0$ . With the aid of the equilibrium constant value, we can record the absorption curve, which results entirely from the charge transfer complex, by balancing the solution of the complex against the concentrations of free donor and free acceptor present in the solution of the complex. For this purpose we used four 1 cm silica cells,



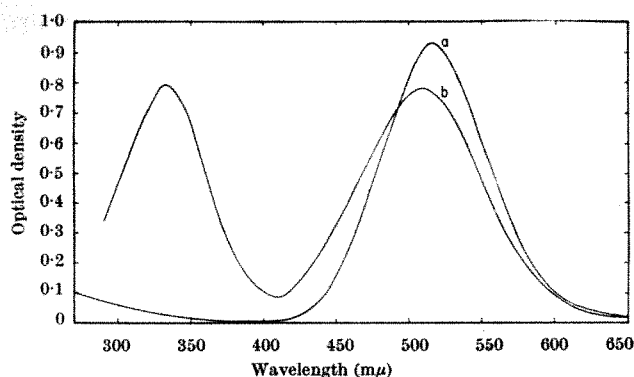


Fig. 2. Absorption spectrum of oxidized cholesterol and iodine in carbon tetrachloride. *a*,  $11.3 \times 10^{-4}$  molar iodine; *b*,  $1.28 \times 10^{-3}$  molar oxidized cholesterol and  $11.3 \times 10^{-4}$  molar iodine. (In *b* the OD shown is twice the experimental OD.)

two in each compartment. The results are shown in Fig. 2.

Because the lipid bilayers are 70 Å thick and about 1 mm in diameter, it was not possible to study spectroscopically their interaction with iodine. As an approximation to the interaction of the bilayer system with iodine, we decided to study the spectra of egg lecithin in water and its interaction with iodine. Egg lecithin, dispersed in water, is thought to have a micellar structure. The micelles are supposedly disk-like structures with a disk thickness of about 70 Å—about the thickness of a lipid bilayer<sup>5</sup>. The polar groups are presumably exposed to the water, whereas the hydrocarbon chains are in the interior. The interaction of this lipid system with iodine should be quite similar to that of the bilayer. We did not attempt to disperse oxidized cholesterol in water.

Aqueous iodine exhibits the spectra of the tri-iodide ion ( $\lambda_{\max} = 351 \text{ m}\mu$  and  $287 \text{ m}\mu$ ) and that of molecular iodine ( $\lambda_{\max} = 460 \text{ m}\mu$ ). When a dispersed aqueous solution of egg lecithin was added to the same solution of iodine, we found that the molecular iodine band at  $460 \text{ m}\mu$  decreased, whereas the tri-iodide ion bands increased. We obtained the same result when we added small amounts of sodium iodide to the aqueous solution of iodine. The results are shown in Fig. 3.

The spectra of thin films of egg lecithin in the solid state were also studied. The films were formed by evaporating the chloroform from a few drops of a 0.02 molar solution of lecithin and chloroform, placed on one flat surface of a 5 cm quartz cell. Because quartz was also the substrate used for the electrical measurements of the thin films (our unpublished work), the two situations should be comparable. The films were dried with helium gas and then a crystal of iodine was placed in the quartz cell and allowed to sublime. The free iodine was evacuated at low temperature. The spectra of iodine–lecithin in the

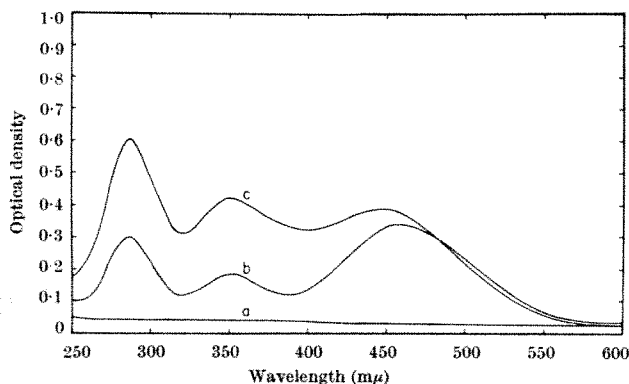


Fig. 3. Absorption spectrum of egg lecithin and iodine in water. *a*,  $1.53 \times 10^{-3}$  molar egg lecithin dispersed in water; *b*, about  $4 \times 10^{-4}$  molar iodine in water; *c*, egg lecithin and iodine in water (concentrations same as in *a* and *b*)

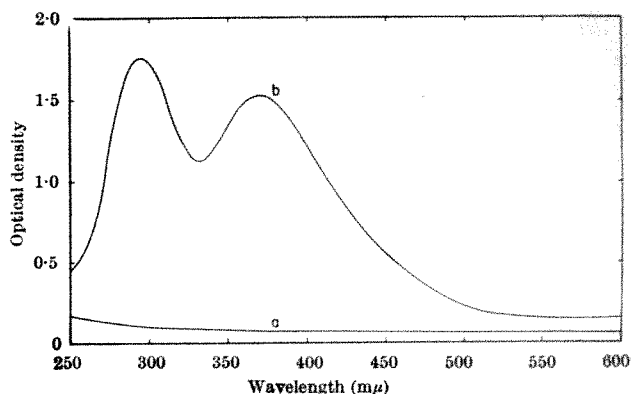


Fig. 4. Spectrum of egg lecithin film exposed to iodine vapour. Film is on quartz. *a*, Egg lecithin film on quartz; *b*, egg lecithin film exposed to iodine vapour, on quartz.

solid state showed two bands, at  $370 \text{ m}\mu$  and  $295 \text{ m}\mu$  (Fig. 4), which are similar to those obtained in the aqueous system—that is, the short wavelength band is more intense than the long wavelength one. From this result and also from the study of spectra in aqueous solutions, it may be concluded that lecithin forms a molecular charge transfer complex with iodine both in the solid state and in aqueous solution. Oxidized cholesterol could not be laid down as a film, unfortunately, and no spectra could be taken.

Because the  $I_3^-$  bands are seen for the lecithins in organic solvents, water and in the solid state, it appears that the same type of interaction occurs between the lipid and iodine in all three cases. This is interesting in view of the proposed states of lecithin in the various cases. The micellar form in water, and the bilayer leaflet in the solid state are similar, but lecithin dissolved in a non-polar solvent does not change the dielectric constant; the lecithin behaves as if its polar groups were neutralized<sup>5</sup>. This suppression of the dipole moment has been explained by the formation of molecular associations, in agreement with the results of molecular weight measurements, and is thought by some workers to indicate some sort of aggregate structure with the polar groups directed toward the interior and the hydrocarbon chains toward the exterior. Nevertheless, the iodine seems to react similarly with the lecithin in the non-polar solvent, in the polar solvent and in the solid state. Moreover, because the interaction of iodine and lecithin appears to be of the same type for a dispersion of lecithin in water (taken as an approximation to the lipid bilayer case) and for lecithin in the solid state film, the changes in electrical conductivity which result may well arise from the complex formation in both cases. If, indeed, these are charge transfer reactions, it seems that electronic conduction should be considered as a possibility in both cases. The same ideas may apply to oxidized cholesterol. The nature of this charge-transfer complex should be the subject of further investigation.

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## Molecular Orientation of Fibres by Polarized Internal Reflexion Spectroscopy

SUCCESSIVE drawing of synthetic fibres increases the molecular orientation of the polymer chains and is a method which is widely used for improving the tensile properties of yarns. Investigations of the changes in the dichroism of individual infra-red modes of the polymer with polarized infra-red radiation have yielded important conclusions regarding the structure and band assignments<sup>1</sup>. The dichroism of each infra-red mode, expressed as a dichroic ratio, is related to its transition moment; the angle of inclination of the bond to the molecular axis; and the degree of molecular orientation. Measurement of the dichroism of nylon fibres by infra-red transmission techniques has been introduced<sup>2</sup> and questioned<sup>3</sup> because sample preparation by microtoming can alter the existing orientation. Internal reflexion spectroscopy<sup>4</sup>, however, provides a non-destructive, rapid and reproducible method for obtaining the surface dichroism of any oriented fibre or film—measurements which are difficult or impossible to obtain by conventional transmission techniques.

An internal reflexion attachment<sup>5</sup> was designed for use with commercial, double-beam spectrophotometers. A trapezoidal germanium prism, providing twenty-one internal reflexions, is placed at a focal point in each beam. Prism holders apply a known and constant hydraulic pressure against the sample and the prism surface to obtain sufficient and reproducible optical contact. A polished germanium reflexion polarizer<sup>6,7</sup> is fixed in front of the monochromator entrance slit. Polarization efficiency in excess of 99 per cent is obtained with a single reflexion from the polarizer surface. First surface mirrors in the polarizer unit return the beam to the optical axis of the spectrophotometer.

To obtain the dichroic ratio, two infra-red spectra are obtained for each fibre at the same contact pressure. Initially, the fibres are placed against the surface of the internal reflexion prism with the incident electric vector parallel to the axis of the fibre. The fibres are then rotated through 90° and the spectrum with perpendicular incidence is obtained. The dichroic ratio is calculated from the ratio of the absorption intensities for the parallel to perpendicularly polarized light at each infra-red mode.

The dichroic ratios for a series of drawn 66 nylon fibres at six frequencies are given in Table 1. The dichroic ratios for a pressed film were obtained for the draw ratio of unity. It was determined that the surface dichroic ratios obtained by internal reflexion spectroscopy are directly related to the bulk birefringence for fibres having a draw ratio of more than 1.4. For values of the draw ratio less than 1.4, the surface of the fibre appears to have greater orientation than the interior.

Table 1. DICHROIC RATIOS FOR A SERIES OF ORIENTED 66 NYLON FIBRES OBTAINED BY INTERNAL REFLEXION SPECTROSCOPY AT VARIOUS INFRA-RED ABSORPTION FREQUENCIES

Dichroism	Frequencies (cm <sup>-1</sup> )	Group assignments <sup>a</sup>	Draw ratios				
			1.00	1.40	2.27	3.76	5.03
Perpendicular bands	3,310	$\nu$ (NH)	1.03	0.69	0.57	0.34	0.36
	3,080	$\nu$ (NH-H)	0.94	0.67	0.56	0.45	0.37
	1,640	$\nu$ (C=O) I	1.01	0.85	0.74	0.46	0.43
Parallel bands	1,538	$\nu$ (NCO) + $\delta$ (NH) II	1.02	1.08	1.40	1.88	1.85
	1,277	$\delta$ (CNH) III	1.03	1.08	1.75	2.20	2.00
	1,200	$\delta$ (CH)	1.01	1.03	1.53	2.01	1.85

$\nu$  = stretching,  $\delta$  = bending, in plane.

Quantitative polarization data obtained in this way are very sensitive to small changes in surface orientation at low draw ratios. The polarized internal reflexion technique therefore complements the X-ray diffraction method for measuring orientation—a method which is most useful for fibres of high molecular orientation. A satisfactory spectrum for each angle of polarization can be obtained without modification or deformation of the

fibre, regardless of its diameter, surface condition, amount of dye or pigment content.

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## THE SOLID STATE

### Substitution by Iron in Kaolinite

KAOLINITE often occurs in admixture with other minerals (for example, micas and iron oxides) in which iron is a legitimate constituent. Because of the difficulties of detection and separation, it has always been doubtful whether iron actually substitutes in the kaolinite lattice. We provide here evidence for substitution of iron(III) in the octahedral (Al) sites.

The materials studied were the fractions of two clays from the St. Austell area with equivalent spherical diameters of 1–2.5 $\mu$ . These were subjected to repeated extraction in an inhomogeneous magnetic field as 16 per cent solid suspensions deflocculated at pH 8.0 with 0.1 per cent w/w tetrasodium phosphate.

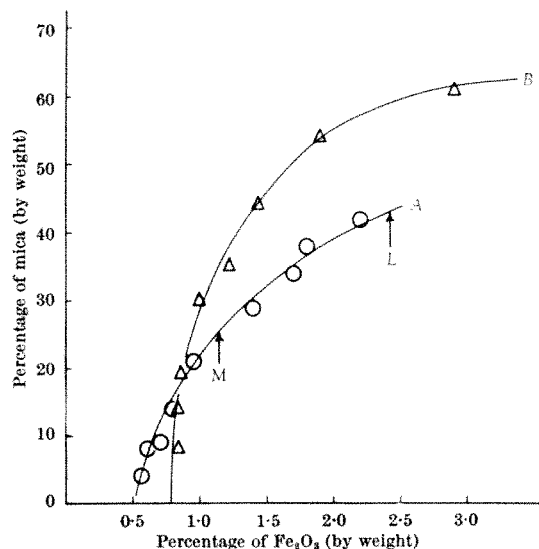


Fig. 1. Plot of the content of mica against the concentration of  $\text{Fe}_2\text{O}_3$  for magnetic extracts of clay samples A and B.

Fig. 1 shows a plot of the content of mica against the total content of iron (as  $\text{Fe}_2\text{O}_3$  determined chemically) for those magnetic extracts of samples A and B which have been analysed. The contents of mica, tourmaline and feldspar were deduced from X-ray powder photographs. A positive intercept on the abscissa is obtained for both clays, indicating that repeated extraction would yield a fraction containing no mica but 0.5 and 0.8 per cent  $\text{Fe}_2\text{O}_3$  for A and B respectively. This residual iron is not extractable by acid washing. Chemical analysis of the highly refined specimens of A and B yields the following:

	Percentage by weight	
	<i>A</i>	<i>B</i>
Fe <sub>2</sub> O <sub>3</sub>	0.31	0.37
FeO	0.002	0.003
K <sub>2</sub> O	0.08	0.28
Na <sub>2</sub> O	0.03	0.09
Ignition loss	14.02	13.96

If it is assumed that the mica fraction of each magnetic extract contains all the K<sub>2</sub>O found, the mean K<sub>2</sub>O contents of the micas in *A* and *B* are found to be 5.8 and 8.5 per cent respectively. On this basis, the mica content of the refined materials would be 1.4 and 3.3 per cent respectively. If this mica contained all the residual iron, the Fe<sub>2</sub>O<sub>3</sub> concentrations of iron in the mica would be 23 per cent and 11 per cent respectively, which are higher values than found for any micas actually extracted from kaolinite in the particle size considered. Furthermore, X-ray diffractometry indicates that the mica content of the refined clays is <0.12 and <0.19 per cent respectively. No mineral other than kaolinite could be detected.

These findings provide *prima facie* evidence for iron substitution in kaolinite and this is confirmed by Mössbauer effect measurements. While these analyses were in progress, Weaver *et al.*<sup>1</sup> published results of a Mössbauer survey of clay minerals including kaolinite. Their kaolinite (from an American source) contains much more iron, however, and the impurity concentration was uncertain.

Fig. 2 shows a typical Mössbauer plot of transmission against velocity for the dioctahedral mica, muscovite (from Lee Moor, Devon). The source used was 10 mc. of cobalt-57, yielding the iron-57 14.4 keV  $\gamma$ -ray. Two quadrupole doublets *XX'* and *YY'* are observed. *XX'*

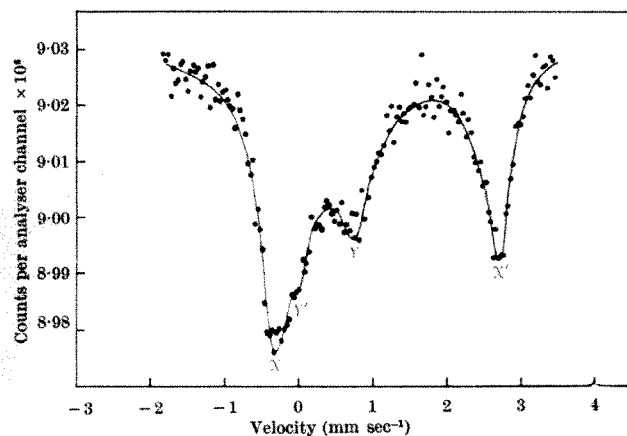


Fig. 2. Mössbauer spectrum of muscovite.

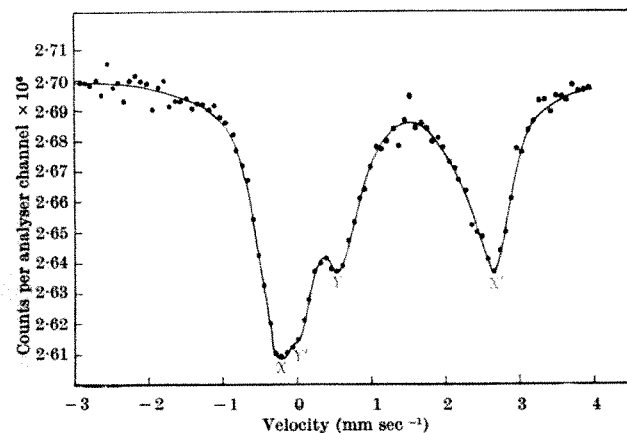
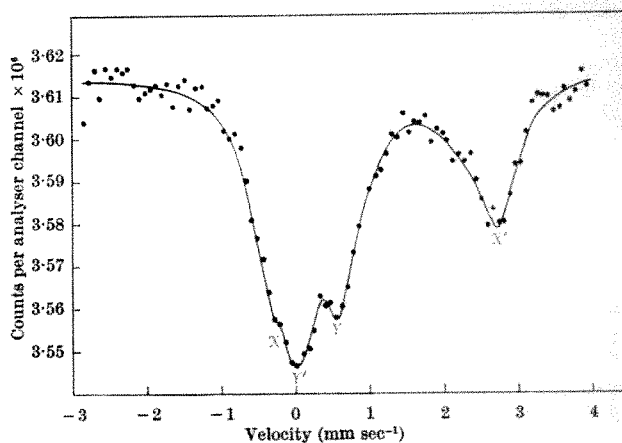
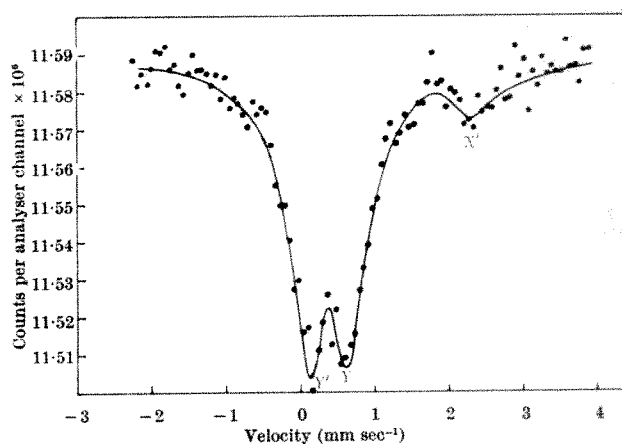
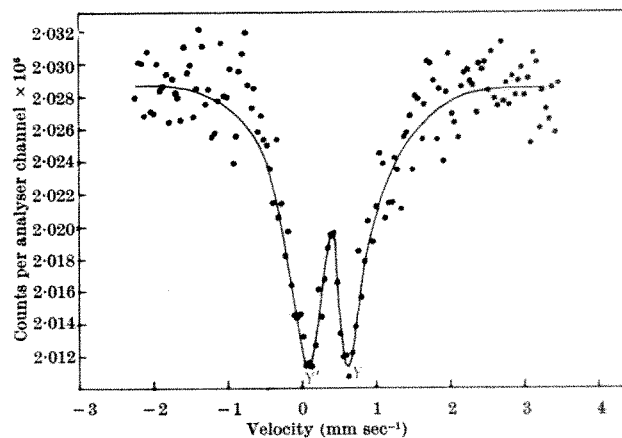
Fig. 3. Mössbauer spectrum of magnetic extract *L*.Fig. 4. Mössbauer spectrum of magnetic extract *M*.Fig. 5. Mössbauer spectrum of refined kaolinite *A*.

Fig. 6. Mössbauer spectrum of gibbsite.

has an isomer shift and quadrupole splitting characteristic of iron(II) and *YY'* is characteristic of iron(III). Figs. 3 and 4 show similar spectra for magnetic extracts of sample *A* corresponding to points near *L* and *M* on the curve of Fig. 1. These show progressive diminution of *XX'* compared with *YY'* as the mica content is reduced (that is, an increase in the ratio of iron(III):iron(II)). Fig. 5 shows a spectrum of highly refined kaolinite *A*. The ferrous doublet has now almost disappeared and the position of the residual iron(II) line *X'* is shifted to lower velocity. The ferric lines (*YY'*) are relatively enhanced and have a smaller quadrupole splitting than those in

muscovite. The spectrum of iron in gibbsite ( $\text{Al}(\text{OH})_3$ ) (Fig. 6) shows a ferric doublet closely similar to that of kaolinite. The results are summarized in Table 1.

Table 1

Material	Iron (II)		Iron (III)	
	Isomer shift	Quadrupole splitting	Isomer shift	Quadrupole splitting
Muscovite	$1.20 \pm 0.05$	$2.98 \pm 0.05$	$0.48 \pm 0.04$	$0.72 \pm 0.05$
Kaolinite		$\sim 2.25$	$0.48 \pm 0.04$	$0.50 \pm 0.05$
Gibbsite			$0.47 \pm 0.04$	$0.52 \pm 0.05$

Isomer shifts are relative to stainless steel. All values are in  $\text{mm sec}^{-1}$ .

The following conclusions can be drawn.

(a) The principal contaminant containing iron in the unrefined kaolinite is mica of the muscovite type, to which the iron(II) doublet in the Mössbauer spectrum is largely due. (Tricathedral biotite shows a smaller iron(II) quadrupole splitting.)

(b) Genuine lattice substitution by iron(III) does occur in kaolinite. Because the iron in refined kaolinite is not extractable in acid, it cannot be due to oxides or to goethite ( $\text{FeO} \cdot \text{OH}$ ) (possibly present in the sample used by Weaver *et al.*<sup>1</sup>). The possibility that the iron(III) doublet arises from small superparamagnetic grains of  $\alpha\text{-Fe}_2\text{O}_3$  (ref. 2) has been rejected because, at room temperature, the spectrum of  $\alpha\text{-Fe}_2\text{O}_3$  particles large enough to give the observed quadrupole splitting should contain a substantial six-line component due to magnetic hyperfine interaction in antiferromagnetic  $\alpha\text{-Fe}_2\text{O}_3$ . Spectra at  $80^\circ \text{K}$  for muscovite and biotite confirm that the iron(III) doublet observed in these materials does not result from this cause.

(c) The similarity of the iron(III) doublet in kaolinite and gibbsite (in which iron(III) ions in the Al sites are certainly octahedrally co-ordinated) suggests that the substitution of iron in kaolinite is also in the octahedral sites.

(d) A small amount of iron(II) substitution in kaolinite is suggested by the fact that the iron(II) line in the kaolinite spectrum is not in the position expected for muscovite.

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## BIOPHYSICS

### Electrically Active Polyelectrolyte Membranes

SEVERAL lipid membranes<sup>1,2</sup> have been described as models for neuronal and other cell membranes, usually based on the lipid bilayer concept of Davson and Danielli<sup>3</sup>. Such membrane models have the characteristics of insulators with a high specific resistivity and dielectric strength. They can, however, be changed with certain protein additives, notably the excitability inducing materials of Rudin and Mueller<sup>4</sup>, to produce membranes with some biological properties. Thus either the protein alone or the protein lipid complex is the important factor for converting the insulator properties of lipids to those resembling biological membranes. This paper describes the preparation and properties of specially prepared polyelectrolyte membranes, containing no lipids, which under a d.c. electric field can spontaneously generate

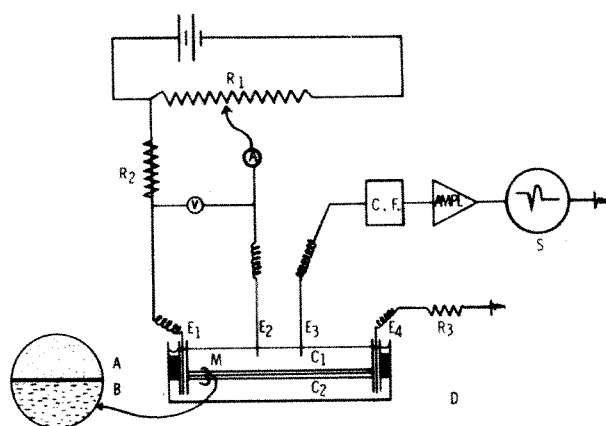


Fig. 1. Experimental arrangement:  $R_1, R_2, R_3$  are 50,000 ohms resistances;  $E_1, E_2, E_3, E_4$  are silver-silver chloride electrodes;  $C_1 = C_2 = 0.15$  normal sodium chloride;  $V$ , voltmeter;  $A$ , microammeter;  $C.F.$ , cathode follower; and  $S$ , scope. Enlargement shows the membrane composition with a polybase layer  $A$ , a polyacid  $B$  and a polysalt interface.

transients with time constants and amplitudes analogous to the spike potentials of neuronal membranes.

The membranes are prepared by reacting a solution of polymeric acid with a polymeric base to give a polymeric salt at the interface. The polyacid, a 10 per cent solution of a co-polymer polyacrylic acid and acrylamide (80/20) in 0.15 normal sodium chloride, adjusted to pH 4.5 with sodium hydroxide, is placed as the lower layer in the Petri dish of the experimental arrangement in Fig. 1 and allowed to react with the polybase, 2 per cent dimethylaminoethyl acrylate, in 0.15 normal sodium chloride, for about 30 min to generate the polyampholyte membrane. The membrane is then carefully washed *in situ* by repeated dilution of the polyacid layer with 0.15 normal saline and draining of the excess fluid. Similarly the upper polybase layer is washed with saline to produce a membrane separating two equal salt solutions (0.15 normal sodium chloride). Silver-silver chloride electrodes are then placed into the electrode holders, and a negative and positive electrode are applied to the polybase and polyacid layer respectively. The system is monitored at the upper surface by means of a third silver-silver chloride electrode, and voltage changes are amplified and displayed as voltage spikes on an oscilloscope. The firing pattern of a given membrane is photographed for further analysis. It is essential that the indicated polarity be maintained and that the membrane should be devoid of imperfections so that it can truly behave as a barrier.

When the d.c. potential is gradually raised, the membrane begins to generate spikes spontaneously at a critical applied voltage. Typical records of the observed membrane activity are shown in Fig. 2. The pattern of firing consists of spikes of varying amplitude (1–120 mV) and duration (the half width of the spikes is between 2 and 10 msec). When the voltage applied to the membrane is raised beyond the critical firing level an increased firing rate is observed and then at a higher applied voltage firing suddenly ceases and there is a constant current flow through the membrane. These voltage variations can be repeated many times without loss of the spontaneous activity properties. Analysis of the shapes of the spikes shows that there is a very fast rise time in the development of a spike followed by a slower decay and inversion of current flow. This reversed current flow usually takes a longer time than the period of the main spike and may last as long as 50 msec.

One of the requirements for the generation of spikes apparently is the presence of salt on both sides of the membrane. Thus distilled water on both sides gives no firing, whereas 0.02, 0.05, 0.15 and 0.2 normal electrolytes on both sides of the membrane give spontaneous spike generation which continues for hours. The critical voltage

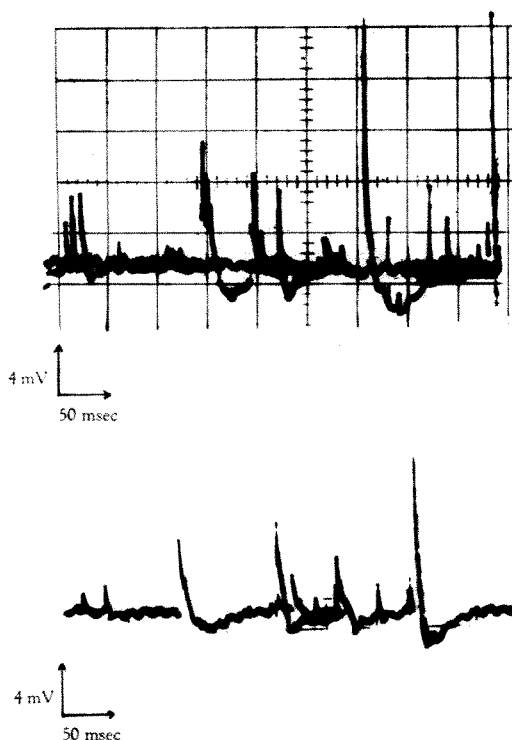


Fig. 2. Oscilloscope traces of spike generated by polyelectrolyte membranes.

for initiation of spikes does not appear to be constant, but varies with each new membrane, which suggests a dependence on the membrane thickness. In a typical experiment, the critical voltage for firing was 0.7 V across the membrane plus electrolyte and the voltage for stopping the firing was 1.3 V. An interesting feature of these membranes is that near the critical firing voltage region, the output of the recording electrode becomes sensitive to mechanical vibrations. This ceases once the firing level is reached.

One variation of this phenomenon is the use of calcium polyacrylate as the membrane component formed by the reaction of a dilute solution of calcium hydroxide with polyacrylic acid at an interface. Such a membrane will also spontaneously generate spikes under a d.c. electric field.

The empirical resemblance of the properties of polyelectrolyte membranes to certain aspects of neuronal membranes is clearly evident. The generation of spikes of the right order of magnitude and shape, the existence of a critical voltage for firing and a higher voltage for cessation of firing suggest that these membranes should be useful models for studying the mechanism of spike generation. Moreover, the simplicity of the model offers a means for independent studies of the "spike" generation mechanisms from those which originate the membrane resting potential of a neurone.

In a recent discussion with Katchalsky, he suggested that current flow through the membrane results in accumulation of salt into the polyampholyte layer, the sodium and chloride ions arriving from opposite sides of the membrane, and at a critical salt concentration the swollen polyelectrolyte membrane shrinks and produces a breakdown region. The electrolyte washes off the excess salt to re-establish the original state of the membrane. Experiments to explore the various physicochemical aspects of these polyelectrolyte membranes and their use as models for biological systems are in progress.

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## RADIOBIOLOGY

### Effect of High Dose Rates on Survival of Mammalian Cells

THE increasing use in radiobiology of pulsed sources of radiation and very high dose rates makes it desirable to study the effects of this type of radiation on biological systems. This paper describes the differences in the survival of cells when exposed to radiation delivered in a single pulse or in two pulses.

The source of radiation was the 15 MeV Mullard linear accelerator at St. Bartholomew's Hospital<sup>1</sup>. It produces an electron beam in pulses lasting 1.3  $\mu$ sec, which can be given either singly or as a series of pulses with a variable repetition frequency. In this work, the output of the accelerator was kept approximately constant and different doses were obtained by placing the samples at different distances from the exit window; thus the exposure time was constant and the dose rate was proportional to the dose. The maximum dose used was 4,500 rads in one pulse, corresponding to a mean dose rate of  $3.5 \times 10^8$  rads/sec.

The dose delivered was measured by using lithium fluoride thermoluminescence dosimetry; this was checked by ferrous sulphate and red 'Perspex' dosimetry<sup>2</sup>. The error in the dose delivered, chiefly caused by the spatial distribution of the beam, was 2 per cent at 200 rads, rising to 10 per cent at 5,000 rads.

The biological system used was a sub-clone of HeLa S-3 cells, obtained from the Radiobiology Laboratory at Oxford. The cells were irradiated in suspension at 25°C, 24 h after collection; they were then diluted and assayed for survival by the Puck technique.

In the first series of experiments, the cell suspensions were corked in polystyrene bottles after routine handling in the presence of air. Irradiations were carried out to give either single pulses or two pulses separated by 2.5 msec. The results are shown in Fig. 1. The solid line is the fitted regression line for the two-pulse data. It can be seen that the single-pulse data follow the two-pulse line very closely up to about 900 rads, but for larger doses the cells irradiated with a single pulse show a greatly reduced sensitivity.

This biphasic nature of the single pulse survival curve is thought to be caused by the removal of oxygen from the relevant site within the cell by its reaction with the radical intermediates formed during the irradiation. As the dose in the pulse is increased the concentration of oxygen will be reduced, but because the cell sensitivity remains almost constant until about 90 per cent of the oxygen in an air-equilibrated system has been removed<sup>3</sup>, the change from oxic to anoxic sensitivity will occur in a relatively small dose range. In this system, the radiobiologically relevant oxygen is apparently removed by a dose of about 900 rads during the 1.3  $\mu$ sec pulse.

In order to check whether the effect observed is indeed caused by the removal of oxygen, a second series of experiments was carried out in which an inert antifoam agent was added to the cell suspension, and either air or



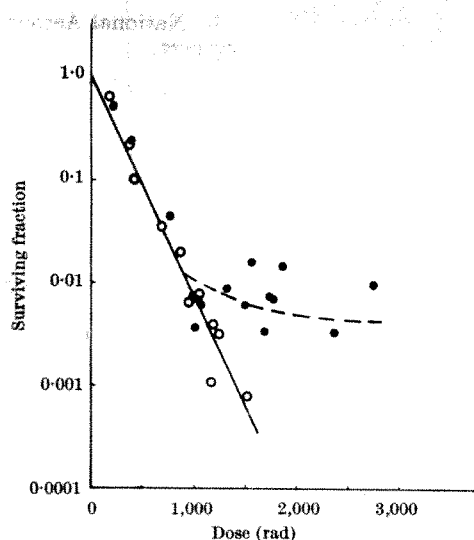


Fig. 1. HeLa cell survival curves following irradiation in static aerobic suspensions with one or two pulses of 14 MeV electrons. (Errors generally smaller than the points.) ●, One pulse; ○, two pulses:  $D_0 = 206$  rads.

nitrogen was bubbled through for 5 min before and during the irradiation. The results are shown in Fig. 2. It can be seen that for irradiation in hypoxic conditions the single and double pulse data both follow the same line which has a slope approximately 2.5 times smaller than that of the line for air. In this case too, the single pulse curve for air changes in slope after a dose of about 1,000 rads.

The fact that the survival curves in hypoxic conditions show no change in cell sensitivity in the range 0–4,500 rads may be taken as confirmation that oxygen depletion during the pulse is responsible for the biphasic nature of the single-pulse air survival curve.

Data from simple air-equilibrated chemical systems<sup>4</sup> suggest that the dose required for oxygen depletion is of the order of 50 krad. The results of Dewey and Boag<sup>5</sup> using pulsed irradiation of bacteria are in agreement with this figure, as also are recent measurements in this laboratory using an oxygen cathode technique. In the mammalian cell system used in the present study, a much smaller dose is required to produce oxygen depletion. This suggests

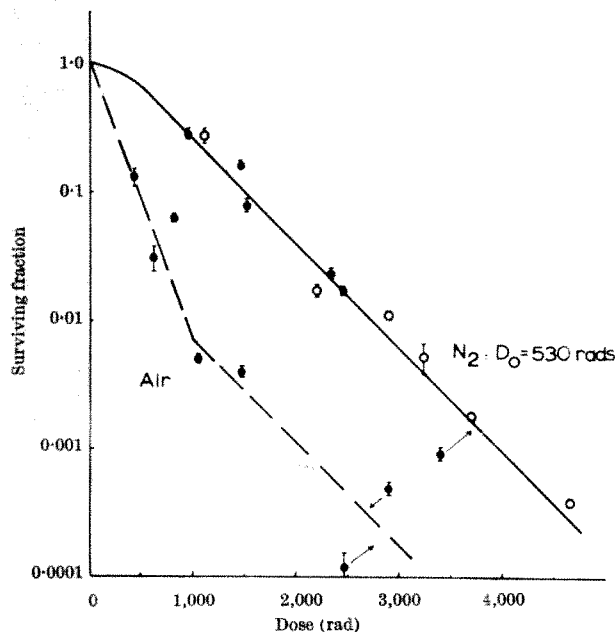


Fig. 2. HeLa cell survival curves under gas equilibration by bubbling air or nitrogen through the suspensions. The initial slope of the broken line is that of Fig. 1, and the second portion was drawn parallel to the nitrogen line. ●, One pulse; ○, two pulses.

that in the vicinity of the sensitive sites within the cell either the equilibrium oxygen concentrations are much lower, or the G-value for the removal of oxygen is much higher than the values in the extracellular medium. In contrast, the results of Dewey and Boag indicate that in the bacterial cell both these parameters are the same as in the surrounding medium. Experiments are in progress to determine total intracellular oxygen content using a Hersch cell.

This investigation was begun in 1965 and it was hoped to extend it by using more intense pulses from a new linear accelerator. This had, however, to be postponed because of a delay in the commissioning of the accelerator.

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### Suppression of Natural Haemolysin Titre in Rabbits exposed Daily to Gamma Radiation

ACUTE whole body X-irradiation of rabbits with 300–700 r. depresses the synthesis of natural anti-sheep (anti-Forssman) haemolysin<sup>1,2</sup>. This serum antibody, which occurs normally, is thought to arise in response to small amounts of related Forssman antigens entering from the rabbit's environment<sup>3</sup>. Recovery begins about 2 weeks after irradiation and normal or enhanced haemolysin titres are usually reached in a few more weeks<sup>2,4</sup>. A second irradiation provokes a second sequence of depression and recovery<sup>4</sup>. This resilience following acute irradiations prompted a study of the extent to which haemolysin production could withstand injury sustained during protracted irradiation with small daily exposures.

Of the twenty-four New Zealand rabbits irradiated, eleven had received an injection of sheep red cells 1 yr before, but the other thirteen had not. The control group comprised thirty-eight non-irradiated rabbits of similar ages and backgrounds. Based on LiF dosimetry, the daily midline air exposure to the cobalt-60 gamma radiation averaged 25 r. delivered at a rate of about 12 r./min. The positions of the rabbits in the radiation field were rearranged daily so that each rabbit received the same total exposure every 24 days. All sera, obtained weekly from each rabbit, were titrated for haemolysin by a microtechnique<sup>5</sup> and some also by a photocolourimetric assay<sup>6</sup>. Titre is given as the  $\log_2$  of the greatest dilution of serum yielding at least 50 per cent haemolysis. All pre-irradiation titres were typical of normal rabbits. Avidity determinations<sup>7</sup> and the slopes of the photocolourimetric titration plots<sup>8</sup> suggested, but did not prove, that the haemolysin present in the previously immunized rabbits was natural haemolysin arising from environmental antigenic stimuli.

Although haemolysin titre usually declined with increasing total exposure, it fell more slowly in the previously immunized rabbits (sub-group A) than in those not previously immunized (sub-group B). Thus in A geometric mean titre declined gradually to 30 per cent of the initial level by 5,000 r.; whereas in B, mean titre decreased abruptly after 1,500 r. to a plateau at 10 per cent of the initial titre (Fig. 1). Titres in six rabbits in sub-group B, but none in A, decreased to less than detect-

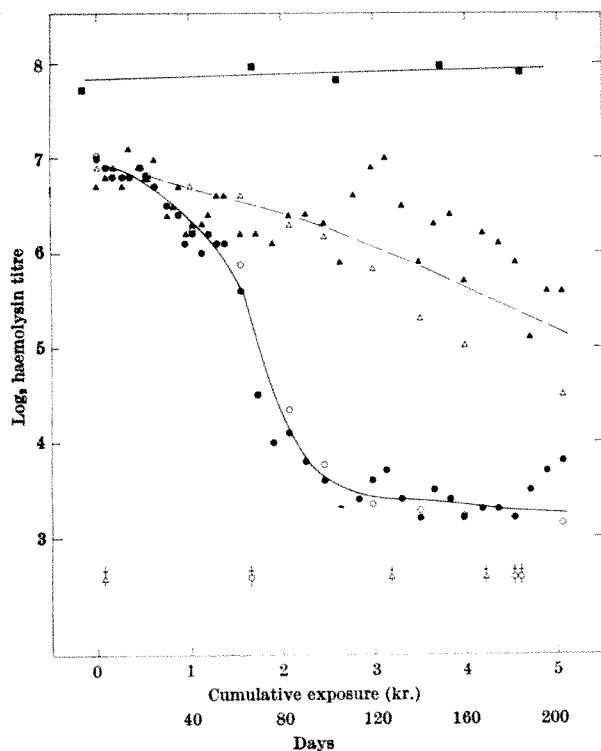


Fig. 1. Mean  $\log_2$  haemolysin titres in gamma irradiated rabbits (25 r./day) previously immunized (triangles) or not previously immunized (circles) with sheep erythrocytes. Squares, unirradiated controls; open symbols, photocolorimetric assay; closed symbols, microtechnique; †, animal deaths.

able levels (although in one antibody reappeared and its titre increased gradually thereafter). Some animals in both sub-groups maintained near-normal titres. The degree of depression was usually independent of initial titre.

Body weight remained fairly constant in eighteen out of twenty-four rabbits which survived 5,000 r. In a separate experiment, the blood lymphocyte count declined 65 per cent after the first 600 r. and decreased only gradually thereafter. There was no marked granulocytopenia.

Five rabbits (two from sub-group A and three from B) had survived an accumulated exposure to 7,400 r. when they were injected intravenously, together with nine controls, with sheep red cells to test their ability to respond to specific challenge. One rabbit from each irradiated sub-group showed no response; the other three produced  $\log_2$  peak titres of 8.5, 10.5 and 11, which were smaller and occurred later than peak titres of most of the controls (mean:  $12.4 \pm 0.9$ ). These results are consistent with those of previous studies involving protracted irradiation and specific immunization<sup>9,10</sup>.

Natural haemolysin titre is probably maintained by a series of small, independent responses in which the meagre influx of environmental Forssman antigens limits antibody synthesis to a level much below the immunological capacity of the rabbit. This minimal demand, together with cellular repair and replacement during the protracted irradiation, could explain the large total exposures needed to depress haemolysin production.

The relative radio resistance of haemolysin production among the rabbits previously immunized with sheep red cells may reflect either an expanded compartment of immunocompetent cells or, through immunological memory, a greater susceptibility to Forssman stimulus. In either case, survival of a smaller proportion of the relevant cell population might have been enough to maintain the greater titres. It is also possible that some of the haemolysin production persisted from the previous immunization.

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## PHYSIOLOGY

### Growth of the Foetus

WE have attempted to formulate a generalized mathematical description of the time course of foetal growth, in order to facilitate comparisons of the metabolic stresses of pregnancy in different species. Previous empirical formulae<sup>1-3</sup> have described parabolic relationships between foetal weight ( $W$ ) and gestational age ( $t$ ) of the type  $W = at^b$ , the value of the exponent  $b$  lying between 3 and 4.

The accuracy of these formulae was greatly improved by McDowell and Allen<sup>4</sup>, who pointed out that embryo age should be calculated not from conception but rather from the end of the "lag" phase of prenatal development. The equation then became

$$W = a(t - t')^b \quad (1)$$

where  $t'$  represents the duration of the lag phase. These authors defined  $t'$  as the time at which the primitive streak is first seen. It might equally well be described, however, as the period of time during which the placenta becomes established because the nature of the nutrient supply must after this be profoundly altered.

Exponential growth equations have been suggested, for example, by Weinbach<sup>5</sup> ( $w = A \exp [k(t - t') - A]$ ), and such forms have been regarded as somewhat less empirical in nature because some attempt is made to endow the constants in the equation with a physical significance, either by reference to a "specific growth impulse" of foetal tissue, or simply by appealing to a universal law of compound interest. The limiting factor in the early stages of growth, however, is likely to be the rate of nutrient supply rather than some internal regulatory mechanism. For this reason, it is interesting to discover to what extent foetal growth can be compared with a model in which (1) the gain in weight is decided by the rate at which nutrients are supplied to the foetus across a surface corresponding to the foetal vascular endothelium; (2) the area of that surface remains in constant proportion to the total surface area of the foetus itself, and will therefore be proportional to  $W^{2/3}$ .

If nutrients are supplied at a constant rate per unit surface, the total rate of supply, and hence the rate of weight gain, will also be  $W^{2/3}$ , that is

$$\frac{dw}{dt} \propto W^{2/3}$$

$$\text{Therefore } \frac{dw}{w^{2/3}} \propto dt,$$

which on integration yields the expression

$$W^{1/3} \propto t$$

or

$$W = at^3$$

where  $a$  is a constant, related to the rate of supply of nutrients per unit of surface area.

Allowing for the lag phase  $t'$  the equation becomes

$$W = a(t - t')^3 \quad (2)$$

It is of interest to note that the same "cubic law" has been used by Klein and Revesz<sup>6</sup> to describe the growth of tumours. In this case also, after an initial lag period, the growth of a mass of tissue cells is controlled by the rate of transport of nutrients across its surface.

To test this relationship, a series of weights of foetuses at different gestational ages for a variety of species was taken from Needham<sup>7</sup>. A Fortran programme was written which used a simple iterative procedure to determine the constants  $a$ ,  $b$  and  $t'$  in equation (2) for each species. (The programme was run on the 'Atlas' computer at the University of London Institute of Computer Science.) The best values of these constants were taken to be those which gave a minimum value for the sum of the squared percentage deviations of observed from predicted weights. Table 1 shows the values obtained by this means. The magnitude of the exponent  $b$  was uniformly close to 3.0 in species the birth weights of which ranged from 0.0014 to 16 kg, and which included the slower growing foetuses of primates.

In Table 1, the values of  $a$  and  $t'$  are those which give the best possible fit to the data when a free choice of

Table 1. CONSTANTS IN THE GROWTH EQUATION  $W = a(t - t')^b$  FOR THE FOETUSES OF SEVERAL SPECIES

Species	$a \times 10^4$	$t'$	$b$
Chick ( <i>Gallus domesticus</i> )	2.6	1.0	3.2
Mouse ( <i>Mus musculus</i> )	1.0	8.0	3.0
Guinea-pig ( <i>Cavia porcellus</i> )	0.6	15.0	3.0
Pig ( <i>Sus scrofa</i> )	0.9	19.0	2.9
Cow ( <i>Bos taurus</i> )	15.0	58.0	2.7
Rhesus ( <i>Macaca mulatta</i> )	5.0	35.0	2.4
Man ( <i>Homo sapiens</i> )	7.0	41.0	2.7

values for  $b$  is allowed in the programme. Further values for  $a$  and  $t'$ , computed with  $b = 3.0$ , that is, with the assumption that the growth equation is cubic, give almost as good agreement with the experimental data. This is illustrated in Fig. 1, in which curves derived from the cubic equation are plotted with the experimental values. It therefore appears that the foetuses of avian and mammalian species, after an initial lag phase, conform to a cubic law of growth, differences in the size of offspring being determined by the rate of transport of nutrients to the foetus, as illustrated in Fig. 1 by the range of values of  $a$ .

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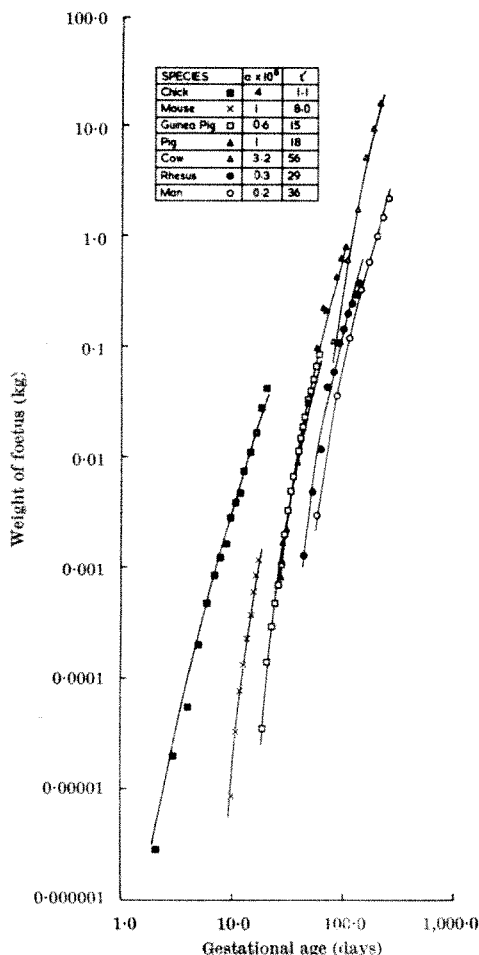


Fig. 1.

## Blocking of Potassium Currents by Pronase in Perfused Giant Axons

ALTHOUGH there is evidence that axonal membrane potentials are related to sodium and potassium movements<sup>1</sup>, there has been little explicit discussion of how these ion movements may be initiated, or of the chemical processes which may permit them to occur<sup>2</sup>. Because it is not possible to identify the molecules involved by measuring potentials and associated ion movements, there is a need for experiments in which the membrane is modified in a known manner and the effects on potentials and ion movements then studied. The molecular dissection of the axonal membrane by internally applied enzymes<sup>3</sup> has shown that most proteases (trypsin, chymotrypsins and papain) block the action potential and induce a considerable increase in sodium and potassium permeabilities<sup>4-7</sup>.

Experiments reported here suggest that the hydrolytic activity of the enzyme pronase (*Streptomyces griseus* proteinase<sup>8</sup>) modify the protein structure of the axonal membrane and that this alteration causes a partial block of potassium currents without affecting sodium currents.

The experiments were carried out on the giant axon of the Chilean squid, *Dosidicus gigas*. Isolated axon fibres were suspended in a bath of potassium-free artificial sea water at a controlled temperature (430 mmolar sodium chloride, 50 mmolar magnesium chloride, 10 mmolar calcium chloride, 5 mmolar *tris* at pH 7.5). The axons were perfused internally by the suction perfusion procedure described in detail elsewhere<sup>9,10</sup>. A steady perfusion rate of about 100  $\mu$ l./min was obtained and an internal electrode was introduced until its tip was half-way along the perfused axon (average length under perfusion about 2.0 cm). The membrane potential was measured between this point and an external reference electrode. Both internal and reference electrodes were silver-silver chloride-potassium chloride cells. The point

control voltage-clamp system was used to measure the ionic currents<sup>11</sup>. A platinum wire was used to supply current along the part of the axon which was being perfused. The wire was insulated except for the 2.0 cm in the perfused zone, which had been plated with platinum black.

Fibres were perfused externally, in potassium-free artificial sea water, and internally with potassium solution (550 mmolar potassium fluoride, 5 mmolar *tris* at pH 7.3) for about 10 min. Next, the fibres were internally perfused with potassium solution containing inactivated pronase at a concentration of 1 mg/ml. (as a control). This solution was kept inside the fibre for about 10 min (in one case for 20 min). The fibres were then perfused with active pronase (Calbiochem, grade A) at the same concentration added to the potassium solution. After 10–15 min (in one case after 30 min) of perfusion with active pronase fibres were perfused again with potassium solution for 10–60 min.

Resting and action potentials were recorded each minute for each experimental condition. Several voltage-clamp series were also run (at least one voltage-clamp series in each case). During the voltage-runs, the membrane was pulsed from the resting potential (about -55 mV) to a series of depolarizing values up to +80 mV.

**Effect of pronase on potentials and on voltage-clamp currents.** The development of plateau action potentials induced by the pronase perfusing solution is shown in Fig. 1. All fibres treated with active pronase (a total of four fibres) developed plateau action potentials and their resting potentials decreased from an average value of -55 mV to -53 mV. Action potential 1A was recorded 1 min before removal of the inactivated pronase (active pronase solution heated at 90° C for 5 min). Action potentials from 1B to 1H were measured every minute after perfusion with active pronase to show the development of the plateau falling phase. Action potential 1H was recorded 30 min after the removal of the active pronase from the perfusing solution.

It should be pointed out here that earlier experiments<sup>5</sup> indicated to us that protein hydrolysis, and therefore protease action on the axonal membrane, could be prevented either by heating the protease solution or by decreasing the temperature of the sea water of the bath while the active enzyme was inside the fibre. The heated protease control demonstrated that the blocking properties observed were not a result of the presence of low molecular

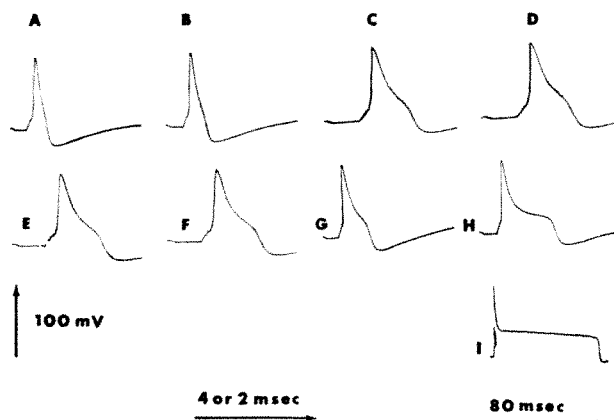


Fig. 1. Membrane action potentials showing the development of a plateau falling phase. Record A was taken 10 min after the start of internal perfusion with inactivated pronase. Record B represents the spike recorded 2 min after the addition of 1 mg/ml. of active pronase to the perfusing solution. For these two records the time scale was 4 msec. Record C was taken 5 min after the addition of active pronase. Records D, E and F show the slow development of plateau membrane action potentials. Each record was taken every minute. The time scale was 2 msec. Records G and H were taken 8 and 10 min after the addition of the enzyme. The time scale was 4 msec. Record I was taken 15 min after the removal of the active enzyme from the perfusing solution. The time scale was 80 msec. The temperature of the external solution was 16° C.

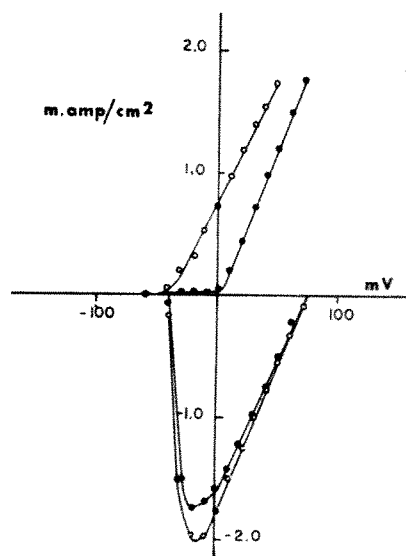


Fig. 2. Current-voltage relationships showing the effect of pronase on potassium currents. Vertical axis represents voltage clamp currents, either maximum sodium inward currents or steady state potassium outward currents. Horizontal axis represents clamped-membrane potentials in mV. ○, Current-voltage relationships measured after 7 min of the addition of the inactivated pronase. ●, Current-voltage relationships measured 25 min after removal of the active pronase from the perfusing solution. The pulse duration was 45 msec. The temperature of the external solution was 16° C.

weight contaminants. It did not show, however, whether or not the enzyme charge (and not the protein breakdown) was responsible for the blocking of the potentials. The low temperature control, on the other hand, seemed to give a good indication of enzyme effect. When looking for a specific inhibitor of the pronase we found that most of them<sup>12</sup> affect the properties of the axonal membrane when applied internally<sup>13</sup>. Although in these experiments it was not possible to use decreased temperatures to inhibit protease, in one axon, kept at a temperature of 10° C (in all others the temperature was between 15° and 17° C), the latency for developing plateau action potentials was 4 min longer.

Two current-voltage relationships in a typical experiment in which the perfusing solution contained first inactivated then active pronase are shown in Fig. 2. This shows current-voltage relationships during internal perfusion with inactivated pronase and during perfusion with potassium solution about 10 min after removal of the active pronase. The potassium currents for depolarizing pulses from 0–60 mV (-60–0 mV absolute potential) are very small. We have measured the "slope potassium conductance"  $G_K$  at different voltages for the current-voltage relationships given in Fig. 2. Plotting the  $G_K$  values obtained as a function of membrane voltage we found that both curves have essentially the same shape. The  $G_K$  curve measured after treatment with active pronase is displaced about 60 mV along the voltage axis.

These data on four giant axons internally treated with pronase demonstrate that this protease induces a block of the "turning on" of the potassium currents without affecting the sodium currents. As a consequence of this, membrane action potentials developed a plateau falling phase as has been shown before in other experimental situations<sup>14</sup>. Pronase is a very unspecific proteolytic enzyme<sup>8</sup>, and little can be learned about the nature of the peptide links involved in this phenomenon. Our results suggest that either the removal of protein or peptides, and/or the splitting of some proteins, modify the structure of the axonal membrane and that this in turn induces a shift of the  $G_K$  voltage function along the voltage axis. In the latter case the appearance of previously unexposed ionizing groups could modify the charge density



of part of the axonal membrane responsible for the  $G_K$  "turning on", thus shifting the  $G_K$  voltage function along the voltage axis. When protein is removed the remaining structural lipids themselves may generate a high potassium resistance membrane, similar to that observed in artificial protein-lipid membranes<sup>15</sup>.

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### Comparison between Tetrodotoxin Effects on Excitation in Mammalian Cardiac Fibres and Squid Giant Axons

BECAUSE tetrodotoxin (TTX) was thought to "selectively" block excitatory inward sodium currents in squid giant axons<sup>1,2</sup>, Dudel *et al.* used TTX as an "independent check" of sodium currents in mammalian Purkinje fibres<sup>3</sup>. Unfortunately, TTX analysis of membrane currents is no longer possible because recent evidence suggests that neither TTX nor the excitation process is specific for sodium.

Excitation of squid giant axons in media free from sodium is not significantly different from excitation in media containing sodium<sup>4-6</sup>. "Heart-like" action potentials with typical spike and plateau phases are observed under many conditions<sup>4-7</sup>, but are characteristic of excitation in external media free from sodium which contain only salts of divalent cations<sup>5,8</sup>. Such action potentials are usually as easily abolished by TTX as those in media containing sodium<sup>5,9</sup>. The presence of sodium is therefore not essential for excitation, and TTX is not specific for any particular ion. It follows that TTX cannot be used to separate the "sodium current" from other ionic currents during excitation.

Because apparently normal excitation takes place in media free from sodium, and because TTX abolishes the inward current regardless of which ions may be carrying that current, a much more general mechanism than a "sodium-specific carrier" or "sodium-specific pore or channel" is required to understand the excitation process. The "macromolecular model" of the excitation process permits such non-specificity of the participating ions, and can thus account for the effects of TTX in both sodium-containing and sodium-free media<sup>9,10</sup>. (Extensive descriptions of the macromolecular model are provided elsewhere<sup>9-11</sup>.)

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### Increase of Cerebral Catecholamines caused by 3,4-Dihydroxyphenylalanine after Inhibition of Peripheral Decarboxylase

N-(DL-seryl)-N'-(2,3,4-trihydroxybenzyl)-hydrazine (HCl) (Ro 4-4602) is a strong inhibitor of decarboxylase of aromatic amino-acids *in vivo* and *in vitro*<sup>1,2</sup>. The drug counteracts the increase of 5-hydroxytryptamine which is induced by 5-hydroxytryptophan less markedly in the brain than in extracerebral organs. Small doses of Ro 4-4602 have even seemed to enhance this transformation in the brain; this effect could not be explained<sup>3,4</sup>.

This communication shows that small doses of Ro 4-4602 markedly enhance the increase of cerebral catecholamines induced by 3,4-dihydroxyphenylalanine (DOPA) because of a selective inhibition of extracerebral decarboxylase.

Male Wistar rats weighing 250-280 g, fasted for 16 h, were injected intraperitoneally with 50 mg/kg of Ro 4-4602 and 30 min later with 375  $\gamma$ /rat of DL-2,3-<sup>3</sup>H-DOPA (specific activity 101.2 mc./mmole). Rats given tritiated DOPA alone served as controls. After decapitation at various times following administration of labelled DOPA, blood plasma was deproteinized with perchloric acid, and the brains and hearts were homogenized in the same acid. The supernatants were brought to pH 8.4 and passed through columns of alumina (Woelm, neutral)<sup>5</sup>. Tritiated DOPA and tritiated catecholamines, eluted with 0.2 normal hydrochloric acid and adsorbed on 'Dowex-50 WX-4' in the sodium ion form (200-400 mesh)<sup>6</sup>, were separated by differential elution using 0.1 normal potassium acetate buffer, pH 6, and 2 normal hydrochloric acid, respectively<sup>7</sup>. In other experiments the action of Ro 4-4602 on the activity of decarboxylase in brain and heart of rats was measured. The procedure involved homogenization of tissue in four volumes of 0.05 molar phosphate buffer, pH 7.4, and incubation of the homogenate in Warburg flasks with  $1.5 \times 10^{-5}$  molar DL-DOPA-1-<sup>14</sup>C (specific activity 4.4 mc./mmole) at 37° C for 1 h. The reaction was stopped with 6 normal sulphuric acid, and the labelled carbon dioxide, trapped in the ethanol amine of the centre well, was measured in a liquid scintillation counter after addition of ethyleneglycolmonomethylether. Ro 4-4602 was either injected intraperitoneally 1 h before killing or added to the homogenate. Fig. 1 shows that the ratio of the concentrations of tritiated DOPA in animals treated with Ro 4-4602 versus control animals is increased in brain, blood and heart for at least 4 h. The maximal ratios are 23, 10 and 7.8, respectively. The ratio of the concentration of cerebral tritiated-catecholamines in treated animals versus controls shows a continuous increase up to 58 after 4 h. In the heart, however, the tritiated catecholamine ratio remains considerably less than 1. The measurements of decarboxylase activity made using small amounts of labelled substrate confirm and complete the earlier findings with the manometric method<sup>2</sup>. Accordingly, the decarboxylase activity in the brain of animals injected with Ro 4-4602 is not significantly inhibited ( $P > 0.05$ ) up to 100 mg/kg of the drug, whereas in the heart there is 83 per cent inhibition with 50 mg/kg (Fig. 2).

These results indicate that relatively small doses of Ro 4-4602 (50 mg/kg) cause a selective inhibition of decarboxylase in extracerebral organs like the heart. This probably leads to a diminished formation of tritiated catecholamines and to an increased accumulation of tritiated DOPA in the peripheral tissues. Consequently, the penetration of  $^{14}\text{C}$ -DOPA into the brain seems to increase, and because 50 mg/kg of Ro 4-4602 do not appreciably inhibit cerebral decarboxylase, the formation of tritiated catecholamines in the brain is probably enhanced. Preliminary findings in animals pretreated with 50 mg/kg of Ro 4-4602 indicated that the cerebral tritiated catecholamines increase to 1.4 and 5  $\gamma$ /g 1 h after 16 mg/kg and 150 mg/kg of DOPA, respectively. Thus the catecholamines consist mainly of dopamine (about 80 per cent to 90 per cent) and to a smaller part of norepinephrine (about 10 per cent to 20 per cent). Preferential inhibition of extracerebral decarboxylase might also explain the unexpected enhancement of the DOPA-induced accumula-

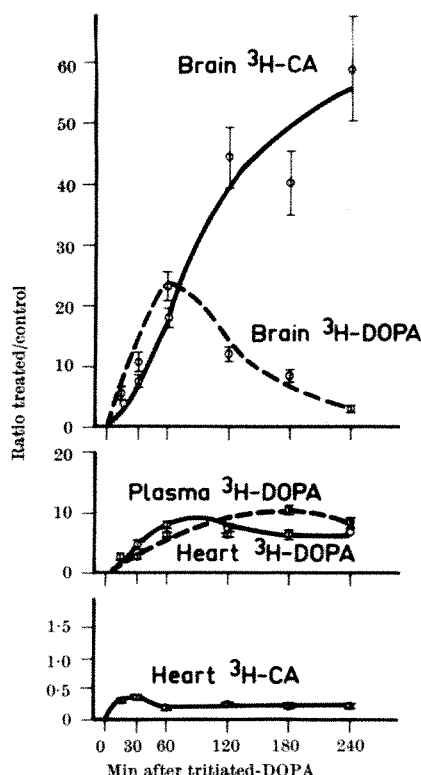


Fig. 1. Ratio of tritiated DOPA and tritiated catecholamines in treated versus control rats. Each control was injected intraperitoneally with 375  $\gamma$  of tritiated DOPA alone; the treated animals were administered 50 mg/kg of Ro 4-4602 intraperitoneally followed by 375  $\gamma$  of tritiated DOPA by the same route after 30 min. The points represent averages of three to five experiments  $\pm$  standard error.

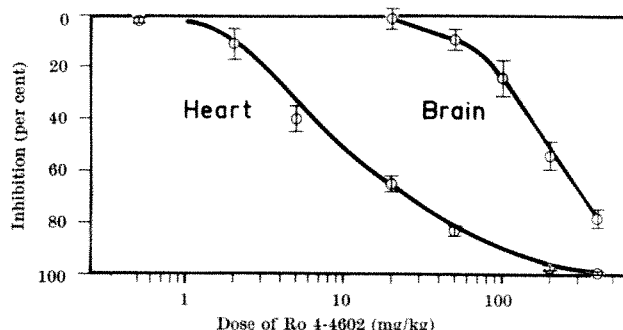


Fig. 2. Inhibition of decarboxylase of aromatic amino-acids in brain and heart of rats 1 h after intraperitoneal injection of Ro 4-4602. Total homogenates (2 ml.) were incubated for 1 h with 3  $\mu\text{g}$  of  $^{14}\text{C}$ -DOPA. The points represent averages  $\pm$  standard error of three to five duplicate determinations of a pool derived from two to five animals.

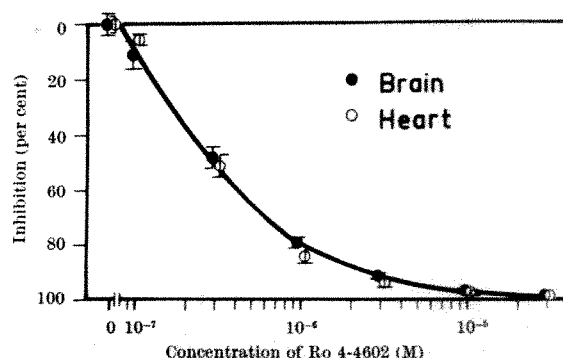


Fig. 3. Activity of decarboxylase of aromatic amino-acids inhibited by Ro 4-4602 *in vitro* (rats). Total homogenates (2 ml.) were incubated for 1 h with 3  $\mu\text{g}$  of  $^{14}\text{C}$ -DOPA. The points represent averages of four to six determinations  $\pm$  standard error.

tion of cerebral catecholamines reported with another inhibitor of decarboxylase, 3,4-dihydroxy-DL-hydrazino-phenylalanine<sup>8</sup>.

The relatively weak inhibitory effect of intraperitoneal Ro 4-4602 on the cerebral decarboxylase possibly results from poor penetration of this drug into the brain. Thus Ro 4-4602, when added to tissue homogenate, causes the same degree of inhibition of decarboxylase in the brain as in the heart (Fig. 3).

In conclusion, Ro 4-4602, owing to a preferential action on extracerebral decarboxylase, increases the content of tritiated DOPA in the blood and consequently seems to increase the supply of the brain with the amino-acid. This probably leads to a marked accumulation of catecholamines (especially dopamine) in the brain but not in the periphery and may have a beneficial effect in neurological disorders such as Parkinsonism.

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### Concurrent Measurement of Renin and Angiotensin in the Circulation of the Dog

It has been known for many years that, following a large haemorrhage, dog plasma develops the ability to stimulate smooth muscle *in vitro*<sup>1,2</sup> and to increase the blood pressure when injected into anephric dogs<sup>3-6</sup>. These experiments suggested that the concentration of renin and angiotensin in the blood might be increased in such circumstances, and this has been confirmed in more recent studies, which have shown that both the concentration of angiotensin in the blood<sup>7-9</sup> and the concentration of plasma renin<sup>10</sup> are increased after heavy bleeding in the dog.

We report here an extension of this work in which changes in the concentration of plasma renin, the rate of generation of angiotensin and the concentration of angiotensin in the blood were measured concurrently.

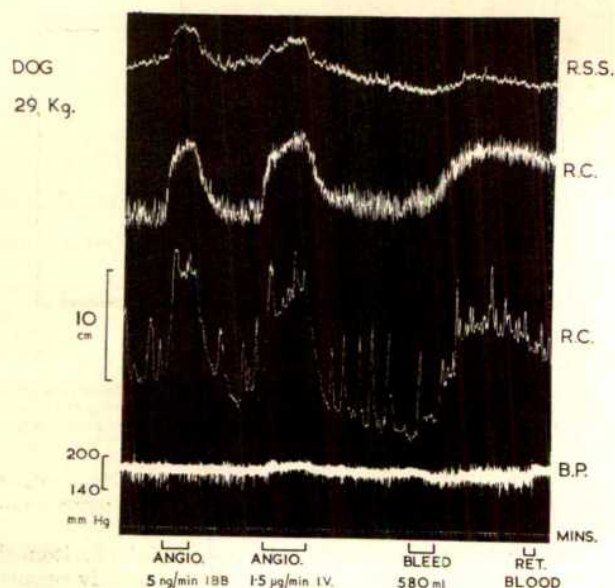


Fig. 1. Dog 2. The responses of three blood-bathed tissues to (i) angiotensin infused at 5 ng/min for 5 min into the assay circuit (rate of flow 15 ml/min); (ii) angiotensin intravenously infused at 1.5 µg/min for 9 min into whole dog; (iii) 580 ml. bled from artery for 5.5 min; (iv) return of shed blood over 2 min. The tissues were a rat stomach strip (R.S.S.) and two rat colons (R.C.), the lower of which was treated with propranolol. The record of arterial blood pressure is also included.

Anaesthesia was induced with ether or halothane and maintained with chloralose and pentobarbitone as in previous experiments<sup>8,9</sup>. The dogs were respired mechanically and given intravenous heparin (1,000 i.u./kg).

The concentration of renin was measured in arterial plasma after an extraction procedure which eliminates detectable endogenous substrate, angiotensinases and accelerators and inhibitors of the renin/substrate reaction. Replicate estimates using this method vary with a standard deviation of 11.8 per cent<sup>11,12</sup>.

The concentration of angiotensin in arterial blood was estimated by the "blood-bathed organ" superfusion technique<sup>13,14</sup>. Changes of angiotensin from the initial concentration were measured, because the absolute concentration can only be derived from the fall in the tissue baseline occurring after bilateral nephrectomy<sup>9</sup>. The response of the assay organs was calibrated either in terms of the concentration of angiotensin in the blood by infusing angiotensin (synthetic asp<sup>1</sup>-NH<sub>2</sub> val<sup>5</sup> octapeptide, CIBA) at a known rate into the blood passing over the assay organs; or in terms of the rate of generation of angiotensin by intravenously infusing angiotensin at a

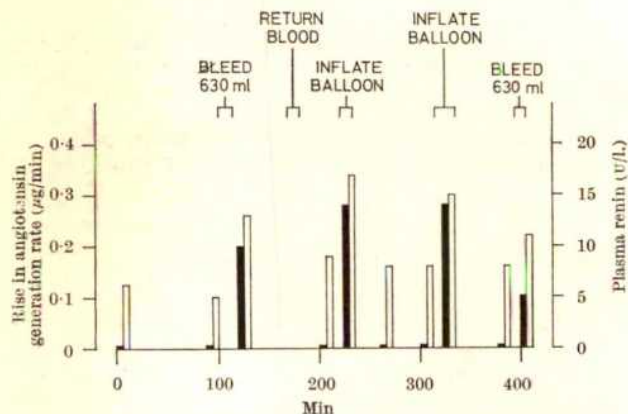


Fig. 2. Dog 6. The changes in the concentration of plasma renin and the rate of generation of angiotensin associated with arterial bleeds of 630 ml., the return of the blood, and the inflation of an aortic balloon just above the renal arteries to reduce renal arterial pressure. (Angiotensin, closed columns; renin, open columns.)

Table 1

Dog	Wt. (kg)	Procedure	Time (min)	Plasma renin (u/l.)	Incr. in angiotensin generation rate (from 1st period) (µg/min/dog)	Incr. in angiotensin blood concentration (from 1st period) (ng/100 ml.)
(1)	33	i.v. Dextran 330 ml.	0	3.5	0	0
			20	175	7.5	0
		Haemorrhage 850 ml.	180-192	200	43.0	0.5
			295	75.0	0.5	6.6
(2)	29	Haemorrhage 580 ml.	0	7.0	0	0
		Return blood	5-11	21	24.5	1.2
			29-31	82	10.5	—
(3)	31	Haemorrhage 600 ml.	0	11.5	0	0
			120-130	140	31.0	—
(4)	25	Dextran 250 ml.	0	9.0	0	0
			4	65	3.0	0
		Aortic balloon inflated; renal art. pressure reduced from 160 mm to 90 mm	130	—	—	—
			140	15.5	0.1	5
		Aortic balloon deflated	142	—	—	—
			218	5.0	0	0
		Haemorrhage 500 ml.	232-245	255	9.0	0
		Return blood	265	—	—	—
		Inflate balloon as before	275	—	—	—
			285	8.0	0	0
		Deflate balloon	295	—	—	—
(5)	25	Dextran 250 ml.	0	4.5	0	0
			5-7	50	4.0	0
		Inflate balloon; fall in renal art. pressure from 150 mm to 70 mm	90	—	—	—
			100	25.0	0.5	13.3
		Deflate balloon	102	—	—	—
			190	15.0	0	0
(6)	21		0	6.25	0	—
			90	5.0	0	—
		Haemorrhage 630 ml. (art. pressure reduced from 160 mm to 140 mm)	100-115	—	—	—
			120	13.0	0.2	—
		Return blood	165-175	200	9.5	0
			208	—	—	—
		Inflate balloon; renal pressure reduced from 150 mm to 50 mm	—	—	—	—
			220	17.0	0.3	—
		Deflate balloon	222	—	—	—
			255	8.0	0	—
			300	8.0	0	—
		Inflate balloon; renal pressure reduced from 150 mm to 80 mm	306	—	—	—
			320	16.0	0.3	—
		Deflate balloon	325	—	—	—
			380	8.0	0	—
		Haemorrhage 630 ml.	385-390	—	—	—
			400	11.0	0.1	—
(7)	17		0	11.0	0	0
			10	16.0	0	0
		Haemorrhage 340 ml.	15-20	—	—	—
			25	16.0	0	0
(8)	27	Dextran 270 ml. i.v.	0	21.0	—	0
			5	13	13.5	0
			65	18.0	0	0
		Haemorrhage to:	85	—	—	—
		420 ml.	113	15.5	0.1	—
		570 ml.	123	15.5	0.15	—
		750 ml.	135	30.0	0.2	13.0
		Return blood	135-140	—	—	—
			160	20.0	0	0
			290	36.0	0.3	0

known rate (Fig. 1). The possibility that this method may overestimate the rate of generation of angiotensin by up to 10 per cent is discussed elsewhere<sup>15</sup>. The superfusion assay method itself also has a random error of similar size.

The experimental stimuli used, as in previous studies<sup>8-10</sup>, were: (a) bleeding from an artery; (b) expansion of the blood volume; (c) marked reduction in renal arterial pressure by inflation of a balloon in the aorta just above the origins of the renal arteries.



The results in the eight dog studies are summarized in Table 1, and are shown graphically in Fig. 2 for the most prolonged experiment (dog 6). In general, there was good correlation between changes in the concentration of rennin, the rate of generation of angiotensin and the concentration of angiotensin in the blood.

Occasional divergences were encountered. Thus in dog 1 an increase in concentration of plasma rennin from 43 U/l. in sample 3 to 75 U/l. in sample 4 was not accompanied by a detectable change in angiotensin. Another discrepancy occurred between the third and fourth samples from dog 8, where the rate of generation of angiotensin increased while rennin fell slightly. Usually, however, in individual dogs, a given change in the concentration of plasma rennin was accompanied by a roughly constant proportional change in the rate of generation of angiotensin and in the concentration of angiotensin in the blood.

The findings suggest therefore that the acute changes in circulating angiotensin observed in these experiments resulted largely from changes in the concentration of the enzyme rennin in blood. The magnitude of the change in angiotensin, however, could well be subject to other influences, which might vary considerably from one animal to another, but which in individual dogs do not alter acutely. Differences in concentration of substrate, angiotensinase activity, and activators and inhibitors of the rennin/substrate reaction may all be important in determining this difference between individuals.

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## Rennin and the Gastric Secretion of Normal Infants

RENNIN is known to be secreted in the fourth stomach of the calf and probably in the stomachs of other young ruminants<sup>1,2</sup>. In the calf it is accompanied by the secretion

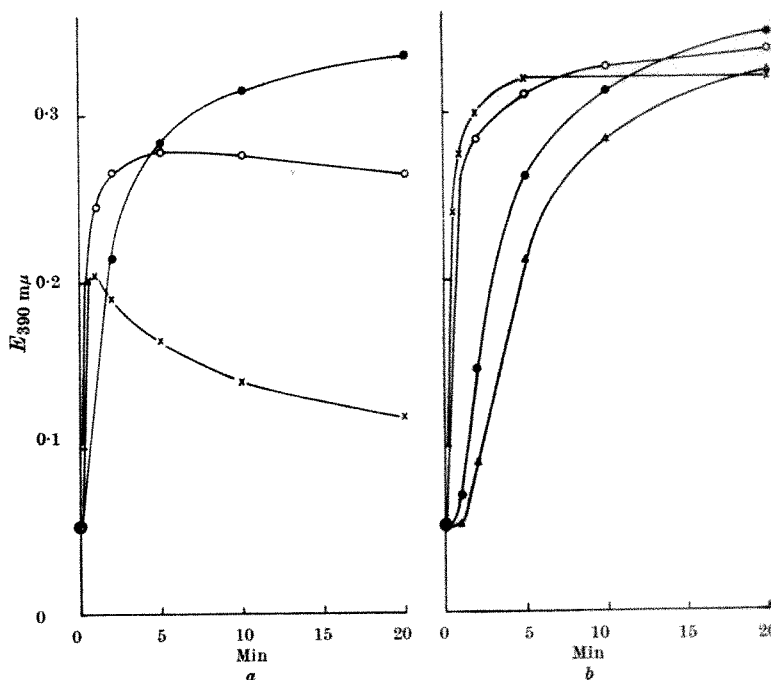


Fig. 1. The action of crystalline pepsin (a) and crystalline rennin (b) on human whole casein. 0.1 ml. pepsin or rennin solution was added to 3 ml. 0.1 per cent casein in acetic acid-sodium acetate buffer (pH 5.65;  $I=0.1$ ) at room temperature. Added enzyme concentrations (per cent):  $\times$ , 0.1;  $\circ$ , 0.01;  $\bullet$ , 0.001;  $\triangle$ , 0.0001.

of pepsin and the ratio of rennin to pepsin decreases as the calf grows<sup>3,4</sup>. The two enzymes can be distinguished by their different pH optima and by the fact that while both have strong milk-clotting powers the proteolytic activity of rennin is comparatively very slight.

The gastric juice of the human adult contains other proteolytic enzymes besides pepsin but none of these has the milk-clotting power which would be expected of rennin<sup>5-8</sup>. By analogy with the secretory pattern in the calf, however, it is in the gastric juice of infants that rennin secretion in the human might be most hopefully sought. Little work seems to have been published in this field apart from a report by Andersen<sup>9</sup> that the ratio of milk-clotting to proteolytic activity in gastric juice samples taken from infants 2-7 months old was the same as that of adults, a finding which suggests that these babies were not secreting rennin.

I have studied the problem of the secretion of rennin in the stomachs of still younger infants, both premature and full term, using human casein as the substrate for both pepsin and rennin. The age range was from birth to 6 weeks old. Activity was measured by the increase in turbidity caused by either enzyme acting on the substrate at pH 5.65; this property is dependent on the  $\alpha$ -casein fraction of whole human casein and does not require the presence of calcium ions<sup>10</sup> (Fig. 1a and b). A differential analysis was based on the different rates of destruction of the two enzymes in mildly alkaline solution (pH 9) at room temperature; although rennin is itself inactivated at pH values greater than 7.0 (ref. 2 and 11), the present investigation shows that the rate of its inactivation is much slower than that of pepsin (Fig. 2). As a result of preliminary tests a standard time of 15 min was adopted for the alkaline treatment. It was established that rennin could coexist for several hours with pepsin in conditions likely to be met in samples of gastric juice and still be readily detected by the method (Fig. 3), and that the identification of 0.0002 per cent rennin in the presence of a fifty-fold excess of pepsin (0.01 per cent) presented no difficulty.

Samples of gastric juice were aspirated from the stomachs of both fasting and fed infants. The samples were freed



from mucus and particulate matter by centrifuging, and the clear supernatants were decanted and used in the experiments. Initial pH measurements were obtained using a glass electrode which entailed the dilution of samples smaller than 2 ml. In nearly every sample examined the initial pH was less than 5, and because Foltmann<sup>2</sup> has shown that rennin is rapidly formed from prorennin at pH

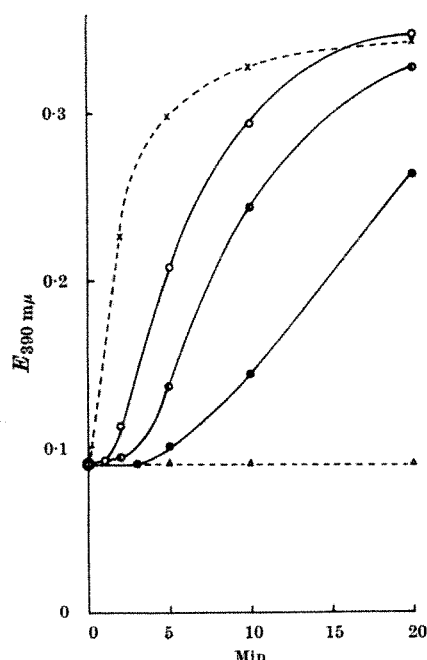


Fig. 2. The action of crystalline pepsin and crystalline rennin on human whole casein before and after adjusting the enzyme solutions to pH 9. The treatment with alkali was stopped at different time intervals by adding aliquots of the enzyme solutions to equal volumes of acetic acid-sodium acetate buffer (pH 5.65;  $I=0.1$ ). Turbidimetry measurements were carried out in the conditions given in Fig. 1. The concentration of added enzyme was 0.001 per cent in all experiments. Duration of alkali treatment: pepsin (---),  $\times$ , 0 min;  $\Delta$ , 10 min; rennin (—),  $\circ$ , 0 min;  $\bullet$ , 20 min;  $\bullet$ , 60 min.

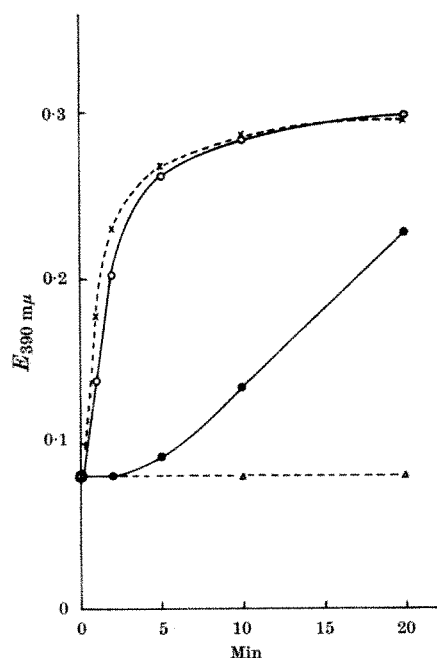


Fig. 3. The action of crystalline pepsin and crystalline rennin on human whole casein; conditions as in Figs. 1 and 2. Solutions were stored overnight at pH 5.65 and room temperature before testing. Pepsin 0.001 per cent (---),  $\times$ , before alkali treatment;  $\Delta$ , after alkali treatment (pH 9; 15 min). Pepsin 0.0005 per cent plus rennin 0.0005 per cent (—),  $\circ$ , before alkali treatment;  $\bullet$ , after alkali treatment (pH 9; 15 min).

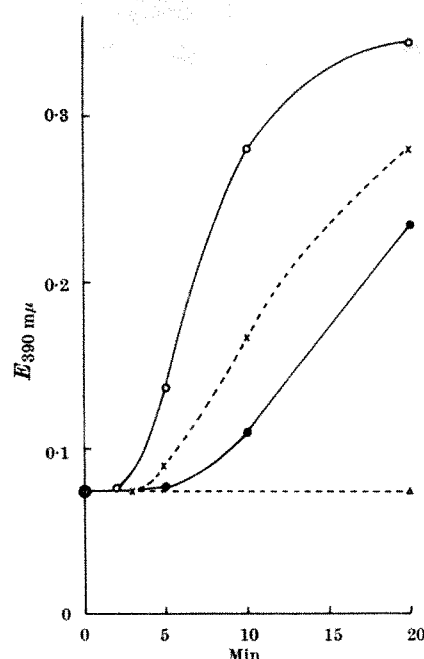


Fig. 4. Turbidimetry response to a gastric juice sample. ---, Native sample;  $\times$ , before alkali treatment;  $\Delta$ , after alkali treatment (pH 9; 15 min). —, Sample plus rennin (final concentration of rennin 0.001 per cent);  $\circ$ , before alkali treatment;  $\bullet$ , after alkali treatment (pH 9; 15 min).

values below 5, this conversion may be judged to have been favoured in the samples reported here.

In no sample was any evidence found for the presence of rennin. Treatment with alkali invariably led to the complete annulment of the turbidimetry response.

The possibility that rennin, although able to coexist with pepsin in pure solution, might be more rapidly destroyed in gastric juice, was tested by adding known amounts of rennin to samples of gastric juice (Fig. 4). Rennin activity was satisfactorily demonstrated, although losses were rather greater than those encountered in pure solution. It was apparent, however, that the accelerated destruction did not invalidate the method and very low concentrations of added rennin ( $<0.001$  per cent) could still be detected with ease.

Results confirming the absence of rennin from gastric secretions of infants were obtained by an alternative method in which the increase in turbidity in casein solutions at pH 7.2 in the presence of calcium ions was used as an index of rennin activity. Under these conditions pepsin is inactive. In practice the method proved less reliable and less sensitive than that which depends on the destruction of pepsin at pH 9; it was not suitable for the detection of rennin in amounts  $<0.01$  per cent.

Twenty-two samples were examined: sixteen by the method of treatment with alkali (seven from full-term and nine from premature babies; age range at sampling, 0–31 days); six by turbidimetry at pH 7.2 (one full-term and five from premature babies; age range at sampling, 0–43 days). No rennin activity was detected in any sample. Non-rennetic activity expressed in terms of pure pepsin, and uncorrected for the dilution by milk in the case of fed infants, ranged from nil (three samples) to about 0.005 per cent. The mean value for thirty-three samples of gastric juice from human adults recently examined by Texter *et al.*<sup>12</sup> was 0.084 per cent.

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### Diurnal Cycle in Serum Concentrations of Follicle-stimulating Hormone in Men

A DIURNAL rhythm in the activity of human follicle-stimulating hormone (FSH) has been recognized by the use of a sensitive and specific radioimmunoassay technique<sup>1</sup>.

In one study we used twenty-three healthy men, aged from 22 to 38 yr. Venous blood (provided by courtesy of Dr George G. Jackson of the Department of Medicine, University of Illinois) was collected on 2 successive days at 5.30 a.m., when each subject was awakened from sleep, and at 2.30 p.m. The subjects for the second study were three healthy hospital interns (24–27 yr) carrying out normal hospital routines. One doctor (K. A.) slept from 1 a.m. to 6 a.m. and his colleagues from 3 a.m. to 6 a.m. Samples were collected at intervals of 4 h throughout the day, and all subjects were awakened for sampling at 5 a.m. All blood samples were allowed to clot and were then centrifuged. Sera were stored at  $-20^{\circ}\text{C}$ . Samples of 0.5 ml. were analysed in duplicate.

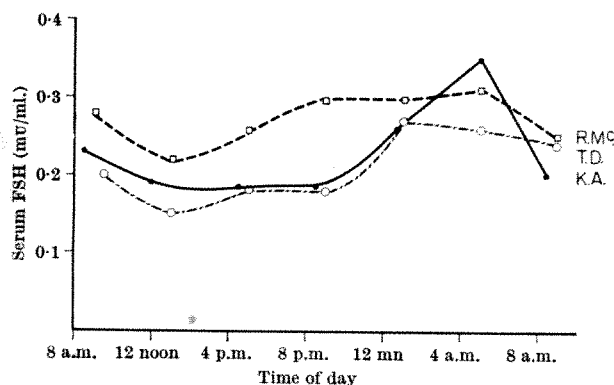


Fig. 1. Variation in serum concentrations of FSH during 24 h.

The results are expressed in mU/ml. of serum (1 U equals the activity in 1 mg of the S1 ovine standard for FSH from the National Institutes of Health, Endocrine Study Section, Bethesda, Maryland). Forty-five pairs of sera from the twenty-three subjects were studied (specimens of one day from one subject were lost). The mean concentrations at 5.30 a.m. were  $0.201 \pm 0.016$  (standard error) and at 2.30 p.m. were  $0.146 \pm 0.018$ , with the overall mean being  $0.174 \pm 0.012$ . The mean error of duplicate determinations was  $\pm 11.1$  per cent. Thirty of the forty-five pairs of sera were greater in the morning, six showed no change and nine were greater in the afternoon. The difference between morning and afternoon concentrations was statistically significant (*t* test for paired data:  $P < 0.05$ ). There were no significant differences in the concentrations at comparable time periods of the 2 days of the study.

The results of the study of the three subjects with multiple samplings during 24 h are shown in Fig. 1. It

can be seen that the concentrations were greatest in the early morning, near 5 a.m., and that they decreased between noon and 4 p.m. These data were examined for the presence of a diurnal cycle by an analysis of variance technique as previously described<sup>2</sup>. The results were very significant ( $P < 0.001$ ). No diurnal cycle was seen in concentrations of luteinizing hormone (LH) measured by a specific radioimmunoassay for human LH similar to the one used for FSH (in preparation).

The timing and amplitude of this FSH cycle resembles that in plasma testosterone<sup>3</sup> and also the well known rhythm in serum concentration of adrenocorticotrophic hormone<sup>4</sup>, but the possible causal connexions among these rhythms are unknown.

The finding of a diurnal cycle in serum concentrations of FSH in men can be added to an already growing list of physiological phenomena showing a diurnal rhythm<sup>2,5</sup>.

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### Carbon Dioxide Laser Hazards to the Eye

LASER radiation in the infra-red, for example from a carbon dioxide laser, is heavily absorbed in the surface layer of any biological tissue. The laser beam interacts strongly with tissue to produce heat, and thermal lesions can easily occur in the bare skin on the hands and the face. Protective reflex mechanisms will prevent long accidental exposures of the skin to a continuous infra-red laser, as they do to any other source of heat.

In the eye carbon dioxide laser radiation is absorbed by the cornea. The corneal blink reflex, released after a latent period of 80 msec, acts to close the eyelids, but it is slow enough to make even low power carbon dioxide lasers dangerous to the eye. Heat coagulation causes irreversible damage to the cornea which can impair vision. Because the infra-red radiation has a very small penetration depth, the damage will be localized chiefly in the surface layers of the cornea. This is also true of damage caused by ultra-violet radiation, which is also heavily absorbed by tissues. On the other hand, the outer parts of the eye are transparent to visible and near infra-red radiation. Laser hazards with such radiation are connected with the focusing properties of the optical system of the eye, and the retina may be damaged.

We have studied eye hazards from carbon dioxide laser radiation ( $\lambda = 10.6 \mu\text{m}$ ) in rabbits. A conventional laboratory model carbon dioxide-nitrogen-helium laser with a maximum single mode power of 4 W was the source. A rabbit was placed about 3 m from the laser exit mirror, and a beam splitter and calibrated bolometer monitored the beam power. The absolute power/unit area at the position of the rabbit's eye could be checked calorimetrically between exposures. The measured intensity distribution across the beam is Gaussian, and we used for the power/unit area its average value inside the  $1/e^2$  intensity circle. The average thus defined is 43 per cent of the maximum value. The power level could be varied in steps, through precalibrated damping filters. A calibrated camera shutter set the short



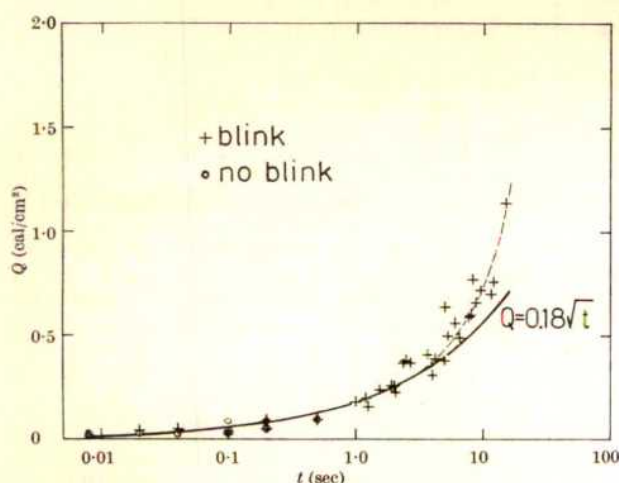


Fig. 1. Energy dose  $Q$  required to release a blink reflex, as a function of exposure time  $t$ .

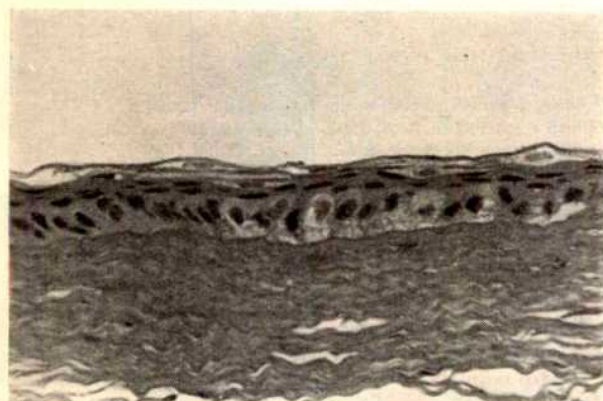


Fig. 2. The corneal epithelium with coagulation of the cytoplasm of the basal cells and some superficial cells. Stained with haematoxylin and eosin.

exposure times at fixed values, and long exposures were measured with a stop watch.

The corneal blink reflex seemed to respond in a very reproducible way to exposure to the laser radiation, and so we used it as an indication of a heat sensation in the cornea. In one series of experiments, we measured the time interval between the start of an exposure and the occurrence of a blink, at various levels of beam power. In another series, we determined the time required for visible damage, caused by heat coagulation, to appear on the cornea.

The energy dose,  $Q$ , sufficient to release a blink reflex is plotted against exposure time,  $t$ , in Fig. 1. In the range 0.01–5 sec the experimental curve follows the relationship  $Q = k\sqrt{t}$ . This fits a simple thermal model, with heat conduction from the surface as the principal means of loss. The release of a blink reflex then corresponds to a definite increase in the corneal surface temperature. With long exposures, the experimental curve deviates from its theoretical course, and we attributed this to the onset of additional loss mechanisms. At very low levels of power such loss mechanisms will prevent the corneal surface temperature from reaching the value necessary for the release of a blink reflex. In the simplest case we expect at long exposures a  $t$ -rather than a  $\sqrt{t}$ -dependence.

The experimental curve relating damage dose and exposure time has a similar shape, but the threshold level is roughly a factor of two above the blink reflex threshold. For an exposure time of 1 sec the damage will be barely visible if the delivered energy is 0.3 cal/cm<sup>2</sup>.

Histology of the laser irradiated cornea showed some features typical of threshold damage which are shown in Fig. 2.

We conclude that the radiation from carbon dioxide lasers must be considered dangerous to the eye, because even low power radiation may cause irreversible changes in the cornea, which lead to impaired vision. Reflexes from metal surfaces, which have very high reflectivity at 10  $\mu$ m, may be nearly as harmful as the direct beam. Ordinary glasses, with side covers, absorb the radiation and are therefore recommended to all personnel working with these lasers. The blink reflex may offer some protection at very low power levels, but should not be relied on.

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### Critical Closing Pressure in Blood Vessels

In considering the control of blood supply to tissues it used to be assumed that the calibre of a blood vessel decreased progressively as tone in the muscle of its wall increased.

In 1951 Burton suggested that, for theoretical physical reasons, there must be a critical level of pressure for each small blood vessel below which the vessel becomes unstable and collapses, thus suddenly closing completely<sup>1</sup>. His concept of critical closing pressure was widely adopted and has been applied in biology and medicine ever since. It is quoted in most larger textbooks of physiology and cardiology throughout the world<sup>2,3</sup>.

Burton thought that the phenomenon explained certain features of shock, of which low blood pressure is characteristic. The concept is fundamental to the understanding of the control of blood supply to vital organs, as well as to the rational management of shock. I therefore wish to point out a fallacy in Burton's logic.

In order that his argument shall stand it is necessary to assume that the muscle in the wall of small blood vessels is able to maintain a constant tension throughout shortening, even beyond the point of zero inert elasticity. But this is not so of muscle in general, and has not been shown to be so in this special case.

Critical closing pressure is therefore not an invariable logical necessity for all small blood vessels, as suggested by Burton. Whether or not it occurs in any particular instance depends on the relation of the Laplacian graph of tension/radius to that of tension/length of relevant muscle. It requires to be demonstrated experimentally in each case.

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## PATHOLOGY

## Excretion of Hypoxanthine and Xanthine in a Genetic Disease of Purine Metabolism

In man uric acid can be produced through a number of pathways which ultimately involve the action of xanthine oxidase on hypoxanthine or xanthine. The principal pathway seems to be through xanthine, which could be formed from guanine or xanthylic acid. Evidence for this is the predominance of xanthine over hypoxanthine in the urine of patients with xanthinuria in whom xanthine oxidase is virtually absent<sup>1</sup>, and of patients with gout<sup>2</sup> or neoplastic disease<sup>3</sup> treated with the inhibitor of xanthine oxidase, allopurinol. Fractionation of urinary oxypurines has been carried out in a disorder recently described<sup>4</sup> in which hyperuricaemia is associated with marked cerebral dysfunction and bizarre behaviour. In this condition considerably more hypoxanthine than xanthine was found in the urine. Treatment with allopurinol decreased this ratio but did not result in an excess of xanthine as it did in cases of gout or other conditions, and increasing the dose of allopurinol resulted in increasing ratios of hypoxanthine to xanthine. These data indicate that in patients with this condition uric acid is produced directly from inosinic acid.

Patients with this disorder are characteristically mentally retarded, with athetoid cerebral palsy, and are prone to self-mutilation, usually by biting the lips and hands. They have consistent hyperuricaemia and markedly increased urinary excretion and turnover of uric acid in the urine. Rates of conversion of glycine- $U^{14}C$  into urinary uric acid are about 200 times those of control individuals<sup>4</sup>. Genetic transmission is as an X-linked recessive trait<sup>5</sup>.

Allopurinol (4-hydroxypyrazolo(3,4-d)pyrimidine) is a potent inhibitor of xanthine oxidase<sup>6</sup>. It is also a substrate, and the product, alloxanthine, is also an inhibitor of the enzyme. In view of experience with allopurinol in hyperuricaemic children, we thought it of interest to investigate the relative proportions of the urinary oxypurines.

Hypoxanthine, xanthine, and allopurinol were determined by a modification of the method of Wyngaarden<sup>7</sup>. Urines collected from subjects on a low purine diet were chromatographed on a  $1 \times 30$  cm column of 'Dowex 50 (AG 50-W X4)'. The purines were eluted with 0.5 normal hydrochloric acid and assayed at 250 m $\mu$ . Alloxanthine was eluted from a  $1 \times 100$  cm column of 'Sephadex G-10' with 0.05 molar sodium phosphate buffer at pH 7.0 and assayed at 260 m $\mu$ . Total oxypurines were also determined by the enzyme method of Jorgensen and Poulsen<sup>8</sup>. Values obtained agreed well with those from the column.

Allopurinol reduced the amounts of uric acid in blood and urine in four hyperuricaemic children and in a control child, similar to findings in adults with gout. At the same time excretion of hypoxanthine and xanthine increased (Table 1). Relatively large amounts of the oxypurines were found in the urine of the hyperuricaemic children before treatment and in each case hypoxanthine was predominant. Molar ratios of hypoxanthine to xanthine

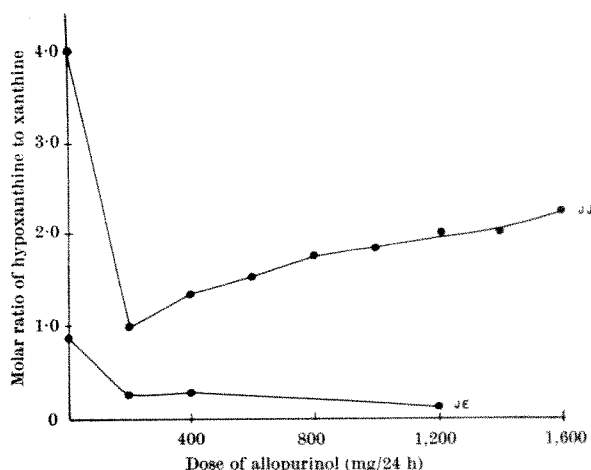


Fig. 1. Effects of increasing doses of allopurinol on the excretion of hypoxanthine and xanthine. The data are the molar ratios of hypoxanthine and xanthine as determined in 24 h urinary excretions. J. J. was a 15 kg boy with the hyperuricaemic syndrome and J. E. was a 15.4 kg control boy with the Cornelia de Lange syndrome.

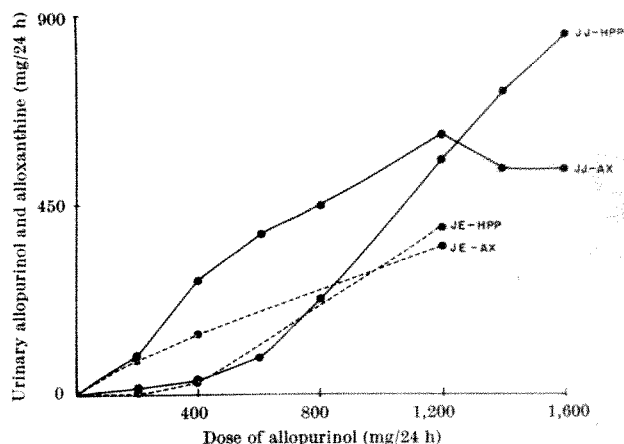


Fig. 2. Urinary excretion of allopurinol (HPP) and alloxanthine (AX) and changes with increasing dose of allopurinol in subjects J. J. and J. E.

were 4.00, 2.28, 2.27 and 1.56. In the control child and the adult with gout, xanthine excretion exceeded that of hypoxanthine and molar ratios of hypoxanthine to xanthine were 0.89 and 0.34 respectively. With treatment, the excretion of the oxypurines increased ten- to thirty-fold. In each instance there was a proportionately greater increase in the excretion of xanthine, so that the ratio of hypoxanthine to xanthine decreased. In the hyperuricaemic children, these ratios still exceeded 1.0, while in the control child and the adult with gout, they were 0.28 and 0.26.

This is more clearly seen in Fig. 1 which illustrates the effects of different doses of allopurinol. In the hyperuricaemic patient, J. J., the ratio of hypoxanthine to xanthine rose with increasing inhibition of xanthine oxidase. This suggests that some of the xanthine found at smaller doses was formed from hypoxanthine. There was also evidence of hypoxanthine predominance in the serum. For instance, at 800 mg of allopurinol, concentrations of hypoxanthine and xanthine in the serum were 3.08 and 1.37 mg/100 ml. respectively. In the control patient, J. E., the ratio in the urine decreased to 0.1 suggesting that, ordinarily, a principal source of urinary uric acid is xanthine which is not derived from hypoxanthine.

The excretion of allopurinol and alloxanthine is indicated in Fig. 2. With a dose of 200 mg the data for J. J. and J. E. were virtually identical for both compounds, and so was the excretion of allopurinol at a daily dose of 400 mg. Alloxanthine is cleared more slowly by the kidney than allopurinol. With a dose of 800 mg, 91 per cent of

Table 1. EFFECTS OF ALLOPURINOL ON THE EXCRETION OF HYPOXANTHINE, XANTHINE AND URIC ACID

Subject	Allopurinol (mg/day)	Urinary excretion (mg/24 h)		
		Hypoxanthine	Xanthine	Uric acid
hyperuricaemic children				
J. J.	0	9	3	330
	200	163	176	52
R. R.	0	34	17	658
	200	319	276	124
A. M.	0	25	18	859
	200	315	249	160
R. S.	0	22	11	553
	200	262	166	55
Control child				
J. E.	0	2	3	194
	200	11	43	99
Adult with gout				
M. G.	0	5	17	700
	600	58	144	423

The weights of the children were about 15 kg.



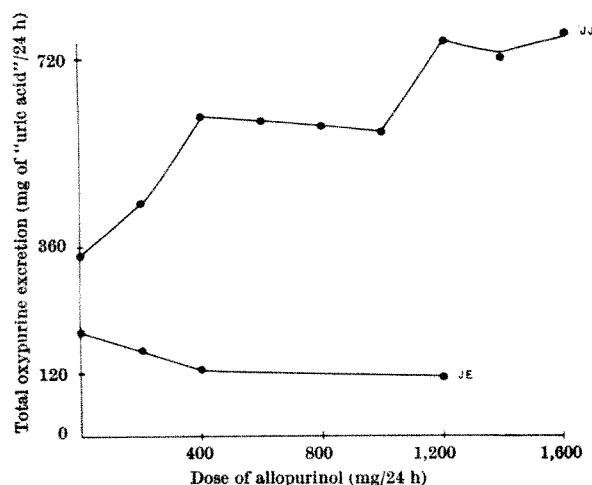


Fig. 3. Effects of allopurinol on the total excretion of uric acid, xanthine and hypoxanthine in the urine. Data for xanthine and hypoxanthine were expressed as mg of uric acid equivalents and the sums of the three purines were plotted at the various doses of allopurinol. Data on additional 24 h urine collections on J. J. confirmed the data graphed at 0, 400, 800 and 1,200 mg. For instance, on 2 separate days on the 1,200 mg dose, the excretion of total oxypurine was 767 and 746 mg/24 h.

the drug in serum was alloxanthine, while in urine only 63 per cent of the total pyrazolopyrimidine was alloxanthine. Levelling off of alloxanthine excretion with larger doses and the linear increase in the excretion of allopurinol with doses greater than 600 mg suggest progressive saturation of xanthine oxidase.

The total excretion of xanthine, hypoxanthine and uric acid decreased progressively with increasing dose in the control J. E. (Fig. 3). A decrease in total excretion was also observed in the adult (Table 1) and has been found regularly in adults in this and other laboratories. This was not true in the hyperuricaemic children. The patient J. J. has excreted more uric acid, but a series of collections for more than a week were in the range of the value charted. When allopurinol was given, excretion of uric acid promptly decreased, but the total excretion of uric acid and the other oxypurines increased.

We conclude that the pathways involved in purine interrelationships in this disease are not the same as those of control individuals or of adults with gout. The data suggest that a principal source of the excessive production of uric acid is hypoxanthine, whereas in others, a large portion of the uric acid produced is formed from xanthine which comes from sources other than hypoxanthine, presumably xanthylic acid and guanine.

The observations are consistent with the recent findings of Seegmiller *et al.*<sup>9</sup> that patients with this disorder lack the enzyme hypoxanthine-guanine phosphoribosyl transferase, which is involved in the re-utilization of purines. If xanthine is not a substrate for this enzyme, absence of the enzyme would lead to an excess of hypoxanthine. In ordinary circumstances most of the purines synthesized might be expected to be used for nucleic acid synthesis, with uric acid excretion reflecting degradation of nucleic acids. The data on controls and on genetic xanthinuria suggest that this leads to xanthine as a principal precursor of uric acid in man. Inosinic acid might be converted to xanthylic acid and then to xanthine, while guanylic acid might be converted to guanine and then to xanthine.

The relationship of these observations to the overproduction of uric acid repeatedly documented in this syndrome is not clear. It seems likely that the overproduction reflects a reduced feedback inhibition, but the feedback inhibitor has not as yet been defined. Decreased total oxypurine excretion in controls after treatment with allopurinol could result from the action of allopurinol as a feedback inhibitor or from re-utilization of oxypurines protected from degradation. Increased incorporation of

hypoxanthine into nucleic acid has been demonstrated by Pomales *et al.*<sup>10</sup> in mice treated with allopurinol. A block in the re-utilization of hypoxanthine might explain a failure to decrease total oxypurine excretion in hyperuricaemic children. There was, however, an increase after treatment with allopurinol. Possibly allopurinol is capable of further reduction of levels of feedback inhibition, or renal clearance of xanthine and hypoxanthine higher than uric acid might lead to excretion in the urine of purine normally excreted in the intestine.

This work has revealed principal differences from the normal condition in the excretion of purines in this syndrome. The elucidation of the mechanisms involved should provide important information on the nature of the mutant individual and could contribute more generally to the understanding of interrelations of purine metabolism in man.

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### Production of Phage-neutralizing Activity in vitro by Cells derived from Burkitt's Lymphoma

THE production of antibodies after exposure to antigens *in vitro* has been reported<sup>1-4</sup> to occur with immune competent cells from nonimmunized animals, but antibody production in response to specific antigens does not seem to have been observed with cultured cells of established human lines. We have now exposed cells of the human lymphoblastoid line P3-J ("Jijoye"), taken from patients with Burkitt's lymphoma, to Coliphage T<sub>2</sub> *in vitro*, and have found that the exposed cells appear to produce a factor capable of neutralizing the phage, probably an antibody. Roswell Park Memorial Institute (RPMI) cell lines<sup>5</sup> No. 41 and No. 212, derived from human osteosarcoma and mesothelioma, respectively, produced no such factor.

The P3-J cells were originally provided by R. J. V. Pulvertaft. Cultured cells, packed by centrifugation, were exposed to Coliphage T<sub>2</sub>; the ratio of cell population to number of plaque-forming units ranged from 1:50 to 1:100. After incubation with T<sub>2</sub>, the cells were washed five times to remove unadsorbed T<sub>2</sub> and were resuspended in spinner flasks containing RPMI medium 1640, a modification of McCoy's medium, supplemented with 20 per cent foetal calf serum and including 100 u/ml. of penicillin. The cell population was maintained at 8-15 × 10<sup>5</sup> cells/ml. by the addition of fresh medium. On the fifth and sixth

days of incubation, the medium was collected and was centrifuged for 2 h at 35,000g. Protein isolates were prepared from the supernatants by addition of sufficient saturated ammonium sulphate solution to provide a final concentration 40 per cent of saturation followed by dialysis. The final volume of every preparation was 1/20 of the original volume of the culture medium.

To assay phage inactivation, 1 ml. samples of protein fractions were each mixed with 1 ml. of fluid containing 500 P.F.U. of  $T_2$  and were incubated for 1 h at 37°C. Plaque counts were determined by the agar layer method and controls were set up with similar preparations obtained from fresh medium and from the medium in which P3-J cells not exposed to  $T_2$  had been cultured. Similar experiments were carried out with cell lines of non-leukaemic origin, RPMI No. 41 and No. 212.

Inactivation of  $T_2$  was expressed as the percentage reduction in the number of plaques formed in experimental samples as compared with control samples. In all experiments summarized in Table 1, the number of plaques was reduced in samples containing  $T_2$  mixed with protein fractions isolated from the culture medium in which P3-J cells previously exposed to  $T_2$  had been cultured.

Table 1. EFFECT OF MEDIUM FROM CULTURES PREVIOUSLY EXPOSED TO COLIPHAGE  $T_2$  ON PLAQUE FORMATION BY COLIPHAGES

Experiment No.	$T_2$ plaque formation*		$T_2$ plaque formation*
	Without CR	With CR	
	P3-J cell line		
1	-9.7	—	—
2	-18.6	—	0†
3	-22.8	—	0†
4	-41.9	—	—
5	-37.1	—	—
6	-5.5	—	—
7	-50.0	—	—
8	-9.8	—	—
9	-15.7	—	-2.5
10	-20.7	—	0
11	-20.3	-2.3	—
12	-24.3	-3.6	—
13	-17.0	-0.6	—
	RPMI cell line No. 41		
1	0†	—	—
2	0†	—	—
	RPMI cell line No. 212		
1	0†	—	—
2	0†	—	—

CR, Cleland's reagent.

\* Effect =  $\frac{\text{experimental plaque count} - \text{control plaque count}}{\text{control plaque count}} \times 100\%$

† Includes cases in which experimental count is greater (within standard deviation) than control count.

The plaque counts of six to ten plates for each group of experimental samples were compared with the counts of an equal number of plates for controls isolated either from fresh medium or from medium in which cells exposed to  $T_2$  had been cultured. The reduction in numbers of plaques ranged from 5 per cent to 50 per cent, and the value of  $P$  for most experiments was less than 0.05. The standard deviation of the plaque counts for each experimental group was less than 7 per cent, and the plaque counts for the two control groups differed less than 4 per cent.

When the protein isolates were treated with Cleland's reagent<sup>6</sup> at a final concentration of 0.02 molar, followed by iodoacetamide at a final concentration of 0.04 molar, a reduction in plaque counts was not observed. When cells that had been exposed to  $T_2$  were assayed with the unrelated  $T_5$ , there was no significant reduction in plaque counts, indicating the specificity of the reaction.

Evidently the P3-J cells previously exposed to  $T_2$  and grown *in vitro* produced a factor that was capable of inactivating  $T_2$ . The effect was specific, because no effect was observed when the assay was performed with  $T_5$  or when the protein isolates were treated with Cleland's reagent. P3-J cells in culture have been shown<sup>7,8</sup> to produce immunoglobulins. The nonhaematopoietic (or nonlymphopoietic) lines RPMI No. 41 and No. 212 do not produce immunoglobulins, and medium in which cells of these two

lines had been cultured did not show any inactivating effect on  $T_2$  when the cells were exposed to  $T_2$  and cultured. These results strongly suggest that exposure to  $T_2$  stimulates P3-J cells to produce specific antibodies in culture.

It is not known whether an antigen or RNA from macrophages<sup>1,9</sup> previously exposed to an antigen stimulates the so-called stem cells to divide into cells producing an antibody, or whether it directly stimulates immune responsive cells. In view of the fact that P3-J cells are a mixed population, the present study cannot give any definite answers to the questions raised. Further studies are in progress to characterize the antibody like factor involved and to elucidate the nature of the cells that produce this factor.

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## Lysosomes in Malignant Skin Tumours

In the course of a study on the histochemistry of experimentally induced carcinogenesis in mouse skin I observed that a few applications of benzyrene to the skin induced apparent activation of lysosomes in the epidermis<sup>1</sup>. I considered it of interest to examine the state of lysosomes in the final tumour stage, especially in tumours classified as malignant.

Tumours were induced in the skin of mice ( $A_2G$  strain) by weekly paintings with 0.5 ml. of 0.8 per cent solution of benzyrene in liquid paraffin. Tumours began to develop at between 30 and 40 weeks. The skin tumours were diagnosed as malignant when on histological examination the epidermal tumour cell masses had infiltrated into the underlying panniculus carnosus muscle<sup>2</sup>.

Frozen sections of the tumour samples were cut by the "controlled-temperature freeze sectioning method" of Cunningham *et al.*<sup>3</sup>. Acid phosphatase was demonstrated by Holt's modification of the Gomori method<sup>4,5</sup>. In normal mouse skin an incubation time of 20 min in the Gomori medium produced well stained particles in the epidermis. These stained cytoplasmic particles have been shown to possess similar characteristics to the biochemically defined lysosomes<sup>6</sup>. After incubation for 1 h, the intensity of staining for the enzyme was so great as to mask completely the presence of discrete cytoplasmic particles.

In marked contrast, however, the tumour cells showed only a few stained particles even after incubation for 1 h. It was only after incubation for 2 h that a definite reaction was produced in the form of numerous stained particles

with some diffuse cytoplasmic staining. It seemed likely that this resulted from the development of resistance of the lysosomal membrane in the tumour cells. Because benzpyrene is soluble in lipids, it is possible that the carcinogen is absorbed by the lipo-protein membrane of the lysosomes. Repeated applications of the carcinogen may result in a stabilization of the lysosomal membrane with a resultant increase in the resistance and impermeability of the lysosomes as evident in the tumour cells. The release of lysosomal enzymes has been suggested as one of the causes of gradual ageing and death of cells<sup>7</sup>. Tumour cells therefore may be protected from the effects of their own lysosomal enzymes by the intractable nature of the lysosomal membranes, and this could be a contributory factor in the continuous growth of such cells.

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## CYTOLOGY

### Correlation between Sperm "Redundancy" and Chiasma Frequency

Most male animals produce a large number of spermatozoa, whereas their females produce a relatively small number of eggs. Classically, this has been related to dilution problems in the external fertilizing medium of the female genital tract, and to synergic effects of super-numerary sperm in dissolving egg membranes or stimulating the female tract. The wide range of sperm redundancy (from 2.0 to  $2 \times 10^{10}$ ), however, suggested that another more immediate cause may be responsible. If a multiple process during spermiogenesis regularly fails in a proportion of attempts, then the number of "perfect" sperm may only be a small proportion of the total, the total produced being related logarithmically to the number of such faulty multiple processes in the production of each sperm. For example, if half such processes are faulty, then it is to be expected that

$$2^P = \frac{R}{n} = r$$

where  $P$  is the number of processes in the production of one sperm,  $r$  is the sperm redundancy,  $R$  is the sperm number divided by the zygote number for one copulation, and  $n$  is the (small) number of "perfect" sperm still required for each egg to meet the exigencies of random collision paths. It was found (Fig. 1) that for those few organisms for which both chiasma frequency and sperm redundancy have been investigated, a logarithmic correlation of the form

$$1.5C = \frac{R}{40}$$

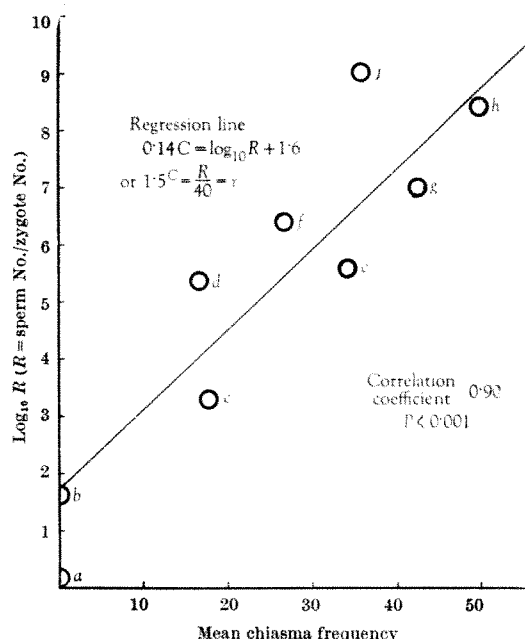


Fig. 1. Sperm number/zygote number and mean chiasma frequency for the species for which both figures have been published. *a*, *Drosophila*; *b*, *Apis*; *c*, *Schistocerca* (preliminary results on sperm number by Miss Judy Kemp); *d*, Chinese hamster (calculated from *f*, on the assumption that the sperm weight is the same and that the proportion from vas deferens is the same); *e*, mouse; *f*, golden hamster; *g*, pig-tailed macaque (chiasma frequency, Egovque unpublished); *h*, man; *j*, bull. Note: Correlation coefficient for chromosome number ( $n$ ) and  $R$  for thirty-nine species is 0.62. Correlation coefficient for DNA/haploid nucleus and  $R$  for thirteen species is 0.12.

fits the points well. This may be taken to imply that about one in three chiasmata has a result which renders the resultant sperm less than "perfect".

Genetic evidence for exactly corresponding breaks and joins in the homologous chromatids is all derived from successful gametes, and leaves open the possibility that failed gametes may have had some inexact chiasmata. Lewis and John<sup>1</sup> made the point that linear exactitude is rare in such biological systems; furthermore, Whitehouse's suggestion<sup>2</sup> that hybrid DNA may initially be formed at chiasmata leads to a theoretical expectation of exactness in only half the resultant reconstituted segments; the others might have an interpolation or deletion of a few nucleotides. He complicates his theory to rectify this.

Implications of this idea are many. Eggs would also be expected to show similar redundancy, and this may therefore be the basis of oocyte atresia in mammals, and in general the production of only a few eggs from large numbers of oocytes by those forms with a large investment (for example, yolk) in each egg. Internal fertilization would then be primarily an opportunity for sperm selection, so that only "perfect" gametes are offered at fertilization. Organisms with large numbers of zygotes in each generation (for example, oysters, plaice) might be expected to show less selection and so to produce a much wider genetic spectrum of zygotes than adults; only a few could survive in normal conditions, but changed environment might result in saltatory evolution. This hypothesis may also have implications for basal mutation rate, the selective value of male haploidy and fertility studies. Notably, gynogamones and other gynogenetic substances which agglutinate sperms may be selective rather than trapping devices.

Preliminary experimental results are promising; investigation of the number of sperms in locust (*Schistocerca*) spermatophore shows it to be of the order of  $2-3 \times 10^5$ . This correlates perfectly with chiasma frequencies of 18-20, assuming 100 zygotes to result. Further work on other organisms, and on sperm competition in mammals, is in progress. Information about sperm number



or chiasma frequency in a variety of organisms is being sought and information will be welcome.

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### Establishment of an Insect Cell Strain persistently infected with an Insect Virus

INSECT tissue culture has received particular attention in the fields of virology, cytology, genetics, developmental biology and insecticide research. The first insect cell line was established by Grace<sup>1</sup> from the ovarian tissues of *Antheraea eucalypti*, and two others were reported to have been established from *Aedes aegypti*<sup>2</sup> and *Drosophila melanogaster*<sup>3</sup>. This article reports the establishment of a cell strain, which has been persistently infected with *Chilo* iridescent virus (CIV), from the haemocytes of the rice stem borer, *Chilo suppressalis* Walker (Lepidoptera, Pyralidae).

The first attempt to cultivate insect haemocytes *in vitro* was made by Glaser<sup>4</sup>, but no one has ever succeeded in cultivating insect haemocytes for long periods or in sub-culturing them. In January 1965 several primary cultures of haemocytes were set up from *C. suppressalis* larvae. A modified sitting drop culture method involving cover glasses and microslide rings<sup>5</sup> was used for the primary cultures. The haemocytes were obtained from diapausing larvae of *C. suppressalis* which were reared on an artificial diet in aseptic conditions. The blood which oozed from a puncture made on a proleg of a larva was allowed to drop on to a Ringer-Tyrode salt solution placed on the bottom of the culture vessel. Most of the haemocytes settled on the bottom of the culture vessel within 10 min and were washed by changing the solution six times. Finally, the solution was replaced by culture medium.

The culture medium (CSM-2F) used consisted of 50 mg of  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ ; 120 mg of  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ ; 160 mg of  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ; 120 mg of KCl; 40 mg of  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ ; 80 mg of glucose; 80 mg of fructose; 520 mg of lactalbumin hydrolysate; 520 mg of Bacto-peptone; 40 mg of choline chloride; 200 mg of TC-yeastolate; 20 ml. of TC-199 medium; 20 ml. of foetal bovine serum; 10 mg of dihydrostreptomycin sulphate; and distilled water added to make the volume 100 ml. The pH was adjusted to 6.2 with potassium hydroxide.

Of the seven different types of cells which constitute haemocytes of *C. suppressalis*, prohaemocytes and plasmatocytes multiplied gradually in the primary cultures. The details of the cell growth in the primary cultures have been reported elsewhere<sup>6</sup>. In most of the primary cultures haemocytes began to degenerate within a month, but continuous cell growth was obtained in one culture, and in this culture the cells began to multiply rapidly 6 months after the culture had been set up.

The first sub-culture was made in July 1965 by taking a part of the growing cell colonies. The primary culture, from which the sub-culture was made, recovered its cell population, and so sub-cultures were repeated more than twenty times from the same primary culture at irregular intervals.

Most of the secondary cultures failed to grow continuously, but some grew very well, and serial sub-cultures were made successfully from one of the secondary cultures. The intervals of serial sub-cultures were more than 20 days during the first 3 months, but were decreased to 10–14 days after December 1965. With the advance of

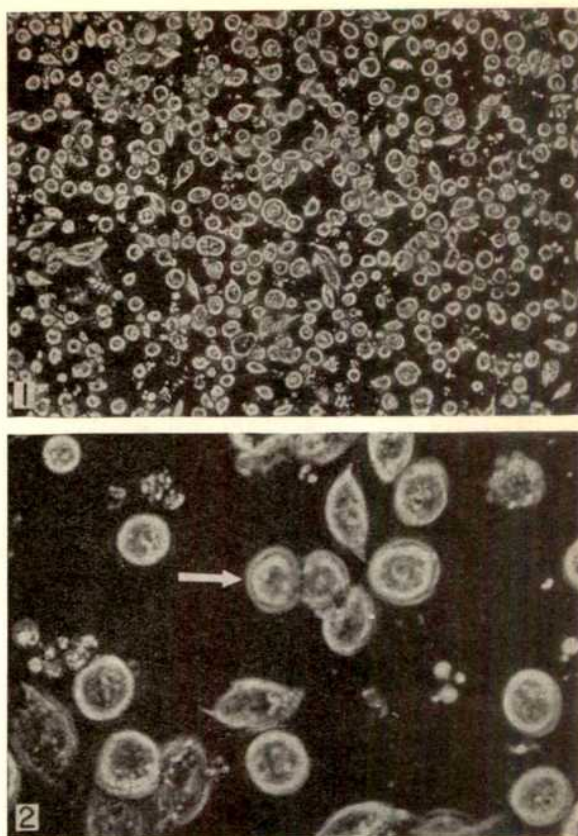


Fig. 1. Cells multiplied from haemocytes of *Chilo suppressalis*. From a culture 27 months old. Bright phase contrast. ( $\times 120$ .)

Fig. 2. A highly magnified phase contrast photomicrograph of the cells shown in Fig. 1. Arrow indicates a cell undergoing mitosis (anaphase). ( $\times 480$ .)

generations, prohaemocytes became predominant, and plasmatocytes gradually disappeared. The cells began to multiply rapidly and I tried to transfer the cells into Carrel flasks, square bottles, T-flasks or culture tubes of various sizes, from small to large containers.

In March 1966 all cultures were accidentally infected with CIV. Experiments with CIV were being carried out in the same laboratory at that time, and CIV is known to be a stable virus, so that the infection may have been caused by accidental contamination of CIV in the culture medium, although care was always taken to avoid contamination. The infected cells were usually granulated and aggregated to form cell masses, which showed typical iridescence by reflected light<sup>7</sup>, and finally degenerated. Most of the cultures were lost through this contamination, but one culture survived. In this some cells still continued to multiply, although most of them degenerated and debris of dead cells was abundant in the medium.

Seven months after infection with CIV, in October 1966, the surviving cells began to multiply again. Sub-cultures were made at 1–2 week intervals, and a cell strain was established. These cells were probably prohaemocytes. In cell strain culture spherical cells,  $18\text{--}2\mu$  in diameter, were predominant, but there were also a few spindle shaped cells  $34\text{--}1\mu$  long (Figs. 1 and 2). In single cell culture some spindle shaped cells became spherical. When the cells were actively multiplying, they became separated from each other, and suspended in the culture medium or lightly attached to the glass. The cells often aggregated to form cell masses when they were overpopulated. If the cells were cultivated by a rotation culture method they attached themselves to the glass surface firmly and took on a very flat form. This transformation of cells resulted in their degeneration.



The degree of cell multiplication was determined by a replicate tissue culture method used on the cells recovered from CIV infection. Samples (1.5 ml.) of the cell suspension were distributed into 15 x 150 mm roller tubes slanted about 5° from the horizontal and stationary culture was carried out at 25° C. The number of cells were determined with a haemocytometer. The average of values obtained from three tubes was taken as the cell number. A growth curve of the cell is shown in Fig. 3. The mean generation time of the cells was estimated as 4.5 days at 25° C.

As well as actively multiplying cells, hypertrophied and granulated cells, small cell masses and debris of dead cells were always present in the culture. The multiplying cells did not show iridescence, but small crystals which showed brilliant greenish or bluish iridescence were often seen in hypertrophied cells and in debris of dead cells when viewed by reflected light by means of the 'Ultropak'. Small cell masses as a whole sometimes showed iridescence. Ultra-thin sections of hypertrophied cells or cells in small cell masses revealed the presence of many virus particles in crystalline arrangement in the cytoplasm (Fig. 4). In addition to the fully developed virus particles, some empty membranes were seen, and these may be a premature stage of virus particles as Smith<sup>8</sup> suggested in TIV.

Ten months after CIV infection, in January 1967, the cells, the cell homogenate or the cell free culture medium were inoculated into the haemocoel of healthy *C. suppressalis* larvae, and all the inoculated larvae became infected. This suggested that CIV also multiplied in the culture of the cell strain.

When the cells in cultures were fixed with Carnoy's fluid and stained by Feulgen's technique, Feulgen positive materials were rarely found in the cytoplasm of the small spherical cells, while some parts of the cytoplasm of hypertrophied cells and the aggregated cells were Feulgen positive. I therefore assume that the actively multiplying cells are not infected with CIV and that they become infected when the culture conditions change unfavourably for the cells, or they carry a latent infection, in which CIV exists in these cells in the Feulgen negative form and is activated when cell growth becomes unfavourable. This was supported by the fact that iridescence was not observed in the new sub-cultures, but many iridescent crystals were seen in the same cultures when they were kept for a long time without a change of medium.

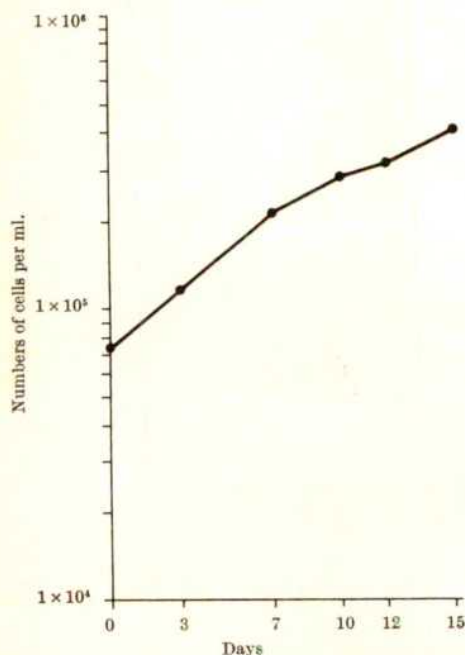


Fig. 3. A growth curve of cells in the CIV carrier culture, 27 months after the primary culture was set up (forty-two transfers). Medium CSM-2F. Temperature 25° C.

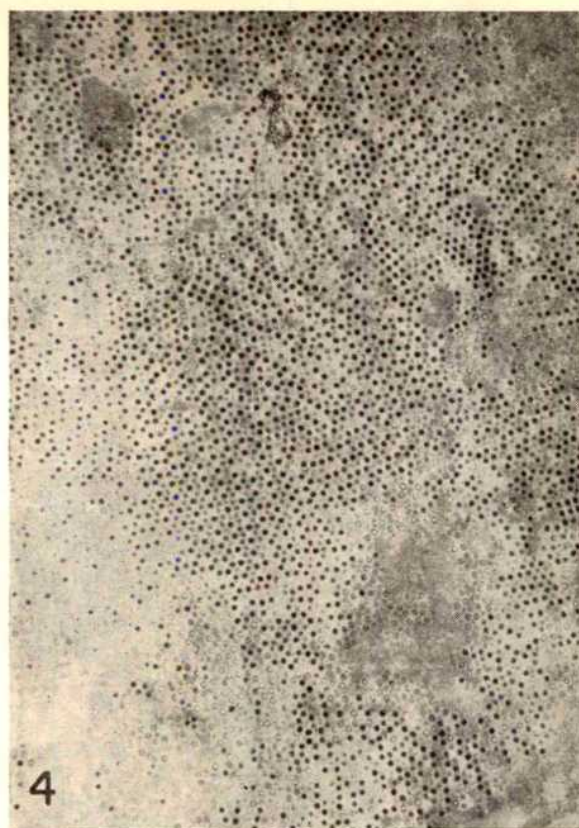


Fig. 4. An ultra-thin section of the cultivated cells infected with CIV, showing virus particles in crystalline arrangement. Empty membranes are also seen. (× 5,000.)

This carrier culture may be used for mass production of CIV, if CIV is used for the microbial control of some insects. It is also possible that the carrier culture can be used for the screening of antiviral agents, because bio-assay of CIV can be carried out easily by injecting the virus into the susceptible insects.

Attempts to eliminate CIV from the cell strain and to establish another virus free cell strain from the primary cultures of haemocytes of *C. suppressalis* are in progress.

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## GENETICS

### Mutable Genes in the Light of Callan's Hypothesis of Serially Repeated Gene Copies

MUTABLE genes have been intensively studied in maize by McLintock<sup>1-3</sup> and Brink<sup>4-6</sup> and they occur widely in higher plants<sup>7</sup>. Among many features of mutable genes, all difficult to explain by conventional mutation theory, the following are especially relevant to the present discussion. (a) One original labile gene often gives rise with high frequency to a whole range of more or less

stable alleles, quantitatively graded in their expression. (b) In addition, a labile gene can frequently mutate to other unstable alleles, differing from the original in the timing or frequency of mutation or in the kinds of stable derivatives most often produced. (c) The frequency of mutation is frequently drastically affected by changes of temperature (in several examples higher temperature causes reduced mutation frequency), or by genes elsewhere in the genotype; such variables affect the mutable gene specifically in the sense that they have no evident effect on mutation in general. All these features are well shown in the *pallida-recurvens* mutable system of *Antirrhinum majus*<sup>9,10</sup>. In the following discussion I shall refer to the *Antirrhinum* mutants because I am familiar with them, although McIntock has documented most of the same effects much more thoroughly in maize.

To explain the almost continuously graded variation seen in derivatives of mutable genes Brink<sup>5,6</sup> has proposed that such variation is caused by different numbers of repeated, probably identical self-replicating units associated with the unstable locus. It has not been clear, however, how the repeated units would be integrated into, or attached to, the chromosome, and there is no obvious explanation for the difference between stable and unstable alleles. A recent hypothesis advanced by Callan<sup>10</sup> opens up new lines of thought and suggests explanations for at least some of the properties of mutable genes.

Callan postulates, as a property of some or all genes of higher organisms, a serial (tandem) repetition of DNA sequences; that is to say, each cistron is regarded as being represented by numerous copies joined end to end. The evidence for this model derives from Callan's own studies of lampbrush chromosomes, from Keyl's<sup>11</sup> demonstration of reduplication of DNA within certain polytene chromosome bands in *Chironomus* and from the well-known gross differences in amount of DNA in each genome between related species of animals and plants. Further evidence pointing in the same direction has been provided in the special case of the bobbed locus of *Drosophila melanogaster*<sup>12</sup>.

Serial repetition of gene copies is only consistent with the known facts of mutation and recombination if there is some over-riding mechanism for keeping all the members of the series identical. This problem has been discussed, and solutions proposed, both by Callan<sup>10</sup> and by Whitehouse<sup>13</sup>, the latter dealing with the problem of recombination. Briefly, it is proposed that within each serial gene there is one "master" gene copy (probably at the end) and that the other gene copies in the same series are "slaves" which are corrected as necessary to conform to the master copy. Callan suggests that, at least in the lampbrush chromosomes of oocytes, transcription of DNA into messenger RNA and the correction of slaves by the master both occur as a consequence of the same process—namely, the uncoiling and recoiling whereby all the DNA of a lampbrush chromosome appears to be exposed in turn in the uncoiled lateral loop extending from the chromomere. In the model the slave copies are transcribed into RNA while they are exposed in the loop and, as they are rewound into the chromomere, they are matched in turn with the master gene copy and corrected if they differ from it. The feature of the model which is essential from the point of view of this paper is that transcription and correction are linked. Although transcription and correction need not both occur in all cells (as Callan points out, correction need only occur once in each life cycle) it seems not unreasonable to suggest that certain mutations or structural changes in the master sequence might interfere both with transcription and with correction. The effect of such a change in the master would then be a hereditary loss of activity of the whole series of gene copies, although the slave copies remained non-mutant.

The essential peculiarity of the kind of mutant just postulated would lie in the internal heterozygosity of the mutant gene series; the normal genetic information lost

in the master copy would be retained in the slave copies, though unable to find expression. In the more usual, very stable type of mutant, on the other hand, the correction process would have ensured that all the slaves carried the same mutation as the master.

To explain how an internally heterozygous gene series could be unstable it suffices to show how a defective segment in the master could be removed and replaced by a normal homologous segment derived from one of the slaves. There seem to be several possibilities here, all involving pairing of homologous genetic segments. Such pairing, at least in meiosis and in so far as it is reflected in the frequency of chiasma formation, is known to be sensitive to temperature, with high temperature frequently causing a reduction in pairing. With the notable exception of Brink's<sup>5</sup> phenomenon of paramutation in maize, highly mutable alleles in higher plants are, in general, more or less autonomous, not depending for their mutability on the presence of any particular other allele on the homologous chromosome. Thus any plausible mechanism must involve intra-chromosomal rather than inter-chromosomal interactions. One obvious possible mechanism is unequal sister-strand crossing-over which, following pairing between the defective master on one chromatid and one of the slaves on the sister, could generate a shortened sequence with a normal master on one chromatid and a longer sequence with a defective master and a defective slave on the other. Of these two products the first should be a stable allele of rather sub-normal (but possibly not perceptibly sub-normal) activity. The second should be an inactive allele, which, if the defective slave segment interfered with transcription, would not be able to mutate in one step to anything approaching full activity. After separation of the sister chromatids and their separate replication in clones of cells the two new alleles should be in adjacent "twinning" sectors. In fact, twinning does not seem to be a usual feature of high mutability. Close examination of mutable *Antirrhinum* petals does reveal some possible examples of twinned sectors, but it is difficult to be sure that these are more frequent than could be accounted for by coincident independent mutations; it is clear, in any case, that most fully pigmented mutant sectors are not accompanied by any phenotypically distinct twins. The scarcity of twinned sectors suggests that intra-chromatid rather than sister-chromatid interactions are the more usual cause of mutability. The most obvious possibility is pairing of repeated gene copies on the same chromatid by loop formation, followed by crossing-over as shown in Fig. 1. This could lead to excision of the defective sequence of the master in a closed loop and the generation of a normal master governing a slave series shortened by one (Fig. 1 A(a)) or more (Fig. 1 B(a)) copies, depending on whether the intra-chromatid pairing involved adjacent or non-adjacent copies. Double exchanges within an intra-chromatid loop could result in conservation of the original number of copies but with the shift of the defective former master copy into a slave position one or more positions down the series (Fig. 1 A(b) and B(b)).

Yet another possibility is to invoke the correction process postulated by Callan. One can imagine that the nature of the defect in the master segment is such that it cannot, or only with great difficulty, be imposed on the slave segments, but that fairly frequent correction can occur in the opposite direction—that is to say, from slave to master. Correction of the master segments without crossing-over would lead to the formation of a normally functional gene with the original number of repeated segments (Fig. 2 A(a) B(a)). Rarely, correction might proceed from defective master to normal slave, resulting in a series with the normal number of segments including a defective slave as well as a defective master (Fig. 2 A(b) B(b)). If correction were correlated with crossing-over, as has been postulated to account for some of the features of gene conversion in fungi<sup>14,15</sup>, the new active master



copies might be associated with different numbers of slaves, in the same way as shown in Fig. 1, thus accounting for the quantitative variation between different stable mutant alleles.

An important fact which must be taken into account is that extreme instability of genes is very much the exception rather than the rule. If normal genes are serially repeated they must in general not show crossing-over between different copies in the same series. This suggests that such crossing-over is a side-effect of correction and does not occur, except perhaps rarely, when master and slaves are properly matched. The high stability of normal genes seems a strong reason for preferring correction, rather than crossing-over, as the essential factor in high mutability.

The mechanism just suggested seems capable of explaining virtually all the different kinds of alleles arising at the *Antirrhinum pal* locus. The most common type of mutant, that with stable and full expression of the gene activity, could be caused by correction of the defective master segment with retention of the normal number of slaves. The next most common type, with stable but more or less reduced expression of the gene activity, could be caused by correction of the defective master copy accompanied by crossing-over resulting in a significant shortening of the slave series, assuming the amount of gene product to be more or less proportional to the number of gene copies. Another comparatively frequent type of mutant, similar to the original *pal-recurrens*, but with greatly reduced mutation frequency, could be caused by the duplication of the defect in the master in the most proximal slave copy (Fig. 2 A(b)), thus closing the door to the most common type of mutation (correction, or replacement, of the master by the proximal slave). The duplication of the defect in the master copy in a slave copy some way along the series (Fig. 2 B(b)), where it could block transcription of the more distal copies, might account for mutable alleles (for example *pal-maculosa*, Fincham and Harrison, 1966) able to mutate only to a low level of gene expression. Mutant alleles showing more or less full gene expression, occasionally reverting to the inactive but mutable condition, (Fincham and Harrison, 1966), are more difficult to account for but could be caused by transposition of the defective copy to the far distal end

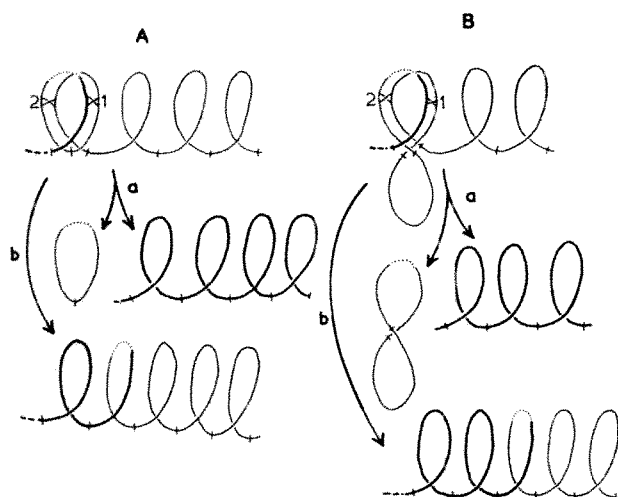


Fig. 1. Possible consequences of intra-chromatid crossing-over. A, pairing of master with first slave segment. (a) Cross-over in position 1 giving excision of defective master copy as a closed loop (lost?) and formation of an active product with somewhat reduced level of activity. (b) Double cross-over in positions 1 and 2 giving product with normal master but defective first slave copy (low level of activity, mutable to higher activity?). B, (less frequent) pairing of master with more distant slave copy. (a) Cross-over in position 1 giving excision of defective master and one or more normal slave copies, and formation of an active product with considerably reduced activity. (b) Double cross-over (positions 1 and 2) giving normal master and defective distant slave copy (intermediate level of activity, mutable to full activity?). Here and in Fig. 2 the thicker lines indicate genetic segments able to be transcribed and thinner lines segments transcription of which is blocked.

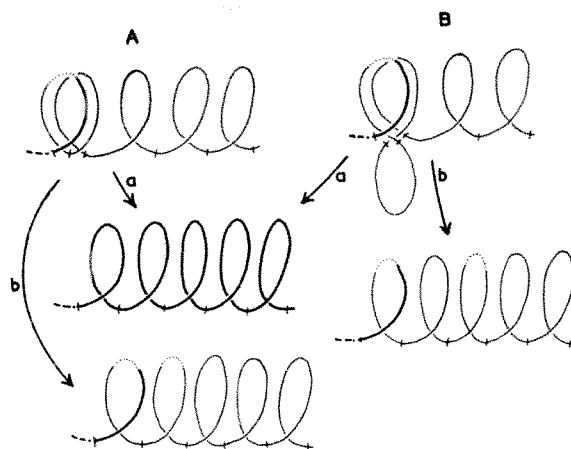


Fig. 2. Possible consequences of master-slave and slave-master correction (conversion) without crossing-over. A, pairing of master with first slave copy. (a) Correction of master to conform to slave giving a fully active product. (b) Correction in the opposite direction to give an inactive product with a greatly reduced frequency of mutation to full activity. B, pairing of master with more distant slave copy. (a) Correction of master to conform to slave giving a fully active product. (b) Correction in opposite direction giving mutable product mutating to partial activity only. Association of conversion with crossing-over would give changes in numbers of gene copies in the products as in Fig. 1.

of the series from where it may be occasionally transposed back to the master position.

Additional assumptions would have to be made to account for some other properties of mutable genes in maize which have not so far been mentioned, including infective paramutation<sup>4</sup> and the induction of new mutabilities and the control of existing ones by transposable elements<sup>1-3</sup>. One of the less extravagant extensions of the theory would be to explain transposition in terms of excision of ring elements by intra-strand crossing-over (Fig. 1 B(a)) and their integration elsewhere in the genome by further crossing-over involving regions of partial homology. The analogy with *F* factors in bacteria is suggestive in this context<sup>16</sup>.

Critical evidence bearing on the speculations made in this paper is lacking at present, and various alternative and equally plausible schemes might be devised. It does seem, however, that if Callan's master-slave concept is acceptable on other grounds, it is possible to construct and test a variety of reasonable hypotheses to account for at least some of the properties of mutable genes. A new dimension is also added to our thinking about the relationships between the members of multiple allelic series in higher organisms.

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## IMMUNOLOGY

## Distribution of Nephric Antigens in Australian Vertebrates

NOTABLE improvements in immunohistological techniques and the availability of a new fauna prompted us to apply anti-kidney sera previously described<sup>1</sup> to further studies of the species distribution of kidney-specific antigens. We used two sera prepared by immunizing rabbits with microsomal material from human and hamster kidneys respectively, and examined their cross-reactivity with the kidneys of other species by immunofluorescence<sup>2</sup>. Contrary to the earlier reports that cross-reactions are limited to mammals, birds and fish, we found that both sera cross-reacted with all kidneys examined from vertebrates of every class. There was also some cross-reaction with seminal vesicle epithelium in the few species studied.

Kidneys removed immediately after death from mammals (including placentals, marsupials and monotremes), birds, reptiles, amphibians and fish, provided blocks 5 mm<sup>2</sup> × 2 mm thick, which were snap-frozen in a slurry of liquid nitrogen-isopentane at -160° C. Blocks were taken from different parts of kidney and from liver, lung and heart of representatives of each class of vertebrate, and from seminal vesicle, testis, prostate and vas deferens of rat, guinea-pig, cat and dog. They were then sectioned at 6μ in a cryostat at about -20° C either immediately after snap-freezing or after storage for up to 6 months in a sealed container at -70° C. More prolonged storage was associated with a variable loss of antigenicity. Unfixed sections were stained by the immunofluorescence sandwich method with each antiserum followed by goat anti-rabbit-globulin labelled with lissamine rhodamine B (RB 200) or fluorescein isothiocyanate.

The failure to stain sections of organs other than kidney or seminal vesicle provided evidence of specificity of the reaction, and this was further confirmed by persistence of staining of kidney and seminal vesicle by sera which had been repeatedly absorbed with homogenates of liver, spleen, lung and testis from humans or rodents; sheep red cell absorptions of the anti-hamster kidney serum were also used to remove Forssman antibody. Absorptions by similar tissue homogenates from other species were used as required to inhibit non-specific staining of tissue sections of those species. Cross-absorptions with kidney from at least two species of each class of vertebrate and with guinea-pig seminal vesicle were also carried out to study their relative neutralizing effect on the sera.

The species studied and the results of the immunofluorescence staining of the kidneys are summarized in Table 1, from which it is clear that both sera reacted with the kidneys of all the vertebrates examined. Human kidney stained very intensely with the homologous antiserum; the staining of hamster kidney was not checked during this investigation because this rodent is not available in Australia. Kidneys from all the other placental mammals tested stained intensely with both antisera. Kidneys from all the other species stained less intensely with the anti-hamster-kidney serum. The anti-human-kidney serum gave staining of similar intensity with the kidneys of marsupials, monotremes, birds and fresh water fish, and moderate staining of the kidneys of reptiles, amphibians and marine fish. The histological distribution of the staining was almost entirely tubular in all kidneys and glomerular staining was negligible; in the mammals it was brightest in the proximal convoluted tubules.

Seminal vesicle epithelium of rat, guinea-pig, dog and cat was found to stain (Fig. 1) with either antiserum with about half the intensity of the staining of kidney tubules. The staining could be completely inhibited by absorption with kidney or seminal vesicle but the latter was not sufficient to neutralize the capacity of the serum to stain kidney. This indicates that the seminal vesicle antigen was either weakly cross-reacting or, if identical with the

Table 1. IMMUNOFLUORESCENT STAINING OF VERTEBRATE KIDNEYS BY TWO ANTI-KIDNEY SERA

	Staining by kidney antisera		Staining by kidney antisera	
	Anti- human	Anti- hamster	Anti- human	Anti- hamster
Mammals:				
Human (numerous)	+++	+++	++	++
Other placentals:	+++	+++		
Pig (3)				
Dog (3)				
Cat (6)				
Horse (1)				
Bovine (3)				
Sheep (3)				
Guinea-pig (numerous)			+	++
Rat (numerous strains)				+
Mouse (numerous strains)				
Scal (2)				
Marsupials:	++	++		
Kangaroo (3)				
Wombat (2)				
Koala (6)				
Possum (4)				
Bandicoot (2)				
Tiger cat (1)				
Rat kangaroo (2)				
Marsupial mouse (2)				
Monotremes:	++	++		
Echidna (3)				
Platypus (1)				
Birds:				
Hen (3)				
Pigeon (2)				
Maggie (2)				
Kookaburra (2)				
Petrel (1)				
Penguin (2)				
Reptiles:				
Blue-tongued lizard (3)				
Red-bellied black snake (1)				
Amphibians:				
Green tree frog (2)				
Queensland cane toad (4)				
Fish:				
Fresh water:			++	++
Trout (4)				
Goldfish (4)				
Tench (1)				
Marine:			+	++
Whiting (2)				
Mullet (2)				
Ling (1)				
Pike (1)				
Toady (1)				
Rock Cod (1)				
Schnapper				
Shark (2)				
Gummy				
Shark (2)				
Sting Ray (3)				

Number tested given in parentheses after each species. Staining graded from very intense (++++) to moderate (+).

kidney antigen, present in smaller amount than in kidney. The demonstration of such antigenicity in seminal vesicles is not unexpected because, like the fish kidney, it is a mesonephric organ. The sharing of antigenicity between metanephric and mesonephric organs, both kidney and seminal vesicle, indicates that the antigens are not merely a product of organ function but a more fundamental component based in ontogeny.

The anti-kidney sera which had been absorbed by liver, lung and spleen gave no staining with any organs other than kidney or seminal vesicles; there was no staining of testis, epididymis, vas deferens or prostate and absorption with testis did not inhibit staining of kidney or seminal vesicle. The absorptions with kidney homogenates of the various species gave results which were complementary to those in Table 1, in that the homogenates had a serum neutralizing capacity which was roughly proportional to the strength of staining of the corresponding kidney sections. Complete neutralization of the antisera could only be achieved by absorptions with the homogenate of the homologous kidney; similar absorption by kidney preparations of other species inhibited all staining of the kidney of that species and reduced the capacity to stain the kidneys of other species to an extent which varied

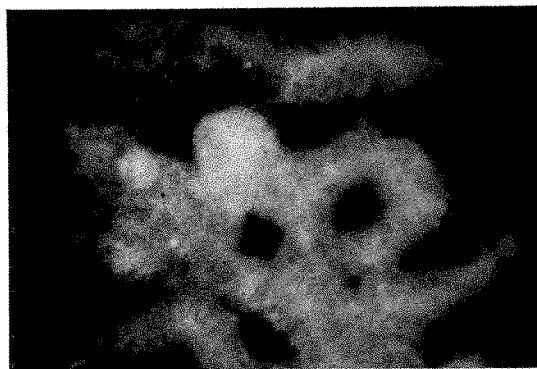


Fig. 1. Fresh frozen section of guinea-pig seminal vesicle treated first with rabbit anti-hamster-kidney serum and then with fluorescein-labelled goat anti-rabbit-globulin. Bright fluorescent staining of epithelium. (×250.)



from total inhibition in close relatives to partial inhibition where the relationship was more distant.

It has been suggested<sup>3</sup> that the present antisera contain antibodies to at least two separate antigens, one distributed throughout the nephron and present also in human kidney cancer, and the other largely restricted to the proximal tubules. Absorption of the sera with human kidney cancer homogenates neutralized the first antibody and left the latter with largely undiminished activity. Such cancer-absorbed antisera still stained, though with slightly diminished intensity, the kidneys of vertebrates of all classes and the seminal vesicles. On the other hand, antisera absorbed with marine fish kidney failed to stain human renal cancer sections. This suggests that both the antibodies cross-react between the species and with seminal vesicle; the "proximal tubular antigen" appeared to be the most abundant in both organs.

The disparity between the present findings that some antigens specific for kidney are common to all vertebrates and the earlier results which suggested a less widespread distribution is attributed to improvements in immunohistological techniques, in particular to the use of liquid nitrogen-isopentane slurry for snap-freezing of tissues. This permits excellent preservation of renal architecture and of labile antigens. Those located by the antisera in this study are fairly easily dispersed in the stained sections which must be examined within 30 min of mounting for accurate interpretation.

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### Antibody Response of Thymectomized Rats after Local Irradiation of the Spleen

THE haemolysin response of rats given a massive dose of X-rays to the surgically exteriorized spleen a few minutes after antigen injection has been found to be essentially normal although delayed for about 2 days<sup>1</sup>. It has also been shown that the antibody response in these conditions can be almost completely prevented either by splenectomy during the latent period or by sub-lethal whole body irradiation within a few hours of the local spleen irradiation<sup>1</sup>. This indicates (a) splenic origin of haemolysin formation and (b) the role of non-splenic lymphoid sites in restoring the antibody response. Histological studies reveal an early repopulation of the locally irradiated spleen by lymphocyte-like cells, followed by the appearance of large pyroninophilic cells and plasma cells<sup>2</sup>. In order to discover the origin of lymphoid cells repopulating the locally irradiated spleen a series of experiments has been performed, and one of them is reported here.

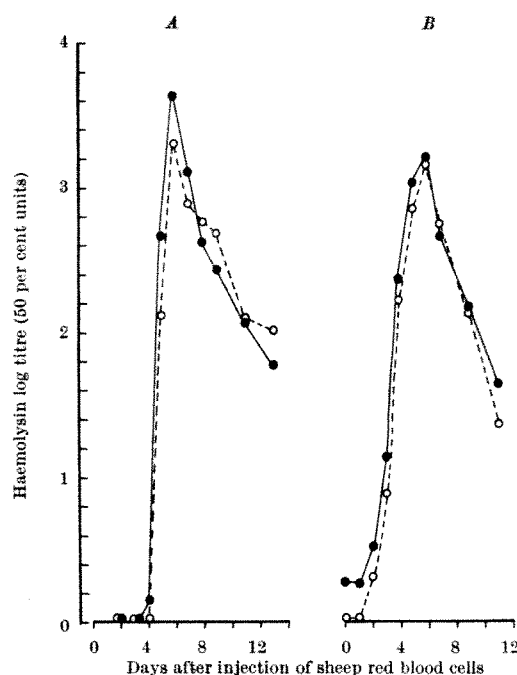


Fig. 1. Mean initial haemolysin response in groups of rats treated as indicated in Table 1. A, Groups 1 (○---○) and 2 (●—●); B, groups 3 (○---○) and 4 (●—●).

Female albino rats were thymectomized at the age of 4 weeks. Three months later one group of the rats was immunized by an intravenous injection of about  $3.5 \times 10^7$  sheep red blood cells and within a few minutes the spleen was surgically exteriorized and locally irradiated with 10,000 R of X-rays<sup>1</sup>. The other group was only immunized and served as thymectomized controls. Two additional control groups of rats of the same age were included: the first was immunized with sheep red blood cells and the second was immunized and locally irradiated as before. Blood samples had been obtained daily for nearly 2 weeks and individual sera were titrated for haemolysins in terms of 50 per cent haemolytic units<sup>1</sup>. At the end of the experiment all thymectomized rats had been autopsied and all those showing macroscopically visible amounts of thymic tissue were rejected. Parameters of the antibody response were individually determined<sup>1</sup> and their means were calculated for each group (Table 1). Average curves of haemolysin titres for each group are shown in Fig. 1.

As shown in Fig. 1A and Table 1, there was no essential difference in the antibody response between rats thymectomized at the age of 1 month and given local irradiation to the spleen 3 months later, and their non-thymectomized controls. This indicates that cells which are responsible for the restitution of the antibody-forming capacity of the heavily irradiated spleen do not come directly from the thymus, and that their response to the antigen localized in the spleen is not dependent on the thymus. This result also suggests that during the first month of life rats are provided with a sufficient number of immunologically competent cells which do not substantially decrease during the next 3 months. This is also supported by the lack of difference in the antibody response between rats thymectomized at the age of 1 month and immunized 3 months later, and their non-thymectomized controls (Fig. 1B and Table 1). In another series, not reported

Table 1. PARAMETERS OF THE HAEMOLYSIN RESPONSE IN RATS AFTER THYMECTOMY AND/OR LOCAL IRRADIATION OF THE SPLEEN

Group	Treatment	Log peak titre	Length in days of		Rate of antibody accumulation	No. of rats
			Latent period	Rise to peak	Initial Average	
1	Thymectomy at 1 month	3.32 ± 0.26	4.2 ± 0.1	2.0 ± 0.1	5.10 ± 0.50	6
	Antigen + 10,000 R to the spleen at 4 months				3.74 ± 0.36	
2	Antigen + 10,000 R to the spleen at 4 months	3.64 ± 0.17	3.9 ± 0.1	2.1 ± 0.1	5.77 ± 0.53	5
3	Thymectomy at 1 month	3.23 ± 0.27	2.3 ± 0.6	3.3 ± 0.3	3.00 ± 0.55	3
	Antigen at 4 months				4.03 ± 0.19	
4	Antigen at 4 months	3.23 ± 0.16	2.3 ± 0.3	3.4 ± 0.2	2.73 ± 0.20	7
	Antigen at 4 months				2.16 ± 0.17	

All values are means ± standard error.

here, no difference in the antibody response was found between thymectomized and non-thymectomized rats when the interval between thymectomy and immunization was extended to 5 months in similar experimental conditions. All these results are in general agreement with reports<sup>3-5</sup> showing that thymectomy of young or adult animals might result in an impaired immunological responsiveness only several months later.

Local irradiation of the spleen somewhat modified the haemolysin response to sheep red blood cells injected immediately before exposure. When compared with unirradiated controls the antibody response after local irradiation of the spleen was delayed for about 2 days, but normal peak titres were reached during a significantly shorter rise to peak and at a much higher rate of antibody accumulation (Table 1). These results corroborate previous ones, describing the effects of local irradiation of the spleen on the antibody response in rats<sup>1</sup>. On the basis of the present results it could be concluded that in the experimental conditions used the thymus does not seem to play a part in the repopulation of the locally irradiated spleen and in the restitution of its antibody-forming capacity.

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### Elimination of Normal Horse IgG labelled with Iodine-131 in Rats receiving Horse Anti-rat Lymphocytic IgG

RECENT reports have indicated that antilymphocytic IgG, in addition to inhibiting the homograft reaction, is capable of suppressing the formation of humoral antibody against a number of antigens<sup>1-4</sup>. Somewhat conflicting results have appeared, however, on the ability of antilymphocytic antibody to suppress the formation of antibody against itself, or against normal IgG globulin obtained from the species in which the antibody was produced<sup>5-7</sup>. We have investigated this problem by studying the effect of prolonged administration of antilymphocytic IgG (horse anti-rat) on the elimination of normal horse IgG, labelled with iodine-131, from rats.

The techniques of raising antilymphocytic serum and preparing purified IgG derivatives have been described previously<sup>3,8</sup>. The labelling with iodine-131 was performed by the method of Hunter and Greenwood<sup>9</sup> and it was found that more than 95 per cent of the radioactivity of the preparation was precipitable with trichloroacetic acid. The rats were females aged 3-4 months (160-210 g) of an inbred hooded strain maintained in this laboratory<sup>10</sup>. Six animals received daily intraperitoneal injections of

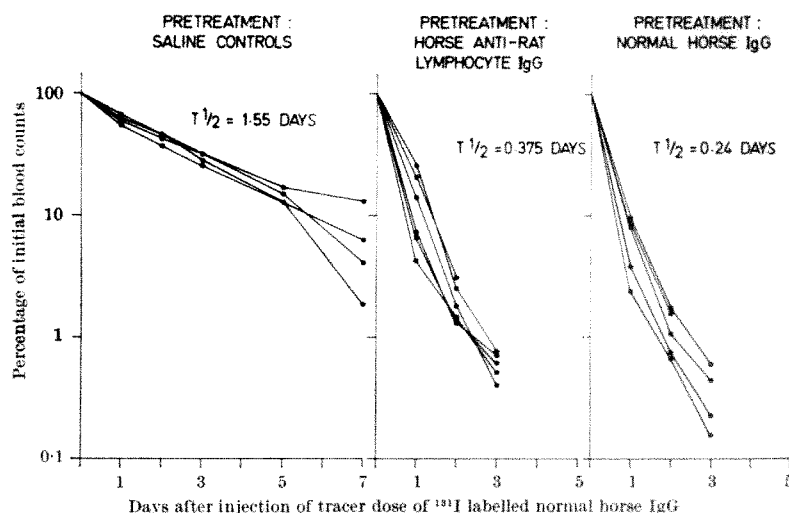


Fig. 1. Clearance of normal horse IgG labelled with iodine-131 from the blood stream of rats pretreated with saline, antilymphocytic IgG and normal horse IgG. Initial counts ranged from 95 to 187/sec. Mean background count 2.5/sec.

antilymphocytic IgG in a dosage which had previously been shown to prolong the survival of skin homografts by a factor of about 2.5 (ref. 8) (1 ml. of 2 g per cent solution daily for 7 days followed by 0.5 ml. daily for 14 days). Six animals received a similar course of normal horse IgG and six others received daily injections of saline. Four days after the last injection of IgG or saline each rat received an intraperitoneal injection of 10 mg normal horse IgG labelled with 10  $\mu$ c. of iodine-131. Sodium iodide (100 mg/l.) was added to the drinking water of all rats commencing 7 days before the injection of labelled protein and continuing to the end of the experiment.

The fate of the labelled protein was investigated in three ways: by counting serial blood samples; by repeated whole body counting; and by counting samples of various tissues removed at autopsy. Blood from a tail vein (0.1 ml.) was diluted with heparinized saline (0.9 ml.) and counted in a well-type scintillation spectrometer incorporating a 2 in. sodium iodide crystal. After tissue samples had been weighed and digested in 6 normal potassium hydroxide, they were counted with the same apparatus. Whole body counting was performed in a ring counter consisting of twelve J26Pb Geiger-Müller tubes shielded with 1.25 in. lead shot which was kindly provided by Dr P. Tothill of the Department of Medical Physics.

The blood and whole body counts are shown in the accompanying graphs (Figs. 1 and 2). It will be seen that rats pretreated with either antilymphocytic or normal horse IgG showed greatly accelerated elimination of the radioactive material from the blood stream (mean biological half-life 0.24 day and 0.38 day, respectively) as compared with the rate of elimination in the control animals pretreated with saline (mean biological half-life 1.55 days). Total body counts showed a similar pattern except that after the initial rapid fall some 2-4 per cent of the original activity remained for up to 5 days. Tissue counts showed that this activity resided principally in the liver and kidneys, where it presumably represents either stages in the breakdown of the injected protein or antigen-antibody complexes.

The results suggest that administration of antilymphocytic IgG in this experimental system does not prevent immunization of the treated animals to horse IgG, thus confirming the hypothesis put forward to account for the failure of initial attempts to achieve immunosuppression with antilymphocytic serum (ALS)<sup>11</sup>. It is now apparent that a high degree of general immunosuppression can be achieved despite immune elimination of the ALS (or IgG) itself, but it is pertinent to ask whether still greater immunosuppression would result if the rate of elimination

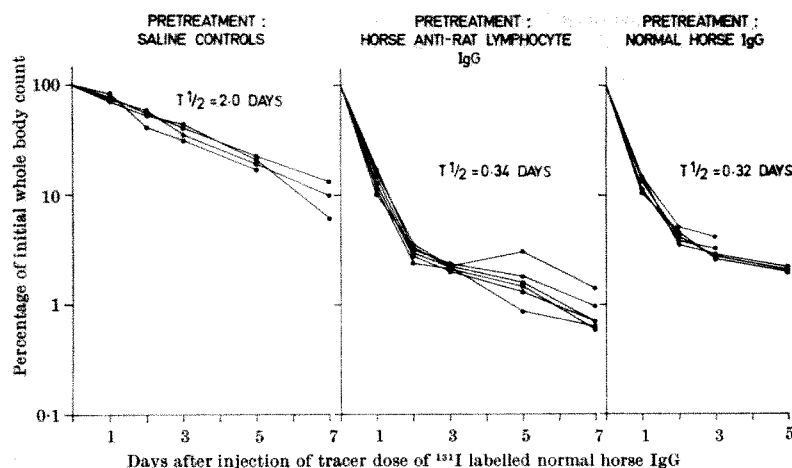


Fig. 2. Elimination of normal horse IgG labelled with iodine-131 as determined by whole body counting in rats pretreated with saline, antilymphocytic IgG and normal horse IgG. Initial counts ranged from 1,572–1,930/sec. Mean background count 15/sec.

of the material could be slowed down. This might perhaps be achieved by previous induction of specific immunological tolerance of this material as suggested by Lance and Dresser<sup>12</sup>, by concurrent administration of chemical immunosuppressants, or by reducing the proportion of non-antilymphocytic IgG in the preparation. These possibilities are being investigated.

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### Effects of *Corynebacterium parvum* on Immunological Unresponsiveness to Bovine Serum Albumin in the Rabbit

THE immunological response of adult rabbits to bovine serum albumin (BSA) can usually be completely suppressed by an intravenous injection of 10 mg of centrifuged bovine albumin (CBA), but this unresponsive state can in turn be prevented by the injection of a heat-killed suspension of *Corynebacterium parvum* at the same time or 6 days in advance<sup>1</sup>. *C. parvum* also augments the production in the adult rabbit of anti-BSA antibody to either CBA or partially aggregated BSA, and at the same time enhances the relative binding affinity of the antibody<sup>2,3</sup>. We have now been able to show that in neonatal as distinct from adult rabbits *C. parvum* does not impede the induction of immunological unresponsiveness to BSA, and that in adult rabbits a suspension of the organism is ineffective as a means of abrogating a state of unresponsiveness already established.

**Experiment 1.** In the first experiment, eleven newborn New Zealand White rabbits were injected intraperitoneally

with 2 mg (dry weight) of a heat-killed suspension of *C. parvum*<sup>2</sup> less than 12 h after birth and they, together with ten control rabbits, were injected intraperitoneally with 100 mg BSA 6 days later. There was no further treatment until the rabbits were 3 months old, when they all received 50 mg BSA intravenously. They were bled every third day for 30 days, when a second injection of 50 mg BSA was given and the rabbits were bled for another 30 days. Sera were tested for anti-BSA antibody by the Farr technique<sup>4</sup> using 0.20  $\mu$ g N BSA\* and 0.02  $\mu$ g N BSA\* antigen concentrations as previously described<sup>2</sup>.

The ten control rabbits, which received 100 mg BSA 6 days after birth, did not produce detectable anti-BSA antibody after the first injection of BSA, and the same was true after the second challenge for the nine rabbits which survived. Of the eleven rabbits which received *C. parvum* 12 h after birth, only one produced detectable

anti-BSA antibody after the primary challenge with BSA but the amount was not sufficient to permit measurement of the antigen-binding capacity. The same rabbit was the only one to respond to the second challenge, but again too weakly for measurement. In other words, the injection of the *C. parvum* suspension did not interfere with the establishment of immunological unresponsiveness in neonatal rabbits.

**Experiment 2.** Six New Zealand White rabbits were injected intraperitoneally with 100 mg BSA less than 12 h after birth, and 3 months later three of them were injected intravenously with 10 mg (dry weight) of a heat-killed suspension of *C. parvum*. Both groups of three rabbits received 50 mg BSA intravenously 6 days later and were then bled every third day until day thirty of the primary response, when they received intravenous injections of 50 mg BSA and were bled every third day for another 30 days. The primary and secondary sera were tested for anti-BSA antibody as in the first experiment.

The antibody responses of the control rabbits and of those treated with *C. parvum* were identical. Two rabbits from each group did not produce detectable anti-BSA antibody after primary or secondary stimulation with BSA. One rabbit from each group produced low levels of anti-BSA antibody in the primary and secondary responses but the amounts were too low to determine antigen-binding capacities. In other words, *C. parvum* does not abrogate the state of unresponsiveness established by the neonatal injection of 100 mg of BSA.

Our findings on the effects of *C. parvum* on immunological unresponsiveness in adult<sup>1</sup> and neonatal rabbits agree with the results of other experiments. In adult animals, the induction of unresponsiveness can be readily blocked by adjuvant materials such as BCG<sup>5</sup>, *Salmonella typhosa* endotoxin<sup>6</sup>, actinomycin D<sup>7</sup>, zymosan<sup>10</sup>, as well as by *C. parvum*<sup>1</sup>. In neonatal rabbits, on the other hand, it has been shown<sup>5</sup> that large amounts of bacterial endotoxin do not block the induction of neonatally induced unresponsiveness to BSA, and that rabbits 1 day old injected with Freund's adjuvant and 5 days later with 100 mg human serum albumin become unresponsive to the HSA molecule<sup>6</sup>.

These results indicate that either the mechanism of the induction of unresponsiveness is different in the neonatal and adult animal, or that neonatal and adult animals may be affected to different degrees by various adjuvant materials. The observed differences could arise from the relatively massive doses of antigen, with respect to body weight, administered to neonatal animals in order to induce a profound degree of unresponsiveness; only relatively small quantities of aggregate-free antigen are

needed to induce a profound degree of unresponsiveness in the adult rabbit<sup>1</sup>. Certainly Paraf *et al.*<sup>6</sup> report that complete Freund's adjuvant does block the induction of unresponsiveness in the neonatal rabbit to 1 mg HSA. Smith<sup>5</sup>, however, indicates that bacterial endotoxin does not block the induction of unresponsiveness to various doses of BSA in the neonatal rabbit. Whether unresponsiveness to high and low doses induced in the neonatal and adult animals arises by different mechanisms or simply results in varying durations or degrees of unresponsiveness remains to be determined. The mechanism of adjuvant action of many substances is still unknown, although with the accumulation of recent data about the induction of unresponsiveness, it would appear that adjuvant materials augment the production of antibodies simply by blocking the induction of unresponsiveness.

The question which now arises is why materials such as *C. parvum*, which act as powerful adjuvants in adult animals, apparently possess no adjuvant activity in neonatal animals. Elucidation of these differences may throw light on the maturation processes of the lymphoid tissues and on the role of different cellular elements in the initiation of the immune response.

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## Histidine Decarboxylase Inhibitors and the Survival of Skin Homografts

CHANG and I (unpublished results) have observed that skin homografting in rats results in a significant increase in urine histamine excretion and that the greatest histamine excretion occurs within a 3 day period of the observed time of graft rejection. The possibility that intracellular histamine metabolism and histamine formation from histidine decarboxylase activity might be involved in the mechanics of homograft rejection led to the present study of the effect of histidine decarboxylase inhibitors on rat skin homograft survival.

Three histidine decarboxylase inhibitors were used—the alpha hydrazino analogue of histidine, semicarbazide and NSD-1055 (4 bromo-3-hydroxy benzyloxyamine). The effectiveness of the alpha hydrazino analogue of histidine and NSD-1055 as potent inhibitors of histidine decarboxylase has been described<sup>1</sup>, while the combination of semicarbazide administration and a pyridoxine deficient diet has been reported<sup>2</sup> to reduce histidine decarboxylase activity to 10–20 per cent of normal.

The histidine analogue (aHH) and NSD-1055 were injected intraperitoneally and the semicarbazide was given

subcutaneously. Control injections of physiologic saline solution were given intraperitoneally twice daily. Injections were started on the day before grafting and each was 1 ml. They were continued daily thereafter.

Skin homografts of standard size were carried out between the inbred P.A. and Lewis rat strains. Circular grafts of abdominal skin 3.5 centimetres in diameter were cut from a pattern and were secured to an area of equal size on the back of the recipient by means of small Michel clips. All of the recipients were females and weighed approximately 200 mg. The grafts were not covered by dressings and the animals were kept in individual cages. The grafts were inspected daily and the percentage of rejection was recorded. Any portion of the graft which was hard and obviously devitalized was regarded as rejected. The use of a comparatively large graft of standard size on animals of uniform size permitted calculation on a daily basis of the percentage of total grafted area rejected in any group of animals as a pooled rejection response. In the calculation of mean survival time of grafts, any graft with 50 per cent or more rejection was regarded as rejected.

Table 1. SUMMARY OF SKIN HOMOGRAFT SURVIVAL TIMES

No. of rats	Treatment*	Total daily dose (mg/kg)	Mean survival time (days)	Range of survival (days)	Change against controls (per cent)	Mortality of rats before completed rejection
P.A. to Lewis						
20	Control		6.4	5–8		1/20
20	aHH	200	13.0	6–29	+103†	2/20
Lewis to P.A.						
20	Control		8.1	6–14		0/20
20	aHH	200	20.9	13–33	+170†	6/20
10	PDD		10.1	7–14	+25‡	0/10
10	SC+PDD	100	35.5	27–63	+340†	4/10
10	NSD	100	13.0	5–20	+60‡	3/10
10	NSD	150	13.5	8–20	+67‡	2/10

\* aHH,  $\alpha$  hydrazino histidine; PDD, pyridoxine deficient diet; SC, semicarbazide, NSD, NSD-1055.

† 0.01 significance level by Wilcoxon rank sum test.

‡ 0.05 significance level by Wilcoxon rank sum test.

Twenty control Lewis rats were grafted with P.A. skin (Table 1). The median survival times of these control grafts was 6.4 days. When 20 P.A. to Lewis grafted animals were given 100 mg/kg of aHH twice daily, the median survival time was increased significantly to 13.0 days.

Twenty control Lewis to P.A. homografts had a median survival time of 8.1 days. Administration of aHH (100 mg/kg twice daily) prolonged the median survival time of grafts to 20.9 days. The inhibition of graft rejection was even more impressive when the cumulative total area of rejected graft in thirty aHH treated rats was compared on a daily basis with the area of rejection in twenty control rats (Fig. 1).

The greatest time of survival for grafts in Lewis to P.A. grafted animals was obtained by giving semicarbazide (SC) to pyridoxine deficient diet rats. The animals received semicarbazide (50 mg/kg) twice daily. The pyridoxine deficient diet (PDD) was started 10 days before grafting. The median survival time of animals on PDD only was 10.1 days. When SC was given in addition to the PDD, the median survival time was increased to 35.5 days. The cumulative total area in which grafts were rejected was less than that of control rats and rats given PDD only (Fig. 1). NSD-1055 was less effective in increasing the survival of Lewis to P.A. skin homografts. The median survival time for 100 mg/kg once daily was 13.0 days and that for 75 mg/kg twice daily was 13.5 days. Work in progress suggests that the dosage level of SC is more critical than the duration of PDD prior to grafting and that PDD also enhances the rejection inhibiting action of aHH.

This investigation does not establish the biological mechanism by which these histidine decarboxylase inhibitors prolong survival of skin homografts. The



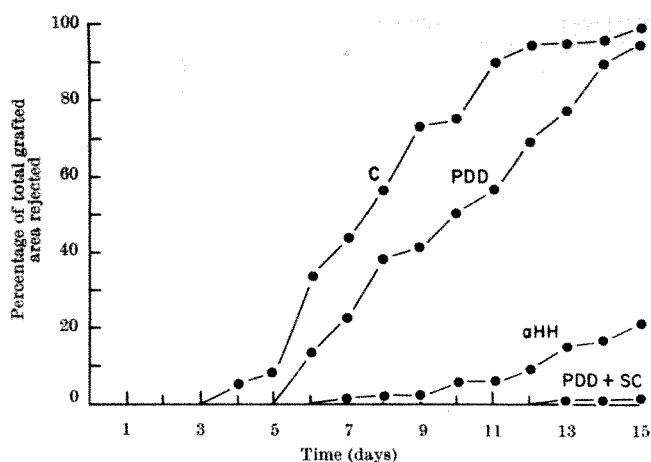


Fig. 1. Graph showing daily percentage of total grafted area rejected in control animals (C) as compared with animals on PDD, aHH and PDD+SC.

action may be an anti-inflammatory one caused by interference with intracellular histamine formation or it may be related to diminished antibody formation. This would be secondary to an enhanced pyridoxine deficiency produced by a pyridoxal trapping action of the histidine decarboxylase inhibitors. Pyridoxal phosphate is the co-enzyme of histidine decarboxylase and Stoerk and Eisen<sup>3</sup> have demonstrated an inhibition of circulating antibody production in pyridoxine deficiency, a suppression not encountered in paired weighed inanition controls and full controls.

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## BIOLOGY

### Gibberellins in the Red Alga *Hypnea musciformis* (Wulf.) Lamour.

GIBBERELLINS, or substances like gibberellin, have been detected in vascular plants belonging to diverse taxonomic groups<sup>1</sup>, and there is evidence for their occurrence in a species of green alga<sup>2</sup> and two species of brown algae<sup>2,3</sup>. We report here the presence of gibberellin-like activity in an extract from a species belonging to a further principal group of the plant kingdom, the red algae.

The extraction method used was essentially one of those previously described for the extraction of acidic gibberellins from higher plants<sup>4</sup>. Plants of *Hypnea musciformis* (Wulf.) Lamour. were collected from coastal reefs near Perth, and deep frozen until required. The thawed material (mass 1 kg) was homogenized in 60 per cent aqueous acetone and allowed to stand in the cold for 24 h before filtration. The acetone was distilled off *in vacuo*, and the aqueous residue adjusted to pH 2.5 and extracted three times with ethyl acetate. The total ethyl acetate extract was reduced in volume and extracted three times with phosphate buffer at pH 6.2; the buffer phase was in turn adjusted to pH 2.5 and extracted three times with ethyl acetate. The ethyl acetate extract was reduced in volume and chromatographed on Whatman No. 3 paper,

using *n*-butanol : 1.5 normal  $\text{NH}_4\text{OH}$  3 : 1 (upper phase) as a descending solvent. After drying, the chromatogram was divided transversely into ten equal strips, and each strip was eluted with acetone/water. Each eluate was dried and taken up in 0.5 ml. of 50 per cent ethanol for assay using dwarf maize of the varieties  $D_1$  and  $D_5$  (ref. 5). Each maize seedling was treated with 0.05 ml. of the eluate, and measured 8 days after treatment. The assay was carried out under fluorescent lights at 25° C. Results are presented in Fig. 1.

It is clear that biological activity was present in the extract, centred in two broad zones at  $R_F$  0.2–0.4 and 0.5–0.8. Comparison of these  $R_F$  and bioassay data with those of gibberellins  $A_1$ – $A_9$  (ref. 6) indicates that activity at 0.2–0.4 could have been caused by any of the gibberellins  $A_2$ ,  $A_3$  and  $A_6$ , while the activity at 0.5–0.6 could be attributed to  $A_7$ . It may be tentatively suggested that the activity observed at  $R_F$  0.0–0.1 and 0.7–0.8, detected in the  $D_1$  but not the  $D_5$  assay, could not have been caused by any of the gibberellins  $A_1$ – $A_9$ . Only  $A_1$  has been found to be more active in the  $D_1$  than the  $D_5$  assay<sup>7</sup>, but this compound has an  $R_F$  in the region of 0.3 in the solvent system used here<sup>8</sup>. It is not possible to estimate the total level of gibberellin activity in the extract, as dose-response curves are not available for comparison with known  $GA_3$ . The general levels of activity are, however, probably of the same order as those found in extracts of shoots of higher plants<sup>1</sup>.

It has been reported that  $GA_3$  stimulates the growth of two species of the genus *Porphyra*<sup>8,9</sup>. These observations, together with the results reported here, suggest very strongly that gibberellins are involved in growth regulation in the red algae.

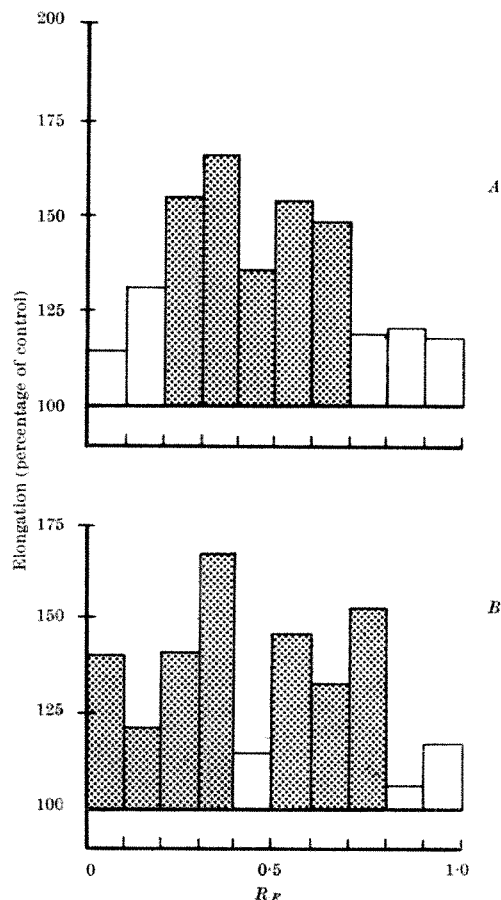


Fig. 1. Percentage elongation of maize seedlings treated with eluates from a chromatogram of a *Hypnea* extract. A, Results using maize variety  $D_5$ ; B, results using maize variety  $D_1$ . Stippled areas represent growth significantly different from the controls at less than the 5 per cent level (t test).

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### Effect of Light on Cell Division in Plant Tissue Cultures

LIGHT strongly influences many aspects of growth in plants. There have, however, been few studies on the effects of light on cell division in non-green plant tissue cultures. In the course of investigating the physiology of cell division in developing callus cultures of *Helianthus tuberosus* it has been observed that light can have an inhibitory effect on cell division.

Observations<sup>1-3</sup> have shown the course of change in cell number with time in explants taken from tubers of the Jerusalem artichoke and cultured in contact with a medium containing 20 per cent coconut milk and  $10^{-6}$  molar, 2,4-dichlorophenoxyacetic acid. It has also been

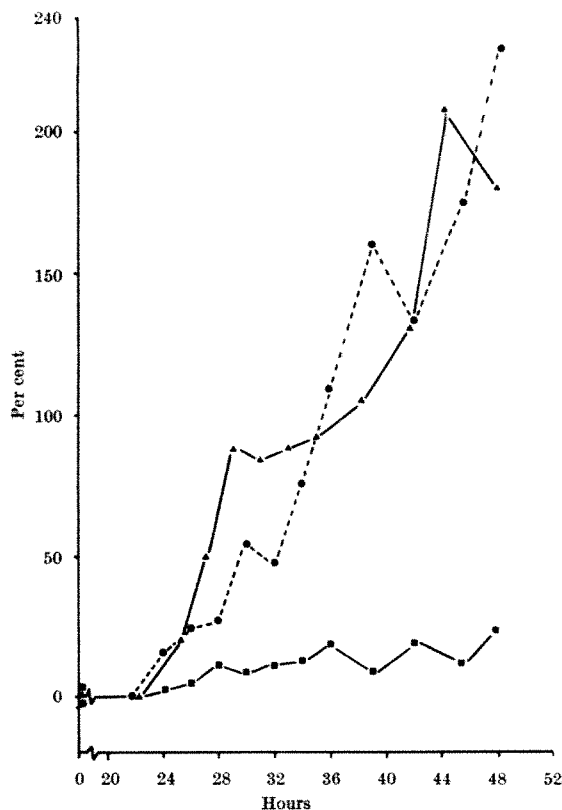


Fig. 1. Percentage increase in cell number per explant with time of incubation. Explants were removed from tubers in green light and cultured in groups of 80 in 12 ml. medium containing  $10^{-6}$  molar 2,4-D, in 9 cm Petri dishes, at 25° C. Agitation was on a reciprocating shaker operating at 50 c/s with a 7 cm displacement. The explants were grown in total darkness (▲), 120 ft.-candles (●) and 450 ft.-candles (■). Both light sources were mixed fluorescent and tungsten. Cell number data were derived from counts on 5 per cent chromic acid macerates of five explant samples.

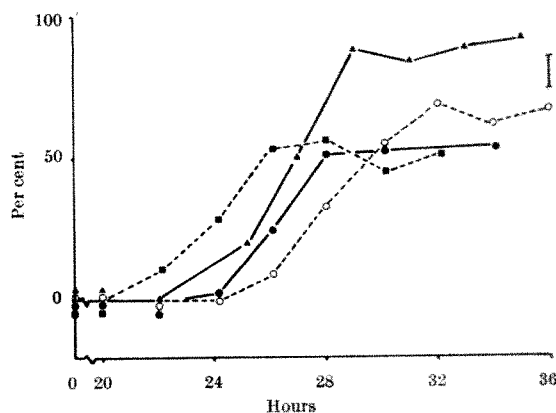


Fig. 2. Percentage increase in cell number per explant with time of incubation. Explants were prepared and cultured as described in Fig. 1. For the first 30 min after excision, the explants were exposed to total darkness (▲), 18 ft.-candles fluorescent light (○) and 120 ft.-candles (●) and 450 ft.-candles (■), mixed fluorescent and tungsten light. Subsequent culture was in total darkness. The vertical line represents the S.E. of the difference between the dark and light treatments.

shown<sup>2,3</sup> that the first few divisions are synchronous. These observations were made with an experimental procedure which involved exposure of the tubers and of the excised explants for periods of 30–90 min to about 18 ft.-candles from a fluorescent source, and exposure to the light from a tungsten lamp when the cultures were being inspected. Subsequently it has been found that the proportion of cells in the explant that divide at the first synchronous division increases from 45 to 90 per cent when the explant is removed in low intensity green light and then cultured in total darkness on a medium without coconut milk but in the presence of 2:4-dichlorophenoxyacetic acid (2,4-D) at  $10^{-6}$  moles/l.

Results of two experiments designed to show the light effect in division are given in Figs. 1 and 2. The explants were obtained with techniques described elsewhere<sup>1</sup>, and grown in bulk culture in a liquid medium.

In the first experiment (Fig. 1), the explants removed in low intensity green light were exposed throughout the period of culture to two intensities of light, and their growth compared with a control in total darkness. In the second experiment (Fig. 2), the explants were exposed to various light intensities for a period of 30 min after excision. They were then cultured in total darkness.

It is clear from the results presented in Fig. 1 that the number of cells formed at the first division is significantly greater in the dark than in the light. Continuous light therefore has a strongly depressing effect on cell division.

Preliminary exposure for a short period after excision has a sharply depressing effect on division at all light intensities (Fig. 2). The explants excised in green light and cultured in total darkness form considerably more cells at the first synchronous division than do those exposed to light for the first 30 min after excision. Clearly radiation other than dim green light has a strongly depressive effect on division in this system and most of the effect is exerted in the period immediately after excision. It may be noted that the synchronous pattern is displayed in all conditions in this experimental series.

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# Rapid Bioassay for Phytokinins based on Transpiration of Excised Oat Leaves

SEVERAL compounds which exhibit phytokinin activity have been reported<sup>1-7</sup>. Current interest in the isolation of this group of compounds indicates that a rapid technique for the detection of low concentrations of phytokinins would be useful. This communication describes such a technique and reports various aspects of transpiration stimulated by phytokinin.

The technique is based on transpiration of excised leaves of a species of *Avena* (oat) induced by phytokinin. This phenomenon is not related to increased uptake of water induced by growth substances<sup>8,9</sup>, nor is it related to cell enlargement of leaf disks<sup>9</sup>. Induced transpiration of excised oat leaves, however, is similar to stimulated transpiration of excised barley leaves<sup>10</sup>.

Initially distinct differences in sensitivity of excised leaves to stimulated transpiration were observed among various genera of the grass family. Species of *Avena*, *Triticum* and *Hordeum* proved to be the most sensitive. These genera were compared to determine the most sensitive species. Results (Table 1) showed that *Avena* species were more sensitive to transpiration induced by phytokinin than species of *Triticum* or *Hordeum*, and species of *Triticum* were more sensitive than species of *Hordeum*. When the quantity of water transpired was expressed in mg/cm<sup>2</sup> of leaf surface, the rates of transpiration of species of *Avena* were about four times that of untreated leaves.

Table 1. COMPARISON BETWEEN TRANSPIRATION RATES OF EXCISED *Avena*, *Triticum* AND *Hordeum* LEAVES, TREATED WITH 2 µg/ml. OF KINETIN

	Transpiration expressed (mg/cm <sup>2</sup> ) of leaf surface*		
	Treated	Untreated	Percentage increase
Oats	292	73	400
Wheat	184	50	361
Barley	117	83	141

\* These calculations involved two surfaces for each leaf.

Because species of *Avena* proved to be the most sensitive to transpiration stimulated by phytokinin, plants of the cultivar 'Fulgrain' (C.I. 4389) were propagated in a growth chamber in the following conditions. Approximately six plants were grown in 8 in. clay pots containing a 2:1 soil-peat mixture. The growth chamber was maintained at 20°-22° C. Plants were subjected daily to a fluorescent-incandescent system of light of about 1,000 ft.-candles for 15 h.

When plants were about 28 days old (the stage of the fourth leaf), the third leaf of each was excised. Later the fourth, fifth and sixth leaves were used. In a given test, however, leaves were taken from the same position on the plant because differences in leaf size and physiological condition influenced the rate of transpiration. Excised leaves were trimmed to either 30 or 38 cm with a sharp scalpel, and basal ends were immersed in various concentrations of the compound to be assayed. In most experiments, two 30 cm leaves were used in each replication; however, one 38 cm leaf gave comparable results. When it was desirable to know precisely the quantity of test material absorbed, excised oat leaves were treated in 15 ml. calibrated centrifuge tubes; the amount taken up could therefore be calculated. Leaves thus treated were maintained in the solutions to be assayed for 24 h at 20°-22° C under continuous fluorescent light (about 400 ft.-candles). Temperature was maintained with an air conditioner; the relative humidity therefore fluctuated between 50 and 80 per cent. Treated leaves were then transferred to calibrated centrifuge tubes containing distilled water and placed under continuous fluorescent light (400 ft.-candles). The amount of distilled water lost from

the tubes after 24 h was recorded. The difference in amount of water lost between treated and untreated leaves was a measure of the quantity of phytokinin in the test solution. The lack of change in leaf weights, after treatment with kinetin, as well as porosity data and visual observations (supplied by Dr J. E. Pallas, jun.), indicated that the water taken up was transpired. Transpiration induced by phytokinin also occurred in the absence of light; however, the rate of transpiration under 400 ft.-candles was about twice as great as that in the dark. Although the optimum assay time is 48 h, the time can be reduced to about 24 h (12 h treatment, 12 h in distilled water). When high sensitivity and quantitative results are desirable the 48 h period is recommended.

Ranges of sensitivity for two cytokinins are shown in Fig. 1. The lower limit of this test was about 0.001 µg/ml. for zeatin (supplied by Dr C. O. Miller) and 0.005 µg/ml. for kinetin. Thus the transpiration test is about ten times more sensitive than the chlorophyll retention<sup>6</sup> and the barley root inhibition tests<sup>11</sup>, but it is less sensitive than the carrot phloem test<sup>12</sup>. It is interesting to note that the order of sensitivity of the carrot phloem method is similar to the oat transpiration test—both are more sensitive to zeatin than to kinetin. Ranges of sensitivity (Fig. 1) for zeatin and kinetin are somewhat different. The effective range for zeatin is about 0.001-0.1 µg/ml. and it is 0.005-1 µg/ml. for kinetin. This difference is not surprising because the toxicity threshold for excised oat leaves is about 5 µg/ml. for kinetin and 0.5 µg/ml. for zeatin.

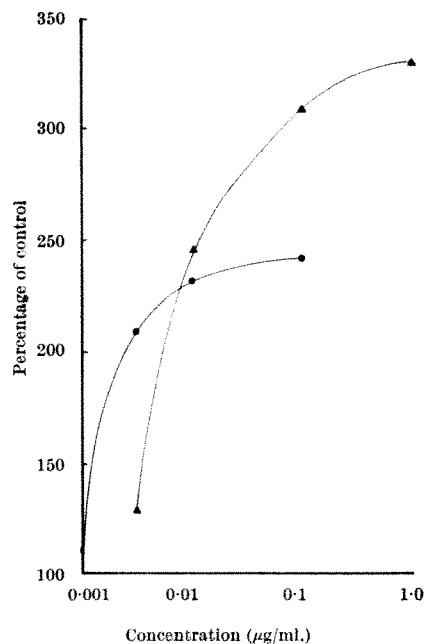


Fig. 1. Effects of concentration of phytokinin on the rate of transpiration of excised oat leaves. ●, Zeatin; ▲, kinetin.

Gibberellic acid stimulates transpiration of excised primary barley leaves<sup>10</sup>. When gibberellic acid was used in the oat leaf transpiration test ( $1.5 \times 10^{-4}$  molar) it did not cause a measurable increase in transpiration. Excised oat leaves therefore exhibit a greater degree of specificity for phytokinins than excised barley leaves.

The advantages of the assay described here can be summarized as follows: (a) short assay time (24-48 h); (b) high sensitivity (zeatin 0.001 µg/ml., kinetin 0.005 µg/ml.); (c) quantitative (see Fig. 1 for approximate linearity ranges); (d) permits accurate measure of amount

absorbed; (e) insensitive to compounds other than phytochemicals.

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### Absence of Nodule Formation on *Ceanothus cuneatus* in Serpentine Soils

YOUNGBERG has reported that *Ceanothus cuneatus* Nutt., a woody shrub native to semi-arid regions of California and Oregon, has clusters of nitrogen-fixing nodules on its roots<sup>1</sup>. During the past 2 years, I have examined the root systems of this species in seven populations in Oregon and California with particular attention to the parent material of the soils involved (Table 1). No plant less than 3 dm tall was examined.

Table 1. POPULATION NODULATION DATA GROUPED BY PREDOMINANT PARENT MATERIAL

Locality and parent material	No. of plants	No. nodulated
3-2 km S. of New Idria, Calif.; serpentine	21	0
Store Gulch, 16 km W. of Selma, Ore.; serpentine	18	1
6-6 km W. of Selma; serpentine	15	0
Star Flat (A), 6-4 km W. of Selma; serpentine alluvium	20	1
Totals	74	2
Mean nodulation = 0.03		
Star Flat (B), 20 m W. of (A); mixed alluvium*	14	11
Rough and Ready Creek Wayside, Ore.; mixed alluvium*	4	3
6-4 km E. of Copper, Ore.; greenschist	11	2
Totals	29	16
Mean nodulation = 0.55†		

\* Alluvium composed of serpentine and other parent materials.

† Differs from the mean of serpentine populations at the 1 per cent level of significance using a *t*-test, and at the 0.5 per cent level of significance by the  $\chi^2$  method. The standard error of the difference between the means is 0.095.

The percentage of plants nodulated on non-serpentine soils ranged from 18 per cent to 79 per cent in separate populations, while the percentage of plants nodulated on serpentine soils ranged from 0 per cent to 6 per cent. The mean percentage of nodulated plants in populations growing on non-serpentine soils is lower than expected. This is a reflexion of both the choice of populations and the low number of plants examined, and would probably be higher in other populations growing in more suitable habitats. The mean is also lowered by sampling populations on soils derived from greenschist, a rock containing minerals which are also found in serpentine rock, and populations on alluvial soils contaminated by serpentine material.

I think that the paucity of nodulated plants growing on serpentine soils results from one or more of three factors:

(1) low concentrations of major (NPK) nutrients; (2) low available molybdenum; (3) toxic concentrations of chromium, nickel, and other heavy metals<sup>2</sup>. Low available molybdenum may be of particular significance because plants which fix nitrogen require more molybdenum than those which do not<sup>3</sup>. Exclusion of free-living<sup>4</sup> and symbiotic nitrogen-fixing organisms from serpentine soils may partly explain the low concentrations of nitrogen in serpentine soils. The few plants that were found nodulated on serpentine soils grew only in microsites where other rock types and soils had been added by colluvial or alluvial mixing. This apparently has had an ameliorating influence on the effects of the predominantly serpentine soil.

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### Spicules in the Skeleton of *Tremacystia d'orbignyi* (Hinde)

THE Sphinctozoa<sup>1</sup> or Thalamida<sup>2</sup> are extinct marine organisms with a chambered calcareous skeleton, which are widely regarded as sponges because spicules like those of existing Calcarea have been figured from Cretaceous species. The best authenticated spicules have been those of *Tremacystia* [*Verticillites*] *d'orbignyi* (Hinde), which were stated by Hinde<sup>3</sup> to be figured from examples detached and mounted separately.

In a paper introducing the treatment of this group in the *Osnovy Paleontologii*<sup>4</sup> Zhuravleva and Rezvoi<sup>5</sup> have claimed that these spicules are imaginary. They maintain that the sphinctozoan skeleton has only a concentrically fibrous structure around radial canals which run through it. When the fibrous sheaths around closely spaced canals are in contact, a tri-radiate space may be left between a group of three canals, and Hinde<sup>3</sup> is believed to have mistaken such spaces for spicules. No authors since Rauff<sup>6</sup> have recorded any spicules, and photographs only show their absence. On these grounds the authors conclude that their skeleton has nothing in common with that of calcareous sponges, and revive the suggestion<sup>7</sup> that they may be related to the Archaeocyatha. In the *Osnovy Paleontologii*<sup>4</sup> volume, which treats sponges and archaeocyathids, it is stated that the skeleton of Sphinctozoa has not even a distant resemblance to those of any existing sponges, and the group is made a class *incertae sedis* between the Porifera and Archaeocyatha.

Spicules of *T. d'orbignyi*, as seen in two of Hinde's original slide preparations (British Museum (Natural History), Nos. S.1425, S.1426) are shown in Fig. 1. His slides contain examples of all the types of spicules which he figured<sup>3</sup>, mounted in balsam as he stated. Spicules are also seen on the surfaces of all his original specimens (Nos. S.8785-8787 and S.8791-8800), as well as in others examined. As he stated<sup>3</sup>, the large forms and their smaller variants (Fig. 1, except bottom right) are sagittal quadriradiates or tri-radiates, like those of existing Calcarea. The finely "fibrous" structure in which these spicules are embedded is also composed of separable spicules (Fig. 1, bottom left and right).

The notion that Hinde mistook spaces in a purely fibrous structure for spicules is thus clearly mistaken. It



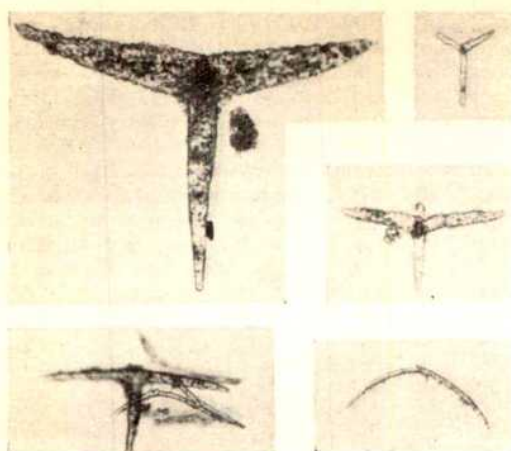


Fig. 1. Spicules of *Tremacystia d'orbignyi* (Hinde), from Hinde's slide preparations S.1425, S.1426 ( $\times 110$ ).

had also little justification, because Hinde could not have mounted detached spicules, as he stated<sup>3</sup>, had they not existed.

I thank the Trustees of the British Museum (Natural History) and Dr H. Ball for permission to study Hinde's material, and Mr S. Ware for locating it.

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### Diurnal Variation of Adrenocortical Function in Hypophysectomized Rats with Multiple Heterotopic Pituitary Homografts

WE have shown that hypophysectomized male rats bearing ten heterotopic, homografted pituitaries under the renal capsules have nearly normal adrenal weight and considerable, though subnormal, adrenal corticosterone production<sup>1</sup>. Because adrenal weight and production of corticosterone was markedly reduced in hypophysectomized rats without pituitary homografts, we concluded that the heterotopic pituitary has adrenal-maintaining activity presumably by means of the secretion of ACTH. Furthermore, because many investigators have shown that a single heterotopic pituitary exhibits only very slight adrenal-maintaining activity<sup>2-4</sup>, we concluded that multiple heterotopic pituitaries maintain adrenal weight by virtue of their collective secretion of individually small amounts of ACTH. The present communication deals with studies of diurnal variation in the function of the cortex of the adrenal glands in rats with pituitary homografts and provides evidence that direct connexion between the brain and pituitary is not essential for the rhythmic activity of the pituitary-adrenocortical axis.

Recipient male rats of the Sprague-Dawley strain, with a history of a relatively high degree of inbreeding in a small, closed colony, weighed approximately 120 g at the time of pituitary grafting. Ten whole pituitaries from 14 day old donor rats of the same strain, but of unselected sex, were transplanted under both renal capsules. The animals were housed in a room of constant temperature with 12 h of light, 12 h of dark cycles beginning at 6 a.m., and were fed water and 'Purina' laboratory chow *ad lib*. Four weeks after transplantation, the animals were hypophysectomized by the parapharyngeal approach. Two weeks later, diurnal variation of adrenocortical function was studied. Blood from the trunk was collected in heparinized beakers after decapitation at 8.30 a.m. and 4.30 p.m. Blood specimens were promptly centrifuged and the plasma analysed for corticosterone by the method of Mattingly<sup>5</sup>, and adrenal glands were weighed on a torsion balance and analysed for corticosterone.

The mean ( $\pm$  standard error) relative adrenal weight of the hypophysectomized, grafted animals was slightly greater than that of the unoperated controls ( $15.7 \pm 0.5$  mg/100 g body weight as against  $13.5 \pm 0.3$  mg/100 g body weight) but the difference was not statistically significant. Furthermore, the adrenal weights of the animals of both groups killed in the morning were not statistically different from the adrenal weights of the respective group killed in the afternoon. The adrenal glands of the animals with pituitary grafts were, however, much larger than those of six hypophysectomized controls ( $6.0 \pm 0.2$  mg/100 g body weight). The adrenal function data are summarized in Table 1. The afternoon concentration of plasma corticosterone was higher than that found in the morning both in the hypophysectomized animals with grafts and in the unoperated controls. Similarly, the adrenal corticosterone content was greater in the afternoon than in the morning in both groups. These data indicate that diurnal variation of adrenocortical function may occur despite the absence of direct connexion between the brain and the adenohypophysis. The data do not, however, shed light on the question of the location of the biological clock regulating pituitary-adrenal rhythmicity.

A location of the biological clock in the brain is supported by several observations including those of Slusher<sup>6</sup>, who found that hypothalamic lesions may interfere with diurnal variation of plasma corticosterone, although they do not alter the response of corticosterone to stress. The finding of Galicich *et al.*<sup>7</sup> who showed in mice that cerebral ablation above the pons, but not above the thalamus, interfered with circadian variation in the content of pituitary ACTH, provides additional evidence that the suprapontine brain participates in the regulation of diurnal variation in pituitary-adrenocortical activity. Finally, the observation that anticholinergic agents block the circadian pattern of plasma 17-hydroxycorticoids in cats<sup>8</sup> supports the hypothesis that the central nervous system is involved in the circadian cycle.

The physiological control of heterotopic pituitary secretion of ACTH also has not been fully explained. One possible mechanism for this control is by secretion of a neurohumour (CRF) from the hypothalamus which is transported through the general circulation to reach the heterotopic pituitary and stimulate secretion of ACTH. Our previous finding that exogenous corticoids depress heterotopic ACTH secretion in hypophysectomized rats with multiple heterotopic pituitary homografts<sup>1</sup> lends tentative support to the hypothesis that the heterotopic pituitary remains under the control of the hypothalamus,

Table 1. DIURNAL VARIATION OF ADRENOCORTICAL FUNCTION

Group	Plasma corticosterone $\mu$ g/100 ml.		P <sup>†</sup>	Adrenal corticosterone $\mu$ g/pair glands		P
	a.m.	p.m.		a.m.	p.m.	
Homografted and hypophysectomized	$5.2 \pm 1.0^*$ (14) <sup>†</sup>	$9.7 \pm 0.8$ (14)	< 0.001	$0.15 \pm 0.03$	$0.27 \pm 0.04$	< 0.01
Unoperated controls	$5.6 \pm 0.6$ (12)	$16.6 \pm 2.5$ (13)	< 0.001	$0.13 \pm 0.01$	$0.26 \pm 0.08$	< 0.02

\* Mean  $\pm$  standard error.

<sup>†</sup> Number of animals per group.

<sup>‡</sup> Probability that the difference between the morning/afternoon mean was due to chance.



because the bulk of current evidence supports the view that corticosteroids suppress secretion of ACTH after a primary action in the median eminence of the hypothalamus<sup>9</sup>. Removal of the entire forebrain, but not cerebral decortication, was also found to diminish adrenocortical function in hypophysectomized rats with pituitary homografts<sup>10</sup>, which suggests that the heterotopic pituitary depends on stimulation by the subcortical forebrain, perhaps by means of a systemically transported neurohumour of hypothalamic origin. Though still speculative, it is attractive to explain our present finding of diurnal rhythm of adrenocortical function in rats with heterotopic pituitaries by postulating that the brain rhythmically secretes a neurohumour which is transported through the general circulation to stimulate the heterotopic pituitary and, in turn, the adrenal cortex.

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### Heritable Resistance to Warfarin in Rats

WARFARIN and other anticoagulants are used for the destruction of rats throughout the world and have been by far the most successful materials ever introduced for this purpose. Since 1960, however, apparently independent outbreaks of resistance to warfarin in *Rattus norvegicus* have been reported from Scotland<sup>1</sup>, Denmark<sup>2</sup> and Wales<sup>3</sup>.

Starting with wild rats caught in Wales, rats resistant to warfarin have been crossed with domesticated albino rats of unknown origin in this laboratory for five successive generations. The offspring in each generation were fed *ad lib.* for six days on medium oatmeal containing 0.005 per cent warfarin, a procedure which is lethal to about 93.4 per cent of rats from our laboratory stock. Table 1 shows that mortality has not differed significantly from the figure of 46.7 per cent expected on the hypothesis that resistance to warfarin depends on a single dominant autosomal gene. A similar result, relating to rats of Scottish origin resistant to warfarin, has been mentioned by Drummond and Bentley<sup>4</sup>, who refer to unpublished work by P. M. Sheppard and D. A. Price Evans of the University of Liverpool.

Twenty-two experimentally naive fifth generation rats, half of which would be expected to possess the inferred gene for resistance to warfarin were subcutaneously injected with 1 mg/kg of warfarin dissolved in dimethyl formamide. Blood samples were taken retro-orbitally under ether or 1:1 oxygen:carbon dioxide anaesthesia immediately before and 24 h after injection, and the one-stage prothrombin times measured using Diagen 'Two-Seven-Ten' reagent. Two weeks after injection the rats were given the six day feeding test. It can be seen from Table 2 that except for rat No. 22 the animals fell into two distinct groups: in one the injected warfarin had little or no effect on the prothrombin time and the animals

Table 1. MORTALITY RATIOS IN FIVE GENERATIONS OF RATS, OFFSPRING OF MATINGS BETWEEN RATS RESISTANT AND SUSCEPTIBLE TO WARFARIN, GIVEN 0.005 PER CENT WARFARIN IN MEDIUM OATMEAL FOR SIX DAYS

Generation of offspring	Parents		Mortality ratios in offspring*		Totals
	Male	Female	Male	Female	
1	Wild type resistant	Albino susceptible	15/31	13/29	28/60
2	Generation 1 resistant	Albino susceptible	10/22	3/16	13/38
	Albino susceptible	Generation 1 resistant	19/59	27/55	46/114
3	Generation 2 resistant	Albino susceptible	43/71	27/56	70/127
	Albino susceptible	Generation 2 resistant	30/62	23/53	53/115
4	Generation 3 resistant	Albino susceptible	11/19	5/12	16/31
	Albino susceptible	Generation 3 resistant	5/12	4/7	9/19
5	Generation 4 resistant	Albino susceptible	15/35	7/17	22/52
	Albino susceptible	Generation 4 resistant	8/26	9/19	17/45
Totals			156/337	118/264	274/601†

\* Sum of 18 chi-squares = 20.6 (18 d.f.); 0.5 > P > 0.3

† Pooled chi-square = 0.3 (1 d.f.); 0.7 > P > 0.5  
Heterogeneity chi-square = 20.3 (17 d.f.); 0.3 > P > 0.2

Table 2. RESPONSES OF FIFTH GENERATION RATS TO WARFARIN GIVEN BY INJECTION AND IN FOOD

Rat No.	Sex	One stage prothrombin time (sec)		Six day feeding test Warfarin eaten (mg/kg)	Died/ survived
		Before injection	24 h after injection		
1	M	14.4	25.0	18.2	S
2	M	15.6	24.9	17.5	S
3	M	16.4	21.8	19.4	S
4	M	15.7	18.9	15.4	S
5	M	16.4	17.9	16.5	S
6	F	17.8	15.1	18.9	S
7	F	14.8	15.4	22.3	S
8	F	13.4	14.6	21.8	S
9	F	15.9	16.2	16.7	S
10*	F	15.8	15.1	23.0	S
11*	F	13.9	15.3	18.5	S
12	M	16.6	183.2	11.4	D
13	M	16.0	192.9	7.3	D
14	M	15.5	86.9	11.2	D
15	M	16.2	98.9	7.8	D
16	M	16.2	73.7	9.5	D
17	M	14.5	141.9	9.2	D
18	M	15.8	91.4	8.4	D
19	F	14.2	67.3	13.2	D
20	F	13.7	49.5	17.3	D
21	F	22.1	51.7	9.6	D
22*	F	19.3	183.7	12.3	S

\* The blood sampling procedure was unsuccessful with these rats before the feeding test; the times given are for blood samples taken before and after a further 1 mg/kg injection given three weeks after the end of the feeding test.

survived the feeding test; in the other there was a large response to the injection and the rats died as a result of the feeding test. Rat No. 22 resembled the susceptible animals and differed from the resistant ones in showing typical signs of warfarin poisoning (congested lungs and bleeding from the nose) and reducing its food consumption during the last three days of the feeding test.

The finding that resistant animals are resistant to both ingested and injected warfarin shows that the resistance can not wholly be accounted for by failure to absorb the anticoagulant from the gut. Further, the difference between the prothrombin times of resistant and susceptible animals injected with warfarin shows that the resistance is related to the level of the blood clotting factors. The underlying mechanisms of resistance may, however, involve reduced capillary fragility, slower turnover of the clotting factors, more rapid detoxication or excretion of warfarin, increased binding of the anticoagulant to plasma proteins, increased storage of vitamin K—which is antidotal to warfarin—or, as in man<sup>4</sup>, more efficient utilization of vitamin K. It is hoped that further research will show in which, if any, of these mechanisms the resistance resides.

The difference between resistant and susceptible rats injected with warfarin, in terms of the activity of clotting factors of the prothrombin complex, also provides a means of distinguishing the two types of rat quickly and reliably,

which should facilitate investigations of resistance to warfarin in the field.

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### Indication of Cyclic Reproductive Activity in Abyssal Organisms

THE difficulty in sampling the sea floor at depths greater than 2,000 m (ref. 1), where the abyssal zone is believed to start<sup>2</sup>, is so great that even today there are not many samples. The yield in specimens from the samples which have been taken is low for any given species of animal and it is seldom that one species has been repeatedly taken. These factors have combined to permit few observations on the biology of any one species. In general, so few ovigerous or gravid specimens of deep sea isopods have been taken that Wolff<sup>3</sup> has reasoned that gravid specimens hide in the mud, thus avoiding capture, or that the breeding period is short and occurs once every second or third year. An example of the absence of gravid female specimens can be found in the report by Menzies<sup>4</sup> on 123 abyssal species of marine isopods representing 447 specimens. In six cases gravid specimens were reported.



Fig. 2. Ovigerous female of *Storthingura birsteini* Menzies, 1962 (ref. 4). (Length 18 mm, maximum width 8 mm) from Scotia Sea, depth, 3,731–3,804 m.

endogenous solar and lunar rhythmicity persists in organisms when they are subjected to constant conditions in the laboratory<sup>5</sup>. We consider it reasonable to suppose that deep sea organisms might show persistent reproductive cycles in the absence of seasonal environmental changes which might reflect their origin from shallow water organisms. The data which we present for species of the genus *Storthingura* support this view (Fig. 1,

Table 1. PERCENTAGE OF GRAVIDITY IN *Storthingura* SPECIES COLLECTED FROM SCOTIA SEA IN TWO OR MORE FINDS

No.	Species	Exped. Cr. and Sta. No.	Depth (m)	Date	Male	Female	Ovigerous female	Gravidity (per cent)	Position
1	<i>S. birsteini</i> Menzies	VEMA 15-114	1,737	March 14, 1959	1	5	0	0	Lat. 55° 02' S. Long. 64° 17' W.
	<i>S. birsteini</i> Menzies	VEMA 15-118	3,776	March 16, 1959	0	2	0	0	Lat. 55° 44' S. Long. 64° 11' W.
	<i>S. birsteini</i> Menzies	ELT 4-126	3,731-3,804	July 29, 1962	2	5	4	57	Lat. 57° 12' S. Long. 62° 45' W.
	<i>S. birsteini</i> Menzies	ELT 11-973	1,920-2,210	Feb. 11, 1964	0	16	0	0	Lat. 55° 18' S. Long. 64° 42' W.
2	<i>S. scotiae</i> *	ELT 6-350	2,450	Dec. 4, 1962	3	1	0	0	Lat. 54° 02' S. Long. 58° 57' W.
	<i>S. scotiae</i> *	ELT 5-268	2,761-2,816	Oct. 20, 1962	0	4	1	25	Lat. 64° 01' S. Long. 67° 44' W.
3	<i>S. robustissima</i> Monod	ELT 4-138	1,290-1,485	Aug. 8, 1962	3	6	1	11	Lat. 62° 02' S. Long. 61° 08' W.
	<i>S. robustissima</i> Monod	ELT 6-432	884-935	Jan. 7, 1963	1	1	0	0	Lat. 62° 54' S. Long. 59° 27' W.

Percentage of gravidity represents the percentage of ovigerous females in the total number of males and females in a population sample.  
\* Manuscript names used by the authors.

There are no known seasonal environmental changes in the deep sea comparable with the magnitude of seasonal change at the sea surface. It might be reasonable to suppose therefore that unusual breeding conditions exist. It has, however, been convincingly demonstrated that

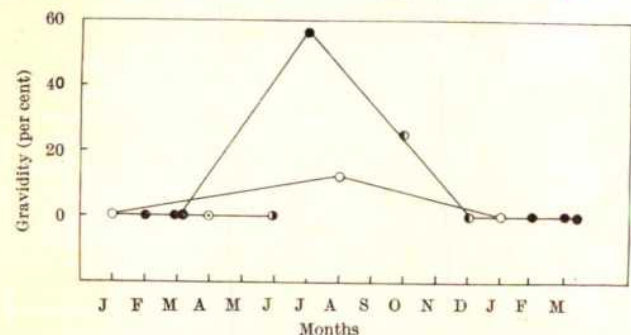


Fig. 1. Breeding activity in sub-Antarctic abyssal species of *Storthingura* (Crustacea, Isopoda). ●, *S. birsteini*; ○, *S. scotiae*\*; ○, *S. robustissima*; ○, *S. eltaniae*\* and *sepioides*\*; ○, *S. triptisipinosa*. (Species with an asterisk are manuscript names used by the authors.)

Table 1). A gravid female is shown in Fig. 2. Quite obviously the data are necessarily scanty. They deny neither of Wolff's<sup>3</sup> speculations, but instead suggest another mechanism—seasonal breeding cycles—comparable with those known for shallow water animals.

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## BIOCHEMISTRY

## Inhibition of Gastric Acid Secretion by Bacterial Lipopolysaccharide

Baume, Nicholls and Baxter report the potent effect of lipopolysaccharide on gastric secretion as if this were a new observation<sup>1</sup>. But the effect was clearly described in 1950 (ref. 2). We have made similar experiments in conscious dogs with vagally denervated pouches of the body of the stomach. Gastric secretion was stimulated by a low dose of histamine given by constant intravenous infusion (100 µg/h of histamine base). Gastric acid secretion was abolished by doses of 10 µg/kg of *Pseudomonas* polysaccharide pyrogen ('Piromen', Flint Laboratories Inc.) given intravenously over a period of about 20 min during stimulation with histamine. Secretion failed progressively over 2-3 h during which time the dogs developed pyrexia as indicated by a thermistor in the rectum (Fig. 1). In control experiments in which saline was given in place of 'Piromen' secretion was maintained.

When gastric acid secretion is stimulated in conscious dogs by feeding, by intravenous 2-deoxy-D-glucose, or by gastrin infusion, atropine sulphate (0.1 mg/kg intravenously) practically abolishes the secretion. Under similar conditions secretion stimulated by histamine is virtually unaffected<sup>3</sup>. Also, secretion stimulated by histamine is much more resistant to depression caused by elevation of the plasma calcium concentration than is secretion stimulated by gastrin<sup>4</sup>. From this we deduce that histamine acts, as is generally supposed, directly on the secreting cells. The potent action of lipopolysaccharide against secretion stimulated by histamine is therefore likely to reflect an effect on the secretory mechanism and not on intrinsic or extrinsic nerves. We agree with Baume *et al.*<sup>1</sup> that alterations in gastric blood flow could be responsible for the effect.

The slow development of inhibition of secretion in our experiments—it seemed to run parallel with rise in body temperature—suggests that inhibition of secretion, like pyrexia, depends on development of a mediator such as leucocyte bacterial pyrogen<sup>5</sup>. It should, however, be mentioned that it has been claimed that in rats lipopoly-

saccharide has gastric secretory inhibitory activity unrelated to its pyrexia effect<sup>6</sup>.

The significance of these observations is two-fold: first, the potency of bacterial lipopolysaccharide in inhibiting gastric secretion merits further investigation; and second, gastric secretory inhibition by biological extracts and other substances should always be accompanied by temperature recordings over several hours.

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CHROMAFFIN cells are present in two separate loci in the adrenal gland of the snake, *Xenodon merremii*. Most are distributed within a homogeneous ribbon on the periphery of the gland; these cells contain<sup>1</sup> and produce<sup>2</sup> large amounts of noradrenaline but little adrenaline. The remainder of the chromaffin cells are scattered among the adrenal cortical cells in the central portion of the gland. These cells contain large amounts of the methylated amine, adrenaline<sup>1</sup>.

The conversion of noradrenaline to adrenaline is catalysed by an enzyme, phenylethanolamine-N-methyl transferase (PNMT)<sup>3</sup>. In mammals almost all of this enzyme is found in the adrenal medulla, where its activity<sup>4</sup> and synthesis<sup>5</sup> are stimulated by the adrenocortical steroids which perfuse this organ in high concentrations. The observation that, in *Xenodon*, there is a high concentration of adrenaline in the central chromaffin cells suggested that PNMT in this species might also be controlled by cortical hormones, so we examined the regional distribution of this enzyme in the adrenals of *Xenodon*.

Four snakes were captured in Argentina in March, 1966. The adrenal glands were dissected into central and peripheral portions, and parts of each were sent to the United States for biochemical analysis. PNMT was assayed by methods described previously<sup>6</sup>, except that phenylethanolamine ( $3 \times 10^{-3}$  molar) was used as substrate, and the <sup>14</sup>C-N-methyl-phenylethanolamine formed enzymatically was extracted into a mixture of toluene and isoamyl alcohol (97:3). The assay depends on the transfer of a <sup>14</sup>C-methyl group from <sup>14</sup>C-S-adenosylmethionine to the amine nitrogen. The resulting radioactive product (*N*-methyl-phenylethanolamine) is separated from the labelled co-factor by extraction into the organic solvent at pH 10.

An average of  $9 \pm 2$  µmoles of *N*-methyl-phenylethanolamine was formed by peripheral

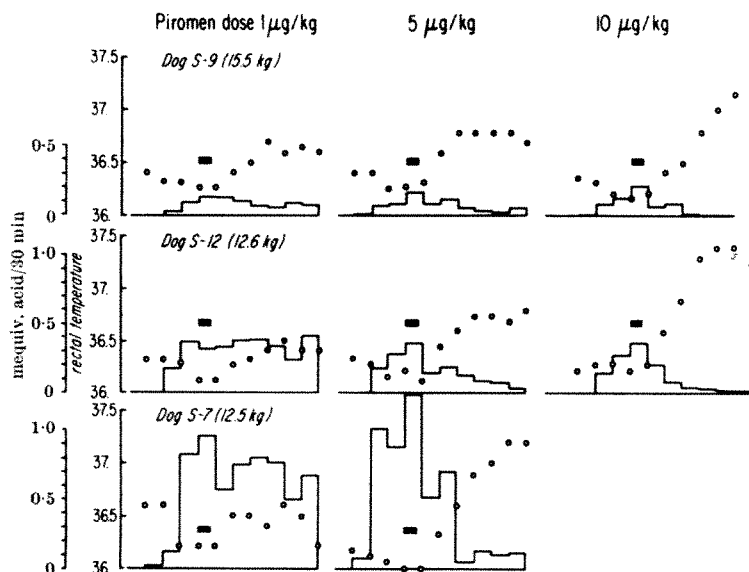


Fig. 1. Effect on acid secretion (solid line) of graded doses of 'Piromen' intravenously injected during the periods shown by black bars. The three dogs were stimulated to secrete from vagally denervated gastric pouches by infusion of histamine base (100 µg/h) beginning at the time of the first temperature recording and continuing till the end of the experiments. Rectal temperature recordings, represented by circles, were taken half-hourly. Dog S-7 was not subjected to the highest dose because 5 µg/kg appeared to cause unpleasant symptoms.



chromaffin tissue, while central tissues were able to synthesize  $104 \pm 28$   $\mu$ moles of the methylated product. Because only a small percentage of the cells in the central zone are chromaffin cells (most are cortical cells which produce steroids), it is likely that the concentration of PNMT in the central chromaffin cells is even greater than ten times that of the cells in the periphery.

Synthesis of adrenaline in the frog, *Rana pipiens*, is catalysed by a variant of mammalian PNMT, which has the same substrate specificity but different physical properties<sup>6</sup>. This enzyme does not appear to be controlled by adrenocortical steroids: unlike the mammalian enzyme its activity does not decline after hypophysectomy<sup>6</sup>. It is possible that snake PNMT is also independent of hormonal control, and that its high degree of localization in the chromaffin cells which are surrounded by cortical tissue is simply a coincidence. This hypothesis could be tested by determining whether central adrenal PNMT declines when the snake is hypophysectomized, or if the peripheral enzyme increases following treatment with large doses of glucocorticoids. A more likely explanation for the distribution of PNMT within the adrenal would be that in reptiles (like mammals, but unlike amphibians) this enzyme is under hormonal control. We suggest that all chromaffin cells in the snake adrenal are potentially able to make adrenaline but that they actually do so only after they have been stimulated chronically with large amounts of adrenocortical steroids.

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### Atassi-Gandhi Sedimentation Coefficient and Molecular Weight Relationships

Atassi and Gandhi<sup>1</sup> have put forward empirical equations for the relationship of sedimentation constant to molecular weight for globular proteins. Their first equation was intended to cover molecular weights in the range 40,000–300,000 and the second had the tested range 12,000–30,000. Data for molecular weights of less than 12,000 were not given and both equations were reported to apply between 30,000 and 40,000. These equations are linear and I intend to show that they are chords to a more general relationship, well known to users of the ultracentrifuge, which relates sedimentation coefficient to molecular weight and is expressed by<sup>2,3</sup>

$$\frac{S[\eta]^{1/3}}{M^{2/3}} = 2.5 \times 10^6 \frac{(1 - \bar{v}\rho)}{\eta_s N} \quad (1)$$

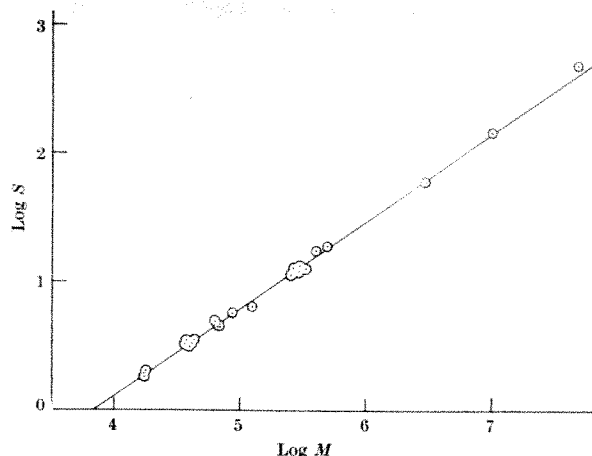


Fig. 1. Plot of log sedimentation constant against log molecular weight. The straight line represents the least squares fitted value. A parabolic least squares did not give a significantly better fit.

where  $S$  is sedimentation coefficient (in Svedbergs),  $[\eta]$  is intrinsic viscosity,  $M$  is molecular weight,  $\bar{v}$  is partial specific volume of the solute,  $\rho$  is solvent density,  $\eta_s$  is solvent viscosity and  $N$  is the Avogadro number.

This equation can be rearranged to a good approximation

$$S = kM^{2/3} \quad (2)$$

or

$$\log S = \log k + \frac{2}{3} \log M \quad (3)$$

Variation in  $k$  is minimized by the use of the sedimentation constant,  $S_{20,w}$ , rather than the sedimentation coefficient, and therefore in testing the accuracy of equation (3), I used the sedimentation constant. The testing involved plotting  $\log S_{20,w}$  against  $\log M$  for a large number of proteins and viruses of  $f/f_0 < 1.30$  and molecular weights ranging from 17,000 to  $49 \times 10^6$  and extracting the slope and intercept by a least squares computer programme. The difference between the theoretical and experimental slopes was not statistically significant (Table 1) and so equation (3) becomes

$$\log S = 3.383 \pm 0.044 + \frac{2}{3} \log M \quad (4)$$

Table 1. THEORETICAL AND EXPERIMENTAL SLOPE AND INTERCEPT VALUES

	Theoretical	Experimental
Slope	0.667	$0.681 \pm 0.009$
Intercept	—	$-2.617 \pm 0.044$

The differences between the theoretical and experimental slopes are not significant at the 5 per cent level.

The equations of Atassi and Gandhi were then converted to a logarithmic form and superimposed on the fitted curve. Their lines (curved in the logarithmic form) lay around the least squares line, but had limitations in that they were only of use up to a molecular weight of 300,000 (as noted by Atassi and Gandhi) and that they were inapplicable at low molecular weights ( $< 9,000$ ). The areas where the equations did not apply lay outside the standard errors of the fitted line.

The limited application of the Atassi-Gandhi relationships can thus be attributed to the taking of chords to the plot of  $S_{20,w}$  against  $M$  and obviously these can only apply over a limited range which depends on the slope of the curve. Because the logarithmic plot is valid for a considerably wider range of molecular weights, and has a sound theoretical basis, it would seem more logical to use this relationship. It must be emphasized that these calculations are necessarily approximate because use of the constant  $k$  implies assumptions about uniformity of hydration, density, and shape. Nevertheless, the approxi-

mation is a good one, as can be seen from the closeness of the theoretical and experimental results, and reasonable values for molecular weights can be obtained.

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### Influence of Urinary pH on the Excretion of Tranylecypromine Sulphate

URINARY pH is an important factor in determining the rate of excretion of amphetamine<sup>1-4</sup>. Tranylecypromine sulphate, an anti-depressant agent, is a related amine for which an assay procedure in urine has been developed<sup>5</sup>, based on its fluorescent properties. This method was at first thought to be only qualitative, but now has been found to be quantitative, and a linear relationship has been shown between fluorescent activity and concentrations of 0.1–4.0 µg/ml. of the drug in urine. Although tryptamine and tyramine reduced the fluorescence of tranylecypromine sulphate in certain concentrations, these were considerably higher than those found in the urine of patients treated with this drug. These amines did not therefore influence the assay procedure, and although it was possible to separate them from tranylecypromine sulphate the yield of this drug was not increased and separation therefore proved unnecessary (our unpublished observations).

Preliminary experiments in three patients given an oral dose of 20 mg of tranylecypromine sulphate ('Parnate') showed that the drug appeared in the urine within 2 h, with a maximum rate of excretion between 2 and 6 h, and disappeared after 12–16 h. The excretion of unchanged drug during the 24 h after administration was only between 1 and 5 per cent. Random urine samples were obtained from twelve patients receiving 10–20 mg of tranylecypromine sulphate daily. The drug was detected in all specimens, but only in amounts of less than 1 µg/ml., except in urine from a patient who had taken an unknown quantity of the drug 24 h before in a suicide attempt, where the concentration was 1.04 µg/ml.

In order to determine whether the excretion of unchanged tranylecypromine sulphate was dependent on the pH of urine, 20 mg was given in tablet form to four healthy subjects twice at weekly intervals. All urine passed during the next 16 h was collected at intervals of 2 h and the content of unchanged drug determined by a method described elsewhere<sup>5</sup>. On the first occasion, the pH of the urine was maintained at about 5.0 by the administration of ammonium chloride tablets before and during the experiment. On the second occasion, an alkaline urine (pH about 8.0) was maintained by giving sodium bicarbonate tablets before and during the experiment.

The results are given in Table 1, and the rates of excretion under both acid and alkaline conditions in one subject are shown in Fig. 1. They demonstrate that the unchanged tranylecypromine sulphate excreted in alkaline

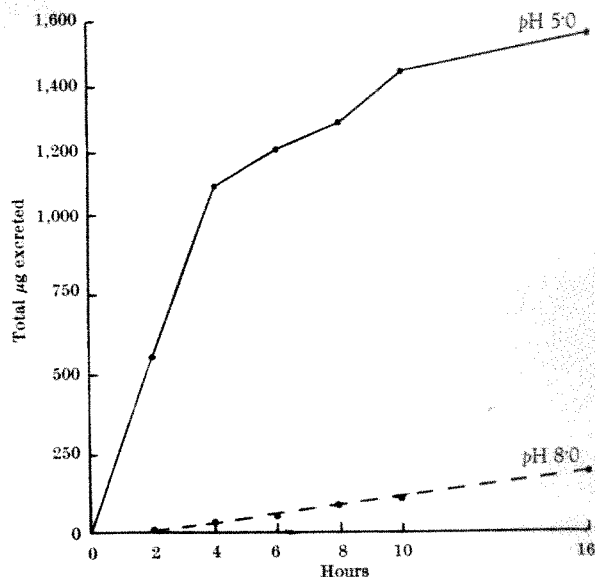


Fig. 1. The cumulative excretion (µg) in one subject of unchanged tranylecypromine sulphate during a period of 16 h after administration of 20 mg by mouth, in acid (pH 5.0) and alkaline (pH 8.0) conditions.

urine after 16 h is only about 1 per cent of the dose, but is increased to about 8 per cent by acidification of the urine. Whereas the maximum rate of excretion under acid conditions was about 46 µg/min, it was only 0.26 µg/min under alkaline conditions. This bore no relationship to the volume of urine.

Despite this low yield of unchanged drug, the difference in rates of excretion suggests that it would be worthwhile to acidify the urine of patients who have taken an overdose of tranylecypromine sulphate. It is possible that the rates of excretion of some of its metabolites may also be increased in a similar way by acidification. If the patient is conscious, ammonium chloride tablets may be given and a 2 per cent solution of ammonium chloride may, with caution, be administered to the unconscious patient. In view of the toxicity of tranylecypromine, caution should be exercised when giving it to patients who may have a defect in urinary acidification, and in whom its action may therefore be increased and prolonged.

It is important to estimate the urine pH whenever the assay procedure for tranylecypromine sulphate is being carried out. If a doubtful result is obtained, ammonium chloride should be administered and the determination repeated when the urine is more acid.

The low yield of unchanged drug is interesting when compared with the relatively high yields of amphetamine under acid conditions<sup>1</sup>. To investigate the possibility that some tranylecypromine might be conjugated as a glucuronide, samples of urine from two patients receiving the drug, and a control urine containing no tranylecypromine were incubated with glucuronidase overnight at 37° C and analysed the next morning for tranylecypromine. Although there was a slight increase in the fluorescence of the control urine, there was a significantly greater increase in the readings from the urine of the patients after incubation with glucuronidase.

It appears therefore that only about 8 per cent of a dose of tranylecypromine sulphate is excreted in the unchanged form in 16 h, even under acid conditions, and that a further small amount may be conjugated as a glucuronide. These figures are consistent with the observations of Alleva<sup>6</sup>, who found that only 4 per cent of a dose of tranylecypromine labelled with carbon-14 was excreted in the rat. He also found that 12 per cent appeared as hippuric acid. There are several possible alternative pathways for metabolism, including cleavage

Table 1. PERCENTAGE EXCRETION OF UNCHANGED TRANYLECYPROMINE SULPHATE IN 16 h AFTER ADMINISTRATION OF 20 mg OF THE DRUG IN FOUR SUBJECTS, UNDER ACID (pH 5.0) AND ALKALINE (pH 8.0) CONDITIONS

Subject	Acid	Alkaline
1	5.8	1.1
2	7.6	1.1
3	11.2	1.7
4	7.7	0.9

of the cyclopropane ring, and these await further elucidation.

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### Biliary Excretion of Aflatoxin in the Rat after a Single Dose

WHEN aflatoxin damages the liver<sup>1</sup> the cells most affected are those which are concentrated around the portal vein and bile duct. There seemed to be no published data on the quantitative pattern of biliary excretion of this toxin and the nature and partition of its metabolites in bile, and so we have investigated the problem.

Our experiments were made possible by the availability of aflatoxins B and G, labelled with carbon-14 in the uridine, and synthesized by a procedure developed here. The method involved the growing of spores of *Aspergillus flavus* in a Czapeck medium for 60 h before the addition to the culture of sodium acetate-2-<sup>14</sup>C. The product has a specific activity of 30 m $\mu$ c./mmole, and the method thus differs from the resting cell technique of Adye and Mateles<sup>2,3</sup>. A male rat weighing 350 g was lightly anaesthetized with ether before the establishment of a biliary fistula as described by Boyland *et al.*<sup>4</sup>. Bile was collected in a saddle-shaped container attached to the back of the rat by 'Elastoplast'<sup>5</sup>. In order to obtain samples of bile at regular intervals, a polythene tube was introduced into the chamber, and through this the bile was removed by suction. A dose of 50  $\mu$ g of labelled aflatoxin B<sub>1</sub> in saline with a specific activity of 30 m $\mu$ c./

mmole, was given by intraperitoneal injection to the rat and bile was collected every 15 min. A sample of bile (0.1 ml.) was transferred to a planchet, dried on a hot plate, and counted at infinite thinness in an end-window Geiger-Müller counter. The rate of excretion of labelled metabolites of aflatoxin B<sub>1</sub> in the bile is shown in Fig. 1.

There was a latent period of about 60 min between the time of injection and the first appearance of these metabolites in the bile samples collected. This was partly a result of the slow rate of flow of bile in the anaesthetized rat. It was, however, clear from the graph that aflatoxin was rapidly excreted through the bile, as was indicated by the observation of Falk *et al.*<sup>6</sup>. Aliquots of the 6 h collections of bile were shaken with equal volumes of chloroform for 20 min. The amounts of radioactivity in the chloroform layer were estimated as described earlier. The distribution of the counts in the various fractions is shown in Table 1.

Table 1. PARTITION OF LABELLED METABOLITES IN THE BILE SAMPLES OBTAINED FROM THE TREATED RAT

Time of collection	Total counts in bile samples (C-B)	Recovery of initial counts (per cent)		
		Total bile	CHCl <sub>3</sub> extract	Conjugates
0-6 h	13,800	30	10	20
6-12 h	770	1.7	0.7	1.0

C-B, counts in sample minus background counts.

Aliquots of the chloroform extracts were run on thin-layer chromatograms of silica gel G, using a mixture of 1 ml. of acetic anhydride; 2 ml. of methanol and 97 ml. of chloroform as the solvent. Each of these extracts contained a mixture of aflatoxin B<sub>1</sub> and aflatoxin M<sub>1</sub>, with similar properties to those described by other authors<sup>7-9</sup>. The principal constituent was a conjugate compound which gave a positive ninhydrin reaction on paper chromatography and was alkali labile. On alkaline hydrolysis of this conjugate, it yielded two fractions, one of which was identifiable with taurocholate on thin-layer chromatography, while the other had a slightly lower *R<sub>F</sub>* value than aflatoxin B<sub>1</sub> and gave a blue fluorescence in ultra-violet light. This last compound may have been a degradation product of aflatoxin B<sub>1</sub>. Work is in process with the aim of elucidating the nature of the aflatoxin bonding in the conjugate. Analysis of urine samples from the treated rat showed that approximately 26 per cent of the dose count was excreted as a glucuronide conjugate in the first 6 h after the injection of aflatoxin.

Our experiments with bile and urine suggest the existence of various pathways of aflatoxin B<sub>1</sub> metabolism in the rat, including that of demethylation proposed by Shank and Wogan<sup>10</sup>, who recovered 14-69.8 per cent of labelled aflatoxin in the faeces of four experimental rats, and Wogan<sup>11</sup> concluded from this that most of an aflatoxin dose was excreted through the bile. Our results show excretion of a glucuronide in the urine and of a taurocholate conjugate in the bile to be of great importance.

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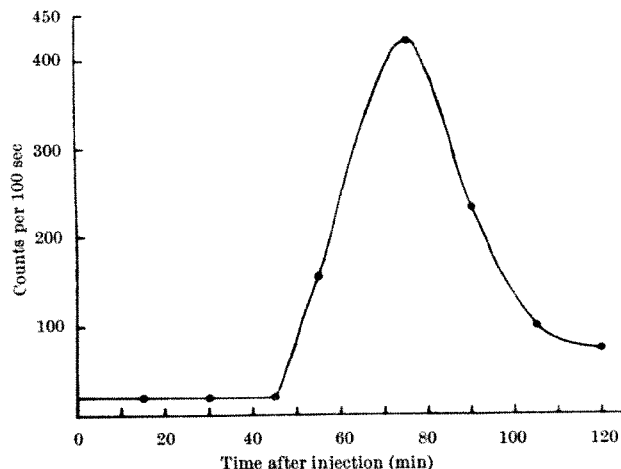


Fig. 1. Rate of excretion of metabolites of aflatoxin B<sub>1</sub> in rat bile.

## Effect of Epinephrine on Insulin Release in Man induced by Secretin

SEVERAL observations taken together support the suggestion that the pancreatic  $\beta$ -cell responds in different ways to an oral glucose meal and to an intravenous infusion of glucose. (1) Oral glucose produces a greater rise in concentrations of plasma insulin than does an identical intravenous load<sup>1,2</sup>; (2) oral glucose provokes a response in plasma insulin four or five times greater than that achieved by infusing glucose intravenously to comparable concentrations of blood glucose<sup>3</sup>; (3) epinephrine blunts the release of insulin usually encountered after intravenous infusion of glucose<sup>4</sup>, but fails to suppress the rise in concentrations of plasma insulin which follows an oral glucose meal<sup>5</sup>.

In the light of these investigations, McIntyre *et al.* suggested that a gastrointestinal factor might allow the  $\beta$ -cell to discriminate between orally and intravenously administered glucose. Such a factor should initiate or augment the release of insulin from the pancreas, and its ability to stimulate the release of insulin should not be blocked by epinephrine.

Secretin has been shown to fulfil the first criterion. When it is intravenously administered, secretin elevates the concentration of portal venous insulin in man<sup>6</sup> and in the dog<sup>7</sup>. Perhaps of greater physiological significance is Dupré's observation that the rate of disappearance of an intravenous glucose load is accelerated by this compound<sup>8</sup>.

We report here that secretin also fulfils the second criterion: it produces an increase in plasma insulin in the face of a sustained infusion of epinephrine.

Four normal female subjects were investigated during the morning hours after an overnight fast. When control venous blood samples had been drawn, an infusion of epinephrine was commenced (4–8  $\mu\text{g}/\text{min}$ ). Forty-five minutes after the start of the infusion, an intravenous injection of 75 U of secretin (Vitrum, Ltd., Stockholm) was given, and venous blood samples were taken every 2 min throughout the next 10 min. Results are shown in Table 1 and a representative investigation is illustrated in Fig. 1. Plasma insulin was measured by the radioimmunoassay technique of Yalow and Berson<sup>9</sup> and plasma glucose by the glucose oxidase method. During the initial period of epinephrine infusion, the concentrations of plasma insulin did not change appreciably from those

before infusion, despite significant elevations in the concentration of plasma glucose in all four subjects (average rise 51.6 mg/100 ml.; range 37.0–70.5 mg/100 ml.). When secretin was administered, concentrations of plasma insulin rose in all four subjects (average rise 28.5  $\mu\text{U}/\text{ml}$ .; range 5–52  $\mu\text{U}/\text{ml}$ .). The greatest increases occurred at 2 min and they rapidly fell to approach pre-injection levels within 10 min. When the infusion of epinephrine was discontinued all subjects showed a marked rise in plasma insulin values which were greatest at 10–30 min.

Table 1. CONCENTRATIONS OF PLASMA INSULIN ( $\mu\text{U}/\text{ml}$ .) AFTER INTRAVENOUS INJECTION OF SECRETIN INTO SUBJECTS RECEIVING EPINEPHRINE

Time (min)	-10	0	30	45	47	49	51	53	55	Highest value after epinephrine
Subject										
B. F.	24	26	24	24	76	64	45	41	41	85
P. D.	15	12	22	28	57	54	50	40	46	78
K. H.	10	6	8	10	38	18	13	18	14	34
F. M.	7	14	16	15	20	20	6	10	10	60
Mean	14.0	14.5	17.5	19.3	47.8*	39.0*	28.5	27.3	27.8	66.5*

\* These values are significantly different from those before the secretin was administered at time 45 min ( $P < 0.05$ , by paired analysis).

75 U of secretin was injected at time 45 min in four normal fasting female subjects who were receiving an infusion of epinephrine (4–8  $\mu\text{g}/\text{min}$ ) commenced at time zero. The last column shows the highest concentration of plasma insulin achieved following discontinuation of the epinephrine infusion.

Two subjects were investigated with an identical dose of secretin when hyperglycaemia was produced by infusion of 300 mg of dextrose/min. Concentrations of plasma glucose rose to 47.9 and 46.5 mg/100 ml., and those of plasma insulin increased 19 and 9  $\mu\text{U}/\text{ml}$ ., respectively, after 45 or 80 min, at which time they were given an identical dose of secretin as in the epinephrine experiment. Concentrations of plasma insulin promptly rose, reaching a peak at 4 min, which represented a further rise of 25 and 26  $\mu\text{U}/\text{ml}$ ., and rapidly fell thereafter.

Using the same batch of Vitrum secretin we were unable to find a significant rise in concentrations of peripheral plasma insulin after intravenous injection of secretin to four normal female subjects, including three of the four referred to earlier, investigated in the basal fasting state (Table 2). This contrasts with the unequivocal rise in the concentration of plasma insulin produced by the same dose of secretin when hyperglycaemia had been produced by epinephrine (even though the hyperglycaemic state itself did not augment the concentration of plasma insulin because of epinephrine-blockage of insulin release) or by infusion of dextrose. These findings point toward a regulating device which may ensure that release of insulin is fully augmented by secretin only in appropriate circumstances (for example, postprandial hyperglycaemic states). However, we wish to emphasize the variations in insulinogenic potency between different batches of secretin. Some Jorpes secretin preparations do elevate plasma insulin levels, even in the normoglycaemic state, as already reported by Dupré *et al.*<sup>6</sup>.

Table 2. CONCENTRATIONS OF PLASMA INSULIN ( $\mu\text{U}/\text{ml}$ .) AFTER INTRAVENOUS INJECTION OF 75 U OF SECRETIN AT TIME ZERO IN FOUR NORMAL FASTING FEMALE SUBJECTS WITH NO PRETREATMENT

Time (min)	-20	0	2	4	6	8	10
Subject							
B. F.	27	26	23	32	30	30	24
P. D.	14	20	14	16	20	16	15
K. H.	—	22	16	13	16	12	10
A. S.	26	31	32	32	30	25	19
Mean	22.3	24.8	21.3	23.3	24.5	20.8	17.0

The effects on the release of insulin of several other gastrointestinal factors have been explored and of these glucagon has perhaps been most thoroughly investigated<sup>10–12</sup>. It is of interest that whereas glucagon undoubtedly initiates release of insulin from the pancreas, this effect is reported to be blocked by epinephrine<sup>4</sup>.

In summary, secretin can, in the presence of hyperglycaemia, cause a significant elevation in concentrations

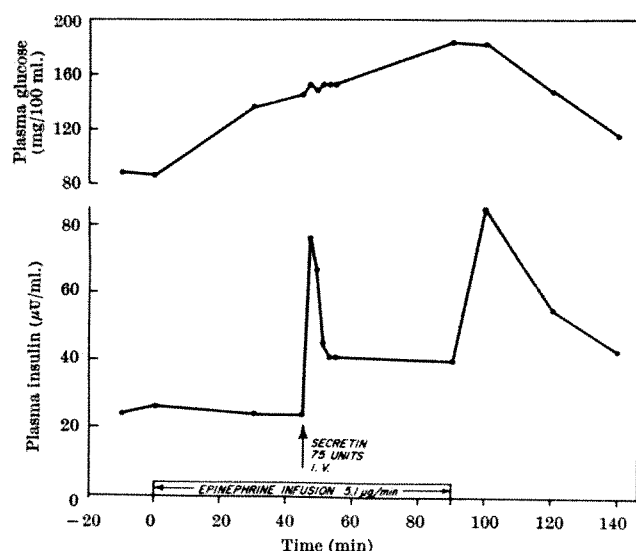


Fig. 1. Changes in concentrations of plasma glucose and insulin in a normal female subject (B. F.) after injection of 75 U of secretin 45 min after commencement of an infusion of epinephrine (5.1  $\mu\text{g}/\text{min}$ ). The rise in concentrations of plasma insulin after discontinuation of the epinephrine is also well shown.



of plasma insulin. This effect is not inhibited by epinephrine, an agent which blocks the release of insulin observed after intravenous administration of glucose. Secretin thus fulfils the requirements as a mediator of the mechanism which produces the augmented secretion of insulin observed after oral, as compared with intravenous, administration of glucose.

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### Concentration of Glucose in New Choroidal Cerebrospinal Fluid of the Rabbit

It is characteristic of mixed cerebrospinal fluid, sampled in the steady state, that the concentration of glucose is deficient compared with plasma. Yet the fluid appears grossly to be neither a source nor a sink in relation to the sugar in the brain because regional differences in concentration have not been disclosed by successive sampling<sup>1</sup>, or by analyses of fluids taken from several sites<sup>2</sup>. In unsteady conditions after elevation of plasma glucose<sup>3</sup>, or during ventriculo-cisternal perfusion with an initially glucose free solution<sup>4</sup>, the flow of glucose into the system is by a facilitated mechanism and at a rate faster than could be accounted for by the contribution through choroid plexuses. Thus the concentration of glucose in fluid might be rapidly imposed by the brain if new fluid were formed with a composition different from that of mixed fluid.

The extent to which the choroid plexuses contribute to the maintenance of the deficiency of glucose was investigated by applying the enzyme method of Lowry *et al.*<sup>5</sup> to its analysis in arterial plasma, a filtrate of plasma and new choroidal fluid sampled by the method of de Rougemont *et al.*<sup>6</sup>.

After anaesthesia with sodium pentobarbitone 40 mg/kg, supplemented when necessary, the concentration of glucose in plasma invariably rose. The mean increase was by a factor of  $1.79 \pm 0.15$  (mean  $\pm$  standard error of the mean, eighteen animals). The hyperglycaemia was very resistant to insulin but was prevented by 2 mg of pentalonium lactate, 1-2 h before induction of anaesthesia. It seems likely that the glycogenolysis is mediated through

Table 1. VALUES FOR THE RATIO CONCENTRATION OF GLUCOSE IN NEW CEREBROSPINAL FLUID : CONCENTRATION IN PLASMA, GROUPED FOR DIFFERENT RANGES OF PLASMA CONCENTRATION

Range of plasma concentration (mmolar)	No. of observations	Mean ratio $\pm$ standard error of the mean
0-8	10	$0.589 \pm 0.015$
8-11	27	$0.589 \pm 0.018$
11-13	34	$0.601 \pm 0.013$
13-15	15	$0.594 \pm 0.020$
15-20	28	$0.519 \pm 0.018$
20-30	30	$0.464 \pm 0.019$
30-40	21	$0.477 \pm 0.013$
>40	13	$0.457 \pm 0.023$

hypothalamic release associated with cortical depression by anaesthesia. The concentration in a filtrate was  $0.98 \pm 0.015$  that of plasma (eighteen observations).

The results of the study are presented in Fig. 1 in which the ratio, concentration of glucose in new fluid : concentration in plasma, is plotted against plasma concentration. Below modestly elevated plasma concentrations (<15 mmolar) the mean ratio was  $0.596 \pm 0.010$  (mean  $\pm$  standard error of the mean, eighty-six observations). When the concentration in plasma was greater than 15 mmolar the ratio was decreased as concentration in plasma was increased. By grouping the observations (Table 1) we found that the change to smaller ratios was rather abrupt between 15 and 20 mmolar in plasma. For less than 15 mmolar in plasma the slope of the calculated best line was virtually zero. For a concentration in plasma of more than 20 mmolar, the mean ratio was  $0.467 \pm 0.018$  (mean  $\pm$  standard error of the mean, sixty-four observations) while the calculated slope was again virtually zero. In Fig. 1b the calculated best lines and 95 per cent confidence limits of the slopes are shown.

The results indicate that at least two processes are involved in the transport. An analysis of the kinetics promises to be difficult and will require further examina-

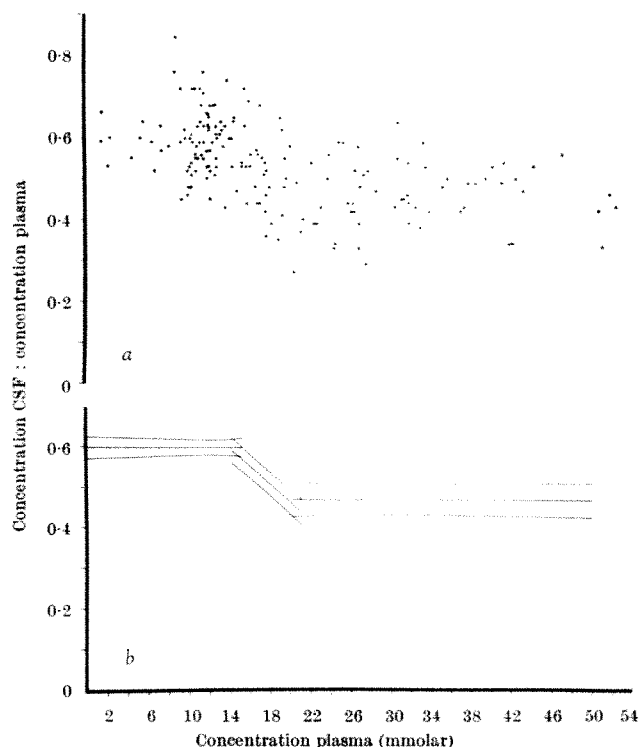


Fig. 1. (a) The ratio, concentration of glucose in new choroidal cerebrospinal fluid : concentration in arterial plasma is plotted against plasma concentration. The variations in plasma concentration were spontaneous and brought about by insulin and/or infusion of glucose. (b) The calculated best lines and the 95 per cent confidence limits for the slopes are shown. The calculated slopes are, for the segment below 15 mmolar,  $5 \times 10^{-4}$ ; for the segment above 20 mmolar,  $2 \times 10^{-4}$ ; and for the intermediate segment which is calculated for points between 14 and 21 mmolar, it is  $-0.022$ .

tion of the behaviour in individual animals and the study of other sugars.

That the new fluid is secreted at approximately the same concentration of glucose relative to plasma as has been measured for mixed fluid in the steady state<sup>7</sup>, shows that in steady conditions the sugar of the fluid enters with the volume inflow. The observation that mixed fluid in the steady state is neither a source nor a sink in relation to the brain<sup>2</sup>, is therefore not explained by a rapid and thus unobserved equilibration between the two compartments. On the other hand, exchange between the two is rapid and, in unsteady conditions, flow of sugar between parenchyma and cerebrospinal fluid is important.

The concentration of glucose in the cerebrospinal fluid is thus doubly controlled, during its secretion and by its relationship with the brain.

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### Specific Activity of Carbon Dioxide expired by Rats after Oral Sucrose and Other Sugars

A COMPARISON of the rates at which the respiratory quotient (RQ) rises after various sugars are given by mouth to human beings or dogs suggests that sucrose is more rapidly metabolized than either glucose or fructose, or a mixture of these monosaccharides<sup>1-4</sup>. Nevertheless, the interpretation of the RQ is not unequivocal. In a more direct investigation of the rates at which oral sucrose, glucose and fructose and certain other sugars are metabolized, sugars labelled with radioactive carbon were fed to rats and the comparative rates at which radioactive carbon dioxide was expired were determined. The radioactive sugars were obtained from the Radiochemical Centre, Amersham, except labelled maltotriose, which was prepared by one of us (A. F. M. M.) by hydrolysing radioactive starch and separating by charcoal adsorption and chromatography on paper.

Solid sugar was fed to the rat in a gelatine capsule which, unless otherwise stated, contained 50 mg of unlabelled and 0.5  $\mu$ c. of <sup>14</sup>C(U) labelled sugar. Samples of the expired air were then collected during consecutive 20 min periods for 1-4 h. The expired carbon dioxide was collected in L-M-hyamine solution in methanol, the total carbon dioxide being determined by titration with 0.1 normal hydrochloric acid in triplicate under nitrogen<sup>5</sup>. Radioactivity present was measured on a liquid scintillation spectrometer, with a non-aqueous scintillator<sup>6</sup>. The specific activity of the expired carbon dioxide was expressed as c.p.m./mmole expired carbon dioxide.

A comparison of the specific activity of the carbon dioxide expired after the oral administration of sucrose-<sup>14</sup>C to a group of ten rats with that after oral glucose-<sup>14</sup>C is shown in Fig. 1. A significantly greater rise ( $P < 0.001$ ) in the specific activity of the expired carbon dioxide was observed during the first 20 min after oral sucrose than after oral glucose. This difference decreased with time, and had disappeared after 1 h. These results agree with

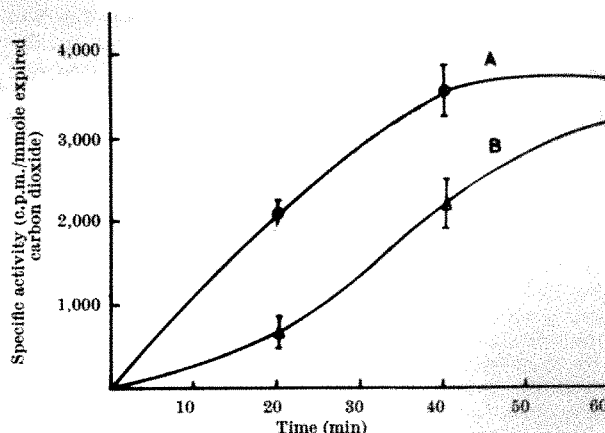


Fig. 1. The vertical lines through each point represent the standard error of the mean value. In each instance a total of 50 mg of sugar was fed, the radioactivity being 0.5  $\mu$ c. A, Sucrose; B, glucose.

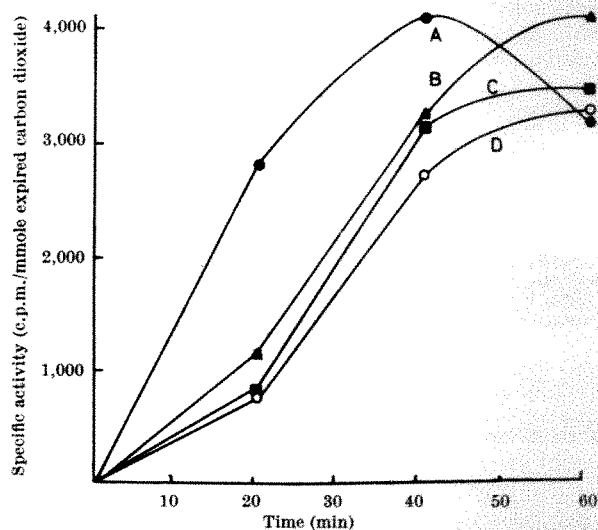


Fig. 2. In each instance a total of 50 mg of sugar was fed, the radioactivity being 0.5  $\mu$ c. A, Sucrose; B, glucose; C, fructose; D, glucose plus fructose.

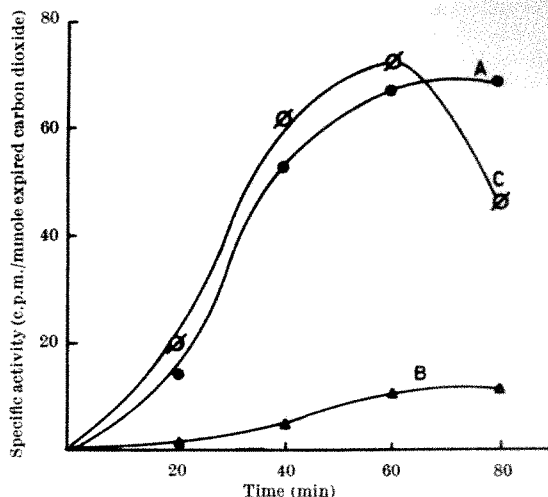


Fig. 3. In each instance a total of 50 mg of sugar was fed, the radioactivity being 6,000 c.p.m. A, Sucrose; B, glucose; C, maltotriose.

the interpretation of earlier workers that oral sucrose is more rapidly metabolized to yield carbon dioxide in the animal body than is oral glucose.

Fig. 2 shows the comparative effects of oral sucrose, glucose, fructose and an equimolecular mixture of glucose

and fructose on the specific activity of the expired carbon dioxide. Oral sucrose produces a greater increase in the specific activity of the expired carbon dioxide than any of the other sugars.

The specific activities of expired carbon dioxide observed after the administration of 50 mg of a number of sugars, namely, sucrose- $^{14}\text{C}$ , glucose- $^{14}\text{C}$  and maltotriose- $^{14}\text{C}$ , to a single rat are compared in Fig. 3. The activity of labelled sugar fed in each instance was about 6,000 c.p.m. The results with oral maltotriose- $^{14}\text{C}$  are similar to those obtained with sucrose- $^{14}\text{C}$ , and the specific activity of the expired carbon dioxide after oral administration of these sugars is considerably greater than that seen after feeding glucose- $^{14}\text{C}$ .

These results support the earlier conclusions of others obtained by different methods that the metabolism of oral sucrose begins more rapidly than that of oral glucose or fructose. The metabolic reason for this interesting difference remains to be investigated.

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### Adenosine Triphosphatase activated by Magnesium and Superprecipitation of Myosin B

THE importance of the divalent cations calcium and magnesium in muscle contraction is widely acknowledged<sup>1,2</sup>. An ordinary preparation of myosin B has been found to contain the "extra proteins" which are responsible for the effects of calcium on the magnesium-activated superprecipitation and adenosine triphosphatase (ATPase)<sup>3,4</sup>. As to the effects of magnesium, however, it is not clear whether this ion acts directly on the protein or indirectly by forming a complex with ATP<sup>5</sup>. How the activity of ATPase is related to the superprecipitation is still not known. We have attempted to ascertain the relationship of the two functional properties of myosin B by observing the effects of magnesium ions on the behaviour of the protein which shows no sensitivity to calcium.

Myosin B was prepared from rabbit skeletal muscle by the method of Perry *et al.*<sup>4</sup>. In order to minimize the influence of potassium chloride on the properties of myosin B and not to complicate the ionic species in test solution, the concentration of potassium chloride was kept at 5 mmolar. The contamination of magnesium ions in the test system could not be avoided even when the reagents were treated with 'Dowex 50' (potassium ion form), and so ethylenediamine tetraacetic acid (EDTA) was added to the system to observe the effect of the smallest possible concentration of free magnesium<sup>6,7</sup>. The rate of superprecipitation was determined by the method described by Levy and Fleisher<sup>8</sup>. In the conditions of the experiment, the turbidity of the suspension of myosin B increased about thirteen times with the addition of ATP, and the extent of the increase was fairly constant (deviation  $\pm 10$  per cent of average), except at the concentration of free magnesium ions of less than  $5 \times 10^{-5}$  molar, where ATP is exhausted before completion of the change in turbidity. The activity of ATPase in the steady state was measured

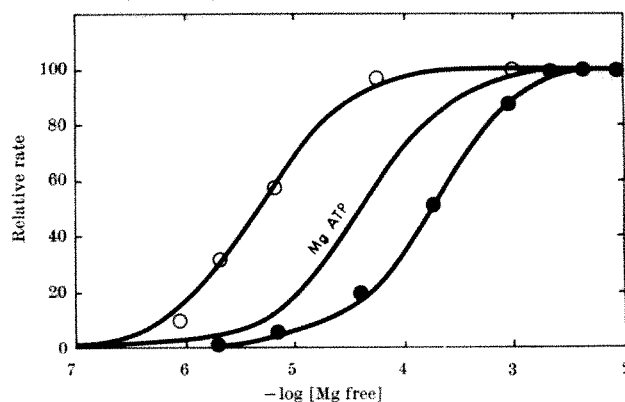


Fig. 1. Effects of magnesium ions on the rates of ATP hydrolysis and superprecipitation of myosin B. A relative rate of ATP hydrolysis (○) of 100 is equivalent to the activity of 0.12  $\mu\text{moles/mg}$  of myosin B/min. A rate of superprecipitation (●) of 100 corresponds to a turbidity change of 0.18/sec (2 cm light path). Reaction mixture: 5 mmolar potassium chloride, 1 mmolar EDTA, 20 mmolar *tris* (pH 7.4), 0.1 mmolar ATP, 0.056 mg of myosin B/ml., and magnesium chloride was varied. Temperature, 25° C. The curve marked MgATP represents the formation of magnesium-ATP complex with the concentration of free magnesium. This curve was calculated according to ref. 9.

by the assay of inorganic phosphate and by pH-stat. The two methods of ATPase analysis were consistent with one another. In Fig. 1 the activity of ATPase and the rate of superprecipitation are plotted against the concentration of free magnesium ions which was calculated as described by Botts *et al.*<sup>9</sup>. We assumed that the total concentration of magnesium consists of those of free magnesium ions,  $\text{MgATP}^-$ , and the complexes of magnesium and EDTA. Each of the experimental curves represented the simple relationship of binding reaction between magnesium ions and a ligand, which was assumed to be myosin B. The apparent dissociation constants were estimated as  $4.5 \times 10^{-6}$  molar<sup>10</sup> for the ATPase curve and  $1.6 \times 10^{-4}$  molar for the superprecipitation. Although there is a region of magnesium concentration where the increase in the rates appears to parallel the formation of  $\text{MgATP}^-$ , the binding of magnesium ions to the protein is apparently responsible for the full activation. The curves of the same type as those in Fig. 1 were obtained with various concentrations of ATP from 0.01 mmolar to 0.1 mmolar. The maximum rates of both ATPase and superprecipitation at varied concentrations of ATP resulted in the curve shown in Fig. 2. The omission of EDTA, in the presence of 5 mmolar magnesium chloride, did not affect the results of Fig. 2. The curve follows the

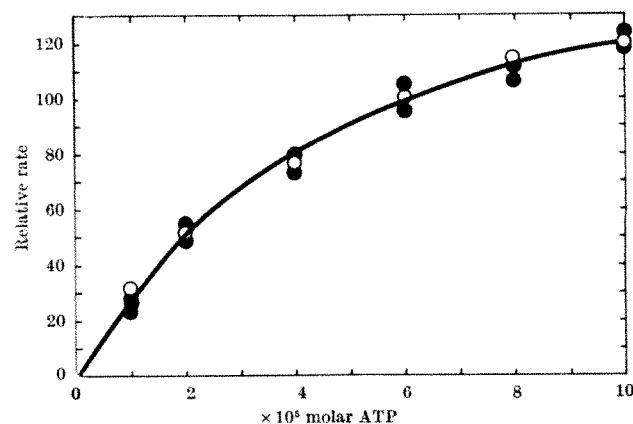


Fig. 2. Change in the rates of ATP hydrolysis and superprecipitation with the concentration of ATP. A relative rate of ATPase (○) of 120 is 0.12  $\mu\text{moles}$  of ATP hydrolysed/mg of myosin B/min. A relative rate of superprecipitation (●) of 120 corresponds to a turbidity increase of 0.18/sec. Reaction mixture contained 5 mmolar potassium chloride, 1 mmolar EDTA, 20 mmolar *tris* (pH 7.4), 5 mmolar magnesium chloride, 0.056 mg of myosin B/ml., and varied concentration of ATP. Temperature, 25° C. The rates of ATPase and superprecipitation in the presence of 5 mmolar magnesium chloride were observed to be maximal at each fixed concentration of ATP.

kinetic formula of the Michaelis-Menten type with the value of  $K_m$  as  $5 \times 10^{-5}$  molar.

Magnesium affects independently the activity of ATPase and the rate of superprecipitation, which suggests that these two effects are not closely related in this respect, whereas the changes in the rates of both functions with the concentration of ATP are parallel to each other. If this parallelism should be an indication of direct coupling of the two functions, the fact that the relationship between the rates and the concentration of ATP follows the Michaelis-Menten kinetics would have some significance. If the value of  $K_m$  represents a dissociation constant of the binding reaction between ATP and myosin *B*, the simplest mechanism which involves the binding of ATP at one site on the protein can be proposed. There are possibly two different types of sites for ATP binding: one for the hydrolysis of ATP and the other for the physical change of protein, with the same affinity to ATP in the experimental conditions. If the value of  $K_m$  for either one or both functions cannot be regarded as the dissociation constant, the same value of  $K_m$  for the two processes appears to be coincidental and no simple explanation for the parallelism can be offered at the moment.

Levy and Fleisher<sup>11</sup> suggested that superprecipitation requires the concerted action of ATP or ITP at two or more sites on the protein complex. These authors determined the rates of superprecipitation in 0.06 molar *tris* buffer (pH 7.4) with 0.03 molar potassium chloride and 5 mmolar magnesium chloride. Our experiments in the same ionic conditions reproduced the curve shown in Fig. 2. The difference between the results reported by Levy and Fleisher<sup>11</sup> and those presented here can be attributed to the extra protein factors, because the preparation of myosin *B* that has not been washed by distilled water shows the results obtained previously. It could be argued that the lack of direct correlation between the rate of superprecipitation and the activity of ATPase observed with natural actomyosin or in certain ionic conditions could result from factors such as ions and extra proteins acting on the specific site(s) that may be involved differently in ATP hydrolysis and superprecipitation.

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### Enzyme Mechanism of Aminoacetone Metabolism by Micro-organisms

ALTHOUGH aminoacetone was first recognized as a microbial metabolite in 1958 (ref. 1), and found to be formed from L-threonine<sup>1-5</sup>, glycine plus a source of acetyl-CoA<sup>1,2,4,5</sup>, or isopropanolamine<sup>5,6</sup> (1-aminopropan-2-ol) by a variety of micro-organisms, it has only recently been found that the amino ketone is further metabolized

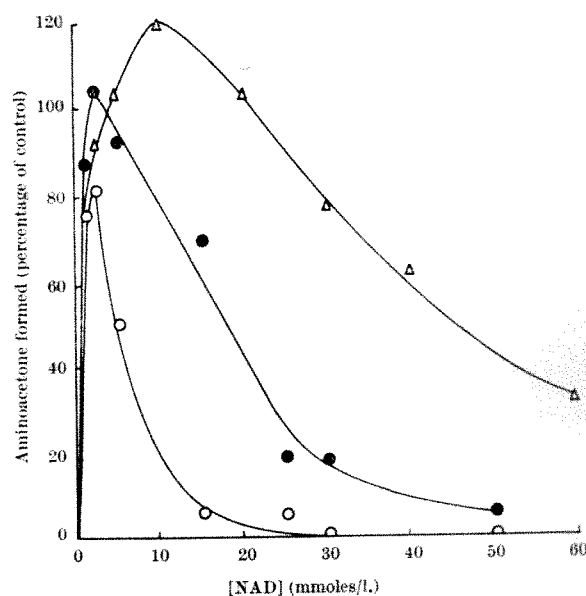


Fig. 1. Effect of the concentration of NAD on the formation of aminoacetone from L-threonine in the presence of 2-oxo acids or malate. Reaction mixtures contained 125 mmolar *tris* buffer (pH 8.5), 400 mmolar potassium chloride, 25 mmolar L-threonine, 2 mmolar 2-oxoglutarate (O), oxaloacetate (Δ) or D,L-malate (●), NAD as shown, 0.1 mg *B. subtilis* cell free extract/ml. Controls were reaction mixtures from which 2-oxo acids or malate were omitted. Incubation was for 10 min at 37° C. The formation of aminoacetone was measured as previously described<sup>4</sup>.

by resting cell suspensions of some bacteria<sup>7</sup>. The rapid utilization of aminoacetone by extracts free from cells has now been demonstrated and information of the enzyme mechanism of aminoacetone metabolism is available.

During a study of the activity of L-threonine dehydrogenase in cell free extracts of *Bacillus subtilis*, grown on nutrient broth, we observed that the accumulation of aminoacetone was inhibited by 2-oxoglutarate, oxaloacetate or D,L-malate. The inhibitory effect of these compounds was marked when nicotinamide adenine dinucleotide (NAD) was present at high concentrations (see Fig. 1), but was completely absent when partially purified preparations of L-threonine dehydrogenase were used. Similar results were obtained when aminoacetone formation was measured during studies of L-1-aminopropan-2-ol dehydrogenase<sup>6</sup> activity. It appeared likely that 2-oxo acids facilitated the further metabolism of the amino ketone and that NAD was also required. This was found to be so using synthetic aminoacetone, and the rate of its utilization by an extract of *B. subtilis* was found to be about 27 μmoles/mg protein/min at 37° C in the system: 125 mmolar *tris*-HCl buffer (pH 8.5), 400 mmolar potassium chloride, 20 mmolar 2-oxo-glutarate, 10 mmolar NAD, 0.125 mmolar aminoacetone and 0.14 mg extract protein/ml. Additional results showed the  $K_m$  for aminoacetone in this system to be about 2.0 mmolar, the optimum activity to be at about pH 10, and that potassium chloride was not required. Maximum rates of about 40 μmoles aminoacetone utilized/mg protein/min at 37° C have been observed.

High rates of utilization of aminoacetone have been observed when cell free extracts of *Pseudomonas* sp. NCIB 8858 are grown on D,L-1-aminopropan-2-ol (ref. 7). Rates of utilization of aminoacetone of about 120 μmoles/mg protein/min at 30° C were found with the system: 60 mmolar potassium phosphate buffer (pH 7.5), 20 mmolar 2-oxoglutarate, 20 mmolar NAD, 0.1 mmolar pyridoxal phosphate, 1 mmolar aminoacetone, 1.0 mg extract protein/ml. At the pH optimum of 7.5, with saturating concentrations of aminoacetone and 2-oxoglutarate, the half-maximum velocity of aminoacetone



disappearance occurred when the concentration of NAD was about 1 mmolar. The  $K_m$  values for 2-oxoglutarate and aminoacetone were similarly found to be about 2 mmolar, and 1 mmolar, respectively. Both oxaloacetate and pyruvate could replace 2-oxoglutarate without marked reduction of activity, and 2'-aminoacetophenone but not 5-aminolevulinate was utilized as rapidly as aminoacetone. The activity of the system utilizing aminoacetone was strongly inhibited by common sulphhydryl reagents, metal-chelating agents and some carbonyl reagents. Methylglyoxal inhibited activity by 50 per cent at 0.25 mmolar and completely at about 1.0 mmolar. The activity of dialysed extracts was increased by a factor of two by the addition of magnesium ions at 2 mmolar.

The results obtained with both *B. subtilis* and the *Pseudomonas* sp. are consistent with an enzyme mechanism for the utilization of aminoacetone involving a coupled aminoacetone-2-oxoacid aminotransferase and 2-oxo-aldehyde-NAD oxidase<sup>8,9</sup> system. The latter enzyme has been studied in extracts of the pseudomonad and found to be distinct from a D-lactate dehydrogenase also present at lower levels of activity. It would seem that methylglyoxalase activities are not involved in methylglyoxal metabolism by cell free extracts. The properties of the presumptive aminoacetone aminotransferase are at present under investigation, but this is complicated by the obligatory requirement for the oxoaldehyde oxidase to prevent any accumulation of the inhibitory product methylglyoxal. No 2-oxo-aldehyde aminotransferase activity with methylglyoxal plus a variety of amino group donors could be detected by assays for aminoacetone formation.

Preliminary results suggest that a number of micro-organisms other than those mentioned here are capable of metabolizing aminoacetone by way of methylglyoxal.

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## Action of Thyrocalcitonin in the Teleost Fish *Ictalurus melas*

HORMONAL control of calcium homeostasis in the lower vertebrates has received relatively little attention. This is particularly true for teleost fish in which there is a definite osseous skeleton but no parathyroid glands. Understanding of control of calcium homeostasis in these animals therefore is a problem of considerable evolutionary and physiological interest. The demonstration of a potent hypocalcaemic factor in the thyroid tissue of certain mammals<sup>1</sup> has now provided a new approach, because teleost fish possess definite thyroid follicles in spite of their diffuse occurrence. We have examined the effects of a porcine thyrocalcitonin extract on concentrations of

serum calcium and phosphate in the catfish and tried to demonstrate the presence of thyrocalcitonin in catfish tissue which contained thyroid follicles.

We designed an experiment to examine the effects of increasingly large doses of the extract on serum calcium and phosphate. Twenty catfish were given randomly four treatments consisting of an acid saline (pH 4) control and three different doses of porcine thyrocalcitonin extract—0.2 ml., 0.5 ml. and 2.0 ml. The extract was prepared as described before<sup>2</sup>. The average weight and length of the fish were 150 g and 23.2 cm. The fish were maintained in large aerated aquaria, filled with water from the river where they had been caught. The pH of this water was 5.8 and the average temperature during the course of the experiment was 25.3° C. The extract was administered intraperitoneally, the fish were bled by heart puncture 60 min after injection, and serum calcium<sup>3</sup> and phosphate (using a modification of the method of Fiske and Subbarow<sup>4</sup>) were determined. The results are summarized in Table 1. Not all blood samples were considered to be large enough for accurate analysis and, where applicable, this is reflected in the reduced number of animals in each treatment group shown in Table 1.

The results in Table 1 show a progressive hypocalcaemia with increasing doses of thyrocalcitonin extract. This stepwise reduction in serum calcium is statistically significant, as indicated in Table 1. The data also show a marked and highly significant hypophosphataemic effect of the extract with maximum response recorded with a dose of 0.5 ml. These responses in the catfish are qualitatively similar to those in the rat<sup>5</sup> and the dog<sup>6</sup>.

Table 1. EFFECT OF INCREASING DOSAGE OF PORCINE THYROCALCITONIN ON SERUM CALCIUM AND PHOSPHATE IN THE CATFISH

Treatment	No. of animals	Serum calcium (mg/100 ml.)		No. of animals	Serum phosphate (mg/100 ml.)	
		Mean	± S.D.		Mean	± S.D.
Acid saline control	4	16.0	± 4.05	4	11.1	± 2.01
0.2 ml. of TC	5	10.4	± 0.84	4	7.4	± 2.10
0.5 ml. of TC	5	9.8*	± 1.34	4	4.8‡	± 1.80
2.0 ml. of TC	5	8.7†	± 0.14	4	5.3‡	± 1.55

TC, Porcine thyrocalcitonin; supernatant fluid from ultracentrifugation step<sup>2</sup>.

\* Significantly different from saline control ( $P < 0.05$ ).

† Significantly different from saline control ( $P < 0.02$ ).

‡ Significantly different from saline control ( $P < 0.01$ ).

In a second experiment, we tried to demonstrate the presence of a hypocalcaemic factor in catfish tissue which contained thyroid follicles. For this purpose a section of connective tissue measuring approximately 2 cm × 5 mm × 5 mm was removed on both sides of the ventral aorta, immediately anterior to the heart, from thirty fish. Microscopic examination of samples of this tissue confirmed the presence of thyroid follicles, but the dispersion of the follicles was rather diffuse. A crude extract of this tissue was prepared by the method of Stahl *et al.*<sup>2</sup>; the supernatant fluid from the low speed centrifugation was used. The extract was then assayed for any hypocalcaemic or hypophosphataemic properties by injecting it into five rats subcutaneously in a dose of 0.6 ml. (equivalent to 120 mg of fresh tissue) to each animal. Serum calcium and phosphate were determined 60 min after injection and compared with a similar control group which had received injections of 0.6 ml. of 0.1 normal hydrochloric acid. The results showed no significant effect of the extract on either serum calcium or phosphate. The serum calcium concentrations remained unchanged at 10.0 mg/100 ml. and the serum phosphate was only slightly depressed from 9.4 to 8.9 mg/100 ml. These results are not considered conclusive in any way, because the diffuse dispersion of the thyroid follicles may require a larger dose to elicit a response. Furthermore, the polypeptide nature of thyrocalcitonin may mean that species specificity is involved in inhibiting the interspecific action of this hormone in certain cases.

Our results have shown that a teleost fish is very responsive to the action of mammalian thyrocalcitonin. This fact in itself is of evolutionary and pharmacological interest and confirms the desirability of pursuing this approach to solve problems of hormonal control of calcium homeostasis in teleost fish.

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### Lysozyme and the Pericapillary Reticulin Network

We have previously observed a heterogeneity in the carbohydrate fraction of human reticulin fibres and basement membranes. This heterogeneity seemed to be related to the biological age of the subject, the age of the network, the metabolic characteristics of certain organs and tissues and the functional dynamics of various cellular groups<sup>1,2</sup>. In a further investigation of the mechanisms of silver impregnation, which will be described in a later publication, we made many enzyme digestions (sialidase, lysozyme, pectinase,  $\alpha$ -amylase,  $\beta$ -glucuronidase, bacterial and testicular hyaluronidase, ribonuclease, pepsin, trypsin, papain and collagenase). During this investigation a peculiarity was revealed after incubation with lysozyme of the samples fixed in Carnoy solution. In the sections treated for 48 h at 37° C with lysozyme (Worthington Biochemicals Corporation, twice crystallized 5 mg/30 ml. of 0.2 molar phosphate buffer, pH 5.3 (ref. 3)) there was a constant disruption and dislocation of the

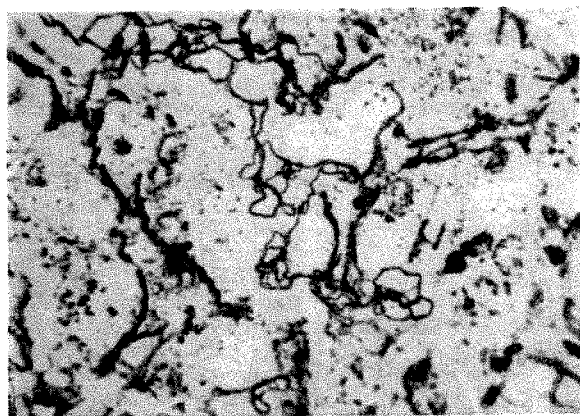


Fig. 1. Human liver. Lysozyme digestion followed by Gomori's silver impregnation. Dislocation of the pericapillary (perisinusoidal) reticulin network. ( $\times$  c. 585.)

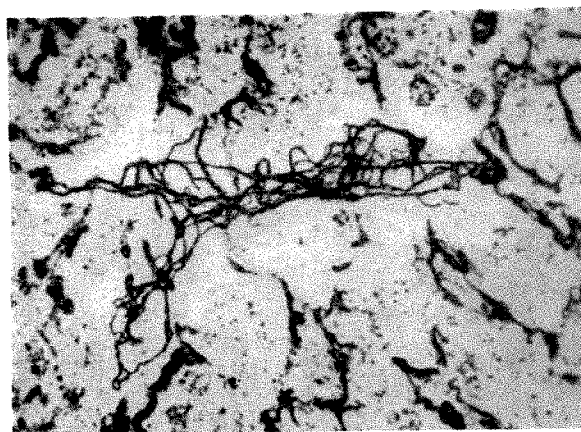


Fig. 2. Human liver. Lysozyme digestion followed by Gomori's silver impregnation. Dispersed reticulin network exhibiting an intense and homogeneous affinity for silver impregnation. Many of the dispersed filaments end in a closed loop. ( $\times$  c. 150.)

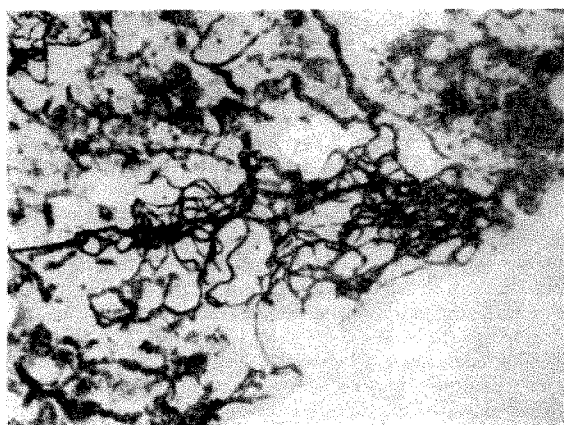


Fig. 3. Human spleen. Lysozyme digestion followed by Gomori's silver impregnation. Dislocation and dispersion of the reticulin network exhibiting aspects of fibrillary balls. ( $\times$  c. 585.)

pericapillary (or perisinusoidal) reticulin network (Figs. 1-4). These aspects have not been seen in control slides incubated in an inactivated lysozyme by Lugol's iodine 1:300 buffer<sup>3</sup> or in the buffer solution. Of all the glycolytic and proteolytic enzymes used, lysozyme alone could bring about this process. If the incubation with lysozyme was preceded by digestion with another enzyme, the changes occurred with the same intensity as those observed with lysozyme alone.

As a consequence of the disruption and dislocation brought about by digestion with lysozyme, fragments of the reticulin network, balls of fibre and isolated fibres were detached from the capillary bed and randomly distributed throughout the whole section. Many of the dispersed filaments were terminated by a closed loop. They exhibited a more homogeneous and more intense affinity for silver impregnation when compared with the untreated pericapillary reticulin network in the control slides. On the other hand, the material located between the pericapillary reticulin network and the endothelial layer, which can be shown by both silver impregnation and the periodic acid-Schiff technique to be an apparent basement membrane, seemed not to be modified by lysozyme digestion.

This process of disruption, dislocation and dispersion of the pericapillary reticulin network determined by the action of lysozyme, was constantly found in human liver (Figs. 1 and 2), spleen (Fig. 3), lungs (Fig. 4), myocardium and kidney, both with Gomori's silver impregnation and McManus periodic acid-Schiff reaction.



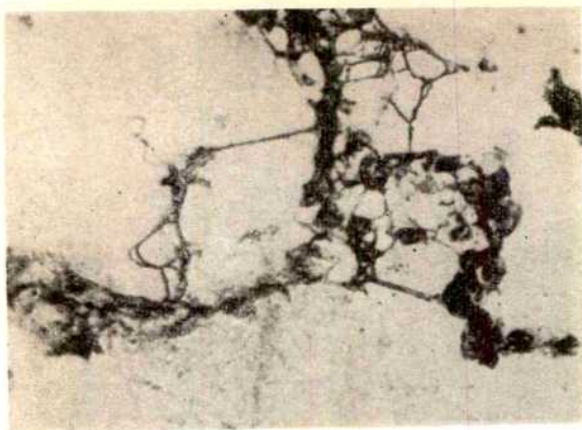


Fig. 4. Human lung. Lysozyme digestion followed by Gomori's silver impregnation. Disrupted filaments of the pericapillary reticulin network of the alveolar wall. ( $\times c. 585$ .)

Lysozyme is a  $\beta$ -glucosaminidase which acts on *N*-acetylated glucosamines in oligosaccharides, hydrolysis  $\beta$ -glycosidic bonds connecting *N*-acetyl glucosamine with other components<sup>4</sup>. The presence of glucosamine in reticular tissue has been mentioned by several authors<sup>2,4,7</sup>. Our results suggest that glucosamine is included in a complex of "cement substances" which connects the pericapillary reticulin network to the sub-endothelial space, structurally organized as an apparent basement membrane. Our results also suggest that glucosamine is a component of the perifibrillar sheet of the reticulin fibre, the elimination of which unblocks chemically and/or sterically some reactive groups, allowing more intense and uniform impregnability with silver nitrate of the dislocated and disrupted network.

Thus there is the possibility of a deeper understanding of the mechanisms which control the visco-elastic resistance of the capillary wall and of the agents which may influence its adequate adaptation to local haemodynamic and metabolic peculiarities.

In conclusion, these data are consistent with the assumption that a material susceptible to lysozyme (probably glucosamine) is included in a "cement" complex which connects the pericapillary reticulin network to the sub-endothelial space, and is organized as an apparent basement membrane.

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### Isolation of an L-Fucose Binding Protein from *Lotus tetragonolobus* Seed

Morgan and Watkins<sup>1</sup> have shown that haemagglutination of group O human red blood cells by an aqueous extract of *Lotus tetragonolobus* seed is inhibited by L-fucose but not by D-fucose. In this communication we describe the isolation from *L. tetragonolobus* of a protein which binds specifically L-fucose and which agglutinates group O human red blood cells. The isolation is based on specific

precipitation of the L-fucose binding protein by a dye containing three  $\alpha$ -L-fucopyranosyl residues. The trifunctional fucosyl dye (1,3,5-tri-(*p*- $\alpha$ -L-fucosyloxyphenylazo)-2,4,6-trihydroxybenzene) was prepared from *p*-aminophenyl  $\alpha$ -L-fucopyranoside<sup>2</sup> by diazotization and coupling to phloroglucinol in a manner similar to some other trifunctional glucosyl dyes<sup>3</sup>. The isolated protein is characterized in terms of its molecular weight and its L-fucose binding isotherm.

Freshly milled seed (weighing 100 g) marketed as 'Asparagus pea' by Thompson and Morgan, Ltd., Ipswich, England, was extracted with a cold salt solution (2-3 l. of 0.85 per cent sodium chloride, 0.02 molar phosphate, pH 6.8 with sodium hydroxide) by stirring for 3 min in a homogenizer immersed in an ice bath. The cleared extract was fractionated by addition of solid ammonium sulphate, first to 30 per cent, then to 60 per cent saturation. The precipitate that formed between 30 per cent and 60 per cent saturation (4° C overnight) was collected and extracted with the salt solution (100 ml. for 100 g of flour). Trifunctional glucosyl dye (1,3,5-tri-(*p*- $\beta$ -D-glucosyloxyphenylazo)-2,4,6-trihydroxybenzene)<sup>3</sup> was added to the clarified extract (50  $\mu$ g/ml.) to precipitate a polysaccharide present in the extract that interferes with the specific precipitation of the L-fucose binding protein by the fucosyl dye. After standing overnight at 4° C, the red precipitate was removed by centrifugation and the fucosyl dye was added to the supernatant to a final concentration of 50  $\mu$ g/ml. The red precipitate that formed (4° C overnight) was collected, washed twice with cold salt solution and then extracted with a cold 2 per cent solution of L-fucose. The dye was removed by treatment with ion exchange resin (AG1-X4, Bio-Rad Laboratory, Richmond, California) equilibrated with the L-fucose solution. After a few hours of dialysis at room temperature against a few changes of salt solution to remove most of the L-fucose, the protein was precipitated by addition of fucosyl dye. The precipitate was washed, dissolved and freed of the dye as before. The protein was dialysed exhaustively against phosphate buffer (0.05 molar phosphate, pH 6.8 with sodium hydroxide) to remove L-fucose. After clarification in a high speed centrifuge the protein solution was frozen and stored at -70° C. From 100 g of flour, 45 mg of protein was obtained.

Some physical properties of the isolated protein in 0.05 molar phosphate buffer, pH 6.8, are:  $\lambda_{\max}$  282 m $\mu$ ,  $E_{1\%}^{1\text{cm}}$  17.5;  $\lambda_{\min}$  250 m $\mu$ ,  $E_{1\%}^{1\text{cm}}$  6.2. The isolated protein was homogeneous in its sedimentation pattern ( $S_{20}$  6.23, for a concentration of 7.5 mg/ml.). The weight-average molecular weight was found to be 107,000 by the sedimentation equilibrium method assuming a partial specific volume of 0.75.

The isolated protein was found to agglutinate group O human red blood cells (0.1 per cent suspension in 0.85 per cent sodium chloride, 0.02 molar phosphate buffer, pH 6.8, 22 per cent C). The minimal protein concentration for agglutination was 38  $\mu$ g/ml., as determined by the two-fold dilution technique. The agglutinating potency of the purified protein was increased, however, to a minimal agglutinating concentration of 2.4  $\mu$ g/ml. by addition to the test mixture of a diluted crude aqueous extract derived from *L. tetragonolobus* as previously described. The dilution of extract used (1:50) caused no agglutination because the highest dilution for agglutination was 1:12.

Inhibition of haemagglutination by a number of saccharides was determined by the two-fold dilution technique. The test mixture contained 38  $\mu$ g of isolated protein/ml of dilute extract (1:50). The minimal concentrations for inhibition were 20  $\mu$ g/ml. of methyl  $\alpha$ -L-fucopyranoside 40  $\mu$ g/ml. of L-fucose, 5 mg/ml. of D-arabinose, 5 mg/ml of D-ribose, and 20 mg/ml. of D-fucose. These data are in agreement with the findings of Morgan and Watkins concerning the haemagglutination inhibition of *L. tetragonolobus* extract by various saccharides. It is thus sug



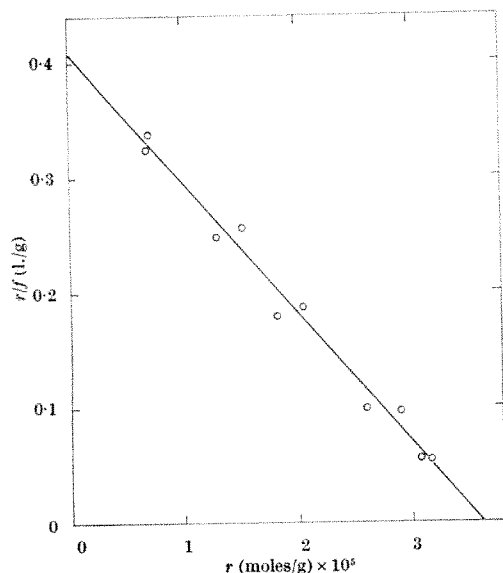


Fig. 1. Plot of binding data. Protein concentration, 0.7–2.7 mg/ml. Temperature, 3° C.

gested that the isolated L-fucose binding protein carries the specificity of the haemagglutinating system.

Binding of L-fucose by the isolated protein was determined by the equilibrium dialysis method using L-fucose labelled with carbon-14 (0.41 mc./mmole, Calbiochem, Los Angeles). In each dialysis cell (Technilab Instruments, Los Angeles) one compartment was filled with 1 ml. of protein solution and the other with 1 ml. of buffer solution. The membrane was made of Visking dialysis tubing. Labelled L-fucose solution (0.5–10  $\mu$ l.) was added to the protein or to the buffer. After 20 h of gentle stirring at 3° C, duplicate samples of 100  $\mu$ l. from both compartments of the dialysis cell were transferred to 15 ml. of Bray's solution for determination of counting rates (Packard 'Tri Carb' scintillation counter). At least 50,000 scintillations were counted for each sample. Counting rates of a blank and of a standard solution were determined as well. The concentration of a stock solution of labelled L-fucose was determined by the method of Park and Johnson<sup>4</sup> and the specific counting rate was found to be 4,056 c.p.m./ $\mu$ g. The concentration of protein was measured spectrophotometrically.

The results of binding experiments at concentrations of protein from 0.7 to 2.7 mg/ml. are plotted in Fig. 1 according to the equation (compare Scatchard<sup>5</sup>)

$$r/f = -Kr + NK$$

where  $r$  is the number of moles of L-fucose bound/g of protein,  $f$  is the molar concentration of free L-fucose,  $K$  is the intrinsic binding constant, and  $N$  is the number of moles of binding sites/g of protein. Fig. 1 shows that the experiments were performed in conditions ranging from a low degree of coverage to near saturation of the binding sites. The fact that a straight line fits the experimental point shows that the binding sites are equivalent and independent, and the  $K$  and  $N$  are independent of protein concentration. From the slope we obtain  $K = 1.1 \times 10^4$  l. mole<sup>-1</sup> and from the  $x$ -intercept  $N = 3.6 \times 10^{-5}$  moles of sites/g of protein or 3.9 sites for each protein molecule ( $\bar{M}_w = 107,000$ , see previously).

Stereospecificity of the binding sites was demonstrated by comparing the ability of the enantiomers, L-fucose and D-fucose (Pfanstiehl Laboratories, Inc., Waukegan, Illinois) to inhibit the binding of labelled L-fucose. A hundred-fold excess of D-fucose does not interfere with binding of the label, whereas L-fucose interferes as expected.

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## MICROBIOLOGY

### Lysis of *Escherichia coli* by Marine Micro-organisms

INVESTIGATIONS of the lethal effect of seawater on *Escherichia coli*, the common indicator organism for pollution by intestinal pathogens, have suggested a variety of possible mechanisms<sup>1–3</sup>. The negative effect of heat sterilization of seawater on its specific bactericidal action indicates a biological interaction which has been neither confirmed nor disproved. We attempt to define here the role of the native microflora in the disappearance of *E. coli* from seawater.

We studied the relationship between the size of the marine microbial population and the rate of death of *E. coli* using seawater concentrates of different population densities. Fresh seawater, sampled at Nahant and Woods Hole, Massachusetts, was centrifuged at 4,000*g* at 10° C and the bacterial count of a series of concentrates was determined by surface plating on a peptone-yeast extract agar<sup>4</sup>. 100 ml. quantities of autoclaved seawater to which different concentrations of marine micro-organisms had been added were placed in 250 ml. Erlenmeyer flasks. Strain K12 of *E. coli* was grown in nutrient broth and

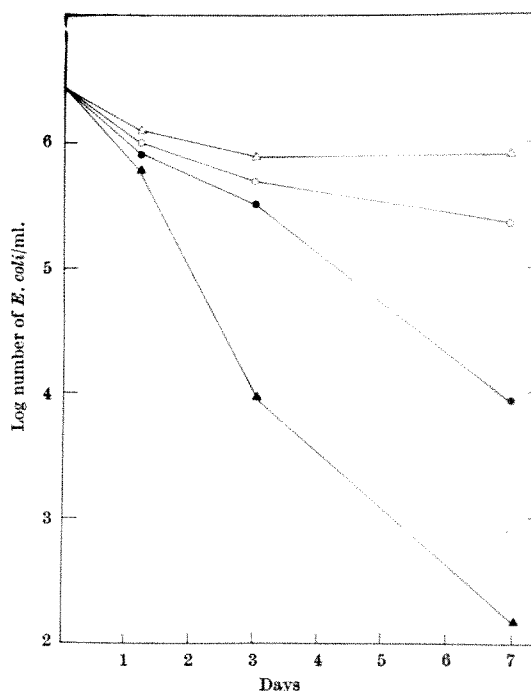


Fig. 1. Plot of the log of the number of *Escherichia coli* present in autoclaved seawater after the addition of different concentrations of marine micro-organisms.  $\Delta$ , Autoclaved seawater;  $\circ$ , autoclaved seawater plus ten marine bacteria/ml.;  $\bullet$ , autoclaved seawater plus  $10^3$  marine bacteria/ml.;  $\blacktriangle$ , autoclaved seawater plus  $10^4$  marine bacteria/ml.



was inoculated into all flasks at a concentration of  $10^6$ /ml. The mixture was incubated at  $26^\circ\text{C}$  on a rotary shaker. Viable counts of *E. coli* were made at regular intervals on eosin methylene blue agar at  $37^\circ\text{C}$ .

The results (Fig. 1) show that the decrease in numbers of *E. coli* was strongly affected by the size of the marine microbial population. Almost no decrease was detected in autoclaved water. As the concentration of marine bacteria in the water increased, so also did the rate and extent of death of *E. coli*.

In parallel experiments we investigated the survival of *E. coli* in samples of seawater taken from the Atlantic Ocean 150 miles off Cape Cod, Massachusetts. Populations of *E. coli* added to the water declined from  $10^9$  cells/ml. to  $10^7$  cells/ml. in 5 days when the total marine bacterial population was about  $10^5$  bacteria/ml. By comparison, the population of *E. coli* declined from  $10^9$ /ml. to  $10^4$ /ml. in 5 days in seawater sampled from the harbour at Woods Hole, Massachusetts, which had a bacterial population of about  $10^6$ /ml.

The development of a specific lytic microbial population after inoculation of *E. coli* into seawater was indicated by the results of later experiments, in which *E. coli* was re-inoculated to natural seawater 5 days after the initial inoculum. As shown in Fig. 2, the lag phase of the death curve of *E. coli* was virtually eliminated in the second curve. The number of *E. coli* cells declined from  $10^6$ /ml. to  $10^2$ /ml. in 4 days following the second inoculation, compared with 7 days following the initial inoculation into seawater. The indigenous marine bacterial population remained constant at  $10^4$ /ml. throughout the experiment.

The stimulation of the lethal activity of seawater by the addition of *E. coli* suggested the enrichment of a microflora parasitic or lytic to *E. coli*. In later experiments, *E. coli* was added to natural seawater in Erlenmeyer flasks as before. Samples of the water were taken at daily intervals and placed on double layer plates containing autoclaved seawater and 1.5 per cent agar in the bottom layer. The top layer contained autoclaved seawater with 0.7 per cent agar and inoculated with  $10^{10}$  cells of *E. coli* K12/ml.

Two kinds of responses were recorded after 2–3 days: (1) the appearance of bacterial colonies surrounded by a

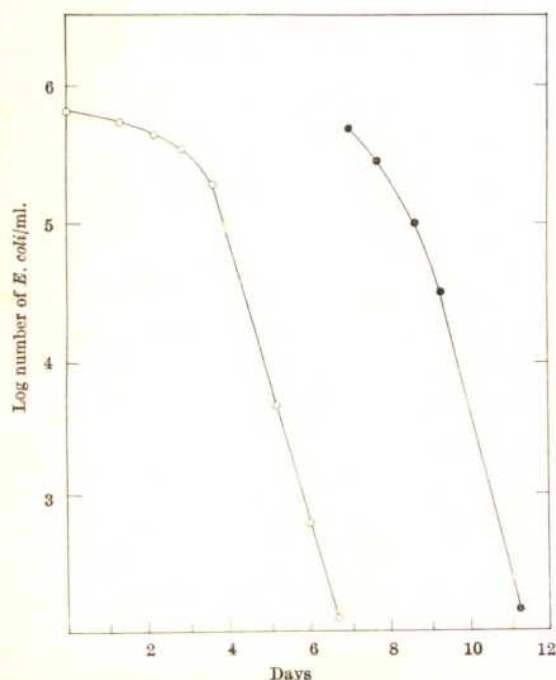


Fig. 2. Accelerated decline of the population of *Escherichia coli* in natural seawater with a re-inoculation of *E. coli* after 7 days. ○, First inoculum of *E. coli*; ●, second inoculum of *E. coli*.

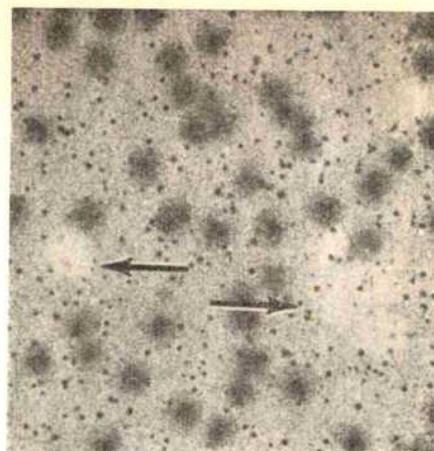


Fig. 3. Plaques observed when seawater exposed to *Escherichia coli* was inoculated onto double layer plates of autoclaved seawater and *E. coli*.

clearing zone in the bacterial lawn; (2) the appearance of clearing zones without visible colonies (plaques). Tests showed that the organisms isolated from the colonies were capable of utilizing the cell walls of *E. coli* as a sole source of organic carbon. Lysis was caused by degradation of the cell walls by extracellular enzymes. In a previous report<sup>5</sup> we described the isolation of similar bacteria from seawater and their activity in killing *E. coli* in artificial seawater.

After exposure of seawater to *E. coli* for 5 days, the number of plaques increased from about 10/ml. in natural water to  $10^3$ /ml.—as measured by the double layer technique. In samples from the plaques, phase contrast microscopy revealed the presence of large numbers of highly motile vibrios ranging in size from 0.2 to 0.5  $\mu$ . These bacteria did not grow on the peptone-yeast agar used in this study and seemed to be obligate parasites. They were also halophilic. Plaques regularly appeared in 3 days on *E. coli* K12 or B double layer plates at  $22^\circ\text{C}$ . Very few of these plaques were obtained with natural seawater, unless previously inoculated with *E. coli*. These organisms appear to belong to the genus *Bdellovibrio* as originally described by Stolp and Petzold<sup>6</sup>, and named by Stolp and Starr<sup>7</sup>. Typical newly formed plaques are shown in Fig. 3.

The fact that the lethal effect of seawater on *E. coli* increases (a) with increasing population size, as determined on a peptone-yeast extract agar, and (b) with an increasing number of lytic bacteria on inoculation with *E. coli* supports our assumption that the indigenous microflora contributes significantly to this effect. A more complex effect is suggested by the results of studies on other qualities of seawater, especially heavy concentration of metal and the presence of algal toxins. It seems reasonable to assume that the nature of the lethal effect of seawater on *E. coli* will vary with seasonal and local changes of the environmental conditions.

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### 'Viractin'

It has been claimed that 'Viractin', a complex mixture of substances obtained from the mother liquors of *Streptomyces griseus* fermentation, reduced the incidence of influenza and other respiratory diseases when it was allowed to evaporate from a gauze pad suspended in the sleeping compartment of patients in a mental hospital<sup>1</sup>. Investigators are agreed, however, that it has no demonstrable *in vitro* antiviral activity and evaporation into the air does not protect mice against experimental influenza virus infection<sup>2</sup>. It has been pointed out that a negative result in experimental animals does not mean that the material is negative in man<sup>3</sup>. A further trial has therefore been conducted among members of the staff of the Post Office Branch of the Treasury Medical Service.

The 'Viractin' was an authentic sample used in earlier studies and was used in the manner and at the rate prescribed by Leach *et al.*<sup>1</sup>. Volunteer members of the staff were recruited and agreed to place impregnated pads in their bedrooms. Half of them (randomly selected) in each section of the trial received pads containing active material and the other half containing a dummy material (benzaldehyde in 90 per cent ethanol with colouring). Each volunteer received a diary card, similar to that used by Hope-Simpson<sup>4</sup>, on which he recorded the following symptoms: sore throat, cold in the head, headache, feverishness and aches in the back and limbs. For the purpose of assessment of the cards only those symptoms recorded for 2 days consecutively or more were regarded as significant. The volunteers did not know the nature of the substance on the pads and the cards were evaluated twice with closely concordant results by physicians who also did not know what material was being used. The first section of the trial included 39 volunteers who were treated and observed between September 26 and November 20, 1966. In the second half, between January 16 and February 24, 1967, all the subjects treated with 'Viractin' were given control material and vice versa. The results are shown in Table 1.

Table 1

Group	No.	Substance	Period	No. of respiratory infections occurring	No. of persons infected	No. of infections/person/week of observation
A <sub>1</sub>	20	'Viractin'	8 weeks	7	6	0.04
B <sub>1</sub>	19	Placebo	8 weeks	22	9	0.14
A <sub>2</sub>	20	Placebo	8 weeks	5	5	0.04
B <sub>2</sub>	19	'Viractin'	6 weeks	15	8	0.13
A <sub>1</sub> and B <sub>2</sub>	39	'Viractin'	Both	22	14	0.04
A <sub>2</sub> and B <sub>1</sub>	39	Placebo	Both	27	13	0.05

Recording continued for a further period of 8 weeks after the solutions were withdrawn, with the following results:

A <sub>1</sub>	20	8 weeks	3	2	0.02
B <sub>1</sub>	19	8 weeks	8	6	0.05

It can be seen that in the first half of the trial those given 'Viractin' fared slightly better than the controls; in the second half the controls fared better. It was concluded that there was no evidence of an effect on virus infections comparable with that reported by Leach *et al.*

It would appear that the original placebo group (B<sub>1</sub>), although randomly selected, had an increased susceptibility to upper respiratory infection which they carried with them throughout all phases of the trial.

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## PSYCHOLOGY

### Binocular Depth Perception of "Julesz Patterns" viewed as Perfectly Stabilized Retinal Images

THE role of eye movements in the promotion and maintenance of binocular fusion and depth perception is not clear, although experiments<sup>1-3</sup> suggest that judgements of depth in a stereoscopic situation may be made with some accuracy in the absence of eye movements. Langlands found reliable perception of depth in normal vision with short exposures of the order of 10<sup>-5</sup> sec, but it seems uncertain whether judgements could have been influenced by after-images. It should be possible in principle to solve this problem by "stabilizing" images on the retina after the method of Ditchburn<sup>4</sup> and Riggs<sup>5</sup>, and in 1963 one of us (C. R. E.) tried to study binocular vision with a contact lens stabilizing system in each eye, but with inconclusive results. Partial destabilization because of poor contact lens fit was a probable cause of this lack of success, as Barlow pointed out in a general criticism of this method<sup>6</sup>. Experiments with an after-image as a "perfectly stabilized image" have been undertaken here<sup>7</sup>, and, because with suitable methods prolonged clear after-images of patterns can be obtained, we decided to try to study complex stereoscopic patterns in these conditions. (Because the after-image is formed as the result of temporary changes in the state of the retinal cells themselves, it can be considered to be a completely "stabilized" image.)

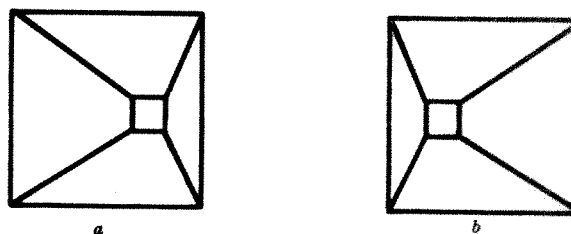


Fig. 1. Stereo pair. Truncated pyramid viewed from above.

Early experiments used patterns such as those in Fig. 1; more than 100 casual subjects described the effects when patterns 1a and 1b were flashed to left and right eyes. Results were ambiguous, approximately 50 per cent reporting that the fused central square was seen "in depth", the remainder stating that the image appeared two-dimensional. The simplest hypothesis to account for the discrepancy seemed to be that the diagrams employed allowed inferences about "depth" to be made from the special nature of the patterns—which could be likened to a corridor or to a truncated pyramid seen from above. Clearly, patterns in which inferences about depth cannot be obtained must be employed. Accordingly, we considered the well known random-brightness patterns designed by Julesz of Bell Telephone Laboratories



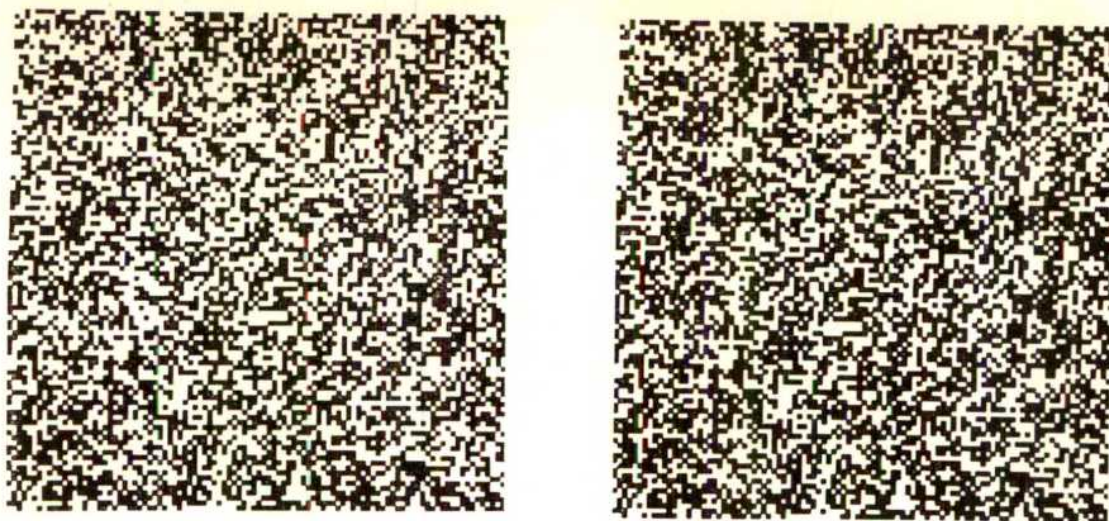


Fig. 2. Julesz stereo pair which gives centre square in front of the background.

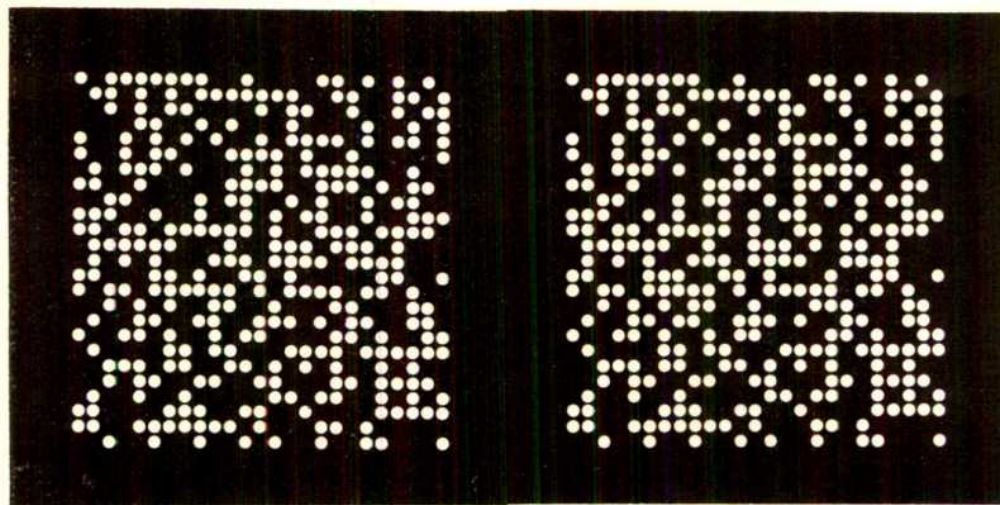


Fig. 3. Stereo pair which gives centre square in front of the background. Our modified Julesz patterns.

(Fig. 2) which when viewed in a stereoscope show a central square standing in depth—this in the absence of any perspective or monocular form cues. Retinal disparity alone, arising from a horizontal shift of the central portion of the otherwise identical patterns, is a sufficient cue to depth<sup>6</sup>. The after-image technique of retinal stabilization consists of viewing a bright flash discharged from behind a cut-out or high contrast photographic slide with its black parts very dense. If a Julesz pattern is so viewed, the very large number of component squares or "picture elements" cannot be resolved in the after-image, and thus, of course, there can be no depth perception. This was solved by devising the pair of patterns shown in Fig. 3—which are basically similar to Julesz patterns but with the number of components reduced. The white circles (picture elements) were placed on the intersections of a  $25 \times 25$  rectangular grid, their position being determined by random numbers. The relative shift of a  $10 \times 10$  central portion is one "picture element".

The patterns were photographed on  $2 \times 2$  in. slides and mounted in a lens stereoscope, at a distance from the lenses equal to their focal length. Infra-red absorbing filters and tracing paper to diffuse the light were mounted behind the slides, and a septum was provided. A single photographic flashlight illuminated both slides.

A fixation point in the form of a black dot on a small white square of paper was attached to each slide, the

points lying in corresponding positions on the outer portions of the slides—specifically, in the row of circles directly above the "inner square" of circles. Experiments were conducted in a darkened room, although subjects were not dark-adapted. The front of the slides was dimly illuminated so that on looking into the stereoscope, a subject could see only the fixation points. Subjects were instructed to fixate, and when the markers were fused, to discharge the flash unit for themselves by pressing a button. The 1 msec flash, provided by gas discharge from a Courtenay 'Major Mark 2' power pack, had an output of 1,000 joules—so bright that detail in the slides at the moment of the flash could not be observed. Within 2 sec, however, the "after-image" became clear, and the detail of the picture elements emerged. The characteristic fading and regeneration cycle of the after-image was forestalled<sup>9</sup> by requiring subjects to face, with closed eyes and at a distance of about 6 in., a stroboscope flashing at approximately 5 cycles/sec. In these conditions, all subjects could see the fused patterns in some detail, and for a period of 10–15 sec.

Without knowing the direction of "depth" in advance of a flash, ten subjects were each given a series of twenty pairs of after-images, randomly ordered with ten "square-in-front" and ten "square-in-back" configurations. Subjects were allowed 15 sec to view the after-image, after which, if they had not already made a judgement, they



were invited to "guess". Few such forced choices in fact had to be made. Subjects' confidence in their choices were reported and noted. There was a 5 min interval between flashes, and no knowledge of results was given until the end of the series. Of the ten subjects, seven were correct seventeen or more times out of twenty, while three were not significantly better than chance ( $P < 0.001$  on the binomial test). Of those subjects who could definitely tell the plane of depth in the after-image, none made an error when confident of his judgement.

In a subsidiary experiment, a number of casual subjects were shown Julesz patterns in a normal stereoscope, and all those making a correct judgement of depth were then given a pair of after-images using our modified patterns; 85 per cent of them made correct judgements on this single observation.

It is therefore evident that stereoscopic depth perception of complex displays can be attained in a perfectly stabilized image, that is in the absence of eye movements, both voluntary and involuntary. Subjective impressions of the strength of the depth effect varied from subject to subject, but to some it appeared striking. The central square was frequently seen to "build up" from its surround. The occasional errors recorded by even the most reliable subjects can probably be attributed to imperfect fixation before the flash, resulting in slight non-correspondence of the stereo pairs.

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## Stimulus Probability and Simple Reaction Time

SIMPLE reaction time tasks usually contain procedures designed to reduce or eliminate the frequency with which subjects anticipate the onset of the stimulus. These measures include varying the interval or foreperiod between the warning stimulus and the subsequent stimulus, and introducing a number of "catch trials" on which the stimulus does not follow the warning. Obviously, one effect of including catch trials is to lower the overall probability that the stimulus will follow the warning stimulus. In view of the known importance of stimulus probability in choice reaction time situations, it is perhaps surprising that, although there have been several studies (for example, refs. 3-5) of the effects of changes in the range and variability of foreperiods, there appears to have been only a single study in which the effect of changing the frequency of catch trials has received systematic investigation. Drazin<sup>3</sup> introduced catch trials to produce three levels of probability of occurrence of the stimulus ( $s$ ):  $P(s) = 1.0$ ,  $0.9$  and  $0.5$ . These levels were allowed to interact with mean foreperiod, so that Drazin's results regarding the effects of  $P(s)$  on reaction time are limited to the demonstration of (1) an inverse relationship between  $P(s)$  and reaction time, and (2) an interaction between  $P(s)$  and the reaction time-foreperiod relationship. The study reported here was designed to examine the relationship of the  $P(s)$  and

reaction time over a wide range of  $P(s)$  values in a sequential, simple reaction time situation.

A metronome-uniselector unit produced a regular series of warning stimuli, each consisting of a brief flash from a 12 V bulb placed at eye-level 1 m in front of the seated subject. Average response rate was held constant by adjusting the rate of presentation of warning stimuli inversely to  $P(s)$ —a procedure which also equates the length of time required to record the same number of reaction times at different values of  $P(s)$ . Mounted below the warning light was a 12 V relay, the arm of which produced a clearly audible click when the relay was energized. The subject responded to this click by closing a microswitch with the thumb of the preferred hand. The construction of the uniselector was such that the overall probability of the stimulus following a warning stimulus could be varied from 0.1 to 0.9 in 0.1 steps. In all conditions of the experiment blanks were randomly positioned in the stimulus sequences. By holding constant the interval between each warning stimulus and the subsequent stimulus (if one had been selected) at 750 msec in all conditions, the uncertainty of the subject as to when the stimulus would occur was reduced as far as possible: the remaining uncertainty was thereby restricted to that associated with the occurrence/non-occurrence of the stimulus. The rate of presentation of the sequence of warning stimuli varied from 1/1.5 sec in the  $P(s) = 0.1$  condition, to 1/13.5 sec in the  $P(s) = 0.9$  condition; the average interval between successive stimuli (and successive responses) was thus 15 sec in all nine experimental conditions.

Table 1. REACTION TIME MEANS, MEDIAN AND STANDARD DEVIATIONS (IN MSEC) OBTAINED IN THE NINE EXPERIMENTAL CONDITIONS

Subject		Probability of stimulus $P(s)$								
		0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
A.G.	$\bar{X}$	217	196	182	173	139	174	136	145	135
	Mdn.	216	190	170	167	136	162	136	138	133
	S.D.	30	36	34	30	15	46	19	25	19
M.R.	$\bar{X}$	266	195	202	196	161	164	162	164	155
	Mdn.	265	181	197	186	155	162	162	151	151
	S.D.	39	43	30	29	25	17	11	48	21
S.B.	$\bar{X}$	236	222	199	193	171	165	188	163	177
	Mdn.	230	212	188	189	165	161	184	163	173
	S.D.	40	46	32	26	27	22	22	8	21
J.B.	$\bar{X}$	274	212	215	181	169	172	158	175	170
	Mdn.	274	212	224	174	158	167	155	165	166
	S.D.	41	40	39	27	42	25	12	35	22
E.T.	$\bar{X}$	299	219	183	200	181	180	168	177	186
	Mdn.	290	208	176	198	177	177	168	174	184
	S.D.	50	56	26	27	25	31	18	17	30
J.I.	$\bar{X}$	318	252	268	215	201	189	167	181	155
	Mdn.	299	253	270	204	197	187	160	175	154
	S.D.	59	37	52	38	35	26	48	32	23
Condition mean		268	216	208	193	170	174	163	168	163

Six male psychology students, all with some experience of reaction time experiments, acted as subjects and were individually tested. All subjects were informed of the  $P(s)$  value appropriate to each experimental condition and were given a short practice period with immediate knowledge of results before each test session. Test sessions lasted for about 12 min each and all subjects were tested under all conditions in a different random order. Subjects were tested under five conditions on the first and four conditions on the second of two consecutive days. A 4 min rest period was allowed between successive test sessions. Fifty reaction times (measured to the nearest msec) were recorded during each test session.

Results obtained are summarized in Table 1. Errors were also recorded but, probably as a result of the instructions given to subjects, were too rare to allow sufficiently detailed analysis: in 2,700 stimulus presentations there was a total of only twenty-nine failures to respond, and only eleven responses which anticipated the onset of the stimulus.

The mean reaction times of the subjects were subjected to a two-way analysis of variance which revealed significant differences between subjects,  $F(5, 40) = 9.8$ ,



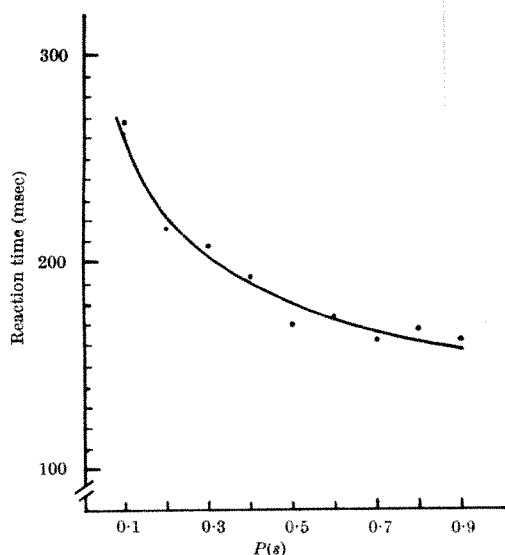


Fig. 1. Simple reaction time as a function of stimulus probability.  
Reaction time =  $154 P(s)^{-0.25}$ .

$P < 0.001$ ; and between the nine experimental conditions,  $F(8, 40) = 29.5$ ,  $P < 0.001$ . The eta-square test was applied to the nine condition means: this yielded  $\eta^2 = 0.726$ ,  $\chi^2 = 15.4$ ,  $P < 0.05$ , indicating that the relationship between mean reaction time and stimulus probability departs significantly from linearity.

A series of curves was fitted to the nine condition means of Table 1, the sum of the squared differences ( $\Sigma d^2$ )

between actual and predicted values forming an index of the goodness of fit of each function. Linear, quadratic and cubic polynomial functions gave  $\Sigma d^2$  values of 2,123, 457 and 313 respectively. A function of the form  $Y = aX^b$  was then fitted, giving  $\Sigma d^2 = 318$ , and it is this curve which is shown in Fig. 1.

The increase in reaction time as a function of decreased stimulus probability occurs as a general shift of the reaction time distribution rather than by the addition of a few very long reaction times: reference to the medians recorded in Table 1 and examination of plots of individual times support this conclusion. Illustrative data are shown in Fig. 2 where reaction time distributions from the fastest and slowest subjects (overall) are plotted as histograms.

These results indicate that the use of catch trials in simple reaction time situations may do more than prevent complete anticipation; it may effect mean latency by shifting the distribution of reaction times. The largest change in mean reaction time occurs at the extreme of the probability range (that is, between the  $P(s) = 0.1$  and  $P(s) = 0.2$  conditions); across other conditions the changes in mean reaction time are fairly small. It follows that in most simple reaction time situations, the customary frequency of catch trials will change  $P(s)$  values over a range where their effects are minimal; however, if catch trials are introduced, together with varied foreperiods and a constant warning stimulus rate, the various sources of slowing may summate or act multiplicatively.

The fact that the mean reaction times of the subjects tend to vary as a function of stimulus probability reinforces the view<sup>1</sup> that the reaction time task may be considered as a pay-off situation. If this view is correct then, even though the present results indicate that catch trial effects are likely to be relatively small, it would seem important in any theoretical analysis of a simple reaction time situation to consider not only the rewards and penalties associated with the behaviour of the subjects, but also the probability of occurrence of those events to which he is set to respond.

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Fig. 2. Distributions of reaction times obtained from two subjects in each of the nine experimental conditions. Each histogram represents fifty reaction times.

### Relationship between Circadian Rhythm of Body Temperature and Introversion-Extraversion

It has frequently been alleged that people can be divided into "morning" or "evening" types (see, for example, ref. 1). Supporting evidence for this view has been obtained by Colquhoun<sup>2</sup> and Colquhoun and Corcoran<sup>3</sup>, who found that the sign of the correlation of a personality measure—degree of introversion—and performance efficiency was dependent on the time of day at which testing was done. At two visual inspection tasks, introverted subjects performed better than extraverts in the early morning; the position was reversed in afternoon tests. The aim of the investigation reported here was to determine whether these differences were associated with underlying differences in circadian physiological rhythm as indicated by body temperature.

Using standard clinical thermometers the sub-lingual body temperatures of seventy-four young men were recorded twenty times during a single 24 h period, on two occasions separated by approximately 1 week. Read-

Table 1. CORRELATION COEFFICIENTS ( $r$ ) OF BODY TEMPERATURE AND INTROVERSION RATING AT TWENTY TIMES OF DAY ( $N=74$ )

Time	0500	0700	0800	0900	1000	1100	1200	1300	1400	1500	1600
$r$	-0.053	0.133	0.435*	0.163	0.043	-0.013	-0.106	-0.054	-0.075	0.006	-0.057
Time	1700	1800	1900	2000	2100	2200	2300	0100	0300		
$r$	-0.042	0.060	-0.016	-0.114	-0.239†	-0.167	-0.229‡	-0.207‡	-0.025		

\*  $P$  (one-tailed)  $< 0.001$ ; †  $P$  (one-tailed)  $< 0.025$ ; ‡  $P$  (one-tailed)  $< 0.05$ .

ings were taken hourly between 0700 and 2300 h, and two-hourly between 2300 and 0700 h during the sleep period of the subject. From 0800 to 1630 h the men were intermittently employed on light duties (mainly short psychological tests); meals were taken at 0700, 1200 and 1700 h. The evening was devoted to light indoor recreation. All subjects were given the Heron Personality Inventory<sup>4</sup> to determine their placing on the personality dimension of introversion-extraversion as measured by their score on the "unsociability" scale. Body temperature at each of the twenty times of day was taken as the mean of the two readings obtained. Although the grand average of these twenty means was not significantly correlated with introversion-extraversion ( $r = -0.044$ ), some significant correlations were observed when individual times of day were considered separately (see Table 1).

Not only the magnitude of the correlation but also its sign appeared to vary in a systematic manner depending on the time of day. Thus the correlation changed from significantly positive (introverts with higher temperatures) to significantly negative over the period from 0800 to 2100 h, that is, over the "active" part of the waking day. During sleep this trend was reversed.

Thirty-nine of the subjects had a score of three or less on the Heron Inventory; the remaining thirty-five subjects had a score of 4 or more (a high score indicates a relatively high degree of introversion). Average tempera-

tures for these two groups were computed separately at each time of day. Two-point rolling means of the resulting values are shown in Fig. 1.

The curve of body temperature through the 24 h period was, in both groups, characterized by a division into three distinct phases: (i) a rapid rise between 0500 and 1000 h; (ii) a further, considerably slower rise between 1000 and about 2000 h; (iii) a rapid fall between about 2000 and 0500 h. Thus the curve is not, as is sometimes suggested, a simple sinusoid.

There was no significant difference in the mean maximal range of temperatures recorded from the two personality groups ( $t=0.178$ ,  $P=0.22$ ); the peak-trough change was about  $1.2^{\circ}\text{F}$  in both groups. Inspection of the curves in Fig. 1 suggests that the temperature of the more introverted subjects rose more rapidly in the early morning and (possibly) started to fall at an earlier point in the late evening. This is brought out more clearly in Fig. 2, in which average temperatures are shown for only those subjects having Heron Inventory scores of either 2 or less (twenty-two cases) or 5 or more (twenty-five cases); again, there was no significant difference in mean temperature range for these more extreme personality groups ( $t=0.186$ ,  $P=0.21$ ). Analysis of variance of the temperatures of the extreme groups revealed a statistically significant interaction between Inventory score and time of day ( $F_{19,555}=1.85$ ,  $P<0.025$ ). It can therefore be concluded that the differences in the circadian rhythms shown in Fig. 2 are reliable.

Although relatively small, this relationship between personality, body temperature and time of day may help to explain the performance differences in introverts and extraverts referred to previously, if it is assumed that temperature reflects the level of "arousal" of the nervous system, and that performance efficiency is related to this level. Comparative measurements with a variety of different groups would be necessary to determine whether the relationship (and also the form of the body temperature curve observed) is characteristic of the particular type of subject used, or whether it is typical of the population at large.

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\* This note was prepared by his colleagues from data collected by Mr Blake before his accidental death in October 1965.

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## GENERAL

### Analysis and Design of Certain Scaling Experiments

IN certain psychological scaling techniques, direct pair comparison judgments of the differences between, or ratios of, magnitudes of attributes of objects (for example, volume or pitch of tones, heaviness of weights, painfulness of electric shocks) are elicited from a single subject<sup>1</sup>. We have investigated the statistical analysis of data thus obtained and the problem of optimal experimental design.

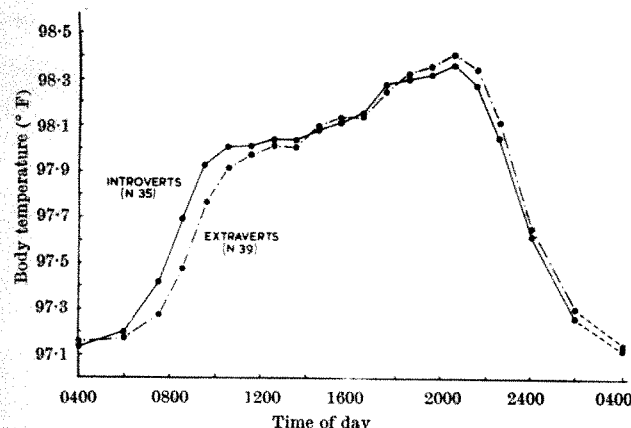


Fig. 1. Mean circadian rhythm of body temperature in introvert and extravert groups.

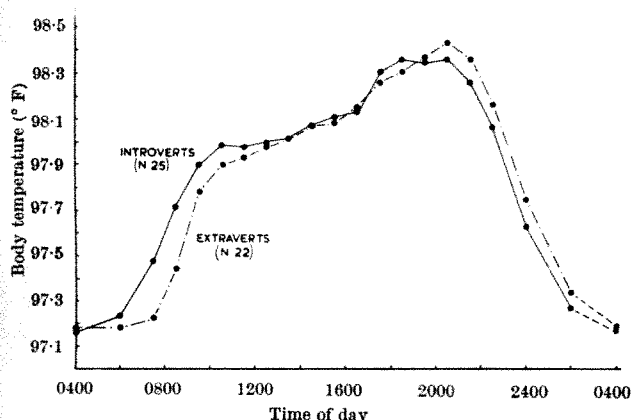


Fig. 2. Mean circadian rhythm of body temperature in relatively extreme introvert and extravert groups.

There are two cases to be considered: (1) the complete case, where all  $\binom{n}{2}$  possible pairs of the  $n$  objects are presented once each to the subject for judgment; (2) the incomplete case, where fewer are presented.

In the complete case it is well known<sup>2</sup> that if each judged difference is treated as consisting of a true difference between magnitudes, and error,  $d_{ij} = x_j - x_i + e_{ij}$ , then convenient least squares (L.S.) estimates of

the magnitudes are given by  $x_i = \frac{1}{n} \sum_j d_{ij}$ , and that ratio

judgments can be treated similarly by first taking their logarithms, and then taking antilogarithms of the average (because such a reduction is always possible with ratio judgments, they will not be mentioned separately any

more). The estimates have variance  $\frac{(n-1)}{n^2} \sigma^2$  and

covariance  $\frac{(-1)}{n^2} \sigma^2$ , where  $\sigma^2$  is the variance of the  $e_{ij}$ .

We suggest the following further analysis of data. The total raw sum of squares of judged differences,  $\sum_{i < j} d_{ij}^2$ ,

with  $\binom{n}{2}$  degrees of freedom (d.f.), is composed of two orthogonal sums of squares (S.S.), due to differences between estimates,  $\sum_{i < j} (\hat{x}_j - \hat{x}_i)^2$ , with  $(n-1)$  d.f., and error,

$\sum_{i < j} e_{ij}^2$ , with  $\binom{n-1}{2}$  d.f. It seems advisable to investigate

systematic distortion in the judgments by extracting from the error S.S. Tukey's S.S. for non-additivity<sup>3</sup>. Then an analysis of variance can be carried out which tests independently both the null hypothesis that  $x_1 = x_2 = \dots = x_n$ , and also that the judged differences are of the form supposed. In certain cases the analysis can be refined by dividing the estimates S.S. into a component attributable to some effect of psychological interest, for example, the psychophysical power law<sup>4</sup>, and an orthogonal remainder.

With more than about fifteen objects the number of judgments required of the subject in the complete case rapidly becomes unmanageable, which leads us to consider the incomplete case. Kaiser has suggested a convenient form of the L.S. estimation equations (personal communication):  $\hat{\mathbf{X}} = \mathbf{K}^{-1} \mathbf{\Sigma}$ , where  $\hat{\mathbf{X}}^{n \times 1}$  is a vector of estimates,  $\mathbf{\Sigma}^{n \times 1}$  is a vector of sums of judgments associated with the row object, and  $\mathbf{K}^{n \times n}$  a matrix of which the diagonal elements are equal to the number of objects with which the relevant object has been compared +1, and the off-diagonal elements are 0 or 1 according to whether the row object has or has not been compared with the column object. It is easily shown that the variance-

covariance matrix of the estimates is  $\sigma^2 \left[ \mathbf{K}^{-1} - \frac{1}{n^2} \mathbf{U} \right]$ ,

where  $\mathbf{U}^{n \times n}$  is the matrix with each element 1.

With appropriate changes in the d.f., the same analysis of variance may be used as in the complete case.

Two principles appear relevant to the selection of experimental designs in the incomplete case: first, each object should be treated in exactly the same way; second, the design should give as accurate estimates as possible.

To formalize the first principle, it is convenient to represent a design as a graph<sup>5</sup>, in which the vertices stand for objects, an edge connecting two vertices indicates that the corresponding objects are compared, and the absence of an edge that they are not. A graph is defined to be transitive if, for any ordered pair of vertices, there is an operation in its group of automorphisms which takes the first into the second, that is, if the group, considered as a permutation group on the vertices, is transitive<sup>6</sup>. This implies that the graph is regular, that is, that each vertex has the same number, say  $k$  (which must be even if  $n$  is odd), of edges, but the converse does not hold.

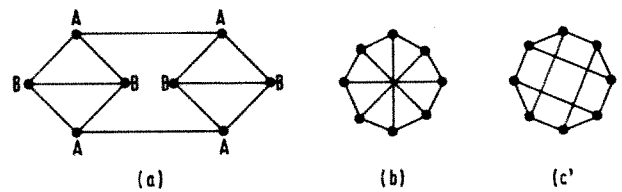


Fig. 1.

For example, in Fig. 1 graph (a) is regular but not transitive (because no vertex will go into another with a different letter), while graphs (b) and (c) are transitive. The first principle is then realized by considering only transitive graphs.

As regards the second principle, a graph gives as good as or better estimates of all linear functions of the object values if, and only if, the difference between their estimation variance-covariance matrices is positive semi-definite. Unfortunately this requirement fails to discriminate very well between different transitive graphs, so instead we use the less stringent condition that the optimal graph shall have a smaller estimation variance for the object values than any other, that is, that the diagonal elements of  $\mathbf{K}^{-1}$  shall be as small as possible.

We have not found any simple method of determining such optimal graphs, and are therefore carrying out a systematic computer-assisted search (which is expected to take quite some time) through all transitive graphs for which  $n \leq 25$  and  $\frac{nk}{2} \leq 100$ , to find those with smallest diagonal elements in  $\mathbf{K}^{-1}$ .

The experimental procedure here is essentially the same as that in Stevens's<sup>7</sup> method of magnitude estimation, in which the subject is presented with a reference object, which is assigned some standard numerical value, and then asked to estimate the magnitude of each of a set of test objects, which are presented one at a time but several times each, because these magnitude estimates are clearly implicit ratio judgments. The design has the following advantages and disadvantages over those discussed here.

(1) In Stevens's design the reference object tends to exert a slight distorting effect on the estimates obtained, which should not occur here, because there is no constant reference object.

(2) Stevens's design makes it impossible to test for distortion in the judgments by means of Tukey's S.S. for non-additivity.

(3) Stevens's design has the advantage that the estimates are not correlated, while in those discussed here they are. (The correlation in general varies from one pair of objects to another, but its average for any transitive graph is  $-1/(n-1)$ .)

(4) The variance of the estimates for an optimal transitive graph over the range of  $n$  and  $k$  we have considered appears to be very roughly half that with Stevens's design and the same number of objects and judgments.

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# BOOK REVIEWS

## INTERPLAY AMONG PRIMATES

### Social Communication among Primates

Edited by Stuart A. Altmann. Pp. xiv + 392. (Chicago and London: The University of Chicago Press, 1967.) \$15; 105s. net.

RECENT literature on primate behaviour has not included a book entirely on communication. This volume is based on the 1964 Montreal symposium, "Communication and Social Interaction in Primates", but while the title of the book has become more specific than this, the subject matter has not. Thus the sections are headed "Reproductive Behaviour", "Agonistic Behaviour", "Causal Mechanisms", "Social Dynamics" and "Communication Processes". There is, however, a well-hidden clue to this apparent discrepancy in the seventeenth chapter, "The Structure of Primate Social Communication", in which Altmann discusses the definition of communication. His solution that "social communication is a process by which the behaviour of an individual affects the behaviour of others" makes the concept a very broad one and enables him to include as communication "the influence of a wrestler on the behaviour of another, of a ballet teacher as she re-positions the body of a student . . .". If one accepts this there is no difficulty about reproductive and agonistic behaviour being communication, and indeed all actions of one animal towards another. It is more usual, however, to confine the concept to those systems where a stimulus is transmitted through the environment, and where the reception of this "signal" is advantageous to all participants, so that selection will increase the efficiency of the system by acting both on sender and recipients. In this narrower sense only a small proportion of the book is devoted to communication, although much of what is described concerns it. Unfortunately, the summarizing discussions at the ends of the sections are not helpful in putting the individual chapters into a cohesive framework.

The first two chapters concern mating behaviour. That by Jolly on the breeding synchrony of a *Lemuratta* troop presents an excellent, clearly written, quantitative assessment of changes in intra-troop relationships before and after the mating period. Social communication is cautiously suggested as the cause of synchrony, but the dangers of interpretation are emphasized in the following chapter on reproductive cycles, mainly of caged and wild baboons. In this chapter Rowell gives a salutary warning against assuming that a particular change in an animal (in this case the appearance of sexual swellings) is a signal because the behaviour of an interacting animal changes concurrently. The chapter contains another, apparently unintentional, warning about the dangers of interpretation: Rowell argues that because the caged male baboons mounted less at the end of the females' sexually swollen periods than in the middle, they were responding to some other aspect of the stimulus situation. Without, however, information about behaviour of the males when more than one female was swollen simultaneously, it is not clear whether the effect was merely one of changing responsiveness to repeated exposure to a stimulus. Further, Rowell's data clearly show for wild baboons a greater percentage of presentings responded to by mounting when the female is at least partly swollen than when not swollen, while the percentage responded to by intention mountings shows the reverse. Inexplicably, swellings are concluded to make no difference to the male's response; perhaps using the interpretative term "intention mounting" has wrongly led the author into

putting these two responses into one category. Thus although Rowell's contention may well be right, the point seems to remain unproven.

Rosenblum and I. C. Kaufman describe the early mother-infant relations of captive group-living pigtail (*Macaca nemestrina*) and bonnet (*M. radiata*) macaques. The data on changes with age of infant in the amount of, and responsibility for, mother-infant contact is interesting but restricted without further information, including interactions with other group members. Species differences are considered but there is no statistical treatment of the data.

Jensen, Bobbitt and Gordon look more closely at the mother-infant relationship in the pigtail macaque. The infants lived alone with their mothers in either a "privation" or "rich" environment (both highly deprived, judging by the illustrations). The authors attempt the important but difficult task of assessing the roles of mothers and infants in their increasing independence of each other. They omit to say, however, how "interactive locomotion" was distinguished from any other, or that because almost all locomotion must have changed the distance apart of the two animals some differences between them may have been simply a measure of their relative activity.

There are three chapters from the section dealing with agonistic behaviour, two chapters from "Social Dynamics" and one from "Communication Processes" which concern aggressive interactions either between individuals of the same social group, or between conspecific groups.

Kummer discusses interactions in *Hamadryas* baboons involving three animals simultaneously, each with a separate role. His interest lies in the development of tripartite relations of adults (male protects female from another group member) from those of infants (mother defends infant against another group member). He suggests that the one-male groups arise ontogenetically by mother-infant relationships becoming male-infant relationships, and that a male is at first maternal toward the female infants who later form his group. While it seems rash at this stage and in such a context to use labels like "maternal" which have motivational implications, this is a thoughtful and thought-provoking chapter.

J. H. Kaufmann's chapter on the social relations of adult males in a free-ranging band of rhesus monkeys contains a wealth of quantitative data. He states that the males constituted a clear hierarchy expressed through spatial displacements and the exchange of aggressive and submissive signals, yet later says "displacements were highly reliable indicators of rank" and that whereas the amount of aggressive behaviour is not a good indicator of rank "the proportion of aggressive acts to submissive ones is more obviously correlated with rank". His criterion seems therefore to be self-correlated. The importance of defining the criterion used for ranking individuals is rightly emphasized by Sade. His clearly presented chapter, also on rhesus monkeys, describes the gestures of attack and flight, shows the linear nature of the relationships, and discusses the importance of a mother's rank in determining her offspring's rank. Both these chapters are of great interest to anyone concerned with the organization of primate groups.

The late K. R. L. Hall (to whom this book is dedicated) has briefly described the behaviour of wild and captive Patas monkeys (*Erythrocebus patas*). Unfortunately, it is difficult to come to grips with this chapter because the inclusion of raw notes on interactions contrasts uncomfortably with the rather general discussion. Nevertheless the author's great enthusiasm and thoughtfulness are apparent throughout and make the chapter well worth reading.

The section on social dynamics also contains an excellent descriptive, and no doubt difficult, field study of the Aye-Aye (*Daubentonia madagascariensis*). The Petters are to be congratulated. Tsumori's interesting chapter



on Japanese macaques, including observations on newly acquired behaviour, completes the section.

The chapters in the section on causal mechanisms stand apart and indicate the sorts of investigation that can follow studies such as those already outlined. Miller discusses the use of instrumental conditioning as a simple language between the experimenter and the animal in a clear and self-critical chapter. Briefly, one of a pair of rhesus monkeys could see the stimulus but not respond, and the other, being able to see its partner but not the stimulus, could only respond. The results were indicative of communication from the former to the latter.

The chapter by Robinson is largely concerned with the quite different technique of tele-stimulation. Data are given on the production of vocalizations in rhesus monkeys, analysed in relation to brain structure. Surprisingly, although Robinson states that the sounds can be categorized into "several recognizable types" this does not seem to have been taken into account in the analysis. Perhaps the author would have found more order in his results if he had looked more closely at the behaviour of the animals than appears to have been the case.

The long and interesting chapter by Ploog is an important contribution to the literature on squirrel monkeys (*Saimiri sciureus*). It includes an attempt to relate specific brain areas to elements of social behaviour, but mostly consists of an extensive study of the genital display and a careful description of vocalizations which seem to belong elsewhere than in a section on causal mechanisms.

Finally, there are the chapters which really are about communication. Struhsaker gives an extensive catalogue of the noises of vervet monkeys (*Cercopithecus aethiops*) which is made especially valuable by careful observations of the situations in which the noises occurred in the wild. Whereas, however, coughing and sneezing are of social significance in other species, and so it may be worth saying they don't seem to be in this one, I have doubts about including vomiting in the catalogue. Indeed, in view of Altmann's statement in the next chapter that "a communicative act is specialized to the extent that its direct energetic consequences are biologically irrelevant to anything but communication", all three should surely be excluded.

Among a good deal that is puzzling in Altmann's chapter is his continued discussion of analogical versus digital systems when he has already concluded that this is a false antithesis. He continually strives to classify events, but taxonomy should be a tool and not an end in itself. It is not clear that the construction of the category "metacommunication" for messages that convey information about other messages is justified, particularly when the use of multimodal signals is well recognized. The theoretical discussion of communication is important but in this instance communication itself seems in danger of breaking down.

As a collection of papers on primates this book contains a great deal of very valuable quantitative data, and covers a wide range both of species (although omitting the apes) and of important topics. It deserves to be widely read.

YVETTE SPENCER-BOOTH

## VISUAL WORLD AND RETINAL IMAGE

### The Nature of Perceptual Adaptation

By Irvin Rock. Pp. x+289. (New York and London: Basic Books, Inc., 1966.) 45s.

PERCEPTUAL adaptation in its simpler forms—adaptation to changes in brightness or loudness of stimuli, for example—has long been familiar, and its physiological basis is beginning to be understood. The phenomenon turns up, however, in a remarkably wide range of more subtle forms, for which at present only the vaguest of speculative

explanations are available in terms of physiology. By the same token, their study may be expected in due course to supply important clues about the mechanisms of information processing that subserve higher perceptual functions. Dr Rock's book is therefore a timely contribution to a discussion which is increasingly bringing psychologists and neurophysiologists into close and fruitful collaboration.

The book is almost entirely concerned with visual perception: with the changes that take place in the appearance of the visual world as a result of a variety of distortions or transformations of the optical image. These range from the drastic inversion of the whole field by means of prisms or mirrors, to the familiar displacements of the retinal image produced by movement of the head or eyes. In each case, it is possible for the appearance of the world to change radically without any corresponding change in the retinal image, so the physical changes responsible are assumed to take place within the visual nervous system. They may on the one hand entail modifications of structure of a "plastic" type, which persist for a time after exposure and give rise to perceptible distorting after-effects; or they may reflect only the presence or absence of concomitant signals from some other part of the nervous system, which need leave no after-effect.

The great merit of Dr Rock's work lies in his clear-headed distinction between perception studied as a form of experience, and sensory-motor co-ordination studied as an aspect of behaviour. He is quite explicit in taking the first as his main concern. "The problems in perception are concerned with how things look . . . Whatever the difficulties . . . we must face the fact that this is nevertheless the subject-matter we wish to explain . . . For example, in adaptation to prismatic displacement of the image, the fundamental question, I should think, is where do objects appear to be located. Yet many workers in this field prefer to speak of the disruption of sensory-motor co-ordination by prisms . . . While it is true that proper co-ordination is often a sign of correct perceptual localization it is by no means necessarily the case" (page viii). Again: "One of the ever-present dangers in contemporary psychology is the tendency to avoid dealing with its unique problems on their own terms, either by hiding behind the skirts of a hardheaded philosophy of science or by prematurely borrowing the paraphernalia of the natural sciences. No explanation of the changes that occur in prism adaptation is going to be satisfactory if it fails to address itself to the question of why things look different following exposure to prisms . . ." (page ix).

I have given these quotations at length to indicate why this book may be rewarding reading for many outside its professional field. It will certainly invite criticism from those to whom talk of subjective experience is either behaviourally defined or (dogmatically) "meaningless"; and in its choice of phrase here and there (for example, "this essentially private world" on page viii, it may be philosophically vulnerable. But its robust insistence on facing psychological problems as such is to be welcomed as yet another sign of health in a field where extreme positions have too long held sway.

Perhaps the main technical theme of the book is that so long as an optical transformation leaves intact "the relationships of components of the image to one another and to the self as object in the field" (page 268), we ought to find it more surprising that the world appears distorted by that transformation than if it appeared normal, for "the essential information conveyed by the retinal image would seem to remain intact". The fact that the optical image is inverted by the lens of the eye, for example, is irrelevant to our ability to see the world "right way up". Rock then suggests that distortions of perception arise because "memories of the specific character of the normal retinal image . . . influence what is now seen". Here the

terminology seems unfortunate, for what is apparently meant is not an intellectual remembering and conscious making of allowances, but rather some kind of automatic and pre-conscious process of association (page 269): "specific visual memory traces (join) with the stimulus to form the underlying correlate" (that is, of visual perception). Although in certain cases (for example, the discussion of adaptation to a displaced image in the fourth chapter) this blows fresh air through a stuffy area of controversy, it has dangers as a general formula. Clearly, unless an optical transformation makes a difference to perception, the subject must be blind to the corresponding distinction. It may not matter which direction of the retinal image becomes labelled as "up"; but if the perception of "upside-down" is to be possible it is surely to be expected by any theory that an inversion of the image should at first make the world look upside down. Moreover, it is far from clear, as seems to be suggested on page 39, that only "memory traces", and not genetically determined connexions, prevent perception from being indifferent to the orientation of the entire image. The evidence from the behaviour of new-born kittens fitted with inverting prisms, cited on page 53, is at least ambivalent on this point. Conversely, it should perhaps not be taken for granted, as it seems to be on page 11 and elsewhere, that all adaptation must be followed by a corresponding negative after-effect, in terms of which it can be measured.

The value of Dr Rock's book does not hang on the resolution of such points as these. The comprehensive survey it offers of a relatively new and active field would alone earn it a welcome; and for the student in particular it has additional merit as an example of careful, painstaking analysis and integration of evidence in an area abounding in logical and philosophical pitfalls.

D. M. MACKEY

## BIOLOGICAL MACROMOLECULES

### Molecular Architecture in Cell Physiology

Edited by Teru Hayashi and A. G. Szent-Györgyi. (A Symposium held under the Auspices of the Society of General Physiologists, Sept. 8-11, 1964, Woods Hole, Mass.) Pp. viii+252. (Englewood Cliffs, N.J., and London: Prentice-Hall, 1966.) 60s.

THE editors of this symposium volume describe its aim as "an attempt to present the thinking and information available at several levels in the organized state of matter of importance to biologists". The eleven contributions vary very widely in meeting this aim. Interesting and stimulating as these papers may have appeared when presented at the symposium itself, in print they make a rather uneven collection without the coherence that might have been imposed by more ruthless editing. The defects of published symposium proceedings have recently been spelt out in *Nature* (214, 46; 1967) and this volume has its share. Above all it is late; more than two years have elapsed between conference and publication. The result is that some of the papers are quite out of date (notably that by Cantoni on sRNA) while in other fields (such as the structure of antibodies on which Edelman writes) there has been such progress that the paper, while still interesting, would now serve mainly as a report on the way.

The article by Caspar on the design of organized biological structures is a valuable statement of principles and is general enough to stand the passage of years. The price paid in this case for avoiding detailed discussion is an abstractness of presentation which might make rather difficult reading for those not already familiar with the subject, but concrete examples are to be found in the articles by Cohen on fibrous proteins and Van Holde on haemocyanin. Katchalsky gives a most illuminating dis-

cussion of the analysis of the thermodynamics of structural transitions in two systems studied by his school—collagen fibres and polynucleotide helices in solution. These examples could very well serve as useful models in discussing the dynamics of more complicated macromolecular systems.

The articles singled out above deal with molecular architecture at the level which would be of most use to a cell physiologist seeking background knowledge. The short paper by Bright Wilson, while strictly in the field of molecular architecture, deals mostly with the topic of hindered rotation about single chemical bonds and would seem very far removed from the concerns of cell physiologists. It does, however, end on a salutary note of warning that the rules gained from the study of small molecules, though useful and indispensable in the field of complex biochemical molecules, should always be applied with caution. This paper provides a nice contrast with the succeeding article by Scheraga on the principles of protein structure in which he seeks to formulate simple models for polypeptide chains and then more complicated ones for polypeptide chains with different side groups that interact with each other, as in a globular protein. The paper provides a useful guide to the large body of developing literature on the attempts to make quantitative estimates of the forces holding a protein molecule together. It also proves to be an illustration of the difficulty of the theoretical problem and perhaps also shows how far there is to go before the most probable conformation of a protein molecule in solution can be predicted.

Three further articles show a very wide variation in the level and range of this symposium. The paper by Shapiro on the elucidation of the base sequence in DNA seems out of place except in so far as it does show how unfeasible the task is at present. There is an interesting article by McElroy and Seliger on the colours of bioluminescence which describes the role of the enzyme and substrate structure, and while this article perhaps goes the furthest in the symposium towards describing the relation between function and the underlying chemical processes, little is known about the structure of biological macromolecules involved. Finally, there is a paper by Inoué and Sato, which is a more or less complete report of an optical analysis of the distribution and orientation of DNA in cricket sperm, and again, while most interesting, would seem more suitable as an ordinary scientific paper than as a part of the printed proceedings of a symposium.

A. KLUG

## PHOTOSYNTHETIC MECHANISMS

### Biochemistry of Chloroplasts

Edited by T. H. Goodwin. (Proceedings of a NATO Study Institute held at Aberystwyth, August 1965.) Vol. 1: Pp. xv+476. 115s. Vol. 2: Pp. xviii+776. 160s. (London: Academic Press, Inc. (London), Ltd.; New York: Academic Press Inc., 1967.)

THESE two volumes contain by far the most complete description so far of the biochemical aspects of the chloroplasts. Some eighty-seven papers are included in this record of the formal proceedings of a NATO Study Institute held at Aberystwyth in 1965. Although the first volume is primarily concerned with structure and chemical composition and the second volume with biosynthesis, there is much overlap. Anyone who needs one volume is likely to require both.

In his introduction, the editor hopes that these volumes "will serve as a basic text for some years for recruits into the field of chloroplast biochemistry". Certainly these proceedings will be most useful to both recruits and veterans, but there are some limitations inherent in this type of collection of individual papers from a meeting. Early obsolescence is one problem, particularly when the

work is in a very active field and appears two years after the oral presentation. For example, achievement of complete photosynthesis (assimilation of carbon dioxide with light energy only) by isolated chloroplasts and at rates comparable to *in vivo* photosynthesis has taken place since the Aberystwyth meeting. Thus the discussions in the first part of the second volume of carbon dioxide assimilation rates which were between 1 and 10 per cent of *in vivo* rates are somewhat dated. Another problem is that the writing is uneven, and the degrees of factuality versus speculation are extremely varied. This variation may be desirable at a meeting, where a principal objective is to stimulate discussion and criticism. Unfortunately, the reader who doesn't attend cannot be a party to these discussions, most of which were apparently carried on in private. The number and length of printed discussions at the end of papers in these books are sparse indeed; there is no discussion at the end of most papers.

The first and second parts of the first volume, on the structure of chloroplasts and chloroplast membranes, contain some excellent electron micrographs, together with interpretations and highly speculative models of the structure down to the level of individual models. There are important differences among the models of chloroplast structure given by Drs Muhlenthaler, Menke, Weier and Benson, and others. Of particular interest are the role and composition of the sub-unit particles seen by all investigators by freeze-etching and staining techniques, and the nature of the binding of sub-units into sheets. A reasonably extended discussion of these interpretations would have been in order. Much useful factual information about the chemical composition of the chloroplasts, lipids, proteins, lipoproteins, nucleic acids and pigments is provided in the balance of the first volume.

The section on nucleic acids, together with the biosynthesis of proteins and nucleic acids from the second volume, provides a most interesting picture of the "autonomy" of chloroplasts within the green cell. One aspect of this is nicely illustrated by a short paper by Dr Gibor. Cells of *Acetabularia* from which the nuclei were removed were grown on  $^{14}\text{CO}_2$ . Subsequent analysis showed that these nuclei-free cells had synthesized DNA labelled with carbon-14.

Dr Wildman provides an extensive discussion of the organization of grana-containing chloroplasts with respect to systems involved in photosynthesis, protein synthesis and RNA synthesis. His proposal that mitochondria-like particles that separate from protuberances of "mobile phase" of the chloroplasts by segmentation are actually mitochondria which have previously fused with the chloroplast should have aroused some discussion, but none is reported.

Other parts of the second volume deal with biosyntheses of carbohydrates, lipids and pigments. These sections contain many excellent factual articles, such as those on lipid metabolism by Bloch and his colleagues, on fatty acids in higher plants by Stumpf, and on the heme and chlorophyll biosynthetic chain by Granick. The fourth part is long and ostensibly concerns photosynthetic phosphorylation, but is actually something of a catchall for electron transport, photophosphorylation, and other aspects of photosynthesis. Much of this is to be found in the proceedings of other photosynthesis meetings.

The second volume returns to structure in the final chapter with a discussion of the relation of biosynthetic mechanism to morphogenesis. As Bogorad points out, although the sequence of structural changes occurring during the development of plastids is well established, the order of biochemical events is still very uncertain. Examples from one promising line of investigation employing biochemical mutants are described by Waller. Other papers deal with more specific structural and chemical aspects of development.

Each volume contains an extensive author and subject index, and the general quality of figures, including electron micrograph prints, is excellent. Any laboratory engaged in research on the biochemistry of green plants will need both volumes.

J. A. BASSHAM

## ZOOLOGICAL NEWS

### International Zoo Yearbook

Vol. 7. Edited by Caroline Jarvis. Assisted by Ruth Biegler. Pp. viii + 410 + 61 plates. (London: The Zoological Society of London, 1967.) 126s.

THE Zoological Society of London took a long chance in 1960 when it started the *International Zoo Yearbook*, but the new publication soon got on to its feet, and is now firmly established as a reference book and as a cross-fertilizer between zoos and zoologists.

The seventh volume of the yearbook opens with a section of nineteen papers on penguins in captivity. Penguins in zoos have great popular appeal from their quaint appearance and behaviour rather than their zoological interest. They are difficult to introduce into captivity; the majority of those captured in the wild die before, or soon after, reaching a zoo, but they are paradoxical in that once established the survivors may live for years. Many die of general stress, starvation or thirst on their journey, but the chief killer of newly arrived birds is aspergillosis for which no cure has yet been found. The birds are very susceptible to infection by moulds, and those that survive appear to develop some sort of immunity or resistance through suffering a non-fatal attack. The papers cover the subject thoroughly, and discuss the advantages and drawbacks of internal air-conditioned exhibits compared with outdoor ones, diet, general management, and breeding. Together they form a valuable guide to the care of these birds in zoos.

The second section describes new buildings and exhibits in various zoos of the world—the trend towards protecting indoor exhibits from the public by glass partitions, and outdoor ones by ditches, continues to bring nearer the ideal zoo with only one bar. The display of mixed species of African large mammals at Borås Zoo in Sweden is particularly striking. The largest part of this section, however, consists of papers on the breeding of various species in captivity, mostly during 1965. It presents a remarkable collection of information which contains much of importance not only to the keepers of zoos but to zoologists in general—reports on breeding behaviour, gestation periods, parturition, parental care and other matters. There is a long list of animals that have rarely, or never before, been bred in captivity, from several primates to kiwis and tuataras. Were it not for the yearbook much of this information would go unpublished or would be so scattered in publication that its retrieval would be difficult.

Several articles on conservation and education are included; it is interesting to see that the larger zoos are concentrating more and more on organized educational tours with preliminary lectures, so that a visit to the zoo is directed away from being a casual inspection of queer creatures towards something of permanent value.

The final part of the second section on husbandry and research, like that on breeding, contains much varied and valuable information, including three articles on the dugong in the wild and in captivity. Veterinary matters cover a wide field, from treating paralysed flamingoes to masturbating a rhinoceros into a funnel. Most of the articles here as elsewhere in the yearbook are of high standard, but a few of the shorter anecdotal ones show a lack of knowledge of the literature.

The reference section contains a list of the zoos and aquaria of the world, with particulars of their contents,

staffing, finance and so on, where ascertainable. Interesting lists of animals bred in zoos during 1965, and of zoological and veterinary research recently published or still in progress, also appear. A census of rare animals in the zoos of the world during 1966 is given so that a co-ordinated international breeding policy for rare animals could be drawn up, and inter-zoo matings or exchanges arranged. The criterion of rarity is the list of animals that are rare, or presumed to be rare, drawn up by the International Union for the Conservation of Nature. The recent attempt to breed from the giant pandas now in London and Moscow, which received such wide publicity, shows the action that the census aims at promoting. Stud books for eleven species of rare animals are now established—for some species, such as the wild horse and the wisent, zoos have reversed their usual role as consumers of rare animals and become producers.

The book ends with several appendices, including a dunce's corner in which is posted a list of the zoos that failed to answer questionnaires, or sent no information for the list of rarities. The volume is well produced, and is illustrated with excellent photographs, line drawings and diagrams. Misprints are few, and, although articles from so many contributors vary in quality, the standard required by the Yearbook Advisory Committee is well maintained.

L. HARRISON MATTHEWS

## ADAPTIVE CONTROL

### Theory of Self-Adaptive Control Systems

Edited by P. H. Hammond. (Proceedings of the Second IFAC Symposium, September 14–17, 1965, National Physical Laboratory, Teddington, England.) Pp. xii + 363. (Pittsburgh: Instrument Society of America, 1966. Distributed by Plenum Press, New York.) \$15.

"ADAPTIVE" is a word with different meanings for different people. During the past decade its use has become widespread among control engineers and many papers about adaptive control systems have appeared in the engineering literature. The proceedings of the 1965 Symposium on the "Theory of Self-Adaptive Control Systems" organized by the control engineers' main international organization, the International Federation of Automatic Control (IFAC), contains a fair sample of the types of problem that control engineers have been describing as adaptive.

The original motivation for engineers to become interested in adaptive systems was the need to solve practical problems that were beyond the scope of classical design procedures. Examples of such problems are the design of safe control systems for very high performance military aircraft and the control, for maximum yield or maximum profit, of highly complex industrial chemical processes. These are problems where some piece of information that is required by the classical design procedure is missing, either because of prior uncertainty or because of unpredictable changes in the environment. The idea was that classical controllers might nevertheless be used if they were made with variable parameters that could automatically be adjusted by a signal based on some measurement devised to compensate for the missing information. Various schemes of measurement and compensation have been proposed and two well-defined problems that have emerged are the problem of identifying the dynamics of an unknown system or "black box" and the problem of extremum control or "hill-climbing". These topics account for about half of the thirty-seven papers in the symposium proceedings.

When they were first proposed it seemed obvious that such systems, where a conventional controller is automatically adjusted so as to improve its own performance, should be classified as adaptive. It was soon pointed out, however, that the instrumentation for automatically adjusting the controller was merely an additional feed-

back loop, and that systems described as adaptive and new in principle could equally well be described as multi-loop nonlinear systems that were in principle no different from, although in practice more complex than, conventional feedback control systems. There was considerable controversy about whether the word adaptive could have any specific meaning for control engineers; one suggestion was that a control system was adaptive if its designer regarded it as being adaptive.

The interest of control engineers in adaptive systems was matched by a corresponding interest of applied mathematicians concerned with the mathematical theory of optimal control. The mathematical theory is not always directly applicable to practical engineering problems, but it does provide a basis for defining and classifying different types of control problem. In this context the name adaptive is reserved for the class of control problems where there is some prior uncertainty that can, with advantage, be reduced by observing the performance of the system as it operates: an adaptive system is thus a learning system. Only two papers at the symposium dealt with this well-defined class of adaptive control problems.

One of the attractions of the field of adaptive control systems is the possibility it offers for collaboration between control engineers and biologists. Engineers can offer techniques for analysing and synthesizing adaptive systems; biologists can describe adaptive systems that are more complex, more efficient and more reliable than any known to engineering. There is a section in the proceedings of five papers on adaptive control in biological systems.

Other papers in the proceedings deal with problems that are related to adaptive control problems. It is clear that there is much research in progress under the title of "Adaptive Control" and that this covers a wide range of related problems. These proceedings show that the IFAC symposium was successful in bringing together a variety of these problems and that there is a continuing need for good understanding of the relationships between them.

O. L. R. JACOBS

## PROGRESS OF GAS DYNAMICS

### Rarefied Gas Dynamics

Edited by C. L. Brundin. (Proceedings of the Fifth International Symposium held at the University of Oxford, 1966. *Advances in Applied Mechanics Supplement 4.*) Vol. 1. Pp. xx + 879. \$18.50. Vol. 2. Pp. xx + 881–1731. 148s. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1967.)

THESE two volumes are made up of the written versions of 103 papers read at the Fifth International Symposium on Rarefied Gas Dynamics; all the papers which were read are represented.

The range of topics covered by such a large number of papers must clearly be very wide, so that the two volumes are organized into five sections, four of which commence with a review paper, written by an eminent contributor to the specialized field in question, and designed to give perspective to the research papers which follow. Divisions between sections (and sub-sections) are not rigid and several papers may as well be in one place as another. This fact is representative of the thread of unity which runs through this very interesting and flourishing branch of modern gas dynamics.

The first section, introduced by F. C. Hurlbut, is devoted to an examination of gas-surface interactions. The subject has long been studied from the experimental point of view, especially to obtain information on energy and momentum accommodation coefficients. The need for a deeper understanding of the complexities of the interaction represented by these coefficients has lately led to the development of theoretical models. Both



classical and quantum theories have been used and both will be found among the papers in the first volume together with the latest experimental information.

The second section, on kinetic theory, begins with a concise account of the mathematical status of the Boltzmann equation by J.-P. Guiraud. The mathematical implications of using a simplified model like the linear Boltzmann equation, the Krook equation and the Mott-Smith approach to strong-shock structure are discussed, together with a brief account of applications of the theory. The next nearly six hundred pages bear witness to the continuing activity in this central field of rarefied gas flow research. Topics dealt with include heat transfer, sound propagation, plane-shock-wave structure and free-jet expansions, and it is interesting to speculate on how many more variations can be wrung from the themes originating with Rayleigh, Poiseuille and the anonymous inventors of rotating cylinders, parallel-plate heat-cells and so on. The analytical use of Monte-Carlo methods in this section is notable. Also noteworthy are the theories of free-jet expansion (supported by good experimental evidence) which indicate the directional character of temperature in certain rarefied-gas conditions.

The third section, and the first in the second volume, deals with (so-called) transition-flows. This very difficult area between the continuum and kinetic theory descriptions of gas flow continues to stimulate a large amount of research. The survey article for this section is by J. L. Potter, and includes, among many other things, some interesting remarks on the subject of slender lifting shapes at very high altitudes. There is evidence in the papers presented that new theories are necessary to account for observations of flows near to the leading edge of sharp flat plates in rarefied hypersonic streams. Much new experimental work on this topic is contained in the papers presented at the symposium.

The section devoted to experimental techniques is comparatively short and has no introductory article. Four papers are devoted to molecular beam developments and six more to progress in other areas. The latter includes the use of a laser for direct determination of molecular velocity distribution functions and the laboratory (wind-tunnel) simulation of the radiation resulting from the release of nitric oxide at high altitudes. The latter has yielded an improved rate constant for the chemiluminescent reaction of nitric oxide with atomic oxygen.

The second volume concludes with a total of ten papers on ionospheric aerodynamics (introduction by J. H. de Leeuw) covering, in particular, basic studies of rarefied plasma and plasma-vehicle interactions.

The editor remarks, in the preface, that the published proceedings of previous symposia in the series have become recognized as reference texts. His hope that the current volumes would continue the tradition must be fulfilled, for it would be impossible to ignore these volumes, not least for their very high quality. While reaffirming this view it may be permissible to express some mild misgivings about the manner in which the material is presented. Both volumes reproduce the papers in ordinary typescript, as submitted by the authors, with frequent rendering of mathematical symbols in manuscript. The variety of type faces, allied to the individuality of the authors' hands, does nothing to increase the atmosphere of either unity or legibility in the books. Most papers carry the diagrams and figures at their end, report-style. The result is constant page-turning. Finding the way about the 1,700-odd pages is not aided by the complete absence of any running heads, save, that is, for the pointless repetition of "Rarefied Gas Dynamics". We are told that the mode of presentation was chosen to expedite publication, and no doubt much time has indeed been saved. It could not, however, have taken very much longer for each author to add (say) his name and/or a short title to each typescript sheet with sub-

sequent benefits for the reader navigating his way through the many pages. No account of discussions following presentation of the papers is given (except in two cases) and the editor remarks that it was found that most verbal questions were answered anyway in the written papers; there must be a moral here somewhere.

In conclusion, apart from the criticisms of presentation just voiced, it must be said that this biennial progress report on research into rarefied gas dynamics will surely be included on the (library) shelves of everyone with an interest in the topic.

JOHN F. CLARKE

## TOWARDS A SCIENCE OF MATTER

### The Origins of Chemistry

By Robert P. Multhauf. (Oldbourne History of Science Library.) Pp. 412+9 plates. (London: Oldbourne Book Co., Ltd., 1966.) 70s. net.

No area of the history of science shows more interesting changes at present than does the history of chemistry, as regards both the accumulation of new evidence and the reconsideration of interpretations. For example, we are radically revising our ideas about the role of atomic theory in mid-nineteenth century chemistry; the Industrial Revolution now looks as much chemical as mechanical in inventiveness; the development of European society over long periods becomes more intelligible as more of the influences of chemical thought and production are detected, and so on. As historical detail is increased it becomes important that the surveys of large problems and long periods should be undertaken afresh, so that we can have alternatives to those basic books of the previous generation on which we relied for instruction and reference for so long. Dr Multhauf, director of the Museum of History and Technology in the Smithsonian Institution, has made a new review of the antecedents of chemical science, taking the mid-eighteenth century as his terminus, in a book which can scarcely fail to be widely read and much used.

The book's value is best seen in a comparison of its title with that of a work with which, as Multhauf himself observes, it most nearly invites comparison, Stillman's *The Story of Early Chemistry*, which was conceived forty years ago. Stillman tried to offer a historical narrative; Multhauf properly and wisely offers an enquiry, in the course of which he informs and, every now and again, soberly entertains.

Multhauf's method is different from both Stillman's and Partington's. Stillman was as conscious as a chemical Macaulay of his duty to narrate. Partington, ever suspicious of speculation, insisted so much on the recording of every fact that all sense of movement is lost in the mass of unconsidered, if carefully verified, trifles. Multhauf examines the evidence in a way which will teach the new student how difficult is the art of writing history and how well it can be done by those who care to learn.

Chemistry to Multhauf is one aspect of a science of matter, "an incongruous edifice, a structure erected by independent schools of architecture—philosophers, occultists and chemists—each working largely independent of the others". He sees three main constituents in early chemistry, which have, unfortunately for our understanding, been examined somewhat in isolation. The philosopher finds evidence of theories of ontology, the occultist admires the labyrinthine wisdom of the alchemists, the modern chemist "sees a kind of technological sub-basement from which his illustrious ancestor, Antoine Lavoisier, was finally to emerge". Multhauf attempts to bring these together from the point of view of a historian of chemistry sympathetic to the history of the other two subjects. He claims no new work on manuscripts. Although there is a vast amount of unexamined, unedited material in the great libraries he considers it probable

that enough material for a broad estimate of classical and mediaeval chemistry and alchemy has already reached print. Multhauf does, however, emphasize the work of some people who, in his opinion, have been neglected in the three important stages of the genesis of Greek, Arabic and Latin alchemy, namely, Agathodaimon, Balinus and Ibn Juljul.

Multhauf does not waste space on summaries of well-known works. For example, his account of Geber concentrates on its style and its relation to other works, so that the reader who wants to know a fair amount of what is in the *Summa perfectionis* will have to turn back to Stillman and others. But the reader who reads the *Summa perfectionis* itself will find Multhauf's few pages an excellent stimulus to making his own analysis of its contents.

The same help to understanding of the original works occurs again and again. In the chapter on medical chemistry: "The history of the chemistry of mercury again raises the question of the sense in which we are to suppose that substances were 'known'. By heating mercury in air Geber tells us that he 'coagulates' it, and in the thermal decomposition of the nitrate he 'prepares it most red'. The author of the *De alumnibus* explains similarly the reaction which led him to corrosive sublimate. We can be reasonably confident that we know what was made in these processes, but can we say that the substances were *known* to their 'discoverers'." The ideas of a substance or a pure substance or a chemical species were all to evolve very slowly, to reach certainty only long after the period of this book (and to dissolve again into uncertainty in our own time).

The chapter on which the book hinges has a title which is very congenial to those followers of Collingwood who want to recover the personal experience in past events: "The Rise of the Chemist". Chemistry is what chemists do, and Multhauf emphasizes this in his chapter on the practice of chemistry by individuals and groups in the century before the genesis of scientific chemistry as we know it.

The economic view must also be presented, and Multhauf does this under the two main heads of "Metals" and "Heavy Chemicals". It is a pity that he does not carry his discussion of metals further into the eighteenth century but, because there is no obvious stopping place after Cramer or Réaumur, it is hard to suggest how else this chapter could have been written. If I felt tempted to say the same sort of thing about the chapter on heavy chemicals, it would be because I forgot that the author claims only to discuss "origins". The heavy chemical industry of revolutionary France is a fascinating subject, but it rested on a long past, and it is for this kind of event, which is still so little appreciated, that Multhauf sets out to prepare us.

Multhauf refers in his preface to the increasing professionalization of the study of the history of science. This is a professional's book for professionals, but like so many of the best books of which this can be said, it will bring great pleasure to anyone, scientist or not by occupation, who can claim to be scientifically educated.

FRANK GREENAWAY

## THEORY OF ADHESION

*Treatise on Adhesion and Adhesives*

Edited by Robert L. Patrick. Vol. 1: Theory. Pp. xi + 476. (London: Edward Arnold (Publishers) Ltd.; New York: Marcel Dekker, Inc., 1967.) 175s. net.

IN a broad appreciation of its meaning, the story of adhesives and adhesion has its origin deep in the veils of time. It is clear both by inference as well as by example that man was engaged in its practice long before recorded history. For his many purposes we may presume that he was moderately content with what he believed possible

and with the success achieved and we may also conclude that his materials and techniques assumed horizons which only slowly extended over many thousands of years. During the present century, however, there has been a dramatic upsurge of interest in the subject, as, of course, there has with many others. The nature of adhesives has now been recognized and consequently many new adhesives have appeared; the role of the interface, the character of material defects and many other aspects are beginning to reveal themselves. This coming of age has drawn on and will continue to draw on a wealth of scientific and technological advances so that what was once often regarded as a black art is now beginning to flourish as a scientific discipline. Indeed, I for one would not be surprised if, in the challenge of this technological age, those academic institutions which offer some specialization within their curricula might very well take it upon themselves to include subjects such as adhesion, combining as they do so many experiences at an advanced level. In this way it could promote a native stimulus, other than the examination system, so desirable for a student preparing himself for the great unknown.

The assembly of hitherto specialized knowledge under one cover has meant an increasing trend towards the edited textbook and the *Treatise on Adhesion and Adhesives* is no exception. The present volume is the first of three on the subject under the general editorship of Dr R. L. Patrick. The second volume, to be published in 1968, will be concerned with materials, including adhesives themselves with special topics such as fibre, high temperature and glass-resin adhesion, as well as with brazing, welding and so on. The third volume is to be restricted to applications. The first volume, which relates to theory, opens with a general introduction followed by chapters on intermolecular and interatomic forces, by R. J. Good, adsorption of polymers, by R. R. Stromberg, the mechanisms of adhesion, by J. R. Huntsberger, role of bulk properties of the adhesive, by Turner Alfrey, jun., rheology of polymers used as adhesives, by D. H. Kaelble, fracture mechanics applied to adhesive systems, by G. R. Irwin, variables and interpretation of some destructive cohesion and adhesion tests, by J. L. Gardon, and surface chemistry, by F. M. Fowkes. The author and subject indices are preceded by a list of symbols used in the text.

It would appear that the editor has given his authors a fair amount of licence and yet despite his inference of "angry young men" no suggestion of prejudice or subtle irresponsibility is dominant or even apparent. To a certain extent, as in any textbook, much of the subject matter is available in separate publications and no doubt, because of this, some readers will find some imbalance in the length of chapters. Again, it may cause some surprise that in a book which speaks easily of stress tensors in one chapter, there is a need to define the imaginary unit in another. Others may regard with gloom the dismissal of non-destructive testing. But these are minor criticisms and the editor and authors are to be congratulated on a most interesting and stimulating production, despite some similarity both in title and perhaps less in treatment with another recent publication. The presentation and style are good, diagrams and tables are adequate, and with the exception of one authoritative chapter, references are ample and surprisingly, for a collated textbook, up to date. In view of occasional but slight duplication, I feel that the internal reference system might have been improved but even this works to advantage in promoting a system of self contained chapters. The book will commend itself primarily to physicists, physical chemists and materials scientists as well as new and established researchers in the field of adhesion itself, and although the price, which is perhaps partly a reflexion of the publishers' well placed confidence, will preclude much personal ownership in this country, it will, as it should, find its way onto many bookshelves, and, I hope, into many more laboratories.

R. P. SHELDON

## University News:

**Bradford**  
DR M. G. MYLROI, at present division instrument manager of ICI Fibres, Ltd., has been appointed to the chair of control engineering.

## Newcastle upon Tyne

DR E. J. FIELD has been appointed to a personal chair in experimental neuro-pathology.

## Sheffield

THE following appointments have been made: Mr H. Nicholson, at present lecturer in the Department of Engineering in the University of Cambridge, to the chair of control engineering in the Department of Electronic and Electrical Engineering; Dr F. A. Benson, at present reader in electronics in the university, to the chair of electronic and electrical engineering; Dr B. B. Argent, at present reader in metallurgy in the university, to the newly established third chair of metallurgy; Dr D. S. Munro, at present reader in clinical endocrinology in the university, to a personal chair of clinical endocrinology in the Department of Pharmacology and Therapeutics.

## Appointments

DR A. KELLY has been appointed superintendent of the National Physical Laboratory's newly organized Division of Inorganic and Metallic Structures.

FREDERIC M. PHILIPS has been appointed director of the Office of Public Affairs of the Smithsonian Institution.

THE following appointments have been made in the Office of Scientific Personnel of the US National Research Council: Dr W. C. Kelly has been appointed director of the office in succession to Dr M. H. Trytten; J. C. Boyce will continue to have the responsibility of deputy director and has been given the additional title of director of associateships; Dr E. W. Scott has been appointed director of special international programmes in succession to S. S. Steinberg.

**CORRIGENDUM.** In Table 1 of the article "Transplantable Mouse Neoplasm Control by Neutron Capture Therapy", by Lee E. Farr and T. Konikowski (*Nature*, 215, 550; 1967), the heading of the first column should read "Neutron flux  $\text{cm}^2 \times 10^{12}$ ".

**ERRATUM.** In the communication entitled "Photochemical Oxidation of Neptunium(V) by Xenon Trioxide" (*Nature*, 215, 732; 1967) the first sentence of the second paragraph

should read: "The neptunium(V) was prepared by solution of neptunium dioxide in concentrated perchloric acid and reduction of the resulting neptunium(VI) to neptunium(V) with hydrogen peroxide". The third sentence of the same paragraph should read: "The ionic strength was maintained constant at 2 molar with lithium perchlorate which was recrystallized six times before use". Reference 2 should be to Koch and Williamson, not Koch and Williams, and reference 4 should be to Cleveland, J. M., *Inorg. Chem.*, 6, 1302 (1967). The spectrophotometer used was GE No. PH/18A/T10P-6V (CPR).

# CORRESPONDENCE

## Voyager Experiments

SIR,—In 1973 the National Aeronautics and Space Administration proposes to conduct the first of the Voyager series of missions to Mars, using the Saturn launch vehicle. We wish to call the attention of the scientific community to the opportunity of proposing experiments for this initial flight.

The primary objectives of the 1973 Voyager Mars mission are to obtain information relevant to the existence and nature of extraterrestrial life; the atmospheric, surface and body characteristics of the planet; and the planetary environment. Orbital reconnaissance, direct measurements during atmospheric entry, and investigations after soft-landing on the Martian surface are part of the mission.

As you may know, funding for the Voyager Program is under formal consideration at the present time by the US Congress, and final funding authority has not yet been obtained for proceeding with Voyager in Fiscal Year 1968. Regardless of the level of funding support obtained for Voyager this fiscal year, it is our plan to begin evaluating scientific proposals on November 1, 1967, and to select scientists for participation in the planning and development steps by February 1968.

Approximately 5,000 copies of the formal announcement have been mailed to scientists on August 9, 1967. If others are interested, additional copies may be obtained from Dr Robert F. Fellows, Code SY, Voyager Program Scientist, NASA Headquarters, Washington, D.C. 20546.

Yours faithfully,

HOMER E. NEWELL

Associate Administrator for Space Science and Applications.

# THE NIGHT SKY IN SEPTEMBER

All times are in Universal Time

		MOON		CONJUNCTIONS WITH THE MOON			
		New Moon	4d 12h	Venus	30d 21h, 10° S.		
		Full Moon	18d 17h	Mars	9d 21h, 1° N.		
				Jupiter	3d 00h, 4° S.		
				Saturn	30d 20h, 4° S.		
					20d 00h, 1° S.		
PLANETS							
Times of rising ( <i>R</i> ) and setting ( <i>S</i> ) during the month							
Name	<i>R/S</i>	Beginning	Middle	End	Mag.	<i>D<sub>g</sub></i> (10 <sup>6</sup> miles)	Zodiacal position
Mercury		Unfavourable for observation			—	121	Virgo
Venus	<i>R</i>	Unfavourable	3h 50m	2h 45m	− 4.0	30	Sextans
Mars	<i>S</i>	21h 00m	20h 25m	20h 00m	+ 0.8	127	Scorpius
Jupiter	<i>R</i>	3h 30m	2h 50m	2h 10m	− 1.3	577	Leo
Saturn	<i>R</i>	19h 55m	19h 00m	17h 55m	+ 0.6	790	Cetus
<i>D<sub>g</sub></i> is the distance of planet from the Earth on the 15th of the month.							
OCCULTATIONS OF STARS BRIGHTER THAN MAGNITUDE +6 AT GREENWICH							
Star		<i>R/D</i>		Time		Mag.	
ϕ Ari		<i>R</i>		23d 01h 15-3m		+ 5.6	
133B. Tau		<i>R</i>		24d 04h 08-2m		+ 5.9	
(D, disappearance; R, reappearance)							

## OTHER PHENOMENA

4d 00h, Venus 10° S. of Regulus.  
9d 21h, Mars occulted by the Moon, visible in Antarctica.  
10d 12h, Antares occulted by the Moon, visible N. Asia.  
20d 00h, Saturn occulted by the Moon, visible Greenland, Iceland.  
23d 07h, Mars 3° N. of Antares.  
23d 18h, Equinox.

## APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

**SENIOR LECTURER/LECTURER** (with a degree in psychology and a main interest in experimental psychology) in the **APPLIED PSYCHOLOGY DEPARTMENT**—The Staff Officer (ref. 582/2), The University of Aston in Birmingham, Gosta Green, Birmingham, 4 (August 24).

**ASSISTANT LECTURER or LECTURER in SOCIAL ADMINISTRATION** in the **DEPARTMENT OF SOCIOLOGY**—The Secretary, The University, Aberdeen (August 26).

**LECTURER** (with good academic qualifications and preferably some teaching experience) in **EDUCATIONAL PSYCHOLOGY/PHILOSOPHY** in the Institute of Education—The Registrar, The University of Keele, Keele, Staffordshire (August 26).

**RADIATION OFFICER** (graduate preferably with a knowledge of radiation protection and electronics) in the **DEPARTMENT OF PHYSICS**—The Registrar, The University of Manchester, Manchester, 13, quoting Ref. 120/67/Na (August 26).

**RESEARCH ASSISTANT** (preferably with an interest or training in electrophysiology, statistical methods or biochemical techniques) in the **DEPARTMENT OF ZOOLOGY** to work with Dr D. M. Guthrie on the physiology of invertebrate nervous systems—The Secretary, The University, Aberdeen (August 26).

**TEMPORARY DEMONSTRATOR/ASSISTANT LECTURER or LECTURER** (preferably with an interest in plant physiology or plant biochemistry) in **BOTANY** at the Hartley Botanical Laboratories—The Registrar, The University of Liverpool, Liverpool, 3, quoting Ref. RV/120/N (August 26).

**SENIOR GRADE PHYSICIST** (with hospital physics experience and preferably experience in therapeutic and diagnostic uses of unsealed isotopes)—Dr H. Miller, Chief Physicist, Regional Medical Physics Department, 21 Claremont Crescent, Sheffield, 10 (August 29).

**CHAIR OF INDUSTRIAL ENGINEERING**—The Assistant Registrar, The College of Aeronautics, Cranfield, Bedford (August 31).

**LECTURER in PHYSICS** to pursue research in experimental high energy physics—The Registrar, University of Manchester, Manchester, 13, quoting Ref. 119/67/Na (August 31).

**LECTURER, SENIOR LECTURER or READER** in the **DEPARTMENT OF SOCIAL AND PREVENTIVE MEDICINE**—The Registrar, The University of Manchester, Manchester, 13, quoting Ref. 125/67 (August 31).

**RESEARCH ASSISTANT** (with a good honours degree and interests in plant ecology, genetics or mineral nutrition) in the **SCHOOL OF PLANT ECOLOGY**, to investigate the use of plant populations tolerant to toxic metals for the reclamation of toxic mine waste—The Registrar, University College of North Wales, Bangor, North Wales (August 31).

**SENIOR LECTURER in the DEPARTMENT OF SOCIAL AND PREVENTIVE MEDICINE**—The Registrar, The University of Manchester, Manchester, 13, quoting Ref. 126/67 (August 31).

**LECTURER** (preferably with a first degree and postgraduate training to Ph.D. level in plant physiology, biochemistry or biophysics) in **PLANT PHYSIOLOGY** in the **DEPARTMENT OF PHYSIOLOGY AND ENVIRONMENTAL STUDIES**—The Registrar, The University, Nottingham (September 1).

**RESEARCH TECHNICIAN** (with experience in histology) in the **UNIVERSITY DEPARTMENT OF ORTHOPAEDIC SURGERY** to work on histo-biochemistry of bone and joint tissues with special interest in Paget's disease—The Secretary, Board of Governors, United Manchester Hospitals, Manchester, 13 (September 2).

**ASSISTANT LECTURER or LECTURER** (with particular interests in economic geology or in fields related to engineering geology) in **APPLIED GEOLOGY**—The Registrar, University of Sheffield, Sheffield (September 4).

**ASSISTANT LECTURER** (preferably wishing to develop an interest in either industrial microbiology, electron microscopy, microbial genetics, or protozoology) in **MICROBIOLOGY** in the **DEPARTMENT OF BIOLOGICAL SCIENCE**—The Academic Registrar (LFG), University of Surrey, Battersea Park Road, London, S.W.11 (September 4).

**ASSOCIATE PROFESSOR OF PSYCHOLOGY** at Macquarie University—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1; or The Registrar, Macquarie University, Eastwood, New South Wales, Australia (Australia and London, September 4).

**LECTURER or ASSISTANT LECTURER** in the **DEPARTMENT OF ANATOMY**, University of Singapore—The Inter-University Council, 33 Bedford Place, London, W.C.1 (September 7).

**BIOCHEMIST**, initially Scientific Officer Grade, in the **PHYSIOLOGY AND BIOCHEMISTRY DEPARTMENT** to join a group engaged in fundamental research into the metabolism of normal and diseased poultry—The Secretary, Houghton Poultry Research Station, Houghton, Huntingdon (September 8).

**SCIENTIFIC OFFICER** (with a good honours degree and postgraduate research experience) at Moor House Field Station for studies on primary production and nutrient circulation in blanket bog—Dr O. W. Heal, Merlewood Research Station, Natural Environment Research Council, Grange-over-Sands, Lancashire (September 8).

**SENIOR LECTURER or LECTURER in BIOCHEMISTRY** in the **DEPARTMENT OF BIOLOGY AND CELL SCIENCE**—The Clerk to the Governors, Woolwich Polytechnic, London, S.E.18 (September 8).

**TEMPORARY LECTURER in CLINICAL PSYCHOLOGY** at the University of Western Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 10).

**LABORATORY SUPERINTENDENT**—The Secretary, School of Agriculture (University of Nottingham), Sutton Bonington, Loughborough (September 15).

**LECTURER in GEOLOGY and GEOGRAPHY** at the University of Natal, Pietermaritzburg—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, September 15).

**RESEARCH ASSISTANT in the DEPARTMENT of BOTANY** to work under the direction of Professor K. Wilson on algal cell-wall structure and growth—The Secretary, Royal Holloway College (University of London), Englefield Green, Surrey (September 15).

**LECTURER** (with a higher degree, preferably a Ph.D. in psychology and measurement in education, or in educational psychology) in **EDUCATIONAL PSYCHOLOGY** at the University College of Townsville, University of Queensland—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 18).

**CHIEF TECHNICIAN or SENIOR TECHNICIAN** (with H.N.C., Final City and Guilds, A.T.M.L.T., F.I.M.L.T., or equivalent qualification) in **HUMAN BIOLOGY**, University of Zambia—The Inter-University Council, 33 Bedford Place, London, W.C.1 (September 20).

**LECTURER** (graduate in pharmacy and preferably holding the degree of Ph.D.) in **PHARMACY** at the University of Queensland, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, September 22).

**SENIOR TUTOR-DEMONSTRATOR in GEOGRAPHY** at the University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 22).

**CHAIR OF PHYSIOLOGY** at the University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 29).

**LECTURER in PHILOSOPHY**; and a Lecturer (qualified in economic, urban or social geography, and preferably an interest in the application of quantitative techniques in geography) in **GEOGRAPHY** at Flinders University of South Australia—The Secretary-General, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1; or The Registrar, Flinders University of South Australia, Bedford Park, South Australia (September 29).

**LECTURER in PHYSICS** at Monash University—The Academic Registrar, Monash University, Clayton, Victoria, Australia; or the Secretary-General, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (September 29).

**POSTDOCTORAL FELLOW** (with a Ph.D. or equivalent degree in physical chemistry or chemical engineering) in **CHEMICAL ENGINEERING** at the University of Canterbury, Christchurch, New Zealand, to carry out full-time research on the thermodynamics of gaseous and liquid mixtures—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (September 30).

**SENIOR LECTURER/LECTURER** (preferably with qualifications in economics including agricultural economics and forestry) in **FOREST ECONOMICS AND FOREST MANAGEMENT**, University of Melbourne—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 30).

**HEAD** (of professional standing or its equivalent, and high veterinary, medical or biological qualifications) of the **ANIMAL DIVISION**—Sir Peter Medawar, F.R.S., National Institute for Medical Research, Mill Hill, London, N.W.7 (October 2).

**CHAIR OF MATHEMATICAL PHYSICS** (preference will be given to candidates whose work is in the field of theoretical quantum physics)—The Registrar, University Registry, The Old Schools, Cambridge (October 21).

**ASSISTANT PROFESSOR or ASSOCIATE PROFESSOR** (interested in cardiovascular pharmacology or biochemical pharmacology) in the **DEPARTMENT OF PHARMACOLOGY**—Professor G. B. Frank, Department of Pharmacology, University of Alberta, Edmonton, Alberta, Canada.

**IMMUNOLOGIST/ZOOLOGIST** (preferably with some immunological experience) for an active research programme in the A.R.C. Cattle Blood Typing Service—The Secretary, A.R.C. Animal Breeding Research Organization, West Mains Road, Edinburgh, 9.

**POSTDOCTORAL FELLOW** (preferably with some experience in magnetochemistry) in the **DEPARTMENT OF INORGANIC AND STRUCTURAL CHEMISTRY**—Professor H. Irving, Department of Inorganic and Structural Chemistry, The University, Leeds, 2.

**PRINCIPAL SCIENTIFIC OFFICER and a SENIOR SCIENTIFIC OFFICER** (citizens of and permanently resident in the United Kingdom or the Republic of Ireland, with a degree in zoology, preferably honours, or a medical degree, and postgraduate research experience in tropical parasitology) at the East African Institute for Medical Research, Mwanza, Tanzania, to plan and carry out research on schistosomiasis and methods of control, and also to train newly appointed science graduates in research methods—The Ministry of Overseas Development, Room 403, Eland House, Stag Place, London, S.W.1.

**PRINCIPAL TECHNICIAN in the DEPARTMENT OF ZOOLOGY** to be in charge of the chemistry teaching laboratories and to be responsible for the supervision of all first-year teaching laboratories in a new building to be ready for occupation in 1969—The Administrator, Department of Zoology, University of Oxford, Parks Road, Oxford.

**RESEARCH ASSISTANTS** (with a first- or second-class (upper or lower) honours degree) in **PHYSICS** to undertake postgraduate research leading to M.Phil. or Ph.D. internal degrees of the University of London—Head of the Department of Physics, Sir John Cass College, Jewry Street, London, E.C.3.

**SENIOR or JUNIOR RESEARCH ASSISTANT in INSECT PHYSIOLOGY** in the **SCHOOL OF BIOLOGICAL SCIENCES**, to join a research project in the field of water relationships—The Administrative Assistant, School of Biological Sciences, University of East Anglia, Wilberforce Road, Norwich, NOR. 77H, quoting Ref. N-X.

**SENIOR TECHNICIAN or TECHNICIAN** (preferably with experience with the electron microscope or in cell biology) to assist in research on the fine structure and function of plant cells—Mr. A. D. Greenwood, Botany Department, Imperial College of Science and Technology, London, S.W.7.

**TECHNICIAN** (preferably with a knowledge of physiological and/or biochemical techniques and experience in another teaching department) in the **DEPARTMENT OF ZOOLOGY** to assist with the preparation of laboratory classes and help with research work—The Deputy Secretary, The University, Southampton.

**ZOOLOGIST** (with ecological interests) to complete a team of Dutch and British freshwater biologists working at Tjeenkemeer in Holland—The Registrar, University of Liverpool, Liverpool, quoting Ref. RV/130/N.

## REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

### Great Britain and Ireland

Political and Economic Planning. Annual Report 1966-67. Pp. 25. (London: Political and Economic Planning, 1967.) [196]

Medical Research Council. Monitoring Report No. 14: Assay of Strontium-90 in Human Bone in the United Kingdom—Results for 1966, Part 1. Pp. iii+14. (London: H.M. Stationery Office, 1967.) 2s. net. [196]

University of Oxford. Third Annual Report of the Delegates of the Science Area for the year ending 31 July 1966. (Supplement No. 6 to the *University Gazette*, Vol. 97, May 1967.) Pp. 137. (Oxford: The University, 1967.) 10s. [206]

The Royal Institute of Chemistry. Index of Chemistry Films: a Comprehensive List of Films, Film Loops and Filmstrips on Chemistry and Related Topics. Pp. xx+300. (London: The Royal Institute of Chemistry, 1967.) 15s. [226]



- Report of the Manchester Museum, 1965-1966. Pp. 20. (Manchester: Manchester Museum, The University, 1967.) [226]
- Timber Research and Development Association. Annual Report for 1966. Pp. 40. (London: Timber Research and Development Association, 1967.) [226]
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- Flora of Tropical East Africa. Edited by E. Milne-Redhead and R. M. Polhill. Cucurbitaceae. By C. Jeffrey. Pp. 156. (London: Crown Agents for Oversea Governments and Administrations, 1967. Obtainable from H.M. Stationery Office.) 18s. [236]
- British Empire Cancer Campaign for Research. Forty-fourth Annual Report covering the year 1966. Part 1: The Chairman's Statement and the Accounts. Pp. i-xi. Part 2: The Scientific Report of the Researches undertaken by the Central Organization and Its Autonomous Councils in the United Kingdom, and by some of Its Affiliated Organizations Overseas. Pp. xii-xlviii+434. (London: British Empire Cancer Campaign for Research, 1967.) [266]
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- Ministry of Transport: Road Research Laboratory. RRL Report LR 70: Driver Behaviour—Safe and Unsafe Drivers. By S. W. Quenaut. Pp. 58. (Crowthorne: Road Research Laboratory, 1967.) [276]
- The Lister Institute of Preventive Medicine. Report 1967. Pp. 39. (London: The Lister Institute of Preventive Medicine, 1967.) [276]
- Building Research Station. Digest 83 (Second Series): Plumbing with Stainless Steel. Pp. 4. (London: H.M. Stationery Office, 1967.) 4d. [276]
- Northern Ireland: Ministry of Agriculture. Leaflet No. 33: Chrysanthemums for Market. Pp. 10. Leaflet No. 100: Carrots. Pp. 12. (Belfast: Ministry of Agriculture, 1967.) [306]
- White Fish Authority. Annual Report and Accounts for the year ended 31st March 1967. Pp. vi+53. (London: H.M. Stationery Office, 1967.) 5s. net. [306]
- An Introduction to Local Natural History. By David A. E. Spalding. Pp. 23. (Sheffield: Sheffield City Museums, 1967.) [306]
- Pp. iv+71+plates 1 and 2. Professional Paper 519: Geology and Uranium Deposits of the Laguna District, New Mexico. By Robert H. Moench and John S. Schlee. Pp. v+117+plates 1-9. Professional Paper 574-A: Theory of Error in Geochemical Data. By A. T. Miesch. Pp. iii+17. \$0.20. Professional Paper 574-B: Methods of Computation for Estimating Geochemical Abundance. By A. T. Miesch. Pp. iii+15. \$0.20. (Washington, D.C.: Government Printing Office, 1967.) [156]
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- Transactions of the American Philosophical Society, New Series, Vol. 57, Part 3: Medical Ethics of Medieval Islam with special reference to Al-Ruhawi's "Practical Ethics of the Physician". By Martin Levey. Pp. 100. (Philadelphia: The American Philosophical Society, 1967.) [166]
- Annales de l'Institut de Physique du Globe de l'Université de Paris et du Bureau Central de Magnétisme Terrestre. Fondée par Ch. Maurain, et publiée par les soins de R. Thellier, avec le concours du Centre National de la Recherche Scientifique. Tome 33: Pp. 281. (Paris: Institut de Physique du Globe, Université de Paris, 1965.) [236]
- Proceedings of the California Academy of Sciences, Fourth Series. Vol. 34, No. 14 (May 9, 1967): Pliocene Non-Marine Mollusks from Contra Costa County, California. By James R. Firby. Pp. 511-524. Vol. 34, No. 15 (May 9, 1967): Review of the Genus *Brachymeloe* (Scincidae), with Descriptions of New Species and Subspecies. By Walter C. Brown and Discoro S. Rabor. Pp. 525-548. Vol. 34, No. 16 (May 9, 1967): Studies on the Cutaneous Innervation of Lizards. By Malcolm R. Miller and Michiko Kasahara. Pp. 549-568. Vol. 35, No. 1 (May 9, 1967): Earliest Tertiary West American Species of *Platyodon* and *Penitella*. By O. S. Adogoke. Pp. 1-22. (San Francisco: California Academy of Sciences, 1967.) [266]
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## SCIENTIFIC RESEARCH COUNCIL, JAMAICA PRINCIPAL SCIENTIFIC OFFICERS

Applications are invited from qualified and experienced persons for posts of Principal Scientific Officer in the Scientific Research Council, Jamaica, to supervise research in the Bioresources and Mineral Resources Sections. The holders of these posts will be section leaders and should possess wide experience in research and the ability to inspire and supervise staff.

Applicants for the post in the Bioresources Section should have a Ph.D. in Biochemistry or Organic Chemistry, or have had relevant postgraduate research experience of equivalent standard and duration supported by satisfactory evidence of research ability.

Applicants for the post in the Mineral Resources Section should have a Ph.D. in Geochemistry, Inorganic Chemistry or Ceramics, or have had relevant postgraduate research experience of equivalent standard and duration supported by satisfactory evidence of research ability.

The salary scale of the post of Principal Scientific Officer is £2,100 by £75 to £2,400 per annum. The maximum salary of £2,400 per annum plus special personal and housing allowances totalling £1,600 per annum will be paid to successful applicants depending on experience and qualifications. Appointments will be on contract for two or three years with a 20 per cent gratuity on satisfactory termination of contract.

Applications in writing, giving full curriculum vitae and the names of three referees, should be sent not later than September 30, 1967, to the **Technical Director, Scientific Research Council, P.O. Box 502, Kingston, Jamaica**, from whom further particulars of the posts may be obtained.

(607)

UNIVERSITY OF SUSSEX

## RESEARCH ASSISTANT

Applications are invited for a post of RESEARCH ASSISTANT in the Experimental Psychology Laboratory for a research project on long- and short-term memory.

The appointment is available from October, 1967, at an initial salary of £800 per annum.

Apply (three copies) stating age, qualifications, research interests and experience, together with the names of three referees, as soon as possible to the Assistant Registrar (Establishment), the University of Sussex, Essex House, Falmer, Brighton, quoting Ref. 937/1.

(597)

UNIVERSITY OF ALBERTA

EDMONTON, ALBERTA, CANADA

## DEPARTMENT OF PHARMACOLOGY

Applications are invited for the post of Assistant Professor or Associate Professor in the Department of Pharmacology from persons interested in Cardiovascular Pharmacology or Biochemical Pharmacology. The salary scale attached to this position is \$9,000 to \$12,450 for an Assistant Professor or \$12,500 to \$16,550 for an Associate Professor. The commencing salary will depend on the qualifications and experience of the successful applicant.

Further particulars of the position and of the concomitant amenities such as travelling expenses on first appointment and pension scheme may be obtained from Professor G. B. Frank, Department of Pharmacology.

(513)

UNITED LIVERPOOL HOSPITALS

## DEPARTMENT OF CHEMICAL PATHOLOGY

Applications are invited from suitably qualified candidates, for the post of SENIOR BIOCHEMIST to assist with the Department's routine work and research programme, particularly in relation to the metabolic ward at the Liverpool Royal Infirmary. The salary scale is £1,833 rising by annual increments (5) to £2,276 per annum. Whitley Council Conditions of Service apply.

Applications, stating age, qualifications, previous experience and the names of three referees should be sent by September 1, to the Secretary, 80 Rodney Street, Liverpool 1.

(628)

## UNIVERSITY OF SURREY TECHNICIAN (CHEMISTRY DEPARTMENT)

Applications are invited from persons aged 21 and over, preferably with City and Guilds or I.S.T. qualifications, for the post of Technician to assist in the teaching and research laboratories of the Chemistry Department. Good holidays and conditions; 37½ hour week; contributory superannuation scheme. Salary at an appropriate point in the scale £683 to £968 per annum plus £60 London Allowance.

The post will initially be at Battersea but will transfer to the new University buildings at Guildford in 1969. Substantial assistance will be given towards travelling expenses from the Guildford area.

Application forms may be obtained from the Staff Officer, University of Surrey, Battersea Park Road, London, S.W.11.

(640)

## EDINBURGH SCHOOL OF AGRICULTURE

### LECTURER IN AGRICULTURAL BIOCHEMISTRY—Grade III

### ASSISTANT ADVISORY NUTRITION CHEMIST—Grade IV

Applications are invited for the above posts.

Applicants should possess an Honours degree in Biochemistry, Agricultural Chemistry or equivalent.

Salary scales: Grade III, £1,396 to £2,193  
Grade IV, £926 to £1,277

Further particulars and application form from Secretary, The Edinburgh School of Agriculture, West Mains Road, Edinburgh, 9.

(602)

**B.F.M.I.R.A.**

Applications are invited to fill a number of vacancies for Scientific Staff in various key areas of the Research Association's activities.

**Membership Services Advisory Officer**

A scientist with experience in food manufacture and technology is required to handle inquiries from manufacturers seeking advice from the R.A.

**Indexer/Abstractor**

The successful applicant will work on information retrieval and production of monthly abstracts. Previous experience is not required. Scientific experience and a knowledge of German is an advantage.

**Biochemist**

An investigation into non-enzymic oxidative reactions and textural changes in fruit and vegetables is supported by an Agricultural Research Council grant. This is a 3-year project suitable for a higher degree. Applicants should hold a good honours degree.

**Assistants**

There are good opportunities for young applicants of approximately A-level or H.N.C. standards in Chemistry, Physics, or Microbiology to enter as Scientific Assistants or Assistant Experimental Officers, depending on qualifications.

Competitive salaries will be offered for all posts.

Apply to:

Director of Research,  
The British Food Manufacturing  
Industries Research Association,  
Randalls Road,  
Leatherhead,  
Surrey.

(613)

**UNIVERSITY OF SUSSEX****THE CHEMICAL LABORATORY  
MICROANALYST**

There is a vacancy for an experienced MICROANALYST to take charge of the Microanalytical Laboratory. This position is graded in the Chief Technician salary range of £1,242 to £1,423 per annum.

Apply, giving the names and addresses of two referees, a brief résumé of previous experience, qualifications and age, as soon as possible, to The Laboratory Superintendent, Chemical Laboratory, The University of Sussex, Falmer, Brighton, BN1 9QJ, quoting Ref. 940/2. (598)

**BATH UNIVERSITY OF TECHNOLOGY**

Applications are invited for a

**SENIOR LECTURER**

in the School of Materials Science. This is a new post arising from the development of the School and suitably qualified established workers in the field of physics or physical chemistry having interests in the study of new materials may be interested.

Salary in the range £2,520 to £3,310. F.S.S.U.

Further particulars and application forms obtainable from the Registrar (S). The University, Bath, please quote reference 67/93. (610)

**IMPERIAL COLLEGE**

Opportunities for postgraduate and postdoctorate research in a large group concerned with physico-chemical properties of materials at elevated temperatures. Specific projects include: (1) Electrochemical and electronic properties of solid oxides; (2) Chemical stability of oxy-carbide and dispersed phase materials. Applicants should possess an Honours Degree in an appropriate subject and for the postdoctorate post either a higher degree or appropriate research experience.

Applications to Professor C. B. Alcock, Metallurgy Department, Imperial College, S.W.7. (570)

**Scientific Civil Service****RESEARCH FELLOWSHIP****MINISTRY OF DEFENCE  
(ARMY DEPARTMENT)****MICROBIOLOGICAL RESEARCH ESTABLISHMENT**

Porton Down, Salisbury, Wilts.

**SENIOR or JUNIOR RESEARCH FELLOW** in the **Experimental Pathology Section** to work on the pathology of virus encephalomyelitis. Programme would relate to work being carried out both in the Virus Section, and in the Epidemiology Section on the transmission of and infection with arboviruses.

**QUALIFICATIONS:** A good honours degree in Medicine or in Veterinary science or equivalent or higher qualification, together with experience or proven interest in histo- or neuro-pathology or histology, and two years' (for J.R.F.) or three years' (for S.R.F.) post-graduate research experience.

**SALARY:** Senior Fellowship between £1,745 and £2,155 annually; Junior Fellowship between £1,180 and £1,575 annually. Both are tenable for three years.

Exceptionally, appointment may be as Principal Research Fellow (£2,250 to £3,105). F.S.S.U. terms.

Application forms for the above post and enquiries about opportunities for fellowships elsewhere, to the Civil Service Commission, Savile Row, London W.1, stating age, qualifications, and research interests, quoting S/570.

**MINISTRY OF AGRICULTURE  
FISHERIES AND FOOD****FISHERIES LABORATORY**

Lowestoft

**PHYSICIST or MATHEMATICIAN** (graded S.S.O./S.O.) required in a team concerned with research in physical and chemical oceanography in relation to fisheries. Projects include the study of the changes in the marine environment likely to result from construction or barrages in estuaries, the forecasting of oceanographical conditions on fishing grounds, and the investigation of near bottom currents in relation to fish behaviour. The Laboratory has four research vessels and is able to moor large arrays of oceanographic monitoring equipment.

**QUALIFICATIONS:** 1st or 2nd class honours degree, or equivalent or higher qualification in appropriate subject and, for S.S.O., at least 3 years' post-graduate experience.

**SALARY:** S.O., £926 to £1,574; S.S.O. (minimum age 26) £1,744 to £2,155. Non-contributory pension, promotion prospects.

**APPLICATION FORMS** from the Civil Service Commission, Savile Row, London W.1, quoting S/52-53/MAF. Closing date September 15, 1967. (600)

**UNIVERSITY COLLEGE OF  
NORTH WALES  
BANGOR****SCHOOL OF PLANT BIOLOGY  
RECLAMATION OF METAL  
CONTAMINATED SOILS**

Applications are invited for the post of Research Assistant to investigate the use of plant populations tolerant to toxic metals for the reclamation of toxic mine waste. The work will include the investigation of ecological conditions on metalliferous mine workings and the development of genetic reservoirs of adapted material. A full-time laboratory technician will be available, and there will be support for travel outside the British Isles. Applicants should have a good honours degree and interests in plant ecology, genetics, or mineral nutrition. Salary, £775 to £825 for a period of three years, with superannuation.

Further details can be obtained from the Registrar, to whom applications should be sent, together with the names of two referees, before August 31, 1967. (574)

**UNIVERSITY OF LEEDS: BOTANY DEPARTMENT:** Vacancy for a technician with experience of scientific and technical photography for interesting and varied duties including a considerable load of enlargement printing for light and electron microscopy, reduction printing for lantern slides etc., using high grade modern equipment for which the technician will be responsible. Must be able to work with minimal supervision, though some tuition at the outset could be available. Good working conditions and pension scheme. Salary according to age and experience on University scale for technicians which is constantly under review. Present range £683 to £968.—Applications, giving full professional details and the names of two referees to Professor I. Manton, F.R.S., as soon as possible. (562)

**LABORATORY TECHNICIAN REQUIRED** with experience of electron microscopy, or school leaver with "A" level in related science subject for training in this work.—Applications, stating age, qualifications and names of two referees, to the Administrator, University Laboratory of Physiology, Oxford. (604)



## MICROBIAL BIOCHEMIST

Ranks Hovis McDougall Limited require a microbial biochemist for the fermentation department at their main Research Centre at High Wycombe.

Applicants should have an honours degree in biochemistry, and a Ph.D. would be an advantage. Post-graduate experience in the field of protein synthesis is essential, preferably using micro-fungi. Age 25-35.

This is an important appointment providing the opportunity for really interesting fundamental research in a new and extremely well equipped laboratory. An appropriate salary will be offered and benefits include first-class pension and life assurance arrangements.

Please write with details of age, qualifications, and career to:

The Research Manager,

**RANKS HOVIS McDOUGALL (RESEARCH) LTD.**

Cressex Laboratories,

Lincoln Road, High Wycombe, Bucks.

(609)

### GRASSLAND RESEARCH INSTITUTE HURLEY, MAIDENHEAD, BERKS PLANT SCIENTIST

An officer is required in the Systems Synthesis Section of the Department of Ecology to work on theoretical aspect of plant and crop growth. This will involve the construction and testing of mathematical models of crop growth, mainly grass, and will be linked with similar studies on animal growth and crop utilization to form husbandry systems based on grassland.

Applicants should have a First or Upper Second Class Honours Degree in Botany, Agricultural Botany or Agriculture with at least three years' postgraduate experience. An interest in the syntheses outlined above and experience in plant or crop physiology are essential, while knowledge of the mathematical techniques would be an advantage as would an interest in the application of this work to agricultural practice.

The appointment will be made in the Scientific Officer (£926 to £1,574) or Senior Scientific Officer grade (£1,744 to £2,155) at a point appropriate to qualifications and experience.

Apply, giving curriculum vitae and names of three referees, to Secretary by August 31, 1967. (648)

### COMMONWEALTH AGRICULTURAL BUREAU VACANCY FOR SCIENTIFIC INFORMATION OFFICER

COMMONWEALTH BUREAU OF PLANT  
BREEDING AND GENETICS, CAMBRIDGE

Duties include preparation and editing of abstracts for the journal for *Plant Breeding Abstracts*, classification of biological literature and dealing with scientific inquiries. Applicants preferred with botanical or agricultural degree, or knowledge of or aptitude for foreign languages. Ability to write good English essential. Salary in scale £926 to £2,431, with provision for superannuation. Starting salary according to age, qualifications and experience. Two extra increments after two years' satisfactory service for applicants aged 32 or under on date of appointment.

Application forms and full particulars from Director of the Bureau, School of Agriculture, Cambridge. Closing date October 14. (584)

### ROYAL HOSPITAL FOR SICK CHILDREN EDINBURGH

#### SCIENCE GRADUATE

required to undertake advanced diagnostic techniques in an expanding department, recently rehoused in a new laboratory block. Experience in immunology or biochemistry would be an advantage. Salary in range £963 to £1,658 per annum, depending on qualifications and experience.

Applications, together with the names of two referees, to the Secretary, Edinburgh Central Hospitals, 1 Rillbank Terrace, Edinburgh, 9. (615)

### UNIVERSITY OF EDINBURGH

#### DEPARTMENT OF VETERINARY PATHOLOGY—LECTURER OR ASSISTANT LECTURER

Applications are invited from registered members of the Veterinary Profession for the post of Lecturer or Assistant Lecturer in Veterinary Pathology. The duties will include the teaching of morbid anatomy and histopathology, and there are good facilities for research. Previous experience in pathology will be an advantage but the post offers good training for the recent graduate wishing to study this subject. Salary Scales, Lecturer, £1,470 to £2,630 per annum; Assistant Lecturer, £1,105 to £1,340 per annum, with placement according to qualifications and experience, and with superannuation benefit.

The successful candidate will be expected to take up duty on November 1, 1967, or as soon thereafter as possible.

Further particulars may be obtained from The Secretary to the University, University of Edinburgh, Old College, South Bridge, Edinburgh, with whom applications (six copies), giving the names of two referees, should be lodged not later than September 16, 1967. (644)

### UNIVERSITY OF LIVERPOOL

#### DEPARTMENT OF ANATOMY

Applications are invited for the post of **Lecturer** in the Department of Anatomy. Candidates should hold a medical qualification. The initial salary will be within the range £1,470 to £2,855 per annum, according to qualifications and experience.

Applications, stating age, qualifications and experience, together with the names of three referees, should be received not later than September 16, 1967, by the Registrar, from whom further particulars may be obtained.

Please quote Ref. RV/142/N. (627)

### RESEARCH BIOCHEMIST (B.Sc.) FOR INDUSTRY

A graduate biochemist is required by the Biochemistry Department of Fisons Pest Control Limited for research in pesticide biochemistry. The work consists of studies on the mode of action of pesticides and involves isolation of mitochondria and chloroplasts, with studies of uncoupling of oxidative phosphorylation and inhibition of photosynthesis. Other areas of biochemistry will be investigated in the future. The graduate will initially work under a Ph.D., but the intention is to develop the graduate's capabilities so that he or she can work independently. The post should therefore be suitable for a newly qualified biochemist. The equipment in the department is good, and publication is encouraged. Although a biochemist will be given first preference, biology graduates with a biochemical emphasis will also be considered. It is essential that the applicant has good practical ability with biochemical procedures.

The general conditions of employment and salary are good. There is an excellent contributory pension scheme and an optional medical provident scheme. The Research Station is situated in very pleasant country about 15 miles from Cambridge and four miles from Saffron Walden. Transport is laid on for staff (free of charge) to cover the areas Bishop's Stortford, Saffron Walden, and Cambridge. There is an excellent subsidized canteen and encouragement is given to various sporting and social activities.

Please write, giving brief particulars of qualifications, to

The Personnel Officer (10),  
FISONS PEST CONTROL LIMITED,  
Chesterford Park Research Station,  
near Saffron Walden, Essex.

(622)

### EAST MALLING RESEARCH STATION AND THE DITTON LABORATORY

**Botanist/Plant Physiologist** for studies on the biological changes accompanying maturation and senescence of fruits under selected storage régimes, with particular reference to the physiological disorders arising during long storage and to the application of the results of basic research. A good honours degree, sound experience in physiological and biochemical techniques, and adequate research achievement. Appointment in Senior Scientific Officer grade, subject to qualifications and experience.

Further details and application forms from: The Secretary, East Malling Research Station, Maidstone, Kent. (650)

**SENIOR TECHNICIAN OR TECHNICIAN** required for Microbiology at Queen Mary College (University of London), Mile End Road, E.1. Previous experience is essential and the possession of the I.S.T. or City and Guilds Science Laboratory Technician's Certificate, preferably advanced, or O.N.C. or similar qualification is desirable though not essential. Salary according to age and ability on the scale £912 to £1,150 or £653 to £938 per annum plus London weighting £60 and possible £30 or £80 special qualification award. Five-day week. Four/five weeks annual leave. Pension scheme.—Letters only to Registrar (B/ST), stating full details of age, experience and present work. (558)

## FARADAY DEAD

MAXWELL was right, of course, when he wrote of Faraday (*Nature*, 8, 397; 1873) that "We are probably ignorant even of the name of the science which will be developed out of the materials we are now collecting, when the great philosopher next after Faraday makes his appearance". If Maxwell had been a fortune teller, he would have known that it was Rutherford who would most thoroughly inherit Faraday's reputation. The similarity of the two men is uncanny. Both of them seemed to know by instinct what experiments to do next, although Rutherford was awkward if not clumsy with his hands. Both were extraordinarily economical in that they did not occupy themselves with trivialities or become caught up in pointless complications—Rutherford's string and sealing-wax was not puritanically a virtue but a proof that most of his experiments were designed in eager expectation of the results. Both of them were astonishingly productive (and Faraday's wife was reconciled to his not sleeping well at nights for the excitement of it all). Each of them earned a dubious reputation about mathematics, and it is probably true that Faraday did not understand the calculus which was in fashion when he died a century ago yesterday. Rutherford was not so much ignorant as disdainful, but seems to have been able to convince a whole generation of students at the Cavendish Laboratory that it is as dangerous to let mathematics take charge of physics as to let an army run a government. Both men were in practice marked out from their contemporaries by their power of handling abstractions, and Faraday's feat was if anything the more remarkable. And then they were, the pair of them, quite unashamedly ambitious. Rutherford would put atomic physics on its foundations, while it is clear that Faraday in the autumn of 1831, with his ring induction experiment behind him, saw the whole of electromagnetism at his feet—and a good deal of atomic physics as well.

That said, it is only fair to acknowledge that the centenary of Faraday's death is not the best time to mark his achievements. The officers of the Institution of Electrical Engineers were right when they laid a wreath at Highgate Cemetery in 1831 to mark the centenary of the first demonstration of electromagnetic induction. For the best part of twenty years thereafter, Faraday was in full flood. After that, some kind of anticlimax was almost inevitable, but it is sad that Faraday should so quickly have lost his sparkle in the 1850s. By all accounts his memory, always bad, became fickle and even treacherous. Yet even in his decline Faraday was able to design prophetic experiments. Professor L. Pearce Williams, in his splendid biography of Faraday (Chapman and Hall, 1965), has pointed out that Faraday went looking for what is now the Zeeman effect as late as 1862, when he burnt

a gas flame doped with sodium between the poles of a magnet and looked unsuccessfully for some influence of the magnetic field on the spectral lines. Zeeman, thirty years later, may well have argued that Faraday would not have attempted such an experiment without good reason. Even Faraday's failures were worth knowing of.

This is only one of the proofs that Faraday was not the kind of country bumpkin he is often supposed to have been—good with his hands but dependent on the Maxwells of his time for support and in particular for mathematics. If anything, the boot was on the other foot. Faraday was the one who formulated—in words—the concept of a field of force; the manipulators of differential equations were so successful in the second half of the nineteenth century that physics was unreasonably saddled with the doctrine of the all-pervading imponderable ether, elastic constants and all. Maxwell himself is entirely explicit about his debt to Faraday, and says quite honestly in the introduction to his classic translation of the doctrine of tubes of force into mathematics that Faraday had already done the physics. But how could somebody who learned his science by apprenticeship, and from Humphry Davy at that, be so capable of abstraction? Genius apart, there is probably something in the view that the informality of his education was more a help than a hindrance at a time when it was above all necessary to find some new conceptual framework within which to accommodate the phenomena of electricity and magnetism and of their interaction with matter. Faraday was more free than his rivals, Ampère and Oersted among them, to admit that lines of force could sometimes be wrapped around in circles. Indeed, much earlier in his career, he had shown hankerings after the doctrine of Bosovich that atoms are not like billiard balls at all, but are more like abstract mathematical points with fields of force attached. It is tempting to ask where Faraday would have been led by a full-blooded devotion to that cause, but that is asking for too much.

The truth is that Faraday's interests, like those of his contemporaries, were curiously prophetic. Looking back, it now seems clear that the roots of the upheaval initiated in the nineties by Lorentz on the mainland of Europe and Thomson in Britain lay in the fifties and sixties, when Faraday's working life was nearly over. He himself tinkered with electrical discharges in gases, which was a fashionable thing to do, but nobody could then make a vacuum good enough to anticipate Thomson's measurement of  $e/m$ . Faraday's laws of electrolysis, linking a specific quantity of electricity with specific quantities of material, could well have been more suggestive of atomic theories than they were. The interaction between light and matter was a common preoccupation, and if it had not been



for the red herring of the ether doctrine, it is hard to believe it would have been necessary to wait for Kramers to put dispersion theory on a solid foundation. In the event, Maxwell's equations showing explicitly the conjugate relationship between electricity and magnetism made further progress possible—at least when they had become familiar. In other words, there is no gulf worth speaking of between Faraday and the modern world. Even the name of field theory has become a science of its own. Given that there have been 100 years, that is a remarkable tribute.

## MODEST CLAIRVOYANCE

THE National Science Foundation has embarked on an interesting and possibly important experiment by setting out to forecast future expenditure on research and development in the United States (*National Patterns of R and D Resources*, NSF 67-7, Government Printing Office, \$0.30). Much of the forecast is based on the figures supplied by federal agencies or is derived from estimates obtained by surveys carried out in industry and the universities. The figures are based on 1965 prices, and the forecast makes the assumption that there will be no drastic change in the economic climate. Although the forecasting consists of little more than intelligent extrapolation, and although the foundation—to begin with, at least—has modestly confined itself to a forecast for 1967 (already half gone) and 1968, anything that smacks of being an objective but realistic forecast of the immediate future could prove to be immensely valuable in the management of scientific resources.

So far as it goes, the forecast for 1968 is reasonably cheerful, given the talk there has been in the past few years about the declining rate of growth for expenditure on research and development and the pressure there has been on the American economy for the past year. Vietnam casts a long shadow. In aggregate, the NSF expects that the United States will spend a total of \$23,800 million on research and development in 1967 and \$25,000 million in 1968—a comparatively modest increase of 5 per cent, or rather less than the 6.9 per cent which is likely to represent the average compound rate of growth between 1965 and 1968. But by now, of course, nobody expects that spending on research and development could continue to double every five years as it did between 1953 and 1958. In reality, total expenditure has increased by a smaller percentage each year since the beginning of the sixties, although it is still moving ahead more quickly than the GNP. Even at this rate, research and development in the United States should reach 5 per cent of the GNP—it is roughly 3 per cent now—long before 1990.

The outlook for basic research is more obscure. The NSF has found it easier to produce a forecast for entire expenditure on research and development in 1968 than to say just what proportion of the total the several agencies involved, in industry as well as the Federal Government, will choose to devote to basic work. It

has been plain for some time that the unprecedented growth rate in the early sixties for expenditure on basic research, which worked out at an average of 17.0 per cent between 1958 and 1965, had fallen to 10.5 per cent in 1965-66. There is nothing in the US budget for the fiscal year which has just begun to suggest a return to the days of plenty. Indeed, the continuing pressure on government expenditure, of which the 10 per cent income tax surcharge is only one symptom, is a real cause for anxiety now that the Government's contribution to basic research has risen to two-thirds of the total. A little parsimony in Congress could do a lot of damage. Yet the NSF forecast takes a comparatively cheerful view of what the Federal Government will do for the universities in the years immediately ahead. If the figures are to be taken seriously, Washington will contribute \$1,600 million to the cost of university research and development in 1968, which implies that the government's contribution will have doubled in rather less than six years and that it will amount, in 1968, to 61 per cent of all that is spent in the universities. It is to be hoped that this cheerfulness will be justified by events. The next few months will be critical, if only because preparations for the budget for the year beginning in July 1968 will have to be made in an unusually sombre atmosphere. The universities will be lucky if they get the extra 10 per cent of federal money which the NSF predicts. The trouble is that they probably need a good deal more than that if the growth of the universities is not to be checked.

The forecast may, however, be more significant than the numbers suggest. For one thing, forecasts are the essential starting point for what is often called indicative planning. In practice this means that it should be possible to make comparisons of, say, the future scale of expenditure on research and development and the future supply of trained manpower in such a way as to decide whether skilled people will be easier or more difficult to find in the years ahead. This could be important if the Federal Government were for some reason to find itself embarking on a huge new programme of research and development, but industrialists may also find the forecasts useful in similar ways. And if the forecasts stretched three or four years ahead, of course, it would be possible for universities to trim their policies—and for students to adjust their choices of courses to follow—so as to win the greatest advantage from circumstances as they develop. Indeed, the benefits which might be derived from these and other comparisons are potentially so great that everybody will now no doubt be hoping that the NSF will soon summon up the courage to take a somewhat longer look into the immediate future. Nobody expects fortune telling, and there are legitimate doubts of the significance of forecasts which rely, as forecasts must, on hazy evidence such as the guesses by businessmen about the scale of involvement in research and development some years ahead, when economic circumstances may be quite transformed. Thus forecasts may be less an indication of future

reality than of present misconceptions. But any forecasts are better than none, especially if their limitations are openly acknowledged. Probably the NSF will want to stick to comparatively modest forecasting until it has won a reputation for clairvoyance, but too much caution would be a mistake. Ironically, the evident value of this beginning will without question set off a clamour for a three or even a five year forecast as well.

There remains the question of how the forecasts can influence the willingness of the Federal Government to spend money on research and development. (Possibly, it is worth recalling that there are limits to the extent to which an agency like the NSF, itself dependent on the Government for funds, can forecast how much its own benefactor will be prepared to spend on agencies like itself.) Pressures may spring up in all directions, and the greatest danger is that Congress and the others holding purse strings may be mesmerized by the figures which have been produced for the various rates of growth. Experience in Britain as well as in the United States shows that treasuries find it almost irresistible to argue that the rates of growth for expenditure on science ought to be linked somehow to the rate of growth of the GNP or to the change of some other economic indicator. This is like putting the cart before the horse. Too much respect for the GNP as a universal yardstick is one way of bringing growth to a halt. In an expanding economy, it is inevitable that some things should grow more quickly than others, and it would be a great surprise if research and development were not among the most vigorous consumers of extra funds. If Congress wants to use the forecasts now produced as a guide to action in the next year or so, it should start from the forecasts of the skilled manpower that will be available in the year ahead and then reckon that enough money must be allocated for these people to be efficiently employed.

## WHERE ARE THE QUASARS?

THE most distinctive property of the quasars is that the radiation from them is shifted enormously to the red, and any attempt to account for their existence must begin with that. But does the red-shift imply recession? And is an apparent recession of the quasars to be interpreted as participation in the general expansion of the universe? This is the train of thought which led, immediately after the discovery of the first of these objects, to the supposition that quasars are for one thing extremely far away, and therefore exceedingly powerful sources of radiation even by the yardsticks of astrophysics. But if red-shift implies distance, and if quasars are distributed more or less randomly throughout the universe, there should be a relationship between brightness and red-shift. The quasars with the biggest red-shifts should, on the average, be the faintest. That is how the argument

began, but the problem of the quasars has so far been enormously complicated and confused by the failure to pick out anything like a significant correlation between the brightness of quasars and the red-shift of their radiation. A year ago, with an air of resignation, Hoyle and Burbidge wrote that "as new red-shift values become increasingly available, the plot of the observed quasi-stellar objects has assumed more and more the aspects of a scatter diagram" (*Nature*, **210**, 1346; 1966). Although there have been several attempts to explain how a real correlation between brightness and red-shift may be obscured by irrelevancies, the absence of a correlation has been the chief reason why people have been energetically seeking ways of accounting for quasars which do not entail rapid recession at the edges of the universe.

It is too soon to know whether the problem will be simplified by the two articles on the red-shift relationship which appear on pages 917 and 919. Now that more data have accumulated, the beginnings of what seems to be a real correlation between brightness and red-shift does seem to be emerging. Horton and Daintree, writing from Jodrell Bank, argue that the relationship is more clearly apparent at higher radio frequencies than those used in earlier comparisons, and they claim that the most compact and the brightest of the quasars in their sample have a brightness and red-shift related by a simple curve not altogether different from the predictions of some cosmological models. In their view, the failure of all quasars to lie on the same smooth curve may be accounted for by processes such as internal absorption of radiation within particular objects. To them, red-shift implies great distance.

Although Longair and Scheuer agree that the newly accumulated data on quasars imply a significant relationship between red-shift and brightness, their interpretation is quite different. They argue that the observed brightness of a quasar with a pronounced red-shift is not itself a measure of the power emitted, but must be corrected to account for several complicating factors—the fact that a red-shift of any origin will reduce the energy of photons and the rate at which they reach an observer, for example. In other words, to them a faintly significant correlation between red-shift and brightness is not a sign that quasars are distant objects but rather a somewhat unsurprising happening which is entirely consistent with the view that the red-shift of quasars has nothing to do with rapid recession or great distance. The most convincing part of what Longair and Scheuer have to say is based on an analysis of the optical brightness of a number of quasars. The difficulty, of course, is that their negative conclusion may not be valid for the data corresponding to the very high radio frequencies at which Horton and Daintree claim the relationship is most apparent. In other words, the two arguments are not necessarily in conflict. The immediate result, no doubt, will be a careful poring over data. The theoreticians anxious to get on with model building will have to wait a little longer.

## NEWS AND VIEWS

### Plans for Kensington

THE small area of London bounded by Kensington Gore, Cromwell Road and Queens Gate contains some of the most important museums and institutions in Britain. The Science, Natural History and Victoria and Albert Museums, Imperial College, the Royal Colleges of Art and Music and the Royal Albert Hall, are all situated there. But this very concentration of collections and colleges, which makes this part of Kensington one of the most important cultural and educational areas in London, is proving disadvantageous. An important event at the Albert Hall, for example, "turns the whole area into a seething mass of motor vehicles", in the words of a letter to the *Times* by the directors of the institutions. They then announced that they have formed themselves into a standing committee to represent to the authorities the unique character of the district and to see that this is taken into account in the planning of buildings and roads.

The institutions have, for example, failed in their separate attempts to have traffic conditions improved. Those students of Imperial College living across Exhibition Road from the main buildings have a great deal of difficulty crossing over the road to work in the mornings. Then, ice-cream vans accumulate outside the Science Museum, causing the pavements to be littered with ice-cream cartons and sticks. The derisory fines that are imposed on the owners of the vans have not stopped them from coming.

When the committee has dealt with the more immediate problems, it intends to "promote a study of the problems and potentialities of the area", in particular to see what might be done to turn the area into an educational and cultural precinct. Since 1959, the University of London has been trying to turn the Bloomsbury area into such a precinct by buying houses in the region as their leases expire and by establishing university offices and departments in them. The original plan of this venture included proposals that certain streets should be closed to traffic, but this has not yet happened.

### Cheerful Association

THE British Association, which is due to begin its annual meeting at Leeds on August 31, has unexpectedly been able to show a small surplus on its accounts for the years 1966-67. On the heels of a deficit of £7,000-odd for the previous year and a gloomy forecast from the general treasurer, this seems a proud achievement. In reality, however, the association is more despondent than delighted, for its balanced budget is more the result of curtailed expenditure than increased income. The Department of Education and Science remains deaf to the plea that the grant of £12,500 which the department has made in recent years for the support of the lecture service should be doubled for a start, and that the department should also match private contributions towards the cost of these social services

up to a maximum of £12,500. The general treasurer does not in this year's annual report repeat his explicit complaints against the department, but he does mention with approval the way in which the Ministry of Technology has decided to make a further grant of £10,000 towards the cost of the propaganda which the association is doing among young people in the cause of the more fruitful application of science. According to the general treasurer, the need for economy has also led to potentially profitable innovations, such as the decision that the association's journal *The Advancement of Science* should from April 1967 be published on a commercial basis.

The chief consequence of straitened circumstances has been a reduction of the extent to which area branches of the British Association have been able to provide lectures on scientific subjects for local organizations. The total number of regionally organized lectures fell from 1,409 in 1965-66 to 998, but the central service provided by the headquarters of the British Association remained more or less unchanged at 311. One striking feature of the comparison between the two years is the decline in the proportion of adults attending lectures provided by the association. Evidently the Women's Institutes have been more seriously deprived of lectures on science than have the schools. There is, of course, nothing in the report to indicate whether the slackening of support from the British Association has been accompanied by an increase of support—not necessarily financial—from other sources. Whatever may have happened, there is obvious comfort to be drawn from the way in which the science fairs, which began as a kind of juvenile sideshow at the annual meetings, have become a continuing hit, first with the *Sunday Times* and now with BBC Television. The association is also modestly pleased about its work with audio-visual aids for education and with attempts to introduce more science lectures to colleges of education, although it is not equally successful everywhere. As it diversifies, the association may have to acknowledge occasional failures.

### Clean Air at Harwell

IN a report issued this week, the National Society for Clean Air urges the Ministry of Transport to fix limits for the contents of car and lorry exhausts. The report, "Air Pollution from Road Vehicles", states that although road and rail transport together use only one-tenth of the fuel consumed in Britain at present, car exhaust pollution so affects city streets that conditions in them could become intolerable as the number of cars increases, if no controls are introduced. Britain will be spared the photochemical smog that afflicts Los Angeles, unless, ironically enough, the skies become clearer. Even then, the most harmful product of the motor car will continue to be carbon monoxide. Already the concentration of this gas in streets can reach dangerous levels—as much as 360 parts per million was recorded in Oxford Circus. It is forbidden to expose industrial workers to a concentration of more than 100 p.p.m. in an eight hour day.

The best methods of reducing pollution, the report concludes, are by installing modified carburettors and fuel injection systems; exhaust after-burners and catalysts are less effective. The report criticizes the

forthcoming British Standards Specification on the level of smoke from diesel engines. Apparently, most existing engines produce smoke at a rate well below that specified, so that the standard is unlikely to prove much use in reducing pollution in the future.

The society has not been the only body with the subject on its mind this week. In a joint statement with the United Kingdom Atomic Energy Authority, the Ministry of Technology announces that scientists at Harwell are to co-operate with their research staff at Warren Spring Laboratory, to investigate problems in "health physics" and the physical chemistry of pollutants.

Of necessity, Harwell has been concerned for some time with radioactive pollution of the atmosphere, and research teams involved in this work have acquired considerable experience of measurement of the properties of aerosols, estimation of maximum allowable concentrations, and other work common to the study of both radioactive and industrial pollutants. At the same time, it is expected that the application to the study of pollution of techniques developed at Harwell will elucidate the life cycles of pollutants, their deposition on various surfaces, and the effect of them on rain.

Last November, the Chairman of the Congressional Joint Committee on Atomic Energy suggested that the United States Atomic Energy Commission should undertake pollution research. Nothing has come of this so far, but it is certain that any American effort would far outweigh that being made in Britain—the Harwell team will never have more than twelve workers. So it is perhaps a little disappointing that no research is to be undertaken into the ecological effects of pollution in Britain, which would not, of course, be covered by an American programme. No one can doubt the need for ecological research of this kind, in which radio tracers might be of great use. According to the National Society for Clean Air, photochemical smog greatly harms plants in America, and in Britain "it is a matter of common experience that some damage to roadside vegetation is caused by motor vehicle emissions".

## Clubs for Colloquia

MECHANICAL engineers in Europe, with help as well as encouragement from the Royal Society, have formed an organization to organize informal specialized conferences on theoretical and applied mechanics. Under the title of EUROMECH, the plan is to organize several conferences each year in various European centres. In future there will be a strict observance of the rule that no more than fifty people shall attend any one conference, although much will depend on the toughness of the chairman appointed to take charge of each of them.

The origins of this scheme lie in the International Congress of Applied Mechanics at Munich in 1964, when a number of the participants set out to organize smaller meetings at which specific subjects could be discussed. By this time a group of chemists had formed an organization with similar purposes under the banner of EUCHEM, chiefly under the prodding of Professor H. W. Thompson, foreign secretary of the Royal Society, so that the mechanical engineers had a useful precedent to follow, and the Royal Society

offered to provide secretarial services for both of them. The new development is that the EUROMECH has now been formed as a permanent organization, with a committee of four (elected from the member countries in rotation) under Professor G. K. Batchelor (Cambridge) and Dr. D. Küchemann of the Royal Aircraft Establishment as secretary.

In the immediate future, EUROMECH plans four conferences on the mechanics of liquids containing bubbles (Grenoble, April 23, 1968), thermoelasticity (Jablonna, October 2, 1967), aerodynamics of rarefied gas flows (Paris, February 7, 1968) and aerodynamics of flows with large velocity fluctuations (Prague, March 27, 1968). Apparently the organization is hoping to run its conferences on a self-financing basis, with participants usually paying their own expenses. Whether this self-denying ordinance will always enable all desirable participants to attend will presumably become clear as experience accumulates. Apparently the Council of Europe has already provided some modest help in this direction, and it is always possible that national academies may be able to provide some help from the funds which they are now accumulating in the cause of European collaboration. Another question outstanding is the extent to which the EUROMECH and EUCHEM will serve as precedents for attempts in other fields to provide some vehicle for communication smaller than a full-blown conference but bigger than a personal visit.

## Species in Stock

LAST week saw the publication of a useful little booklet, a revised "Directory of Collections and List of Species maintained in Canada 1967" (HMSO). This directory is one of a series published on behalf of the Permanent Committee of Commonwealth Collections of Micro-organisms. The organization, established in 1957 with headquarters in London, is under the auspices of the Commonwealth Science Committee and provides for the co-ordination and exchange of information on maintaining cultures between the member nations: Australia, New Zealand, Canada, India, Ghana, Trinidad, the United Kingdom and lately Jamaica. It fosters the maintenance and expansion of cultures. Recently, for example, it provided funds to maintain a collection in Jamaica that would otherwise have been lost, and it tries, by way of the directories, to make the cultures accessible to anyone who needs them.

The directories of all the member nations, last published in 1960, are being revised during the next two years and the new Australian directory has already appeared (HMSO, June 1967). The information for the directories is collected by national committees which keep an eye on all the collections maintained and endeavour to make them as complete as possible. It is surprising therefore to find that the only species of algae listed in the Canadian directory is *Chlerella vulgaris*. Is there really no culture of *Chlamydomonas* anywhere in Canada?

## Grass Technology

BRITAIN has never really understood that plant breeding is a technology—this at least is the opinion expressed by Mr N. W. Simmonds, director of the Scottish Plant Breeding Station, in the station's annual



report for 1966-67. He says that the results have been small scale of operation, swamping by foreign varieties in several crops, the absence, or near absence, of serious commercial effort, and consequently, poor service to the British farmer. Mr Simmonds says that much good science has been done in Britain, for example, on genetic studies, but it is likely that the continental or American breeders will profit from the research, and Britain will find itself paying royalties on the products of its own inventions. If these fundamental studies are to be put to use, a sustained and applied effort will be required.

In his view, the passing of the Seeds Act (1964) was a crucial event followed by the introduction under the act of schemes relating to the various crops. The act makes it possible for breeders to levy royalties on their own varieties in commercial cultivation and should stimulate commercial plant breeding in Britain. If this fails, then there would have to be a great expansion of state breeding. If the act does succeed, Mr Simmonds thinks that the official breeding stations will be pressed in two directions—partly towards fundamental studies and partly towards breeding crops which are not commercially attractive to the breeders. Mr Simmonds thinks the suggestion that the fundamental studies should be adapted to the universities is wrong in that the studies demand facilities such as land, glasshouses and assistants which could rarely be achieved by British universities, and the studies are basically economic in orientation and would therefore be regarded as "impure" in university circles.

## More Accidents

THE number of accidents in British factories and at building sites rose again last year, according to the Annual Report of the Chief Inspector of Factories (HMSO, 12s. 6d.). The increase was less than over the previous two years, but the fact that almost 300,000 people were injured and that fatal accidents increased from 627 to 701 can be pleasing to no one. The number of workers under eighteen involved in accidents did, however, fall slightly, from 18,200 to 17,400.

The Chief Inspector, Mr R. K. Christy, emphasizing the need for safety consciousness in industry, said that every establishment should have a trained safety officer, and that managers should plan for safety just as they plan their production or research and development. More specific recommendations are contained in a chapter concerned with the electrical industry in which, however, the accident rate of 25.9 per 1,000 workers is comparatively low. A new testing service is to be set up by the Ministry of Technology to certify the safety of electrical equipment used in flammable atmospheres.

Some interesting comparisons can be made between accident rates in various types of industry. Working in a jute factory, for example, appears to be dangerous: 61 workers in every thousand were involved in an accident last year, twice as high a rate as that in the cotton spinning industry, and nine times as high as that in the manufacture of stockings and knitwear. Or again, there were ten accidents per thousand workers in office machine factories, but the rate was thirty-five among those working on electrical domestic appliances. How can it be so much safer to make a typewriter than an iron, a duplicator than a washing machine? And an inherently dangerous occupation like the manu-

facture of explosives is, in fact, roughly four times as safe as working in a brickworks. Even more mysterious is the incidence of accidents in certain regions. In the Sheffield area, 63 in every thousand workers are injured; the figures for Birmingham and Nottingham are 25 and 28. The inspectorate cannot explain this, and comes to the conclusion that "numbers of reported accidents by themselves are not a reliable guide to accident prevention performance". It would be interesting to know if large establishments give more consideration to safety than small ones, and if the large number of accidents in the building industry (45,600, of which 288 were fatal) is due as much to this cause as to the nature of the work.

The report also discusses the difficulty of classifying and enumerating accidents. At present an accident is described as an injury that disables a person from working for three days. It is the duty of the employer to inform the inspectorate on the fourth day after the injury, but the extent of the injury may not then be clear. A system of classification based on the hours of work lost will not distinguish between restricted, but permanent, injuries, such as the loss of a finger, and general, but temporary, injuries, such as a badly sprained back. More than three-quarters of all the accidents that happened resulted in cuts, abrasions and strains. It is difficult to say how serious these injuries were, or how easily they could have been avoided.

The report does give details of the causes of fatal accidents. Of the 372 fatal accidents in industry, 95 involved a clear breach of the law by the occupier of the factory, and only 6 by the worker himself. In building, of the 288 deaths, 160 were caused by the employers breaking the law, 22 by the worker. The introduction of highly technical processes and sophisticated plant seems to be an unimportant factor in the increase in the number of accidents. "There is little evidence to suggest that industry is inadequately equipped to deal with the hazards that technological changes may involve; there is, however, abundant evidence to show that in some factories the most obvious dangers continued to be ignored."

## The Sutton Hoo Ship

THE British Museum has reopened the excavation of the Sutton Hoo ship and in the next decade hopes also to open the twelve remaining royal graves at the site on the banks of the Deben in Suffolk. When the museum began preparing a four volume definitive description of the Sutton Hoo burial ship so many questions arose that it decided to re-excavate the site.

The original discovery of the ship and its priceless treasures was made in 1939 and with the greatest generosity the landowner Mrs Edith Pretty gave the treasure to the nation; it is now in the British Museum. Mrs Pretty initiated the first excavation and when the complexity of the mound and richness of the treasure were realized Mr Charles Phillips, a Cambridge archaeologist, was invited to take over the excavation and handle the emergency. Considering the imminence of the Second World War and the lack in Britain of any archaeologist with experience of such a rich and complex site, the operation was remarkably successful. Unfortunately, however, at the end of the dig the site was, with lack of foresight, written off as of no further value. No effort was made to cover the ship, which was filled

with bracken to the gunwales and left two years exposed to frost and silting. It was to suffer much worse. By some lamentable failure of communications, the site was not scheduled as an ancient monument and, with Mrs Pretty dead, the land was taken over by the War Office as a battle training ground. It suffered accordingly.

When the British Museum team arrived this summer under the direction of Dr R. L. S. Bruce-Mitford of the Museum and Mr P. Ashbee, there was no trace of the ship. It was under five feet of silt with a machine gun emplacement dug through the stern and an unexploded bomb alongside which the Royal Engineers had to deal with.

Exactly why are the British Museum re-excavating the site? There are several reasons, all stemming from the fact that the original dig was such a hurried affair. Most importantly, complete details of the ship's construction were not recorded and these are of major importance. It is not every day that there is an opportunity of learning how a seventh century ship was built and this ship was no mere cenotaph—it was old and repaired and must have seen considerable service. The museum also wanted to look under the side of the ship for traces of the missing forty oars and steering paddle, to find the dimensions of the mound which had not been recorded and to sift through the mound of debris from the previous excavation looking for small fragments missing from the pieces of treasure.

The hunt has met with some success. Although all that was under the ship was two split logs supporting it, with no traces of the oars, the museum has found in the debris missing fragments from the treasure, notably the third of the boat's head decorations from the largest of the three hanging bowls and parts of the helmet and shield. These fragments are to be restored at the museum. A complete plaster cast of the hull has been made with great skill, and a fibre-glass positive will be taken from this. All that prevents the museum from making a full scale sailing replica of the ship is lack of money. It would cost £20,000.

There has always been a mystery about who, if anyone, was buried in this ship. Was it a grave or a cenotaph? Numismatists disagree over the dating of the thirty-seven gold coins in the ship's treasure, so it is not known who was reigning about the time of the burial and no skeleton was found in 1939. Mr H. Barker of the British Museum Laboratory is trying to determine if a body was ever in the burial by analysing the 2,000 iron rivets and samples of adjacent soil for deposits of iron phosphate.

In 1950 Mr Barker reported (*Nature*, **166**, 348; 1950) that the only fragment of material originally excavated in 1939 and then thought to be bone was, in fact, amorphous ferric phosphate. It did, however, contain carbon particles distributed as in calcined bone. Since the soil at Sutton Hoo is acid, the subsoil sand has a pH of 4.5, and would decompose bone. Mr Barker suggests that phosphate liberated from bone reacts with any iron to produce ferric phosphate. This summer the position of the 2,000 or so rivets in the ship has been mapped and each will be analysed for deposits of ferric phosphate. Even though many of the rivets were displaced by the tanks that rolled over the ship in the 1940s, it is hoped that the pattern of distribution of any ferric phosphate may give a clue as to whether or not a body was buried with the ship.

## Crystal Chemistry of Proteins

from our Molecular Biology Correspondent

THE pursuit of three dimensional protein structures by X-ray analysis has given rise to a number of highly profitable by-products. Among these is the discovery, chiefly associated with F. M. Richards and his co-workers, that protein chemistry can be made to go on in the crystalline state and that X-ray diffraction can be applied dynamically to its observation. Richards and his associates have explored the diffusion of various reagents into ribonuclease and carboxypeptidase crystals, and have shown, for example, that substrates can be turned over in the crystal, unreacted substrate diffusing in and the reaction products out. By the introduction of a bifunctional reagent, intermolecular cross-links can be formed, so that an insoluble, but still enzymically active, crystal results.

The latest article (Wyckoff *et al.*, *J. Mol. Biol.*, **27**, 563; 1967) describes elegant techniques for observing physical and chemical processes in protein crystals, gives a number of applications to the study of ribonuclease-S (pancreatic ribonuclease in which a peptide bond has been cleaved by exposure to subtilisin), and indicates how the methods have helped in the determination of the structure to 3Å resolution.

The method involves mounting the protein crystal (less than 1 mm in size) in a flow cell, which is placed in a diffractometer. Any suitable reflexion can then be selected for observation, and its intensity monitored as a function of time while an appropriate reagent is allowed to diffuse into the crystal. Rate curves are given for diffusion of ammonium sulphate and of inhibitors through the crystal. Dilution of the ammonium sulphate mother liquor leads to an intensity diminution in a half-time of 90 seconds. By contrast the diffusion of an inhibitor (an iodouridine phosphate) involves a half-time of 11 hours and the process is still measurably incomplete after three days. This enormous difference in the rates of penetration of small species is not explicable simply in terms of their diffusion coefficients, which differ only by a factor of about two: on the one hand the crystal lattice probably operates as a molecular sieve, impeding the progress of the larger nucleotide molecule; on the other, the binding of the inhibitor to the protein leads to a retardation (as in adsorption chromatography). A similar effect—titration of ionizing groups—is evidently responsible for the slowness of the response to a change in pH outside the crystal; in this case a change in the unit-cell dimensions is observed. The same system has been used to obtain binding curves for various inhibitors, to determine the number of sites—for the iodouridine phosphate, for example, a secondary binding site is surmised to exist—and to ascertain whether different ligands compete for the same site.

Since ligands can be washed out of a crystal with fresh ammonium sulphate solution, it has been possible to use a single crystal without disturbing its mounting in the goniometer for the comparison of a series of heavy-atom derivatives and the like. A single crystal was used in the same way for the collection of reflexions in the presence and absence of the isomorphous substituent, so that small intensity changes could be recognized with a high degree of confidence. The end-product of this work—the structure in detail of the enzyme-inhibitor complex—is promised soon.

## How Colchicine Works

by our Cell Biology Correspondent

ANY attempt to explain how colchicine functions must account for all the diverse and seemingly unrelated effects it has on cells. For example, apart from its well known ability to block mitosis by interfering with the structure of the mitotic spindle, colchicine reversibly induces the retraction of the axopods of protozoa, disrupts myofibril morphogenesis, interferes with the deposition of cellulose fibres in plant cell walls, causes acute poisoning in mammals primarily by its toxicity to the central nervous system and, most surprising of all, relieves the pain of gout.

The important discovery, made by Borisky and Taylor (*J. Cell Biol.*, **34**, 525 and 535; 1967), that colchicine binds specifically and reversibly to a sub-unit protein of microtubules has led them to propose that colchicine acts by preventing the formation of microtubules—a hypothesis which provides plausible explanations of all the diverse effects of colchicine.

What Borisky and Taylor have found is that tritiated colchicine administered to HeLa and KB cells can be recovered, after homogenization of the cells, in a soluble fraction in which the colchicine is specifically and non-covalently bound to a protein with a sedimentation coefficient of 6S. A similar reversible binding, with the same equilibrium constant and kinetics, takes place *in vitro* when the soluble extract from the cells, both at interphase and in mitosis, is incubated with the colchicine. Furthermore, the colchicine is not chemically modified when it forms the complex.

The problem then becomes one of identifying the source of 6S protein, the target site of colchicine. Two pieces of evidence show it to be a sub-unit protein of microtubules. First, a survey of the colchicine binding activity of several cell types and isolated organelles has revealed a strong positive correlation between the amount of colchicine bound and the abundance of microtubules. Mitotic cells, tritiated and flagellated cells, sperm and nerve cells all bind large amounts of colchicine and the only structures known to be common to these cells are microtubules. Slime moulds, on the other hand, have few if any microtubules and bind very little colchicine. It is interesting that, although recent evidence (see *Nature*, **215**, 345; 1967) suggests not all microtubules are identical, they all seem to share the property of strongly binding colchicine.

Second, when isolated sea urchin mitotic apparatus is extracted at low ionic strengths, the microtubules disappear and the soluble extract contains the 6S colchicine-binding protein. Borisky and Taylor conclude that the 6S material is a constituent of the microtubules, and Shelanski and Taylor (*J. Cell Biol.*, **34**, 549; 1967) present other evidence that the microtubules of sea urchin sperm tails also contain this 6S protein.

Thus Borisky and Taylor propose that colchicine acts by binding to sub-unit protein of the microtubules and preventing its polymerization into microtubules. They envisage that this polymerization is an equilibrium process, possibly nucleated by centrioles, and the equilibrium can be shifted in favour of the monomer by complexing monomers with colchicine. They are apparently in the process of testing this hypothesis directly by an *in vitro* experiment.

All the known effects of colchicine can be plausibly explained in terms of the hypothesis—even its toxicity to the central nervous system and its relief of gout pains. Nerve cells contain microtubules and doubtless their depolymerization would have deleterious effects. It would be of great interest to know whether neurofilament protein, as well as neurotubule protein, binds colchicine because a possible relationship between the two structures has been the source of much speculation. In gout, the colchicine may well give relief to the pain by disrupting the microtubules in leucocytes, thereby inhibiting phagocytosis and relieving the inflammation that accompanies it.

## Soft Rot Enzymology

by our Microbiology Correspondent

A WELL known response of plants to microbial infection is an increase of phenol oxidase activity followed by the deposition of dark oxidation products. Phenols and their derived pigments usually suppress the growth of phytopathogens *in situ* and limit the area of diseased tissue. In turn, certain phytopathogens are able to counter such host defence mechanisms. The factors which determine plant resistance or microbial virulence are extremely complex and, although many attempts have been made to correlate the disease condition with microbial activities, understanding in this area remains fragmentary.

The activities of phenol oxidase, pectinase and dehydrogenases during the progress of potato soft rot have been reported recently by Lovrekovich, Lovrekovich and Stahmann (*Phytopathology*, **57**, 737; 1967). High concentrations of the causative bacterium *Erwinia carotovora* were inoculated into healthy tubers and after 24 hours the infection locus was surrounded by a large zone of white rotted tissue. This latter zone was bounded by a narrow black margin which delimited the spread of the pathogen. Disease development was very rapid for the first day, but thereafter the rotting was contained by the host. Phenol oxidation in the rotted area was clearly inhibited by the bacteria because phenol oxidase and potential substrates were present. Furthermore, when bacteria were added to pigmented potato sap, the melanoid colour was lost. The bacterial inhibition of phenol oxidation was studied in model systems containing various preparations of the enzyme and catechol as the substrate. The bacteria were able to inhibit the oxidation only when glucose was supplied to the reaction mixture, a result suggesting that the sugar acted as a source of reducing power in the inhibition. Rotted tubers possessed very high levels of dehydrogenase activity in comparison to healthy tubers, and this activity was associated with the bacteria rather than with the host tissues. The authors conclude that the white rot can be explained in terms of the action of cell bound bacterial dehydrogenases maintaining the potato phenols in a reduced state even in the presence of active phenol oxidase. How then is the spread of the rot confined?

The black infection margin comprises macerated tissue, and Stahmann and his colleagues showed that extracellular bacterial pectinase was responsible for this development. Evidently at sites removed from the pathogen, activity of the host phenol oxidase is

unimpaired and it is reasonable to postulate that the black ring constitutes a host defence mechanism. Indeed, tubers pretreated with pectinase had much increased resistance to subsequent soft rot infection. The authors suggest that the pectic enzymes that diffuse into the potato tissues induce the phenol oxidase. The actual "induction" process is not discussed, but it is unlikely to involve *de novo* synthesis of the oxidase because healthy tissues are abundantly supplied with this enzyme. Rather, the process may be one of oxidase release or activation, phenomena known to occur under a variety of abnormal conditions in lower and higher organisms.

Significant as these findings are, they comprise only some of the factors affecting disease resistance and virulence. For example, below the white rotted tissue, black ring formation cannot be detected but the pathogen is restricted in its spread none the less. Here the decisive factor is the supply of oxygen and, when it becomes limiting, both the growth of the pathogen and the oxidation of the phenolics are repressed. The dehydrogenase activity of *E. carotovora* is, however, an important factor in its virulence and, in a wider context, the higher reducing capacity of diseased tissues is characteristic of fungal as well as bacterial host-parasite interactions.

## A Possible Relationship between Radio Flux Density and Red-shift for Quasars

by

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Demonstration of a relationship between the flux densities and red-shifts of quasars suggests that the red-shifts give an indication of their distances.

ASSUMING the red-shifts of quasars to be an indication of their distances, it might be expected that there would be a relation between their flux densities and red-shifts. Hoyle and Burbidge<sup>1</sup> found no such relation in the available experimental data from the revised 3C survey at 178 Mc/s. Bolton<sup>2</sup> suggested that this relation was obscured by a large dispersion in intrinsic luminosities, and Longair<sup>3</sup> pointed out that the relationship would be further modified by any cosmological evolution in the intrinsic luminosity. It now appears possible to demonstrate this relationship experimentally, partly because further data are available but more particularly because we are using flux densities at high frequencies. We compare the results for emitted frequencies of 500 Mc/s and 5,000 Mc/s, obtaining the flux densities at the relevant frequencies by interpolation from the observed spectra. New observations at 2,695 Mc/s and 4,995 Mc/s (Horton, Conway and Daintree, in preparation) were used to extend the spectra of quasars in the 3C catalogue. The spectra of more quasars were taken from the Parkes surveys<sup>4,5</sup> and red-shift data from refs. 6 and 7.

The observed flux densities in Figs. 1 and 2 were divided by  $(1+z)$  to normalize the values to a standard emitted bandwidth.

The points in Fig. 2 all fall on, or to the left of, a smooth envelope curve. Although the data may be incomplete for values of  $z$  approaching 2, it is thought to be substantially complete up to  $z = 1.5$ . At this value of  $z$ , an "envelope" source has a flux density of about five flux units at a frequency of 2,000 Mc/s. Consequently such a source even with a spectral index as low as 0.25 would be above the flux density limits of both the revised 3C catalogue and the Parkes surveys and so is likely to be included already. If this curve is really an envelope it indicates an upper limit to the intrinsic luminosity; the shape of the curve and the distribution of points along it would be related to the cosmological geometry and the evolutionary properties of the quasars.

The quasars lying on the envelope curve—their reference numbers are tabulated on the right hand side of Fig. 2—have similar characteristics. In general they have small angular size<sup>8</sup> and flat spectra; properties thought to be

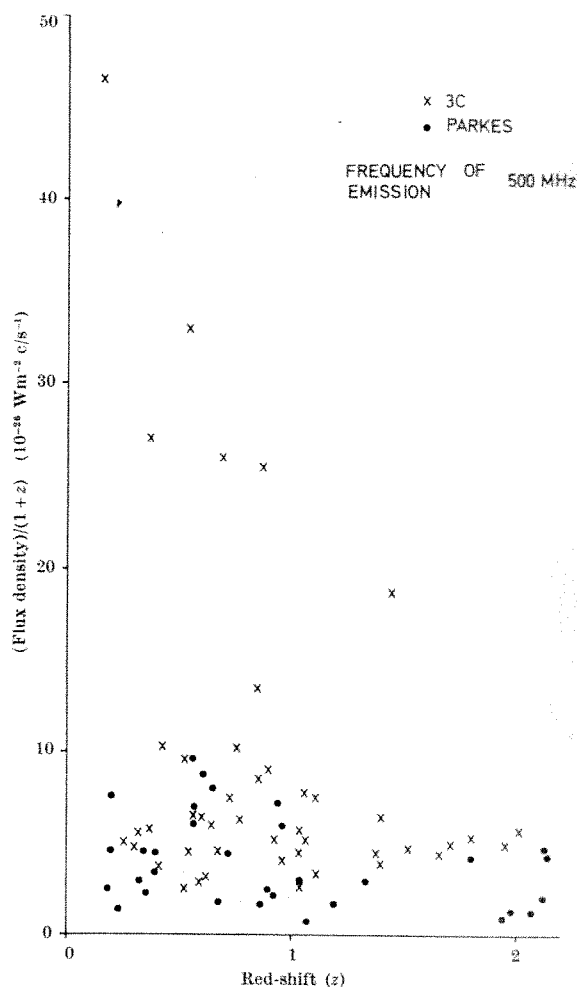


Fig. 1. Bandwidth corrected flux densities versus red-shift for an emitted frequency of 500 Mc/s.



associated with young sources. This gives added weight to the existence of the envelope since such sources may well be in their most luminous phase.

It is interesting to note that the same relation does not appear so clearly at an emitted frequency of 500 Mc/s (Fig. 1). At lower frequencies, self-absorption will modify the luminosity and greater scatter can be expected. Presumably our relation demonstrates that the flux density at 5,000 Mc/s more accurately reflects the intrinsic properties of the emitter. A search for red-shifts greater than 2 might profitably be directed towards sources detected at frequencies above 1,000 Mc/s, the flux densities of which may be near the limit of sensitivity of the 4C catalogue at 178 Mc/s.

The shape of the envelope has been compared with the flux density variation, obtained from constant luminosity sources at different distances, in various cosmological models. With suitable choices of absolute luminosity, all models gave equally good fits to the data (see Fig. 3 for example). The absolute luminosities required, however, varied by up to a factor of 5. In all cases 3C 273B, which is beyond the scale of Figs. 2 and 3, lies to the left of the theoretical envelope.

We have examined the consequences of the model proposed by Longair<sup>9</sup> to fit the number counts at 178 Mc/s,

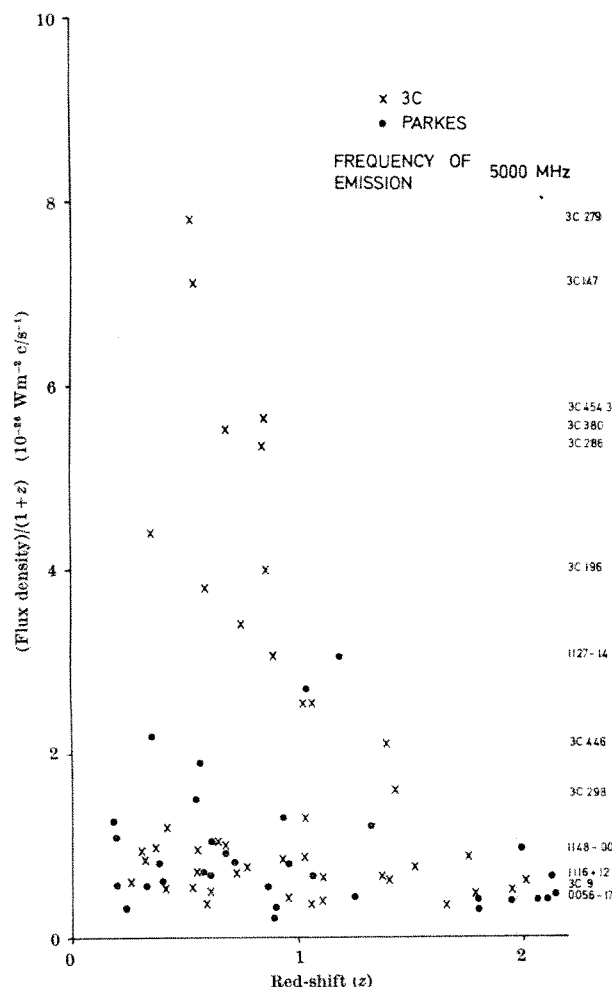


Fig. 2. Bandwidth corrected flux densities versus red-shift for an emitted frequency of 5,000 Mc/s.

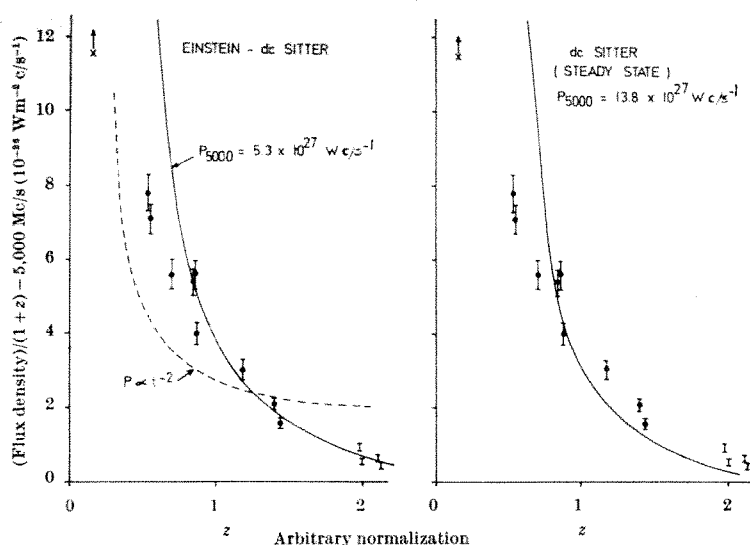


Fig. 3. Comparison of the envelope data with luminosity variations in two cosmological models.

in which the mean intrinsic luminosity varies with epoch as  $P \propto t^{-2}$  in an Einstein-de Sitter universe. Fig. 3 shows the effect of including this variation in the flux density-distance variation, together with the "envelope" sources and the constant luminosity curve for an Einstein-de Sitter universe. We suggest that Longair's model is not applicable to our data, either because the upper limit to luminosity does not behave as the mean, or because the dependence on epoch is less at higher frequencies.

For an Einstein-de Sitter universe the upper limit to luminosity at an emitted frequency of 5,000 Mc/s appears to be about  $5 \times 10^{27} \text{ W c/s}^{-1}$ . This may be compared with the analysis by Ryle and Longair<sup>10</sup>, who suggest that quasars have a constant luminosity—at an emitted frequency of 1,407 Mc/s—for about  $10^5$  years and that there is only a small scatter in luminosity at this stage. The luminosity then declines rapidly. Our work confirms this, and shows that the scatter may be even less at high frequencies.

The data used for the "envelope" sources are given in Table 1.

Table 1. DATA FOR THE "ENVELOPE" QUASARS FREQUENCY OF EMISSION: 5,000 Mc/s

Source	$z$	Frequency of observation (Mc/s)	Flux density $10^{-28} \text{ W m}^{-2} \text{ c/s}^{-1}$
3C 9	2.012	1660	$1.7 \pm 0.2$
0056-17	2.125	1600	$1.4 \pm 0.2$
3C 147	0.545	3240	$11.0 \pm 0.7$
3C 196	0.872	2670	$7.5 \pm 0.5$
1116 + 12	2.118	1610	$2.0 \pm 0.25$
1127-14	1.187	2290	$6.5 \pm 0.6$
1148-00	1.982	1680	$2.8 \pm 0.3$
3C 273	0.158	4320	$34.0 \pm 1.9$
3C 279	0.536	3260	$11.9 \pm 0.75$
3C 286	0.848	2710	$9.9 \pm 0.65$
3C 298	1.436	2050	$3.9 \pm 0.35$
3C 380	0.691	2060	$9.5 \pm 0.6$
3C 446	1.402	2080	$5.1 \pm 0.4$
3C 454.3	0.859	2690	$10.5 \pm 0.7$

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# Red-shift Magnitude Relation for Quasi-stellar Objects

by

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A red-shift magnitude correlation for quasi-stellar objects exists, but it does not imply a red-shift distance relation. If the red-shifts of quasi-stellar objects are cosmological their mean optical magnitude has changed with epoch.

THE apparent magnitudes of the brightest galaxies in clusters, or of radio galaxies, are extremely well correlated with their red-shifts. On the other hand, when the apparent magnitudes of quasi-stellar objects (QSOs) are plotted against their red-shifts, a broad scatter of points is found, and no correlation is obvious from inspection of the scatter diagram<sup>1</sup>. This lack of correlation has been variously interpreted as indicating that the red-shifts are not of cosmological origin, or merely that the QSOs have a wide dispersion in absolute magnitude. Red-shifts are now known for about 100 QSOs, and we thought it worth while applying some statistics to the problem. We found that, despite the large scatter, there is a highly significant correlation between apparent magnitude  $m_v$  and red-shift  $z$ .

There are three possible causes of a correlation between apparent magnitudes and red-shifts. (1) A red-shift (whatever its cause) changes the amount of radiation received from a source. (2) In the cosmological interpretation (and some of the local theories too) large red-shifts imply large distances, and therefore, through the inverse square law, large apparent magnitudes. (3) The luminosity of a source may be correlated with its red-shift. In the cosmological interpretation there could be a change in the mean luminosity of QSOs with epoch. In the various forms of local hypothesis there could be a physical connexion; for example, if the red-shifts are gravitational it might be that the sources with the deepest potential wells release the greatest energies.

After presenting the data we shall eliminate the first of these effects; then we shall investigate whether the data allow us to postulate the second without cancelling it out by the unknown effects of the third.

As the sample of quasi-stellar objects we have used all seventy-five objects in E. M. Burbidge's list<sup>2</sup> which lie north of declination  $-7^\circ$ . (There is now no good reason for the restriction  $\delta > -7^\circ$ , but as it excludes only nine of the eighty-four objects little harm has been done.) We are well aware that many selection effects occur in the processes of identifying QSOs and choosing those for which red-shift measurements are attempted—for example, bright QSOs, QSOs with strong radio emission, QSOs at high galactic latitudes and QSOs with strong emission lines are favoured. We have not, however, heard of or thought of any selection effect which would introduce a correlation between red-shift and apparent magnitude, without having any genuine astronomical significance.

Before proceeding to the correlations with red-shift, we note that there must be a wide dispersion in either the radio luminosities or in the optical luminosities of QSOs, for there is little correlation between their optical and radio magnitudes (Fig. 1). In fact there is probably a wide dispersion in both, for some individual objects vary by more than a magnitude at optical as well as at

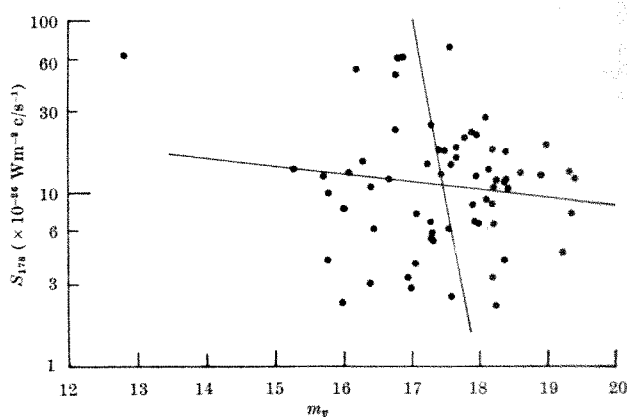


Fig. 1. Optical and radio magnitudes of QSOs; this diagram shows only the 178 Mc/s radio flux densities. The two lines are the regression lines; the correlation coefficient is  $-0.149$ , and is not significant at the 5 per cent level.

high radio frequencies. Thus we should not expect a very obvious correlation between magnitudes and red-shifts, even if red-shifts are good distance indicators.

## Correlations between Red-shifts and Apparent Magnitudes

The red-shifts  $z$  of the QSOs in our sample are plotted against their apparent  $V$  magnitudes  $m_v$  in Fig. 2, their flux densities at 178 Mc/s,  $S_{178}$ , in Fig. 3, and their flux densities at 1,400 Mc/s,  $S_{1400}$ , in Fig. 4. The regression lines of  $\log z$  on  $m_v$  (or  $\log S$ ) and vice versa are also shown, and the correlation coefficients have been computed. A few objects of the sample are missing from Figs. 3 and 4, because some have been observed as radio sources at 1,400 Mc/s but not at 178 Mc/s, some at 178 Mc/s but not at 1,400 Mc/s, and a few ("radio-quiet") objects have not yet been observed at any radio frequency.

The correlation coefficient between  $\log z$  and  $m_v$  is  $+0.489$ , and the application of an  $F$  statistical test (see, for example, ref. 3) shows that the correlation is significant at the 0.1 per cent level. The correlation coefficients of  $\log z$  with  $\log S_{178}$  and  $\log S_{1400}$  are  $-0.198$  and  $-0.197$  respectively, and are not significant even at the 5 per cent level.

It has been reported that a correlation of radio magnitude with red-shift is found if one considers only sources with "flat" radio spectra<sup>4</sup> or only sources with small angular structure<sup>5</sup>. As there is a well known connexion between small source size and a deficiency in low-frequency radio emission (see, for example, ref. 6), we may consider these as one phenomenon, being in some sense a measure of the compactness of the radio source. We have tried re-plotting

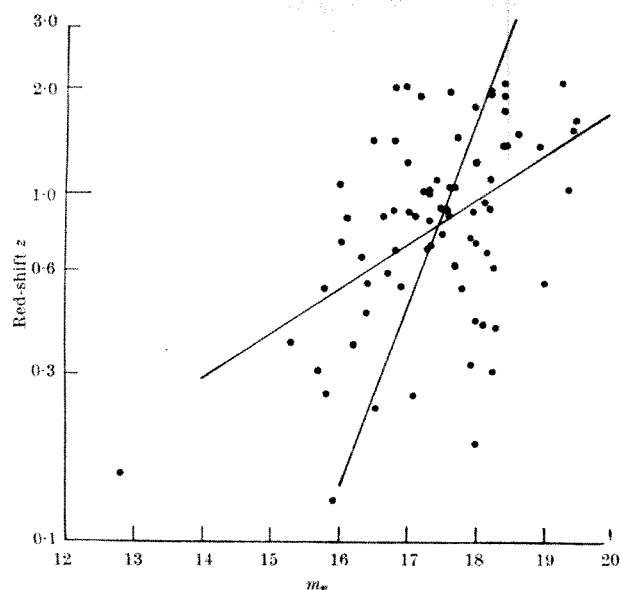


Fig. 2. Red-shift versus  $m_v$ . The regression lines are shown; the correlation coefficient is +0.489, and is significant at the 0.1 per cent level. The values of  $m_v$  are taken from ref. 2.

Figs. 3 and 4 using different colours to represent sources with the ratio  $S_{178}/S_{1400}$  in various ranges, but without achieving any obvious departure from the appearance of chaos. We have also selected those sources for which observations with long baseline interferometers<sup>7</sup> or of interplanetary scintillation (L. T. Little, private communication) have shown that at least 50 per cent of the flux comes from a region much smaller than  $1 \times 1$  sec of arc; such sources do show correlation, the correlation coefficients in Figs. 3 and 4 being -0.611 and -0.815 respectively, both significant at the 1 per cent level.

### Eliminating the Effects of Time Dilation

The general formula for the flux density, at frequency  $\nu$ , of a source (or the apparent optical intensity observed through a given filter) in a homogeneous isotropic universe may be written<sup>8</sup>

$$S(\nu) = \frac{L(\nu)}{4\pi \left( \frac{\sin Ar}{A} \right)^2} \frac{L(\nu(1+z))/L(\nu)}{(1+z)} \quad (1)$$

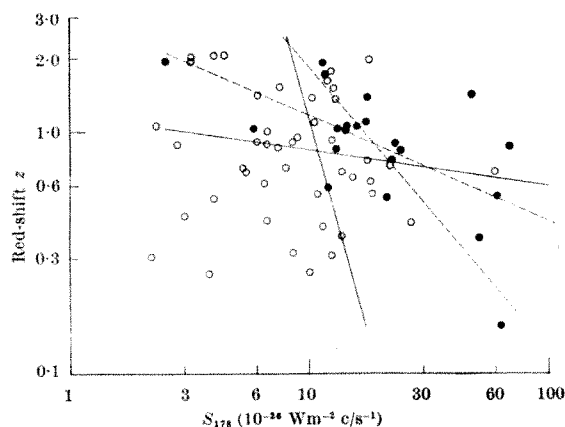


Fig. 3. Red-shift versus  $S_{178}$ . The 178 Mc/s flux densities are taken, in order of preference, from refs. 15, 16 and 17. Full circles represent compact sources, in which more than 50 per cent of the flux comes from a region less than 1 sec of arc in diameter, as shown by interferometry or by interplanetary scintillation. Open circles represent other sources. The regression lines for all sources are shown as full lines; the regression lines for compact sources alone are shown dashed. Correlation coefficients: all sources -0.198 not significant at 5 per cent level. Compact sources -0.611 significant at 1 per cent level.

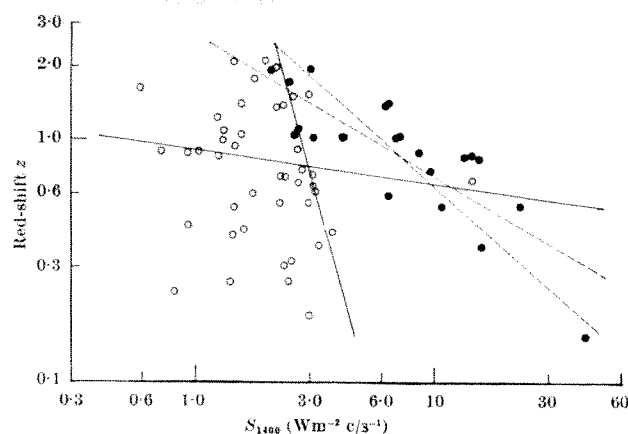


Fig. 4. Red-shift versus  $S_{1400}$ . The 1,400 Mc/s flux densities are taken, in order of preference, from refs. 18, 19 and 15. Symbols as for Fig. 3. Correlation coefficients: all sources -0.197 not significant at 5 per cent level. Compact sources -0.815 significant at 0.1 per cent level.

where  $L(\nu)$  is the power emitted per unit bandwidth, at frequency  $\nu$ ,  $r$  is the co-ordinate distance of the source and  $A$  is the curvature of the cosmological model. In this formula, the first factor represents the fact that the radiation is spread out over a spherical surface of area  $4\pi(\sin Ar/A)^2$ , and the second factor represents the change in the radiation due to the time dilation between source and observer. The latter factor includes: (a) the fact that radiation received at frequency  $\nu$  was emitted at frequency  $\nu(1+z)$  [ $L(\nu(1+z))/L(\nu)$ ]; (b) the diminished energy of each photon [ $(1+z)^{-1}$ ]; (c) the diminished rate of arrival of photons [ $(1+z)^{-1}$ ]; and (d) the contraction of the observed frequency band relative to the band in which the radiation was emitted [ $(1+z)^{-1}$ ]. What we now wish to emphasize is that the formula (1) is true whether the red-shift is cosmological or not; all the effects of time dilation remain the same, whether the red-shift is "cosmological", or an ordinary Doppler shift (as in Terrell's model<sup>9</sup>), or arises because the source is a puddle of hot gas at the bottom of a deep gravitational potential well (as in Hoyle and Fowler's model<sup>10</sup>), or a combination of these and other effects. The difference between the "cosmological" and other hypotheses appears only in the relation between  $r$  and  $z$  (see footnote). Each cosmological model predicts a definite relation between  $r$  and  $z$ ; if the red-shift is attributed to some other cause, there may be a different relation between  $r$  and  $z$ —for example, if QSOs were ejected from one local explosion and travel with constant velocity,

$$r = \text{constant} \times \frac{z(z+1)(z+2)}{(z+1)^2 + 1}$$

or no relation at all (for example, gravitational red-shifts).

Thus some correlation of  $\dot{S}$  with  $z$  must be expected, because of the second factor in (1), whether  $z$  is related to distance or not. The fact that there is a significant correlation between apparent magnitude and red-shift cannot be regarded as evidence in favour of the notion that  $z$  is a distance indicator. Fortunately the second factor in (1), which represents the effects of time dilation (or red-shift), involves only the observed red-shift and the observed spectrum of the source and can be computed without introducing any particular theory. It is therefore

In the "local hypothesis" the distances are so small that we can safely use the approximation  $(\sin Ar/A) = r$ . If the red-shifts are due to gravitation or to unknown causes, no ambiguity arises in the interpretation of  $r$ , but if the sources are moving and the Doppler effect makes a noticeable contribution to the red-shift, we must specify with great care the time at which the distance  $r$  is to be measured. If the source moves with constant velocity, the value of  $r$  which must be used in (1) is the distance from the source to the solar system, measured in the rest-frame of the source at the time when the radiation reaches us. If the source has a non-uniform velocity,  $r$  must be calculated as for a source which has moved since the radiation was emitted with the velocity which the real source had at that time. With this definition of  $r$ , the validity of (1) can be verified directly, but laboriously, by applying the rules of special relativity. It is obvious, however, that the value of  $r$  defined above is the radius of the sphere over which the radiation is spread, in the source's frame, when the radiation reaches us.

possible to investigate whether  $z$  is related to distance by plotting  $z$  (or  $\log z$ ) against

$$\begin{aligned} S'(v) &= S(v) \cdot (1+z) \cdot L(v)/L(v(1+z)) \\ &= S(v) \cdot (1+z) \cdot S\left(\frac{v}{1+z}\right)/S(v) \\ &= (1+z) S\left(\frac{v}{1+z}\right) \end{aligned} \quad (2)$$

or the corresponding average over the band covered by the  $V$  filter ( $m'_v$ ) in the optical case. (Note that the factor introduced in (2) is not the same as the "K correction".)

The radio spectra needed for computing  $S'(v)$  were provided by Dr P. J. S. Williams (private communication). For the optical spectra, the smoothed mean energy distribution derived by Sandage<sup>11</sup> from a study of the colours of forty-three QSOs has been used.

Figs. 5, 6 and 7 show  $m'_v$ ,  $\log S'_{178}$ , and  $S'_{1400}$  plotted against red-shift and the regression lines for these distributions of points. The most striking result is that the correlation between apparent magnitude and red-shift has disappeared completely, the regression lines being almost exactly perpendicular. The  $m'_v$ - $\log z$  correlation coefficient is +0.054, and is not significant even at the 5 per cent level.

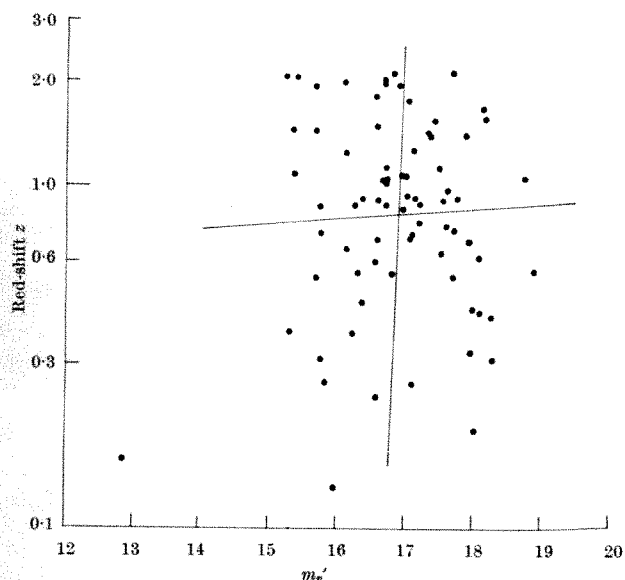


Fig. 5. Red-shift versus  $m'_v$ . Correlation coefficient +0.054.

The radio data plotted in Figs. 6 and 7 show that high corrected flux densities ( $S'(v)$ ) are, if anything, correlated with large red-shifts. This result is not entirely unexpected; the source counts for QSOs indicate an excess of faint QSOs which, on the cosmological hypothesis, for example, must be explained as a cosmological evolutionary effect<sup>12</sup>.

The question which immediately arises is whether such apparently complete independence of  $m'_v$  and  $z$  is compatible at all with the idea that  $z$  is a distance indicator. We ask first of all whether the large dispersion in luminosities could obscure the distance dependence of  $m'_v$  sufficiently.

### The Effect of Luminosity Dispersion

The limit at about 19.5m imposed by observational selection, which is a vertical line in Fig. 2, is transformed by (2) into a curved line in Fig. 5 and can therefore affect the correlation in Fig. 5. In view of this complication, the simplest way to test the possible effects of a broad dispersion in luminosity on Fig. 5 is to compute analogous diagrams for model distributions of sources. Such calculations would be worthless, however, if we were to pick a

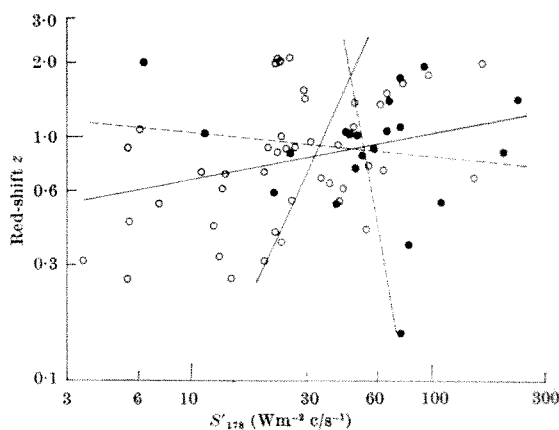


Fig. 6. Red-shift versus  $S'_{178}$ . Symbols as for Fig. 3. Correlation coefficients: all sources +0.295 significant at 5 per cent level. Compact sources -0.134. Not significant at 5 per cent level.

set of arbitrary models based on some tenuous theory, with numerous adjustable parameters. What we should like to do is to preserve the observed distribution of sources in  $z$ , and also to preserve the observed distribution in  $m'_v$ , and to introduce as an assumption only that  $m'_v$  and  $z$  are correlated through a relation between  $z$  and distance. This aim is achieved as follows. We assume that  $z$  is a good distance indicator, and that the form of the luminosity function (but not necessarily the density of sources) is independent of distance. We must also assume a particular relation between  $r$  and  $z$ , but the precise form of this relation is not critical. After all, we are hoping to discover whether or not  $r$  increases with  $z$  and we cannot expect finer details to have much effect on a diagram with a broad scatter of points. We have in fact used the relation

$$r = \text{constant} \times (1 - (1+z)^{-1}); A=0 \quad (3)$$

which is the correct relation if the red-shifts are cosmological and we live in an Einstein-de Sitter universe, but it would change the results little if we had simply used  $r=Cz$ . The remaining steps in the calculation are completely determined by the condition that we require (a) the same distribution in  $z$  as the observed distribution and (b) the same distribution in  $m'_v$  as the observed distribution.  $L(v)$  is computed for each observed source in Fig. 5, using equations (1) and (3), and a table of relative probabilities of  $L(v)$  is compiled by giving each source a weight  $W$  inversely proportional to the volume within which a source of its luminosity would have  $m'_v < 19.5$ . (This table does not show the true luminosity function for QSOs, for it is not derived from a complete sample. It does reflect the same selection effects as the sample in

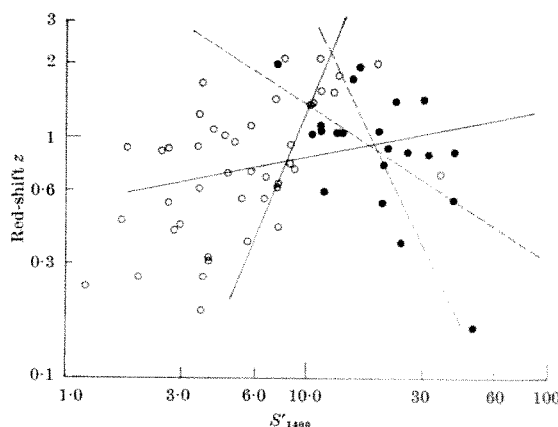


Fig. 7. Red-shift versus  $S'_{1400}$ . Symbols as in Fig. 3. Correlation coefficients: all sources +0.282 significant at 5 per cent level. Compact sources -0.558 significant at 1 per cent level.



Fig. 5, and this is just what is required to satisfy condition (b).) To produce a diagram for comparison with Fig. 5, we take each of the seventy-five observed values of  $z$  in turn (ensuring (a)), and select a value of  $L(v)$  at random, a value of  $L(v)$  being selected with the appropriate probability  $W/\Sigma W$ . Then  $m_v$  is calculated; if  $L(v)$  leads, for the current value of  $z$ , to an apparent magnitude  $m_v > 19.5$ , we select another  $L(v)$ , and so on, until one  $L(v)$  is selected for which  $m_v < 19.5$ . Plotting the points ( $m'_v$ ,  $z$ ) found in this way, we obtain a diagram with identically the same distribution of red-shifts as the observed sample, and with the same mean distribution of  $m_v$ , but with the correlation between them implied by equation (3). The whole process was performed many times by computer and automatic curve plotter; the regression lines and correlation coefficients of the "model" diagrams were computed at the same time.

The procedure was tested by using a model diagram as if it were Fig. 5, to generate a luminosity function and a second generation of model diagrams; this was repeated for a number of first generation model diagrams and it was found that the distribution of correlation coefficients was the same in first and second generation model diagrams. The procedure was also tested by repeating it to higher and higher generations from the same initial diagram. If the procedure is sound, there should be no change in the statistical properties of the diagrams after the first generation, though first generation diagrams may differ from the observed diagram if (3) is not valid for real QSOs. In fact, some increase in correlation coefficient was noticeable after ten generations; this is readily explained, however, because if a particular luminosity class is missing from one diagram it never reappears in subsequent generations, so that the luminosity function gradually becomes narrower.

The remaining uncertainty in the procedure is that the luminosity function is computed from one sample of QSOs, so that there is an unknown uncertainty in the luminosity function. In particular, the correlations are fairly sensitive to the number of intrinsically faint QSOs.

Correlation coefficients were computed for many model diagrams (without physically plotting them), and Fig. 8 is a histogram showing their distribution. Correlation coefficients as low as that observed in Fig. 5 occur in only 17 out of 274 trials, and we conclude that the width of the luminosity distribution is unlikely to be a sufficient explanation for the lack of correlation between  $m'_v$  and  $z$ .

We have not attempted to compute model distributions for comparison with the correlations between red-shift and radio flux density for compact sources, shown in Figs. 6 and 7, because the numbers of sources are considerably smaller.

## Conclusions

There is a significant correlation between the apparent magnitudes of QSOs and their red-shifts. There is also a significant correlation between radio flux density and red-shift among QSOs which are very compact radio sources.

The correlations are largely accounted for by the effects of red-shift alone, without any dependence of distance on red-shift. The correlation with visual magnitude disappears entirely when  $\log z$  is plotted against  $m'_v$  instead of  $m_v$ . The correlations with  $\log S'$  incline to positive or negative values according to whether we take all QSOs or only the compact radio sources.

It is unlikely that the lack of correlation between the red-shifts and the apparent magnitudes  $m'_v$  corrected for the direct effects of red-shift can be explained by the luminosity dispersion alone.

There appear to be only two possible interpretations of these results. Either the distance is unrelated to the red-shift (at least the dependence is much weaker than the relation (3) over the range  $0.2 < z < 2.2$ ), or the luminosity increases systematically with red-shift so as to make

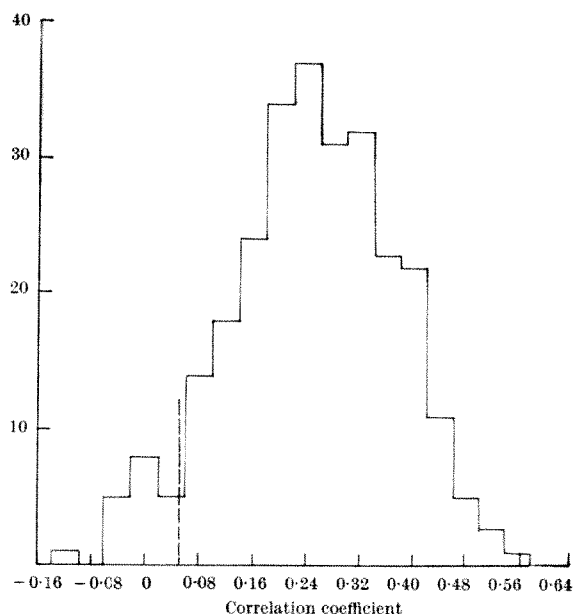


Fig. 8. Histogram of correlation coefficients computed, as described in the text, for models which have the observed distribution in  $m_v$ , and the observed distribution in  $z$ , but have the correlation between  $m_v$  and  $z$  required if  $z$  is a distance indicator, and the distribution of luminosities does not vary with epoch. The observed correlation coefficient is shown by a vertical dashed line.

$L(v)/(\sin Ar/A)^2$  roughly independent of  $z$ . In particular, if the cosmological interpretation of the red-shifts is correct, the optical luminosities must have been greater at earlier epochs; a similar evolution of radio luminosities has already been found necessary to account for the radio source counts<sup>12-14</sup>.

In principle it should be possible to test the dependence of  $r$  and  $z$  without the additional possibility of a dependence of  $L$  on  $z$ , by restricting the analysis to QSOs with small red-shifts. At present the number of QSOs with known red-shifts below, say, 0.3 is too small to make such a test significant.

We thank the director of the Mathematical Laboratory of the University of Cambridge, Professor M. V. Wilkes, for permission to use the TITAN computer, and Mr L. T. Little for communicating his observations of interplanetary scintillation in advance of publication. One of us (M. S. L.) is indebted to the Commissioners for the Exhibition of 1851 for the award of a research fellowship.

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<sup>8</sup> Davidson, W., and Narlikar, J. V., *Rep. Prog. Phys.*, **29**, 539 (1966) (Equation 3.21).

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# Spawning of Haddock in Captivity

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The courtship behaviour and spawning habits of haddock have been observed for the first time. Whether the behaviour of haddock in the sea is the same remains to be determined.

KNOWLEDGE of the spawning behaviour of the haddock *Melanogrammus aeglefinus* is slight, despite its commercial importance. The time of spawning and the approximate areas in which it occurs are known from studies of the distribution and abundance of sexually mature fish and the planktonic eggs and larvae<sup>1-4</sup>. The conditions under which they spawn, however, usually at depths exceeding 100 metres, have so far prevented the direct observation of reproductive behaviour.

Recently, a small group of haddock kept in the aquarium of this laboratory have spawned and we are able to report on their behaviour. These fish, obtained by hand line fishing at Loch Ainort, Isle of Skye, in February 1967, were maintained in a 700 gallon glass-fronted aquarium tank supplied with recirculated seawater from a large reservoir. The temperature was maintained at 4–6° C until the end of March, when breakdown of the cooling unit resulted in a slow rise to 10–13° C. During April a single female fish paired with one of a group of three males and spawned repeatedly.

The behaviour within this group of fish immediately before spawning was striking, involving aggressive displays between male fish, and courtship displays between what appeared to be the dominant male and the single mature female. Throughout this preliminary behaviour all the male fish produced intense sound. We have previously reported on sound emission during aggressive encounters between haddock outside the breeding season, and have described the pulsed sounds produced in those circumstances<sup>5</sup>. Similar sounds were produced in this group of fish during frontal and lateral displays by males, and were accompanied by the extension of the vertical fins. The sounds and displays were made in response to the proximity of both male and female fish. The intense sound production throughout the spawning period correlates with the observation of Templeman and Hodder<sup>6</sup> that the sound producing muscles of this species are more highly developed in sexually mature fish, particularly in males.

The sounds produced during this study were recorded on magnetic tape, and oscillograms of these recordings are given in Fig. 1. We have previously remarked on the difficulty of avoiding acoustic distortion of low frequency sounds in enclosed bodies of water, and it should be noted that these oscillograms are affected by multiple reflexion of the sounds from the tank walls and water surface.

During aggressive behaviour the dominant male fish produced sounds for extended periods, moving about the floor of the tank with all fins extended. This appeared to initiate courtship by stimulating the female to approach. Although only one of the male fish courted and mated with the female, the other males present were sexually mature and showed signs of mating behaviour. With the female advancing on the dominant male, her vertical fins held against her body, there was an increase of the repetition frequency of the pulsed sounds produced by the male. As courtship proceeded, with the female following the dominant male about the tank, the repetition frequency

of the sounds increased further to give a humming sound. This leading behaviour by the male involved greatly exaggerated swimming movements and was accompanied by a marked change in pigmentation. Two accessory pigment spots developed along each flank behind the prominent 'thumbprint' characteristic of the species (Fig. 2).

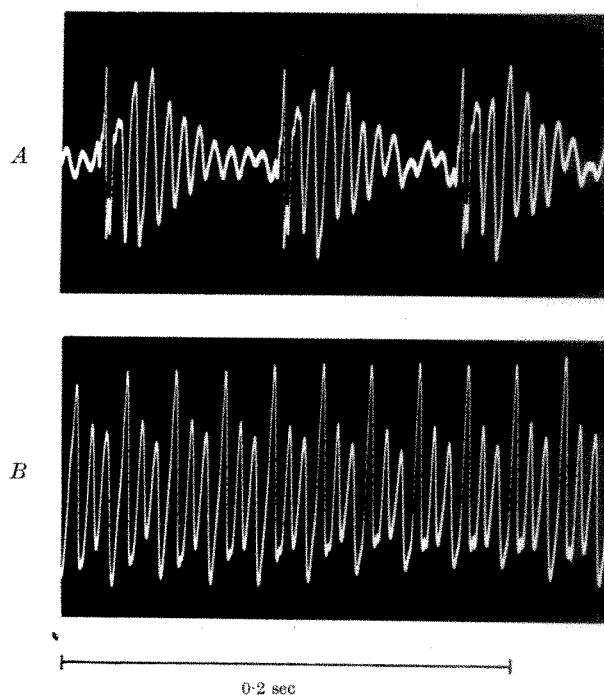


Fig. 1. Oscillograms of recorded sounds produced by a male haddock during courtship. A. Sounds produced during solitary display by a male fish, heard as a fast knocking sound. B. Sounds produced by a male fish leading a female around the floor of the tank in a flaunting display, heard as a humming sound.

The flaunting display culminated in a sexual embrace. By a quick turning movement, the male came alongside the female and mounted her either dorsally or laterally. With both fish swimming rapidly around just off the bottom of the tank, the male then slid round the female to a ventral position (Fig. 3) and, in this attitude with the ventral surfaces of the two fish apparently held together by their swimming movements, they swam vertically upwards while releasing eggs and milt. The production of sound ceased during this embrace. After this brief spawning, the female fish retired to a secluded corner of the tank and the male returned to the floor, and started making a noise again.

The spawning act was repeated at nearly regular intervals, essentially in the same way, as summarized in Table 1. The interval between successive spawnings appeared to be determined by the female fish. Immedi-



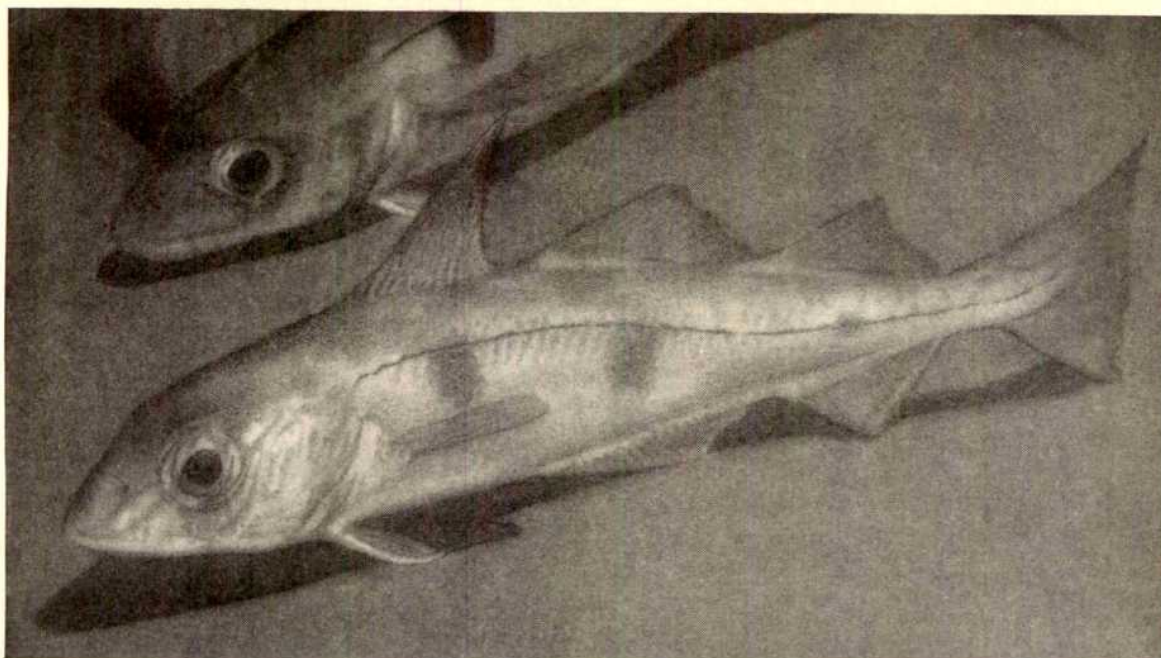


Fig. 2. Flaunting display by a male haddock (in foreground) to a female, showing the development of accessory pigment spots on the flank of the male.

ately after eggs had been shed, all contact with male fish was avoided until the period immediately before the next spawning. By contrast, the dominant male fish showed no diminution of aggressive or courtship behaviour and on several occasions attempted to embrace the female within several minutes of spawning.

After each spawning, the pelagic eggs were collected from the tank using a fine mesh net. These eggs were retained for subsequent incubation but on five occasions their numbers were estimated. The estimates ranged between 7,500 and 16,500 eggs per spawning, with a mean of about 12,000, which gives an estimated total production of 168,000 eggs. This compares with a mean fecundity of 224,000 for a female of similar size and age, obtained by counting the number of developing ova in the ovaries<sup>2,7</sup>.

Estimates of the proportion of eggs fertilized were also

made by counting random samples taken from the tank immediately after spawning, and at intervals thereafter. The eggs were placed in a fine mesh bag and thoroughly washed with clean seawater to remove excess sperm, and then allowed to develop normally in fresh seawater up to the early stages of cleavage, when fertilized and unfertilized eggs could readily be distinguished.

A sample of eggs taken from the sixth spawning within 15 minutes of being shed showed that 93 per cent had been fertilized. A sample from the seventh spawning, however, taken within three minutes of being shed showed only 53 per cent fertilized. This increased to 83 per cent for eggs allowed to remain in the tank for 10 minutes, and 88 per cent for eggs left for 15 minutes. Samples taken from a later spawning (13th) showed in the first few minutes a reduced percentage of fertilized eggs (19 per cent after two minutes), though this increased later (59 per cent after 10 minutes, and 91 per cent after 13 minutes). Under the conditions in the tank, the immediate result of the spawning embrace is the fertilization of only a fraction of the eggs; many appear to be fertilized later by sperm in the water. It is possible, however, that our method of treatment of these eggs after liberation washed off sperm adhering to the outer membranes and thus reduced fertilization. The observation may not therefore provide a true indication of the efficiency of the spawning embrace in ensuring a high rate of fertilization.

The repetitive spawning we have observed for the female haddock is in accord with previous suggestions by several authors<sup>7,8</sup>, and similar repetitive spawning, though with longer intervals, has also been reported for the cod *Gadus morhua*<sup>9</sup>. Raitt has reported<sup>7</sup> the frequent presence in the lumen of ripe haddock ovaries of a number of large translucent ova (mean diameter 1.2 mm), while the ovary as a whole contained a larger number of smaller opaque ova (mean diameter 0.7 mm). He encountered very few ova of intermediate size and suggested that the larger ova were ripe and ready for shedding. The difference in volume between these ripe hyaline ova and the rest, together with the high fecundity of this species, suggests that only a limited number of ova can be allowed to ripen before shedding must occur. It is possible that the interval between successive sheddings is in part determined by the time of ripening of a number of the ova.

Table 1. SUMMARY OF SPAWNINGS BY CAPTIVE HADDOCK (*Melanogrammus aeglefinus*)

Date (April 1967)	Time (B.S.T.)	No.	Time since previous spawning	Estimated No. of eggs released	Remarks
9	—	1	—	—	Fertilized eggs collected from tank on 12/4 at 0900 h
11	—	2	—	—	Fertilized eggs collected from tank on 12/4 at 0900 h
13	0100 h	3	—	No estimate made	Spawning observed
14	c. 0245 h	4	25-75 h approx.	No estimate made	Fertilized eggs collected from tank on 14/4 at 0630 h*
15	0800 h	5	29-25 h approx.	11,000	Spawning observed
16	1100 h	6	27 h	No estimate made	Spawning observed
17	1420 h	7	27-25 h	16,500	Spawning observed
18	1937 h	8	29-25 h	No estimate made	Spawning observed
20	c. 0245 h	9	31-25 h approx.	No estimate made	Fertilized eggs collected from tank on 20/4 at 0830 h*
21	1420 h	10	35-5 h	13,000	Spawning observed
22	1835 h	11	28-25 h	12,000	Spawning observed
24	0100 h	12	30-5 h	No estimate made	Spawning observed
25	1037 h	13	33-5 h	7,500	Spawning observed
27	c. 0230 h	14	40 h approx.	No estimate made	Fertilized eggs collected from tank on 27/4 at 0900 h*
29	—	—	—	No estimate made	Small number of infertile eggs found in tank

\* Time of spawning estimated from developmental stage reached by eggs. Female fish, aged 5 years (1962 year class), length 39 cm. Male fish, aged 4 years (1963 year class), length 36 cm.  
B.S.T. = British Summer Time, or Greenwich mean time + 1 h.



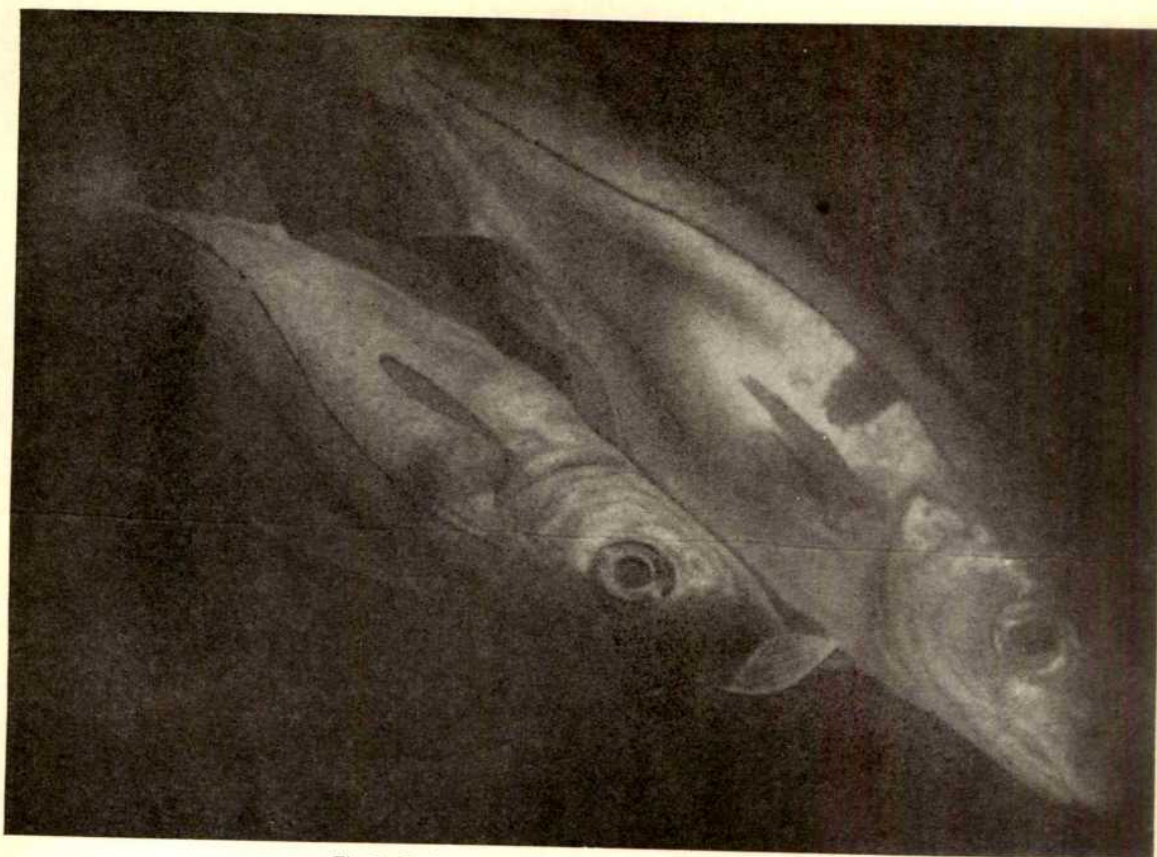


Fig. 3. Embrace of a female haddock by a male (lower fish).

The spawning of these fish in captivity has been valuable in suggesting that the reproductive behaviour of haddock in the sea is complex, involving aggressive and courtship displays and culminating in a sexual embrace. The pigment change in male fish and the prominence played by sound production in courtship, together with the increased development of the sound producing apparatus in males during the breeding season, suggest that this preliminary behaviour plays an important part in reproduction. This could be verified by listening for these sounds in the sea on haddock spawning grounds. Our observations were necessarily limited by the small number of fish involved and the limited volume of water available, and it would be premature to suggest that courtship occurs just off the sea bottom. It is of interest, however, that Trout<sup>10</sup>, from the

evidence provided by trawl catches of this species, has suggested that spawning may be benthic or at least bathypelagic.

We are indebted to Mr R. Jones for providing us with the live fish used for these observations.

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## Man, Vegetation and the Sediment Yields of Rivers

by

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Is it possible to reconstruct erosion rates for past epochs when modern rates of erosion all reflect the hand of man to a greater or lesser extent?

MANY attempts have been made to estimate the rate at which the continents are worn away by measuring the sediment loads carried by rivers at the present time<sup>1,2</sup>. The validity of such attempts depends on the accuracy of the determination of modern erosion rates and on the extent to which modern conditions reflect those which

existed in the geological past. It is now realized that the annual sediment yield of any river can vary by as much as a factor of five from one year to the next<sup>3</sup> and that reliable estimates of erosion rates depend on long term records. Little account has been taken, however, of the impact of human beings on their environment and the



increases in sediment yield which are thereby produced. My reconnaissance studies of rates of erosion in a wide range of climatic conditions in eastern Australia suggest that present sediment yields of many rivers draining areas of long human settlement are far in excess of those which may have prevailed in the geological past.

I have estimated the rate of removal of suspended sediment by twenty-one streams in eastern Australia from observations of stream flow and suspended sediment load. These observations were made over a period of two years at infrequent intervals but over a wide range of hydrologic conditions. A relationship between discharge and sediment yield was established for each river and was used in conjunction with a flow duration curve based on data from a continuous water level recorder to estimate the annual suspended sediment yield. The annual suspended sediment yield of a river is expressed as the volume of sediment lost ( $\text{m}^3$ ) per unit area ( $\text{km}^2$ ) of the surface area draining to that river; this area is designated the catchment area. Similar calculations were made for another nine rivers in south-eastern Australia using long term records maintained by reservoir authorities. The general relationship between mean annual suspended sediment yields and mean annual run-off for these rivers is shown in Fig. 1.

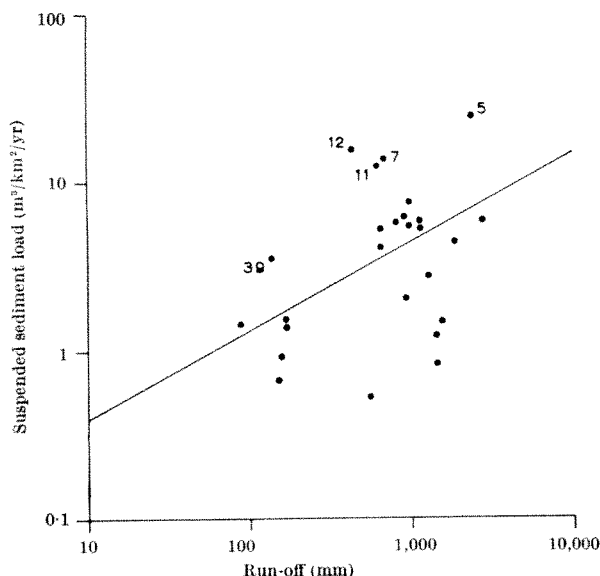


Fig. 1. Relationship of suspended sediment load to run-off for the catchments studied in eastern Australia.  $\log_{10} Sy = 0.527 \log Qy + 0.0668$ .  $r = 0.499$  with 25 degrees of freedom and is significant at the 0.01 level.

The reliability of these estimates of sediment yields in Australia is confirmed by the earlier estimates of  $4.3 \text{ m}^3/\text{km}^2/\text{yr}$  as the rate of erosion of the area tributary to the Burrinjuck reservoir in New South Wales<sup>4</sup> and of  $11 \text{ m}^3/\text{km}^2/\text{yr}$  as the suspended sediment yield during flood periods from the Collie and Preston catchments in south-west Western Australia<sup>5</sup>. Both Fournier<sup>6</sup> and Strakhov<sup>7</sup> have produced maps showing rates of sediment loss from the land surface and Fournier indicates a rate of removal of suspended sediment from the north-east Queensland areas of  $750 \text{ m}^3/\text{km}^2/\text{yr}$  and for south-east New South Wales of about  $80 \text{ m}^3/\text{km}^2/\text{yr}$ , while Strakhov indicates rates of  $20\text{--}40 \text{ m}^3/\text{km}^2/\text{yr}$  for both districts. Although wide variations from these average rates would be expected, Fournier's values are a whole order of magnitude greater than those based on Australian data. Even bearing in mind the short period of investigation in Australia, it is unlikely that the values that I have obtained are erroneous to that extent.

The Australian rates compare with erosion rates from experimental plots under natural vegetation of  $1.0\text{--}$

$40.0 \text{ m}^3/\text{km}^2/\text{yr}$  quoted by Fournier and of  $10.5 \text{ m}^3/\text{km}^2/\text{yr}$ , measured on an experimental plot near Aix-en-Provence<sup>8</sup> and with rates of  $5.5\text{--}9.9 \text{ m}^3/\text{km}^2/\text{yr}$  from forested catchments in Oregon<sup>9</sup>. These results, together with an examination of the sources of Fournier's data, suggest that Fournier used streams draining areas much affected by changes in vegetation and soil stability induced by human activity and that the Australian observations provide closer estimates of natural erosion rates than do Fournier's world wide estimates. I therefore agree with Tricart<sup>10</sup> that Fournier's values are much too high for estimates of natural erosion and suggest that they represent accelerated erosion—the results of intense anthropic modification of the natural landscape. Because the Australian catchments were selected so as to avoid as much human disturbance as possible, the rates at which they are eroded are probably closer to the rate of natural erosion, even though some of them do contain areas modified by man. The effect of this modification can be seen in Fig. 1, where points 7, 11, 12 and 39 represent higher mean annual suspended sediment yields than those of other rivers with similar mean annual run-off. All these rivers have relatively large catchments in which the modification of vegetation and agricultural activity is greater than in the other catchments examined.

### Relief and Sediment Yield

Rates of erosion in mountainous areas are often greater than those in plains country. They may be as much as forty times greater in periglacial conditions<sup>11</sup>, where vegetation affords little protection to the soil, and up to fifty times greater in the mountains of Georgia (in the United States) than in the coastal plain<sup>12</sup>. Mal'tsev<sup>13</sup>, on the other hand, has found that the silt loads of Central Asian rivers increase downstream, and the volume of sediment removed per  $\text{km}^2$  diminishes with increasing altitude. Much the same happens in the Irrawaddy catchment<sup>14</sup>, where the silt load passing Mandalay is  $75.5 \text{ m}^3/\text{km}^2/\text{yr}$ , but below Mandalay  $347.0 \text{ m}^3/\text{km}^2/\text{yr}$  are derived from the Chindwin and a further  $507.0 \text{ m}^3/\text{km}^2/\text{yr}$  from the non-perennial streams of the dry zone, thus producing a total load at Prome of approximately  $310 \text{ m}^3/\text{km}^2/\text{yr}$  (ref. 15). On a much smaller scale in north-east Queensland, I have found that most of the load of Davies Creek was acquired after the creek had emerged from the steep upper catchment with its rain forests onto drier more sparsely vegetated plateau surfaces. In all these three examples, the streams are flowing from densely vegetated mountain areas into less densely vegetated, drier areas downstream and the effects of channel gradient and catchment slope on sediment yield are overshadowed by the difference in vegetation.

Thus although in some areas good correlations are found between the relief and maximum length of a catchment (expressed as a ratio of relief and length) and suspended sediment yield<sup>16</sup>, there is no generally applicable relationship of this kind. The factors which are significant in one area, or on one scale of investigation, may be of minor importance in another area or at a different scale. Relief factors are thus considered of little significance in the contrast between the sediment yields of the Australian catchments and those described by Fournier and others.

### Sediment Yields and Run-off

Comparisons of run-off and sediment yields show the combined effects of relief and vegetation. Although the correlation coefficient of  $0.499$  for the suspended sediment yield run-off relationship for the Australian data in Fig. 1 is significant at the  $0.01$  level and suggests that sediment yield increases with increasing run-off, data presented by Langbein and Schumm<sup>17</sup> suggest that sediment yield is a result of the influence of precipitation. Thus the yield of sediment increases with the amount of precipitation but this effect can be offset by the influence of vegetation, which increases in bulk with

effective annual precipitation. The data used to construct the upper curve in Fig. 2 lead to the approximate result

$$S = \frac{1.631 (0.03937P)^{2.3}}{1 + 0.0007 (0.03937P)^{3.3}}$$

where  $S$  is annual sediment load in  $\text{m}^3/\text{km}^2/\text{yr}$  and  $P$  is effective precipitation in mm defined as the amount of precipitation required to produce a known amount of run-off under specified temperature conditions. Following the same procedure as Langbein and Schumm, the lower curve in Fig. 2 has been calculated by the use of data from a wide range of published sources. Langbein and Schumm acknowledge that few of the rivers they have studied drain areas with their primeval environment and that most of the basins studied are in the centre of the United States—the very area in which Leopold<sup>18</sup> has reported that human activity has increased sediment yields by between two and fifty times. Langbein and Schumm's assertion that the shape, if not the quantities, of their curve may represent the natural balance between sediment yield and run-off may be questioned. The peak of the curve at a run-off of 30 mm may be exaggerated, for in these hydrological conditions the removal of vegetal cover permits great soil erosion by the few storms which produce significant run-off. This is described by Mundorff<sup>19</sup> in the Kiowa Creek Basin, Colorado, where mean annual run-off ranges from nothing to 15 mm and run-off and sediment yields from grassland soils are moderate to high and depend on the density of vegetal cover maintained during grazing or cultivation, whereas on forest soils the combination of surface litter, high infiltration rate and interception of the precipitation by the forest cover results in low run-off and sediment yield.

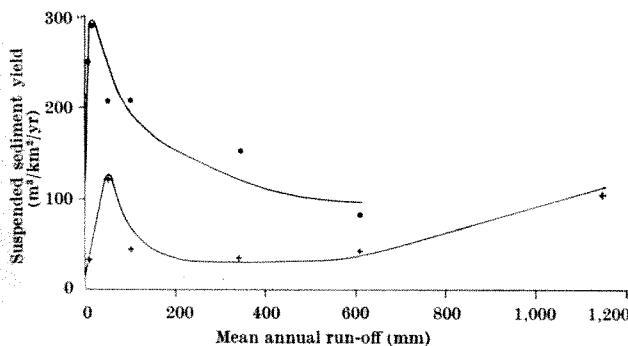


Fig. 2. General relationships between suspended sediment and run-off for streams studied by Langbein and Schumm and for a selection of rivers, including some major Asian rivers. ●, Catchments studied by Langbein and Schumm; +, catchments from other published data.

The shapes of the two curves in Fig. 2 may therefore reflect the "historical hangover" described by Chorley<sup>20</sup> and Anderson<sup>21</sup> in which the effects of changes in the equilibrium between climate, soils and vegetation, are delayed. For example, after forest clearance the roots hold the soil together until they rot some 5–10 yr later. In many of the semi-arid areas which now experience high sediment yields, the natural vegetation is a relict formation from wetter climates. Its failure to regenerate and the erosion of associated relict soils may be important causes of the high sediment yields. In more humid areas faster re-growth of vegetation and more regular precipitation make the effects of destruction of vegetation less drastic. The frequency of climatic oscillations in the recent past makes the present time an unsuitable period in which to make observations for extrapolation into longer periods of geological time.

#### Sediment Yield and Precipitation Distribution

Although the correlation coefficient of 0.499 for suspended sediment yield with run-off for the Australian catchments (Fig. 1) is significant at the 0.01 level, there

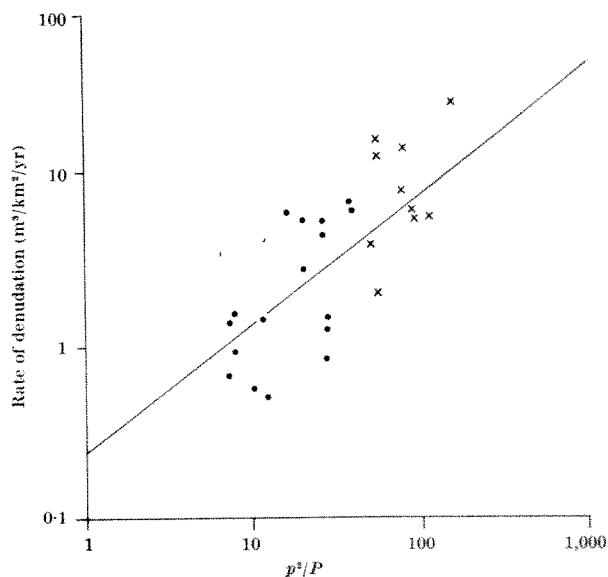


Fig. 3. Relationship of suspended sediment load to  $p^2/P$  for the catchments studied in eastern Australia.  $\log_{10} Sy = 0.737 \log p^2/P + 0.380$ ,  $r = 0.687$  with 25 degrees of freedom and is significant at the 0.001 level. ●, New South Wales catchments; x, Queensland catchments.

is a correlation coefficient of 0.687, significant at the 0.001 level, between these suspended sediment yields and the ratio of the square of the maximum mean monthly precipitation to the mean annual precipitation ( $p^2/P$ ) (Fig. 3). Fournier<sup>6</sup> has used world-wide data to demonstrate highly significant correlations between this measure of the amount of precipitation and the distribution and sediment yields of four classes of large catchment areas (Fig. 4). This measure of precipitation seasonality reflects not only the erosive impact of intense rainstorms

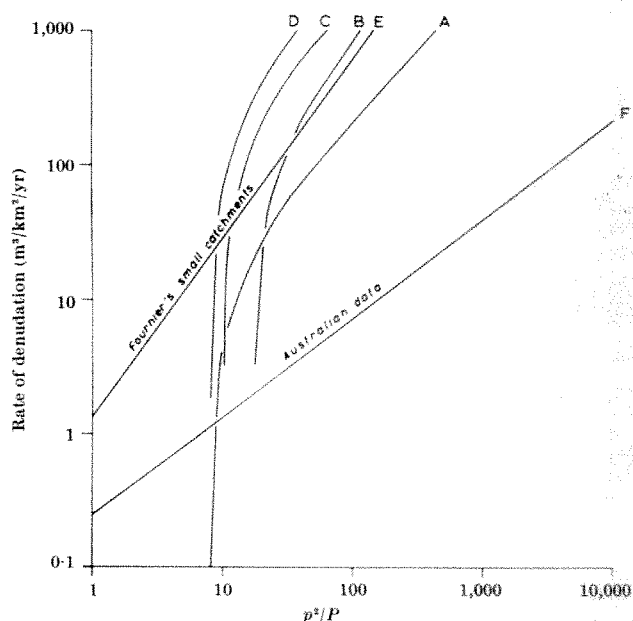


Fig. 4. Regressions of mean annual suspended sediment load against  $p^2/P$  ratio for catchments in eastern Australia and small catchments listed by Fournier, and Fournier's curves A, B, C and D, expressing general relationships between suspended sediment load and  $p^2/P$  ratios. Curve A applies to catchments of little relief when  $p^2/P$  is less than 20. Curve B applies to catchments of little relief when  $p^2/P$  is more than 20. Curve C applies to catchments of pronounced relief in all climates except semi-arid climates. Curve D applies to catchments of pronounced relief in semi-arid climates. The respective equations and correlation coefficients are: A,  $Sy = 6.14 - 49.78$ ,  $r = 0.800$ ; B,  $Sy = 27.12x - 475.4$ ,  $r = 0.960$ ; C,  $Sy = 52.49x - 513.21$ ,  $r = 0.950$ ; D,  $Sy = 91.78x - 737.62$ ,  $r = 0.980$ ; E,  $\log_{10} Sy = 1.356 \log x + 1.130$ ,  $r = 0.830$ ; F,  $\log_{10} Sy = 0.737 \log x + 0.380$ ,  $r = 0.687$ . All six correlation coefficients are significant at the 0.001 level.

such as that experienced during wet season storms in north-east Queensland, but also the nature of vegetation which becomes attenuated in areas of seasonal drought.

The contrast between the effects on sediment yields of evenly distributed precipitation of New South Wales and the highly seasonal rainfall of north-east Queensland is readily apparent from the distribution of points in Fig. 3. These Australian sediment yields are less than one-tenth of those in catchments with less than 2,000 km<sup>2</sup> in the USA, France and Syria examined by Fournier<sup>6</sup>, for which there is also a highly significant correlation with ( $p^2/P$ ) ( $r = 0.829$ , significant at the 0.001 level).

Vegetation growth and catchment erosions are both related to the magnitude and frequency of precipitation events. Irregularly distributed rainfall occurring in storms of high intensity and short duration fails to ensure sufficient water supply for the growth of dense vegetation and is also very effective in eroding the soil. The Fournier ratio ( $p^2/P$ ) expresses this variation well because the value of the ratio increases with both seasonality and total annual precipitation. High sediment yields are therefore to be expected in areas with marked wet and dry seasons, typical of the monsoon climates. Thus the observations from the Middle West of the United States and from Asia which have been used to construct Fig. 2 may be reconciled, both the relatively dry climates and the monsoon climates having higher ( $p^2/P$ ) ratios than temperate climates where the precipitation is more evenly distributed throughout the year. Thus the typical curve of the sediment yield run-off relationship is bimodal with a peak at 40–50 mm mean annual run-off and another at about 2,000 mm mean annual run-off (lower curve, Fig. 2).

These peaks become much more accentuated, however, when data from catchments with considerable human interference are used—the data from the USA, for example. Human interference also produces high sediment yields in the humid tropics, for example, 2,500 m<sup>3</sup>/km<sup>2</sup>/yr from the small Tjatjaban catchment in Java<sup>22</sup> which is greatly affected by cultivation of steep slopes. Although agriculturalists have long been aware of the effects of human interference in the humid tropics<sup>23</sup>, its implications for calculations of sedimentation during the geological past have been insufficiently examined. The assumptions made by Gilluly<sup>1</sup>, Judson and Ritter<sup>24</sup> and Mackenzie and Garrels<sup>25</sup> that the present sediment loads of rivers may be taken to represent those during periods of crustal evolution may be entirely unwarranted. The observation of present rates of erosion may provide us with no information directly relevant to the past, for human interference is so extensive and has spread so rapidly that we cannot be sure that observations relate to natural conditions. There is also the problem of the lag between weathering during cold periods in the Quaternary

and the removal of this material from slopes and valley floors. Recent investigations by Kirky in Scotland<sup>26</sup> and by myself in the Southern Tablelands of New South Wales have shown that much material now being removed from slopes and catchment areas is debris which accumulated during periods of Pleistocene periglacialation.

Without examining the complex physiographic, pedologic and hydrologic factors which affect erosion rates, there is sufficient evidence from the simple relationships between precipitation and sediment yield to demonstrate that the use of modern erosion rates for the reconstruction of past rates of sedimentation is full of difficulty. It is to be hoped that the present concern for nature reserves and the preservation of wild life will include sufficient areas of wilderness which can be used to measure geological processes and thus to enable the extent of man's role as a geological agent to be determined.

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## Single Stranded DNA in Lymphocyte Chromosomes

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The distribution of single stranded DNA in lymphocytes has been examined by an immuno-fluorescence technique. Chromosomal genes seem to be active in these cells throughout the cell cycle.

In mammalian cells, single stranded DNA probably serves for replication<sup>1</sup>, as a primer for its own polymerase<sup>2</sup> and possibly as templates for the formation of RNA<sup>3-5</sup>. The location of such DNA in the nucleus, according to the

metabolism or life cycle of the cell, is therefore of interest because it may provide a structural foundation for some functions of chromatin. Conventional histochemical techniques, however, are not sufficiently specific<sup>6,7</sup> to

demonstrate those structures of chromatin which reveal its function. Antinucleotide antisera, on the other hand, are directed solely against short segments of single stranded DNA and, if labelled with fluorescein, provide alternative, stoichiometrically reliable, tracers of this substance in chromosomes.

Human peripheral blood lymphocytes<sup>9</sup> were grown in short term primary culture with phytohaemagglutinin. No antibiotics were used. Partial synchrony of initial mitoses<sup>10</sup> was improved by incubating cultures for 8 h at 37° C, keeping them at 20° C overnight and then re-incubating them. The first cell cycle reached mitosis after 48–50 h of incubation. Similar synchrony occurred in DNA synthesis, shown by the uptake of tritiated thymidine (Fig. 1).

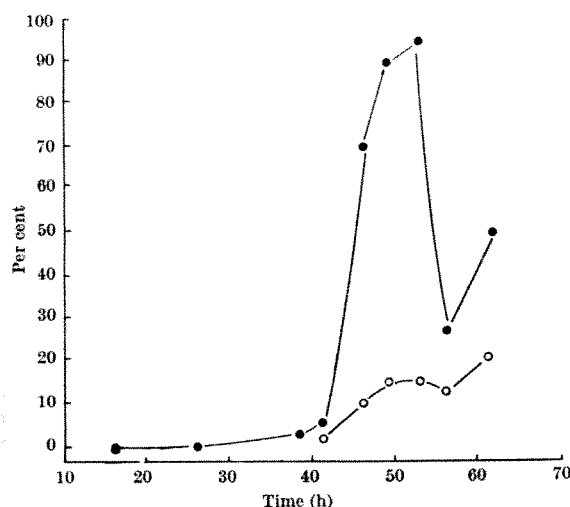


Fig. 1. Rate of uptake of tritiated thymidine as a measure of synchronization in initial cell divisions of lymphocytes in primary culture. ●, Percentage of labelled mitoses; ○, mitotic index.

Isolated nuclei were prepared by hypotonic swelling at 100 mOsmoles for 20 min. They were fixed in Carnoy's fluid, preserving nucleic acids in a configuration vulnerable to attack by nucleases<sup>11,12</sup> and probably retaining antigenic sites similar to their natural form. They were air dried for 30 sec on a hot plate at 55° C. The slides were washed briefly in phosphate buffered saline (PBS, 0.14 molar sodium chloride; 0.0175 molar phosphate, pH 7.0) before subsequent treatment.

Rabbit antiserum against heat denatured DNA, electrostatically complexed with basic, methylated bovine serum albumin, was supplied by Drs Werner Braun and Otto Plescia of the Institute of Microbiology, Rutgers University.

The DNA contained mixed proportions of all bases, and the antiserum reacted in complement fixation systems as an antitetra-hexanucleotide<sup>8</sup>. Crystalline fluorescein isothiocyanate (Baltimore Biological Laboratories) was conjugated to the antiserum, using a fluor : protein ratio of 1 : 100. This ratio was critically necessary to avoid an alteration in the isoelectric point of rabbit globulin<sup>13,14</sup> sufficient to obscure results by non-specific electrostatic attraction between conjugate and chromatin. Labelled globulin was separated from other proteins and unbound dye by chromatography on 'DEAE-Sephadex', using PBS as eluate<sup>15</sup>.

Slides were stained in damp chambers for 1 h at 37° C, and washed in 500 ml. of PBS for 15 min with gentle magnetic stirring. Control slides were treated with DNase for 1 h at 37° C, in PBS containing 0.05 mg of magnesium chloride/ml. and 0.1 mg/ml. of enzyme. Control sera included antiserum absorbed with native or

denatured DNA together with standard immunofluorescence controls.

Specimens were illuminated by a Sylvania L 50 mercury vapour lamp through a dark field condenser. They were viewed through apochromatic objectives containing iris diaphragms and immersed directly in buffered glycerol, using a Zeiss UG2 (4 mm) exciter and No. 47 barrier filters, and were photographed through a UG2 exciter and No. 47 barrier filters on 'Agfachrome' film using 3 and 6 min exposures.

Interphase nuclei (Fig. 2) presented two patterns. Most frequently there was a collection of spots of various sizes throughout the nucleus on a very faint uneven background. This appeared in very few cells collected before 20 h, but the proportion increased thereafter and by 45 h most cells contained many spots. Their number increased with the age of the culture rather than with nuclear size. In early cultures the nucleolus was rarely stained (Fig. 2b), but older nuclei usually showed nucleolar fluorescence, occasionally multiple (Fig. 2c).

The second pattern occurred in small nuclei with a bright diffuse fluorescence often with a particularly bright zone at the nuclear membranes. Some cells were an irregular composite of both diffuse and spotted patterns, and this suggested that the diffuse reaction was an artefact. More intense heating of slides (such as 80° C for 1 min) promoted diffuse fluorescence and confirmed this suspicion.

Chromosomes in prophase fluoresced uniformly (Fig. 3a). There was some variability in intensity; certain chromosomes had brighter fluorescence at the periphery, and others contained intenser segments within the body of chromosomes (Fig. 3b). It was not possible, however, to distinguish given chromosomes from cell to cell by their reaction, though in each cell all chromosomes fluoresced to a greater or lesser degree.

In metaphase, there was bright fluorescence in discrete areas along the arms of chromatids with weak fluorescence between (Fig. 3c). In some spreads bright fluorescence appeared throughout all chromosomal material, but, as in interphase, heat promoted this diffuse reaction (Fig. 3e). Preparing slides without heating preserved the discrete reaction, but also gave poor separation of chromosomes.

The discrete fluorescent areas were irregularly shaped, some being small and circular, and others extending some way along the chromatid (Fig. 3d). Between one and five spots appeared in most chromosomes. In each nucleus most chromosomes reacted, but from cell to cell the distribution of reactions varied. Within a chromosome the spots were asymmetrically dispersed, and chromatids of a single chromosome (isologous) often reacted differently (Fig. 3d). The most frequent sites of attachment were telomeric or beside secondary constrictions. Centromeres were occasionally stained, but secondary constrictions and satellites were only reactive in strongly heated preparations (Fig. 3e).

There was no fluorescence if serum was absorbed with denatured DNA, but it occurred if native DNA was used. Nuclei treated with DNase no longer reacted (Fig. 3f). Methyl green (at pH 3.8) stained discrete areas densely in interphase nuclei, but not in chromosomes (Fig. 2d and e).

Ambivalence of such enzyme and histochemical controls has been encountered previously<sup>6,7,11,12</sup> and attributed to problems of their specificity and dependence on polymerization as well as chemical content of condensing chromosomes. If the strong attachment to localized spots is interpreted as specific reaction with areas rich in single stranded DNA, however, the distribution should agree with chromosome replication, and, possibly, template formation of RNA. Nucleolar fluorescence in young cells is consistent with template provision for active RNA synthesis found in phytohaemagglutinin cells during the *G*<sub>1</sub> period. Other nuclear and nucleolar reactions occurred as frequently as the uptake of labelled



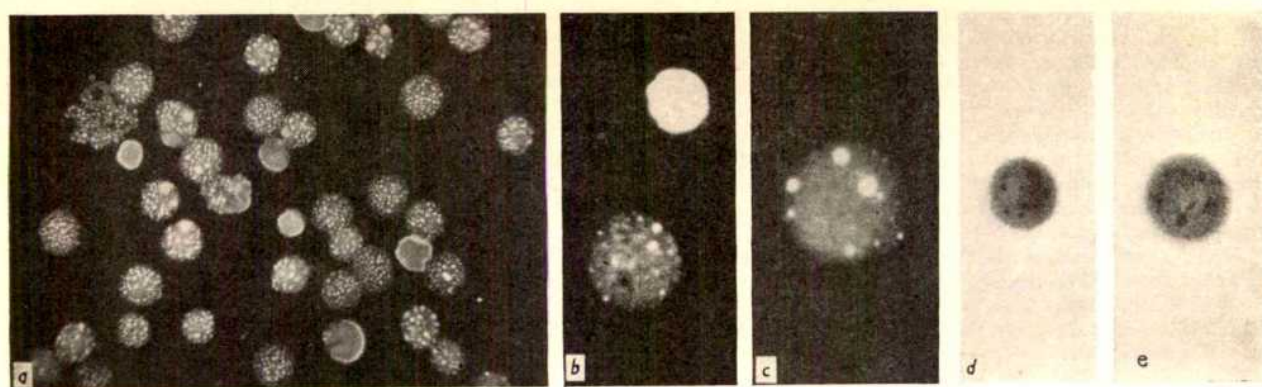


Fig. 2. Distribution of fluorescein-labelled antinucleotide antiserum in interphase. (a) General view of spotted and diffuse patterns. Some cells are a composite of both patterns and the diffuse reaction is probably a heat artefact (see text). A metaphase complement is present at the top left. (b) Nuclei from a young (30 h) culture, one showing a spotted pattern with an unstained nucleolus, the other reacting diffusely. (c) Nucleus from a mature (48 h) culture with several large nucleolar-like areas showing fluorescence. (d) and (e) Methyl-green stains of similar nuclei with chromocentre-like areas staining densely.

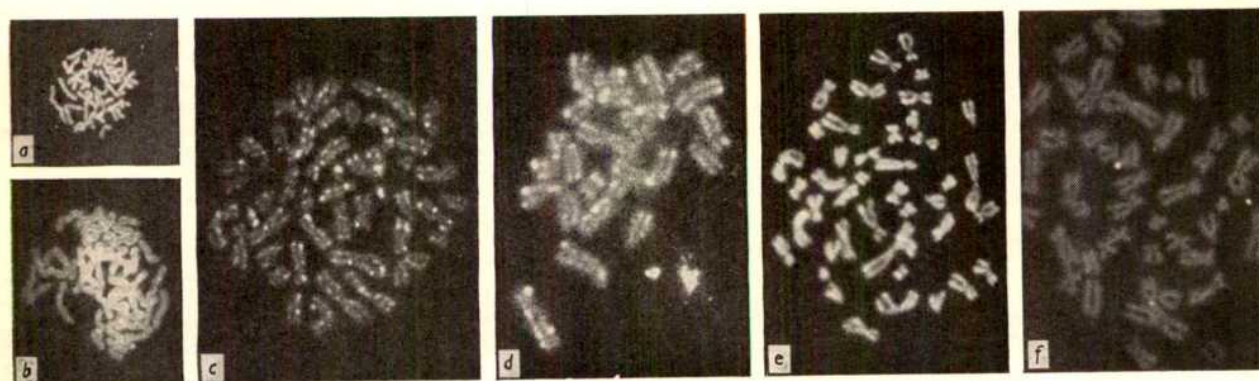


Fig. 3. Distribution of fluorescein-labelled antinucleotide antiserum in mitosis. (a) Prophase chromosomes fluorescing throughout, with particular intensity at the periphery. (b) Prophase chromosomes showing general fluorescence but with some variation within their substance. (c) Metaphase chromosomes with discrete areas of fluorescence against a faint background. (d) Metaphase chromosomes at high magnification to show asymmetrical distribution of fluorescence. (The brighter material at lower right is cell debris fluorescing blue.) (e) Increased fluorescence in metaphase chromosomes which have been heated. (f) Decreased fluorescence in metaphase chromosomes which have been treated with DNase.

thymidine in the *S* period, and probably represent replication both among nucleolar organizers and other chromosomal segments. The faint background attachment is interpretable as a weak cross reaction between native DNA and antibody primarily directed against denatured DNA. The intensification of the background by heat, a phenomenon noted in other immunofluorescence work<sup>16</sup>, is probably, in this case, an artefact of heat denaturation.

These findings imply that the substrate is DNA in a template forming or replicating mode, during which short segments of the polymer reveal, *en passant*, successive antigenic sites. The presence of single stranded DNA in mitosis indicates a residuum or continuation of replication and template activity during condensation and segregation. The possibility of residual replication does not agree with autoradiographic evidence of incorporation of thymidine which ceases before the  $G_2$  period<sup>17</sup>. On the other hand, the possibility of continuing template formation concurs with the presence of uridine detected<sup>18,19</sup> in prophase and, possibly, metaphase; and would also be likely because of the genetic control of karyokinesis implied by non-disjunctional genes<sup>20</sup>. If chromosomal genetic activity continues during metaphase, furthermore, it seems reasonable that the active loci should occur telomerically or at heterochromatic junctions, because these parts of compact chromosomes may offer favourable sites for DNA to uncoil freely. Reactive sites vary between sister chromatids as well as between whole chromosomes or complements. This unbalanced content of single

stranded DNA must influence chromosomal dimensions and is reflected in certain asymmetrical characteristics of chromosomal behaviour, such as differential condensation rates within and between chromosomes<sup>21,22</sup>; unequal chromatids, such as seen in endoreduplication<sup>23</sup>, and dissimilar replication patterns in homologues<sup>17</sup>. Another consequence would be the statistical nature of homologue pairing by morphological<sup>24</sup> and autoradiographical appearances<sup>25</sup>.

These observations modify the proposition<sup>26</sup> that only a minor portion (about 20 per cent) of lymphocyte chromatin is active. In dividing cells, metabolic demands change through the cell cycle, and the active fraction may not always contain the same genes. Thus although there were complements in which all groups, including X chromosomes, fluoresced in both arms, this only reflects the replication implied by presence of RNA, detected before<sup>27</sup> throughout all chromosomes including both members of the X pair, and cannot be used to estimate the extent of inactivation implied by dosage compensation<sup>28</sup>. Fluorescence of interphase sex chromatin could not be distinguished, and activity in mitosis, where a minimum of gene control for self-condensation and spindle attachment may be required of each chromosome, need not imply activity during other periods of the cell cycle. Dosage compensation may represent an extension of the inactive phase in a cycle common to all chromosomes, but not necessarily maintained throughout mitosis. In much the same way, the needs of cell division in regenerating tissues<sup>29</sup> call into expression gene groups, detectable by

hybridization as single stranded segments of chromosomal DNA, which otherwise remain inactive.

In the circumstances of these experiments, it appears that in lymphocytes at least chromosomal gene activity is dynamically distributed in time and site. Its randomness suggests that there may be a more or less constant oscillation between repression and depression. Possibly dynamic genes are more easily modulated, and are more open to structural interchanges which occur somatically in normal<sup>30</sup> as well as aberrant cells. A structural basis for such behaviour is offered by the observation<sup>31</sup> that flexibility of hydrogen bonds within DNA allows thermodynamically feasible twists between open and coiled states. In the uncoiled state, phosphate repulsion between strands would serve to separate many fluctuating single stranded segments as they surface, without disturbance of the overall structure. Such a system would reveal, transiently, the short segments of single stranded DNA seen in the experiments represented here.

Continual gene availability is a pre-requisite of genetic systems in which control and structural elements are separated<sup>32</sup> and in which epigenetic control over differentiation<sup>33</sup> or phenotypic expression in heterokaryons<sup>34</sup> is mediated through extra-chromosomal factors, possibly similar to ribosomal regulators of bacterial RNA and protein synthesis<sup>35,36</sup>. Similarly, the observations reported here provide a possible basis for systems in which the DNA molecule acts as a coding device only, while mutation resides in euchromatin-heterochromatin transitions<sup>37,38</sup>, and gene expression is under the permissive control of a cytoplasm in contact with the demands of the environment<sup>39-42</sup>.

Finally, these antisera offer resolution and specificity not available with natural antinuclear sera from autoimmune disease. The valuable work of previous workers<sup>16,43,44</sup> showed that the use of natural antisera in cytological analysis was only limited because they contain unknown mixtures of antibodies. Artificially specified antisera may prove of greater interest in tracing chromosomal constituents and their relation to chromosomal aberration<sup>45,46</sup>.

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## A Limiting Microfibril Model for the Three-dimensional Arrangement within Collagen Fibres

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Consideration of evidence of the mode of polymerization of collagen monomers provides a more detailed picture of the three-dimensional organization of the fibrils in a collagen fibre.

In his classic review Bear<sup>1</sup> described the hierarchies of structural order within a macroscopic collagen fibre in terms of aggregates of fibrillar units of increasing size. Several investigators have tried to give a more detailed

picture of the three-dimensional organization of the fibrils in terms of the packing of the fundamental macromolecular units, the collagen monomers. These attempts, based primarily on X-ray diffraction and electron microscope



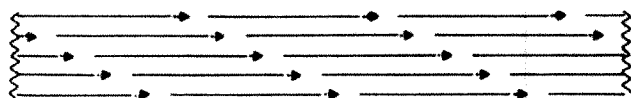


Fig. 1. The quarter-stagger, end-overlap arrangement of collagen monomer units according to the proposal of Petruska and Hodge<sup>10</sup>.

observations, have been very fruitful, but have not been entirely successful. Further consideration of evidence on the mode of intermolecular polymerization of collagen can provide still further insight into this problem.

Although the point has been questioned recently<sup>2</sup> we begin with the assumption that the collagen monomer unit has the same rod-like form in the native fibril that it has in solution<sup>3-5</sup>. The rods, approximately 3000 Å long and 15 Å across, are asymmetric or polarized in the sense that the regions containing polar and apolar side chains are not distributed uniformly along the length of each rod. This is shown quite clearly in electron micrographs<sup>6-8</sup> of positively stained segment long spacing precipitates. Hodge and Schmitt<sup>9</sup> demonstrated that the native fibre was composed of monomer units oriented so that all monomers had the same polarity but with each monomer displaced by one-quarter of its length from its nearest neighbours. More recently, Petruska and Hodge<sup>10</sup> showed that each molecule is 4.4 times the length of the fundamental native repeat of 695 Å and modified the "quarter-stagger" arrangement with the suggestion that there was no head-to-tail association of chains in a continuous array. There was, rather, a "hole region", 0.6 times the length of the basic repeat distance ( $D$ ), between monomers in the same axial disposition. It was proposed that an end-region overlap, 0.4  $D$  plausibly, as illustrated in Fig. 1, stabilized the fibre assembly, in agreement with the observations of Olsen<sup>11</sup>. This arrangement very plausibly explains the cross-striation pattern seen in electron micrographs of native collagen fibres in a two-dimensional representation. As Smith<sup>12</sup> and others<sup>13,14</sup> have shown, however, one cannot make a three-dimensional arrangement which maintains a quarter-stagger relationship between every nearest neighbour monomer unit. Smith<sup>12</sup> deduced that only about 67 per cent of monomer contacts in the native structure could be in quarter-stagger agreement. Grant *et al.*<sup>15</sup> arrived at the similar conclusion that parallel monomer units could not have quarter-stagger arrangements throughout a fibril. On the other hand, the appearance of a systematic set of holes within a collagen fibril seems well established<sup>10</sup>.

These representations treat the collagen monomer as if it were a rigid rod which has a radial symmetry of its properties at any particular point along the rod, that is, in directions perpendicular to the rod axis. This is obviously not the case. Each monomer unit is composed of three peptide chains, two of which (designated  $\alpha 1$ ) are essentially identical while the third peptide strand,  $\alpha 2$ , has a different composition. Each chain is wound as a left-handed helix,

but the three helices are wound into a compound right-handed helix which makes a complete turn every 90 Å. At any particular level along a rod, the chains may be disposed as indicated in Fig. 2a or 2b.

As the individual strands wind up the compound helix, the positions of the chains shift with respect to the external co-ordinates of the monomer rod, so that, as shown in Fig. 2c, the side chain dispositions also vary. The monomer rod can be thought of, in essence, as a helically striped pole in which one band (the two  $\alpha 1$  chains) is twice as wide as the other (the  $\alpha 2$  external projection). Positive staining with cationic and anionic electron dense stains shows that both positive and negative side chain functional groups appear in the same regions in native fibrils. Hence, to assure fibril stability, the packing of the monomer rods must be such that attractive electrostatic interactions between rods are maximized. To achieve this, the side chains of the adjacent molecules must be suitably disposed and this implies that a rotational phasing perpendicular to the monomer unit long axis must exist in addition to the axial quarter-stagger.

Insight into the nature of the rotational phasing comes from studies of the intermolecular polymers found in native collagen fibres and on the distributions of molecular species. The presence of intermolecular cross-linkages in mature collagen has been amply demonstrated<sup>16,17</sup>. Two features are particularly important for the present discussion. First, the intermolecular polymers fall into distinct weight classes:  $2 \times 10^5$  ( $\beta_{22}$ )<sup>16</sup>  $3 \times 10^5$  ( $\gamma$  components)<sup>16,18,19</sup>, and  $1.2 \times 10^6$  ( $\delta$  components). Veis *et al.*<sup>18</sup> pointed out the fact that polymers with molecular weights intermediate between  $\gamma$  and  $\delta$  were not detected and suggested that there was some natural unit, corresponding to a tetramer of collagen monomers, which was highly internally cross-linked and hence particularly stable. Subsequent work<sup>19</sup> using high resolution disc electrophoresis, ultracentrifugation and carboxymethyl cellulose chromatography confirmed the absence of intermediate size polymer species.

The second point concerns the nature of the  $\gamma$  components. Veis and Anesey<sup>19</sup> showed that all four possible  $\gamma$  components were present in extracts of mature collagen:  $\gamma_{111}$ ,  $\gamma_{112}$ ,  $\gamma_{122}$ , and  $\gamma_{222}$ . Of these, the intermolecular species  $\gamma_{111}$  and  $\gamma_{222}$  were quantitatively the principal components, and they appear to be polymerized in distinctly different ways. Chromatography of gelatines rich in these components have been carried out on carboxymethyl cellulose according to the technique of Veis and Anesey<sup>19</sup> and the appropriate fractions taken. After desalting, the  $\gamma_{111}$  and  $\gamma_{222}$  fractions were dissolved in dilute, salt free 0.1 molar acetic acid and renatured by cooling solutions to 4°C. Addition of salt free ATP to the  $\gamma_{111}$  solutions resulted in precipitation of copious amounts of segment long spacing collagen, Fig. 3, which in both positive and negative staining shows the presence of spools of monomer length,  $\sim 3000$  Å. In sharp contrast to these results, the identical renaturation and precipitation procedures did not lead to SLS collagen when applied to  $\gamma_{222}$  gelatin. Fibrous

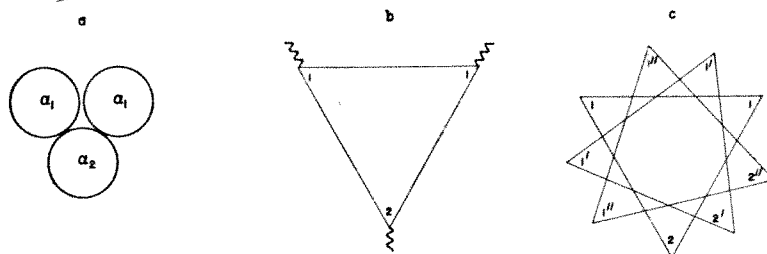


Fig. 2. Cross-section representations of a collagen monomer. a, The conventional representation at a particular level, showing the two  $\alpha 1$  chains and the  $\alpha 2$  chain; b, a triangular representation of the triad cross-section. The corners are marked with chain type designations. The lines emanating from the corners indicate the positions of the side chains; c, three successive residue levels in the compound helix, showing how the relative chain and hence side chain orientation vary with respect to a fixed frame of reference as one progresses up the compound helix.

precipitates were obtained in this case which gave the native type of periodicity, Fig. 4.

One conclusion drawn from this set of observations is that  $\alpha 1$  chains can be aligned and intermolecularly cross-linked with their lengths in register. On the other hand, the  $\alpha 2$  chains must be aligned and intermolecularly cross-linked in the quarter-stagger array. In aligning helices of the same pitch, it can readily be seen that if there is some regular periodic variation in property at any particular helix position in each helix, then alignment of the two helices to bring these sites together at some common point will also align all other equivalent sites. To abut common sites, however, one helix must be rotated about its long axis. These considerations indicate that if a particular pair of  $\alpha 2$  chains on adjacent molecules are properly disposed for cross-link formation, then  $\alpha 2$  chain sites must come together periodically at other places in equally suitable juxtaposition. There are therefore no problems in constructing intermolecular polymers of  $\gamma_{222}$  type in a quarter-stagger structure.

The appearance of  $\delta$ -component polymers with no species intervening between it and the  $\gamma$ -type polymers, as pointed out earlier, suggested that a tetramer of monomer rods represented a fundamental packing unit within a fibril. Olsen<sup>20</sup> noted that native fibrils in negative stain electron micrographs appeared to be composed of sub-filaments with diameters representing one to four monomer molecules. A suitable structure, preserving the quarter-stagger-overlap ordering, is a right-handed helical arrangement of four monomer units displaced successively from the origin by 0, 1, 2 and 3 fundamental repeat distances,  $D$ , along the axis and  $90^\circ$  about the axis. A second  $\delta$ -unit

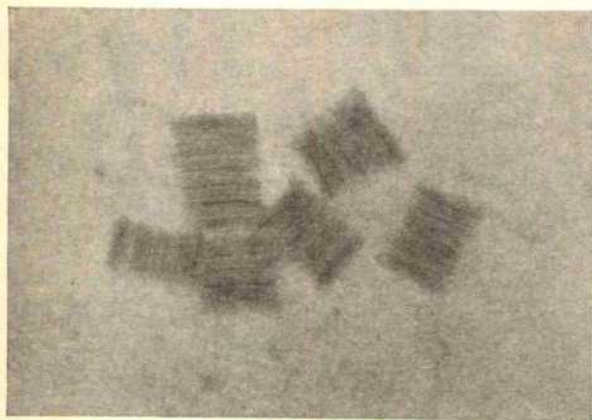


Fig. 3. Segment long spacing collagen prepared from  $\gamma_{111}$  gelatine isolated by carboxymethyl cellulose chromatography. Stained with phosphotungstic acid, pH 2.0, and saturated uranyl acetate. ( $\times 45,000$ .)

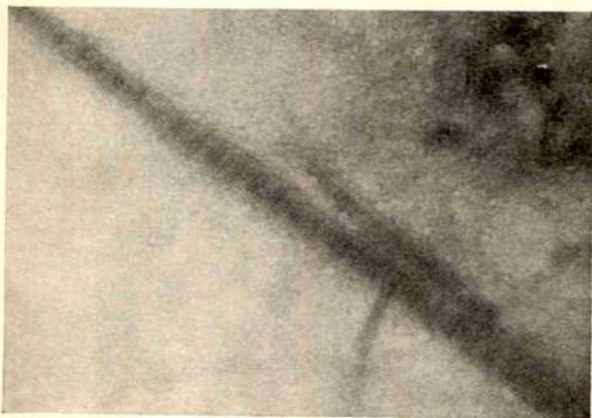


Fig. 4. Native type fibres produced from a  $\gamma_{222}$  rich gelatine fraction isolated by carboxymethyl cellulose chromatography. Stained with phosphotungstic acid, pH 2.0, saturated uranyl acetate. ( $\times 45,000$ .)

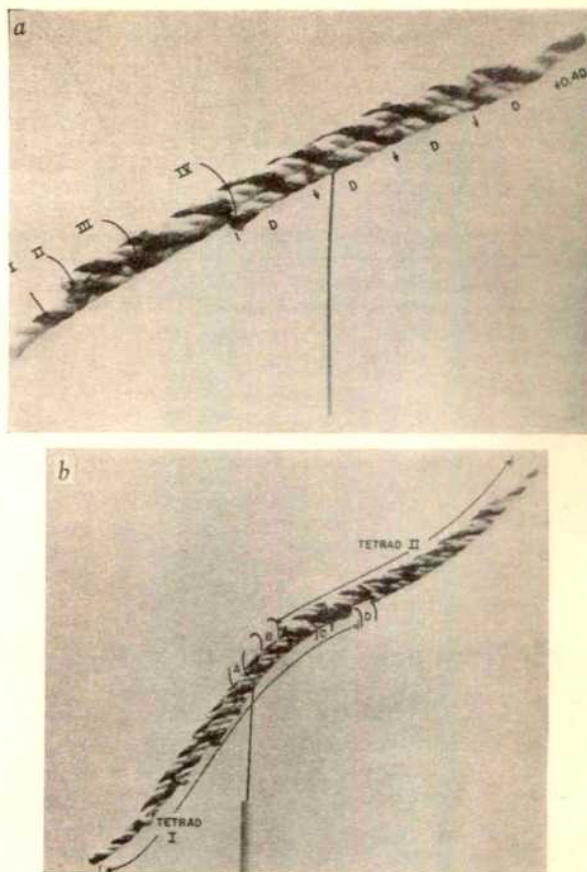


Fig. 5. Models of the limiting microfibril. *a*, The fundamental tetrad of collagen monomer units. The  $\alpha 2$  chains have been blackened. Each monomer is displaced by  $1D$  from the  $A$  end of the adjacent monomer unit; *b*, two tetrads packed together into a continuous filament showing the positions of the  $0.6D$  holes or spaces and the overlap between tetrads.

tetramer then fits directly into the first tetramer, leaving a  $0.6D$  unit hole and a  $0.4D$  overlap, and this process may continue to make microfibrils of indefinite length in which each monomer is in the quarter-stagger with respect to its neighbours. The objections of Smith<sup>12</sup> and Grant *et al.*<sup>15</sup> to the overall quarter-stagger structure can be accounted for by the side by side packing of the limiting fibrils. The in-register packing of fibrils has the advantage that in its interfibril cross-linkages can lead to the  $\gamma_{111}$  type of side by side polymer. The model is illustrated in Fig. 5.

This model appears to be in accord with the behaviour of collagen fibres. In contrast to the sheet-like strictly quarter-stagger arrays, it provides for the filament character of the collagen fibril. When fibrils are swollen in acid one sees at the swollen fraying ends readily separable small discrete fibrils. There is also the proper variation in fibril density as the system of holes pervades even the smallest unit so that the density differences described by Grant *et al.*<sup>15</sup> are present at all structure levels. Acid-swelling of the collagen can be assumed to occur by solvent imbibition and separations of the limiting microfibrils without disruption of the basic spacings. Burge *et al.*<sup>21</sup> showed that in spite of 400 per cent increases in width and 100 per cent increase in volume of the fibres the first order X-ray diffraction equatorial spacing perpendicular to the fibre axis increased from only 13 to 15 Å in the pH range 2–12. The constraints preventing unlimited swelling and dissolution of the fibrils would be the interfibril bonds. In one of the earliest studies of the depolymerization of insoluble collagen, Veis and Cohen<sup>22</sup> suggested that there had to be at least two independent systems of transverse



intermolecular cross-linkages in mature insoluble collagen. The present model represents these two modes of polymerization, one set (the  $\alpha$  2-system) joining the tetramers into the limiting microfibrils and the other set (the  $\alpha$  1-system) joining the fibrils into the infinite insoluble network characteristic of insoluble collagen. Finally, while fibrils reprecipitated from salt or acid solutions of the soluble collagens may well appear to have aggregated in an essentially random fashion as suggested by Grant *et al.*<sup>15</sup>, it seems unlikely that the *in vivo* aggregation of collagen could be random as well. Tissue specific fibril packing, degree of polymerization and modes of cross-linking all appear to be too well controlled to have resulted from an initial random monomer axial alignment.

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## A Membrane Effect of Basic Polymers dependent on Molecular Size

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Albumin penetration into animal cells is stimulated by basic polyamino acids and DEAE-dextran, and increases with the size of the polymers. This dependence on size suggests that multiple attachment of a macromolecule to the membrane is necessary for biological function.

TUMOUR cells in culture will take up protein molecules such as albumin and ferritin<sup>1-4</sup>, and this transport can be enhanced by the presence of protamine, histones and basic polyamino-acids in small concentrations<sup>5</sup>. Studies of the uptake of albumin by cells of sarcoma S-180 suggest that polymers of L-ornithine, L-arginine, L-histidine and D-lysine of high molecular weight can act in amounts of 0.01  $\mu\text{g}/\text{ml}$ . and can produce a ten- to thirty-fold increase in the uptake of albumin in concentrations of 3  $\mu\text{g}/\text{ml}$ ., but in the same conditions free amino-acids and diamines such as spermine and spermidine with a small molecular weight have no effect on albumin uptake<sup>5</sup>. The question therefore arises as to whether a minimum molecular size is necessary to cause stimulation. We have set out to examine the supposed relationship between biological activity and molecular size, and have also examined the possibility that basic macromolecules which are not peptides—DEAE-dextran, for example—may possess a similar ability to stimulate albumin uptake. (DEAE-dextran (diethylaminoethyl-dextran) was tested in the sizes of molecular weight 2,000,000 (commercial preparation) and molecular weight 80,000. The latter was a gift from the Research Division of Pharmacia, Upsala. The ratios of glucose:DEAE of these two preparations were 2:1 and 2:7 respectively.)

We used the technique developed in this laboratory<sup>1,5</sup> to follow the uptake of labelled albumin into tumour cells. Sarcoma S-180 cells were grown in monolayer cultures rinsed with serum-free Eagle's medium and incubated for 60 min at 37° C in 1 per cent horse serum in the presence of albumin labelled with iodine-131 and basic polymers. (The activity of the incubation mediums varied between  $2.5 \times 10^5$  and  $5.2 \times 10^5$  C.P.M./ml.; the specific activity in 1 per cent horse serum was of the order of 5–10  $\mu\text{C}/\text{mg}$ .) They were then rinsed once with Earle's saline, detached by trypsinization and the cell suspension was washed

twice in saline and treated with 5 per cent trichloroacetic acid. The acid insoluble fraction was washed, counted on 'Millipore' filters, and its specific radioactivity was determined<sup>1</sup>.

The effect on albumin uptake of poly-L-ornithine and poly-L-lysine with different molecular weights in concentrations of 3  $\mu\text{g}/\text{ml}$ . is shown in Table 1, column A. (This is ten times less than the concentration which, in the same conditions, produces the first signs of abnormal cell

Table 1. CORRELATION BETWEEN SIMULATION OF ALBUMIN UPTAKE AND MOLECULAR WEIGHT OF POLYMERS BELONGING TO FOUR HOMOLOGOUS SERIES

Homologous series	Molecular weights ( $\times 10^{-3}$ )	No. of experiments	Effect in % increase above control uptake	
			A (3 $\mu\text{g}/\text{ml}$ .)	B ( $1.5 \times 10^{-6}$ molar)
Poly-L-ornithine	200	(9)	2,680	2,680
	130	(4)	2,200	1,430
	90	(3)	1,718	774
	45	(5)	976	220
	4*	(7)	509	10
Poly-L-lysine	225	(5)	872	960
	125	(4)	796	496
	50	(2)	417	104
	20	(5)	600	60
	6-7	(2)	528	17-7
Poly-D-lysine	3-1	(2)	597	8-2
	113	(9)	1,912	1,078
	65	(9)	1,211	360
DEAE-dextran	19	(9)	590	58-3
	2,000	(3)		890
Spermine ‡	80†	(3)	33-7	13-5
	0-20	(14)	4	0

The effect is expressed as per cent increase above the uptake of control cells. Column A gives the stimulations caused by 3  $\mu\text{g}/\text{ml}$ . of each compound. Column B gives the effect of a  $1.5 \times 10^{-6}$  molar solution of each compound.

\* Poly-D-L-ornithine.

† Because of its slightly lower degree of DEAE substitution and its greater ash content, this dextran was used in the corrected concentration of 3-78 instead of 3-0  $\mu\text{g}/\text{ml}$ . The dextran MW  $2 \times 10^6$  was tested directly at the concentration of  $1.5 \times 10^{-6}$  molar.

‡ Includes data obtained with 10, 30 and 100  $\mu\text{g}/\text{ml}$ .

reaction, that is, a beginning detachment from the glass. Unless otherwise mentioned, all concentrations of basic polymers were  $3 \mu\text{g/ml.}$  On a weight basis ( $3 \mu\text{g/ml.}$ ) the effects of the different poly-L-lysines are rather similar, but those of poly-L-ornithine decrease sharply at a molecular weight of less than 90,000. It had already been observed<sup>5</sup> that identical amounts of poly-L-lysine varying in molecular weight between 15,000 and 70,000 do not have markedly different effects. When, however, the effects of the L-lysine and L-ornithine polymers are expressed on a molar basis (column B), they decrease sharply with decreasing molecular weight and spread over a range of more than  $10^3$ . Column B is derived from column A by correcting for the differences in molarity. (This conversion is justified by the finding that the dose-effect curves of homopolymers of different sizes converge towards the same threshold and that in all cases  $1.5 \times 10^{-8}$  molar lies well above threshold. Furthermore, an identical relation between effect and size was observed when poly-L-ornithines of different sizes were used in the same experiment in concentrations of 0.3 and  $30.0 \mu\text{g/ml.}$ ) When plotted on a double log scale against the molecular weight, the figures in column B fall on two straight lines (Figs. 1 and 2, solid lines). Several molecular sizes of poly-D-lysine were available for testing. Their effect is also shown in Figs. 1 and 2 (closed circles). It is evident that they fit the curve for poly-L-lysine better than the curve for poly-L-lysine. This confirms the earlier observation that poly-D-lysine is a more powerful stimulant of albumin uptake than poly-L-lysine, and is comparable in effect to poly-L-ornithine<sup>5</sup>.

The intersection of the solid lines of Figs. 1 and 2 with the abscissa indicates that the minimum molecular weights required to elicit the membrane effect are approximately 500 and 900 for  $1.5 \times 10^{-8}$  molar solutions of the poly-amino-acids; they correspond to tetra- and octa-peptides. They are greater than the molecular weight of the polyamines spermine (203) and spermidine (146), which may explain why those amines are inactive in the stimulation of albumin uptake<sup>5</sup>.

In spite of the totally different nature of its repeating unit, DEAE-dextran is able to mimic the effect of basic polyamino-acids. Table 1 gives some results obtained with DEAE-dextran of two different molecular sizes. Both on a weight and molar basis, the substituted dextrans are less active than the polyamino-acids, but as with the polyamino-acids, the DEAE polymer of smaller size has a smaller effect. The relation between action and size of the two dextrans is represented graphically in Fig. 1 (interrupted line to the right). Obviously the same general

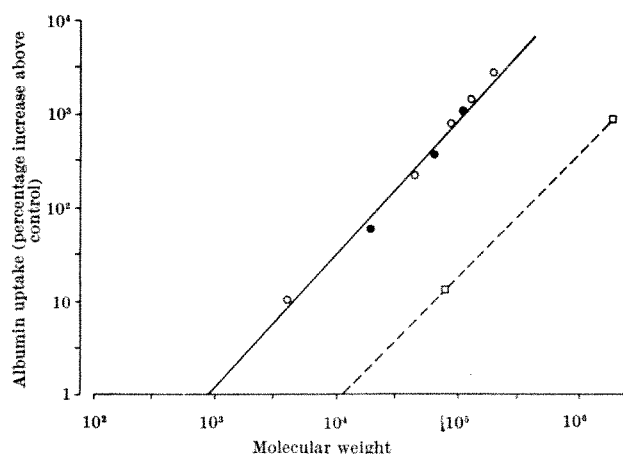


Fig. 1. Correlation between activity and molecular weight of polymers in homologous series of poly-L-ornithine (open circles), poly-D-lysine (closed circles) and DEAE-dextran (squares). Ordinate: stimulation of albumin uptake expressed in per cent increases above control uptake. The lines of best fit intersect with the abscissa at MW of 900 (DP, 8 ornithine) and 11,000 (DP, 52 glucose).

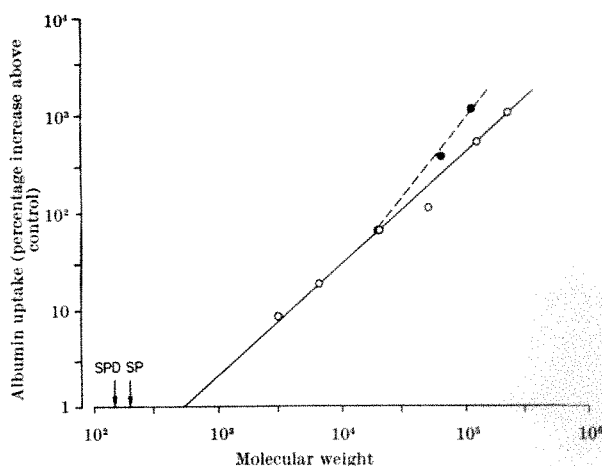


Fig. 2. Same correlation as in Fig. 1 for a homologous series of poly-L-lysine (open circles). The poly-D-lysine values of Fig. 1 are redrawn for comparison (closed circles). Intersection at molecular weight 500. Tested in amounts of  $3 \mu\text{g/ml.}$  or more, spermine (SP) and spermidine (SPD) had no significant effect.

correlation exists for both classes of compounds, although they differ markedly in the molecular size required for comparable effects. Thus the smallest effective molecular weight of DEAE-dextran is of the order of 11,000, corresponding to fifty-two glucose units, compared with 500 to 900 for the polyamino-acids.

These observations represent an interesting example, possibly the first, of a biological effect on the cell membrane which is clearly related to the molecular size of the agent responsible. It is reminiscent of the data of Kat-chalsky *et al.*<sup>6</sup> concerning the concentrations of polylysine required to agglutinate red blood cells; five times as much polylysine was required when the degree of polymerization of homopolymers was decreased from 200 to 16. The simplest way to account for the relation between the size and activity of polymers in a homologous series is to assume that stimulation of albumin uptake requires the attachment of the polymer molecule by more than one basic group and that the effect increases with the number of bonds between the polymer and the cell membrane. In the conditions of our experiments, the polyamino-acids are in the form of flexible random coils<sup>7,8</sup>, so that the number of basic groups likely simultaneously to reach the plane of the cell surface can be expected to increase with molecular size.

If the notion of multiple attachment is correct, the activity of a polymer should decrease when the spacing between amino groups is increased in the primary structure and when the molecule as a whole becomes more rigid because of its tertiary structure, and this expectation appears to be borne out by the data on DEAE-dextran. The dextrans used in this study have a glucose to DEAE ratio of 2:1 and 2:7, which fixes the distance between basic groups at approximately twice that in the amino-acid polymers. This doubling of the interval, together with the rigidity caused by the intramolecular cross-linking characteristic of dextrans, may explain why a larger molecular size is necessary for action.

The same structural factors may also explain why histones are considerably less active than homopolymers of most basic amino-acids<sup>5</sup>. Because only between a quarter and a third of the amino-acids in histone molecules are basic, the average distance between  $\text{NH}_3^+$ -groups will be correspondingly longer than in homopolymers of basic amino-acids. Furthermore, parts of the histone molecules are considered to be in the  $\alpha$ -helical form<sup>9</sup>, which, with the appreciable content of hydrophobic amino-acids, confers a degree of rigidity on the histone structure that is lacking in basic homopolymers. The ineffectiveness of a strongly charged diamine such as spermine suggests that a three-point attachment to the membrane may be

necessary to elicit a response. This requirement could be met by a tetrapeptide.

The nature of the interaction between basic polymers and cell surfaces is not known, but recent physico-chemical studies on isolated tumour cell membrane<sup>10,11</sup> are relevant. Thus part of the membrane protein is in the  $\alpha$ -helix form, and it has been suggested that some helical segments of the molecule have orientation normal to the plane of the membrane and reach into the lipid layers, whereas other segments reside in the surface layer. A polybasic compound attached to the cell membrane may be able to influence the conformation of membrane proteins by changing the charge distribution on segments of peptide chains. Multiple attachment to neighbouring points of the membrane could strain the membrane structure, and one basic macromolecule could be expected to interact in this way with several structural proteins and to have a profound effect on membrane structure and function. Although new data on protein orientation and conformation in the cell membrane<sup>10,11</sup> offer an improved basis for understanding the effect of basic polymers on cell membranes, there is also evidence of small amounts of RNA in the plasma membrane of tumour cells<sup>10,12</sup>. Because of the physiological association of basic proteins and nucleic acids in the nuclei, it is worth suggesting that a comparable interaction may occur between polyamines and membrane-RNA.

The relation between activity and molecular size described previously also throws new light on the mechanisms underlying pinocytosis. Previous electron microscope studies have shown that cells of Ehrlich ascites carcinoma and sarcoma S-180 take up large proteins

such as ferritin by pinocytosis, and there is evidence that in other tissues labelled albumin is taken up by the same mechanism. Electron microscopic studies in progress have shown that poly-L-ornithine in the condition of these experiments increases the uptake of ferritin and labelled albumin in pinocytotic vesicles (unpublished results of Revel and Ryser). It is suggested that the multiple attachment of a large basic polymer to membrane constituents and the conformational changes resulting from this interaction constitute a stimulus sufficient to induce pinocytosis.

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## Tolerated Infection with the Sub-bacterial Phase of *Listeria*

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Auto-immune and malignant disease in man may be the result when tolerance to the mycoplasma phase of *Listeria* is disturbed.

DESCRIPTIONS of micro-organisms associated with the lesions of auto-immune and malignant diseases of man have been, and still are, so frequently made that only a small selection of the published literature can be included in the references of this paper. Claims that these may represent the causative agents of the diseases have been treated with great reserve. In similar diseases of animals, however, where experimental studies can more readily be made, there is much evidence for the role of infective agents.

There are several reasons for this sceptical attitude, but an important one has been the lack of an acceptable hypothesis to replace the classical theories of microbial or virus infection in these diseases. Such a hypothesis must account for the apparent anomalies, and especially for the failure to identify a distinctive pathogen, regularly associated with each condition; for the frequent absence of a demonstrable immune response; and for the obvious roles of carcinogens, hormones, shock effects and similar non-infective agents. Suspicion has been fostered by evidence both of contamination of tumours *in vivo* and of cultures *in vitro*, especially, in the latter case, with micro-organisms such as mycoplasmas and diphtheroid bacteria that are commonly presented as potential pathogens. These are found in eggs, animal sera and tissue cultures, all of which have been extensively, but not exclusively, employed in the studies in question.

I believe that these difficulties and apparent anomalies can be explained. Comparison of published evidence with the results of investigations in this laboratory provides evidence to support the hypothesis that some—possibly but not necessarily all—forms of auto-immune and neoplastic disease in man can be mediated by disturbance of immune tolerance between the host and a widely distributed micro-parasite.

Such a hypothesis need not depend on or require the association of a specific pathogen with each pathological condition. The requirements for an infective agent in the role of mediator of auto-immune or neoplastic disease are: (a) that the infection be either congenital or acquired in early infancy, so that immune tolerance is achieved; and (b) that the base sequences of host and parasite nucleic acids be similar, so that episome units may be transferable. In effect, among the micro-organisms that might play this part, the sub-bacterial phase of *Listeria* (*Listerella*) seems best qualified to fulfil the requirements, and it is apparent that many of the best-attested descriptions of suspected infective agents in these diseases refer to phases of *Listeria*, the widespread occurrence and potential importance of which have only recently begun to be understood<sup>1-3</sup>, and are still far from fully recognized.

There is already a theoretical background for this argument. I have reasoned<sup>4</sup> that auto-immune disturbance can result from upset of tolerance, caused by sub-

cellular elements of bacteria, acting as episomes in body cells and Hotchin<sup>6</sup> suggested that "docile human agents" could cause chronic disease in late life, as the lymphocytic choriomeningitis virus does in mice. These refer to auto-immune disease, but suggestions that infective agents in neoplastic disease may act in a similar manner have also been made. There is evidence that defective rather than complete viruses are associated with malignancy<sup>6</sup>, that is, they act as episomes; they have appropriate base sequences<sup>7</sup>, and most, if not all, of them are either congenital or chiefly affect very young animals<sup>8,9</sup>.

*Listeria* can be found in the blood and tissues of a very high proportion of humans (as well as other animals) in a form very difficult to detect and recognize; it seems often to be congenitally acquired and is thus tolerated immunologically. The base ratios of its DNA are similar to those of its human host, in the region of 40 per cent<sup>10-12</sup>. In addition to the results of investigations made in this laboratory, and of other surveys of *Listeria* as such<sup>1,2,13</sup>, it is apparent from the descriptions of latent blood infections with micro-organisms, unidentified but exactly corresponding to *Listeria*, that it occurs in a large proportion of even apparently healthy persons<sup>14,16</sup>.

Murray<sup>2,13</sup>, a discoverer of *Listeria*, gives as general characters of this inadequately studied micro-organism that it is widely distributed among the individuals of at least twenty-seven species of animal; that it is liable to be confused with other bacteria or completely overlooked; and that it exhibits certain very unusual, puzzling and obscure appearances in its host-parasite relationship. These include a tendency to cryptic persistence of infection and to immune tolerance by the host. The classic sign of *Listeria* infection is monocytosis, but this is recognizable only in a minority of cases. Infective mononucleosis in man is believed by some authorities to be caused by a combined infection of bacillary *Listeria* with a virus<sup>17</sup>, which may well be the bacterial and the mycoplasma-like phases of the same organism. The term mycoplasma may be regarded, in this context, as synonymous with the *L*-form, because the sub-bacterial phase of *Listeria* possesses the characters of a mycoplasma, in its ability to form filaments of typical size and appearance, in its tendency to parasitism, and in its antigenic constitution, which is discussed later in this article.

The occurrence of what I now believe to be *Listeria* has been very widely reported from cases of rheumatic and neoplastic disease but its identity has not been established, and because of the very diverse identifications that have been made, the significance of these findings has not been recognized by bacteriologists and pathologists in general. Bacteria, especially cocci, diphtheroids and mycoplasma, have been isolated from arthritis and other auto-immune diseases<sup>14,15,18-23</sup>. Acid-fast bacilli, actinomyces, spirochaetes, even fungi and sporozoa, as well as viruses and, once more, mycoplasmas, have been reported in, and sometimes isolated from, neoplastic disease including leukaemia<sup>14,21,24-33</sup>. Most if not all the morphological appearances described, including the viruses (Fig. 1), are within the range of variation of *Listeria*, and in some cases these investigators have given recognizable descriptions of its morphological cycle<sup>14,16</sup>.

Comparison of strains of *Listeria* from eight cases of infectious mononucleosis, and two of *L. monocytogenes* from the National Collection of Type Cultures, with the illustrations and descriptions of previous workers, made it apparent that the appearances previously recorded are characteristic of newly isolated *Listeria*. The same organism was isolated from all the ninety-five human subjects examined, but there was much variation in the ease with which this could be done, depending on the apparent degree of infection. Isolations from serum or serous fluid could most easily be made by adding 1-5 ml. of the inoculum to 10 ml. of a simple fluid medium (for example, Brewer's medium) so that the inoculum itself provided the necessary enrichment; but it must be emphas-

ized that the early stages of culture would not be recognized as bacterial growth by anybody unfamiliar with the practical problem.

From the lesions of twenty-one cases of rheumatoid arthritis and eight of mitral stenosis, and from the blood of nine cases of malignant tumours and six of leukaemia and Hodgkin's disease, it was relatively easy to isolate *Listeria*, frequently in the bacterial phase (that is, "diphtheroids"). These figures are small, but are presented for the purpose of confirming the identity of the micro-organisms that have been described from similar sources in the past. From the blood of forty-nine healthy subjects it was isolated almost invariably in the mycoplasma phase, and the apparent degree of infection was less consistent, being so slight as to be detected only with difficulty in at least two-thirds of these. The remainder were moderate, except for three, not clinically ill, who were heavily infected and from whom the bacterial phase could be isolated. All specimens of the serum of the horse, ox and calf, and of the eggs of the domestic fowl, were infected in some degree, but these were chiefly pooled samples, and so the true incidence is not known. It is thus presumably congenital in the fowl, and may be so in man, because it is a common cause of abortion<sup>1</sup>, and was isolated, during this investigation, from the organs of four out of four miscarried infants.

It must be emphasized that this high degree of infection in healthy subjects is in accordance with current theories of infectious mediation of auto-immune disease<sup>4,5</sup>, and some oncogenic viruses have also been shown to be comparably widely distributed in healthy members of the affected species, and to cause tumours only exceptionally<sup>8,9,34</sup>. The healthy subjects should not be regarded as controls, as in classical infective theory.

The mycoplasma phase was isolated, either alone or with the bacterial phase, sometimes (as in healthy subjects) in very small quantities, as cysts, about 4  $\mu$  in diameter, staining poorly with basic dyes, but showing weakly Gram-positive granules in a pale, Gram-negative matrix. These are readily mistaken for dried debris and thus overlooked, especially if scanty, but in the electron microscope they can clearly be recognized as aggregates of very tiny spheres (Fig. 1). In culture, growth sometimes proceeds rapidly, so that in the course of about 48 h typical mycoplasma filaments develop from the cysts, or this process may be delayed for several weeks. Cultures that are both initially scanty and then slow to grow are liable to be discarded as negative. There is little doubt that this has happened many times in the past. Transformation into the bacterial phase sometimes occurs in a few days, or may do so only on prolonged culture. About 20 per cent of the isolates made on this occasion eventually reverted to typical *Listeria* morphology.

This transformation has been figured by other workers, notably Villequez<sup>16</sup> and Alexander-Jackson<sup>14</sup>, who did not, however, identify *Listeria* but regarded their isolates as new species associated principally with cancer, although they both also recognized a possible link with arthritis. The clearest and most complete illustration is that given by Turner<sup>35</sup> in his description of the life cycle of the organism of bovine pleuropneumonia; the chief difference being that the bacterial phase, whereby *Listeria* is usually recognizable, is more completely suppressed in the pleuropneumonia organism.

The inclusion of egg or serum to culture media is desirable for the isolation of this organism, but this presents a serious problem, because all such material is liable to be contaminated, and may show visible cysts even if these have been killed by inspissation, thus confusing the reading of early or slow growing cultures. Tissue cultures or other media containing native serum, even if filtered, are quite unsuitable for either isolation or growth purposes, because the chance of their containing living, filter-passing elements of this or similar mycoplasmas is very high. To achieve



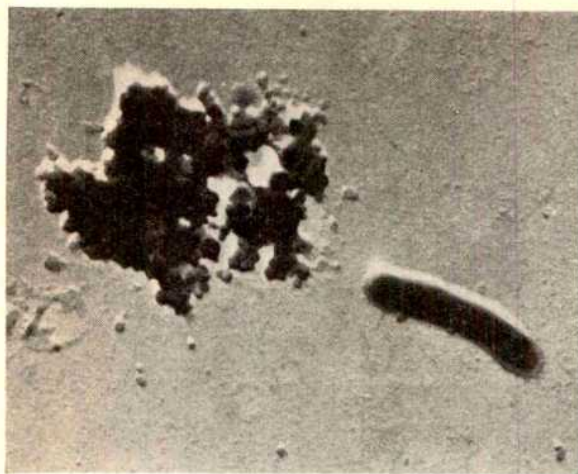


Fig. 1. Two phases of *Listeria monocytogenes*. Partially disrupted cyst, composed of tiny granules, resembling oncogenic viruses in size and form, and bacterial form. Electron micrograph, gold-palladium shadowed. ( $\times 12,500$ .)

satisfactory control, the only serum that can be used is that under examination on each occasion.

### Causation Hypothesis

Infection by *Listeria* seems to be universal, at least in certain communities, and because the diseases concerned are chronic and affect human beings, the suspected pathogen cannot be related to the pathological conditions by classical infective theory, nor can Koch's postulates be satisfied. In fact, a hypothesis for infective mediation of auto-immune and neoplastic disease does not require that they should be. Any infective agent that fulfils the requirements could, in theory, play the role equally well. *Listeria* is a clear candidate, because, in addition to being tolerated immunologically, and showing evidence of nucleic acid similarities with man, it is so notably widespread; and because the appearance of its smaller elements (Fig. 1) is very similar to that of the tumour viruses illustrated by Gross<sup>8,9</sup> and others. This should be considered in the light of Alexander-Jackson's suggestion<sup>10</sup> that the Rous sarcoma virus is the mycoplasma phase of a bacterium.

Thus using *Listeria* as an illustration, and the tumour viruses<sup>8,9,34</sup> and LCM virus<sup>5</sup> as models, the hypothesis may be stated in the following terms.

The mycoplasma phase of *Listeria*, lacking the strong antigens of the cell wall, and probably congenital in the host, is tolerated; it is widespread in the body, so that any tissue can be affected when tolerance is disturbed. Loss of tolerance could cause auto-immune disease, by the mechanism suggested by me previously<sup>4</sup>, taking the form of rheumatic disease if the invading episome produced streptococcal antigens, which do occur in *Listeria*<sup>1</sup>. In contrast neoplastic disease could involve too complete a tolerance, with the episome acting the part of the defective viruses or mycoplasmas that are believed to be associated with malignancy<sup>6</sup>.

Evidence of a connexion between *Listeria* and neoplastic disease is afforded by its possession of I blood antigens<sup>35</sup>. Disturbance of the normal balance between adult I and foetal i antigens has been observed in these conditions<sup>37</sup>. It is apparent that an excess of the semi-tolerated I could result in such disturbance of balance, resulting in a tendency towards suppression of I and selection of the foetal i. Thus an embryonic type, especially of blood cell, may be permitted to develop in the immunologically mature person. Increased tolerance could also result in the removal of suppression of the bacterial phase, so that recognizable bacteria appear in the lesions of neoplastic disease, as other people<sup>14,25,26</sup> have noted.

It has been reported that stimulation of the immune response by transduction can produce a regression of tumours in experimental animals<sup>38</sup>.

As I have already pointed out, the hypothesis does not demand that a specific pathogen should be associated with each disease. The possibility that *Listeria* may be the infective agent mediating these types of pathological condition has been fully examined in this paper, but, in fact, the common human mycoplasmas (whether or not these are actually related to *Listeria* phylogenetically<sup>39</sup>) are well adapted to play the same part. They also possess streptococcal antigens<sup>39</sup>, and their infections are marked by the occurrence of I:i antigen disturbance<sup>37</sup>.

The role of carcinogens in this hypothesis is extended rather than reduced, as might at first be imagined. Carcinogens are believed to act on the nucleic acids of the host<sup>40</sup>. If so, they must be equally capable of affecting those of the parasite, and producing a defective, potentially malignant episome. Carcinogens and hormones are known to induce malignancy in previously benign infections with oncogenic viruses<sup>8,9</sup>. It must be accepted that environmental and heritable factors are important principal or concomitant causes of auto-immune and neoplastic disease, but the evidence suggesting that an essential infective element exists in some such conditions is now very considerable. This can be correlated with the other known facts by a hypothesis such as is now proposed.

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# Quinoid Secretions in Grain and Flour Beetles

by

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Quinoid secretions are found to have an unexpectedly wide distribution in tenebrionid insects which live on stored food. The seventeen species involved include the common grain, flour and fungus beetles.

A VARIETY of insect secretions which comprise toxic chemicals have received attention in recent years<sup>1,2</sup>. Our interest is specifically directed to those of common stored-food pests. We report on the occurrence of quinones in grain, flour and fungus beetles of the family Tenebrionidae: *Alphitobius diaperinus* (Panz.) and *laevigatus* (F.); *Alphitophagus bifasciatus* (Say); *Gnathocerus cornutus* (F.) and *maxillosus* (F.); *Latheticus oryzae* Waterh.; *Palorus ratzeburgi* (Wissm.) and *subdepressus* (Woll.); *Tenebrio molitor* L. and *obscurus* F.; *Tribolium anaphe* Hint., *brevicornis* (Lec.); *destructor* Uyttenb.; *castaneum* (Herbst); *confusum* Duv.; *confusum* mutant *msg*; and *madens* (Charp.). These species are the principal surface feeding insect pests of cereal staples and related food products. We have developed a polarographic technique which has enabled us to detect quinoid toxin in all these beetles and to define the secretions electrochemically. The method, which permits the quantitative determination of as little as  $10^{-7}$  g of quinone, will be described elsewhere.

Insects were made available from stocks at various laboratories in Canada, Great Britain, and the United States. They were maintained on a mixture of wheat flour and wheat germ until used.

Fig. 1 shows polarographic curves obtained from insects *Tribolium madens* and *brevicornis* (upper waves). The half-wave potentials of  $-12$  mV and  $-14.5$  mV versus saturated calomel electrode agree with that for tetratoxin from *Tribolium confusum* and *castaneum* and *Latheticus oryzae* (Table 1), as well as for synthetic ethylparabenzoquinone<sup>3</sup>. *T. madens* and *brevicornis* are found in mills and warehouses, infesting wheat, barley, oats, chicken feed and honeycomb, among other foods. They are similar in appearance, size and habits to *T. confusum* and *castaneum*, which belong to the same genus. They have, however, a surprisingly high quinone content, amounting to as much as  $384$   $\mu\text{g}/\text{beetle}$  (Fig. 1, Table 1). The chemical constitutes as much as 5 per cent of their body weight. Catalytic reduction performed in the polarographic sample compartment with  $\text{Pd-H}_2$  proceeded with ease, was complete and produced a clear pattern of reversibility of the electrode process (lower waves in Fig. 1). Log plots of  $i/(i_d - i)$  versus mV (not shown in Fig. 1) were straight and revealed a two electron transfer. The reciprocals of the slopes were 0.031 and 0.030, respectively.

Polarograms for two species of pests, *Gnathocerus cornutus* and *Latheticus oryzae*, belonging to other genera, are pictured in Fig. 2. These beetles, slightly less than an eighth of an inch long, are widespread throughout the world, infesting rice and flour mills, also milo, beans, sorghum, raisins mixed feeds, and other ordinary foods.

Table 1. QUINONES IN STORED FOOD INSECTS

	$E_1$ , mV versus saturated calomel electrode	$\mu\text{g}/\text{insect}$						$\mu\text{g}$ of $Q + HQ$ , average/ insect
<i>Tribolium madens</i>	$-14.5 \pm 1.5$	Q: 197	146	216	123			
		HQ: 6	9	8	5			179
<i>Tribolium brevicornis</i>	$-12 \pm 2$	Q: 384	309	262	254	318	295	
		HQ: 10	11	8	10	14	12	329
<i>Tribolium castaneum</i>	$-13 \pm 1$	Q: 71	43	52				
		HQ: 5	7	9				62
<i>Tribolium confusum</i> (wild type)	$-13 \pm 1$	Q: 57.9	69.0	53.1	36.6	62.1		
		HQ: 1.2	2.4	1.5	2.4	3.0		57.8
<i>T. confusum</i> (mutant <i>msg</i> )	$-13 \pm 1$	Q: 0.0	1.1	0.0	2.2	0.3		
		HQ: 1.1	1.9	1.8	2.3	1.4		2.4
<i>Gnathocerus cornutus</i>	+58	Q: 0						
		HQ: 6						6
<i>Latheticus oryzae</i>	$-12 \pm 1.5$	Q: 3	1					
		HQ: 8	2					7

Figures were computed from polarographic data (consult text). Q, quinoid; HQ, hydroquinoid. Reciprocal slopes,  $\log i/(i_d - i)$ , in all cases  $0.031 \pm 0.002$ .

The straight line log plots, both with reciprocal slopes of 0.030, again evidence reversibility of the electrode process, with a two electron transfer. For these beetles, however, the waves are partly or entirely anodic (hydroquinone) and the diffusion currents are less than  $1.5$   $\mu\text{amp}$ . The

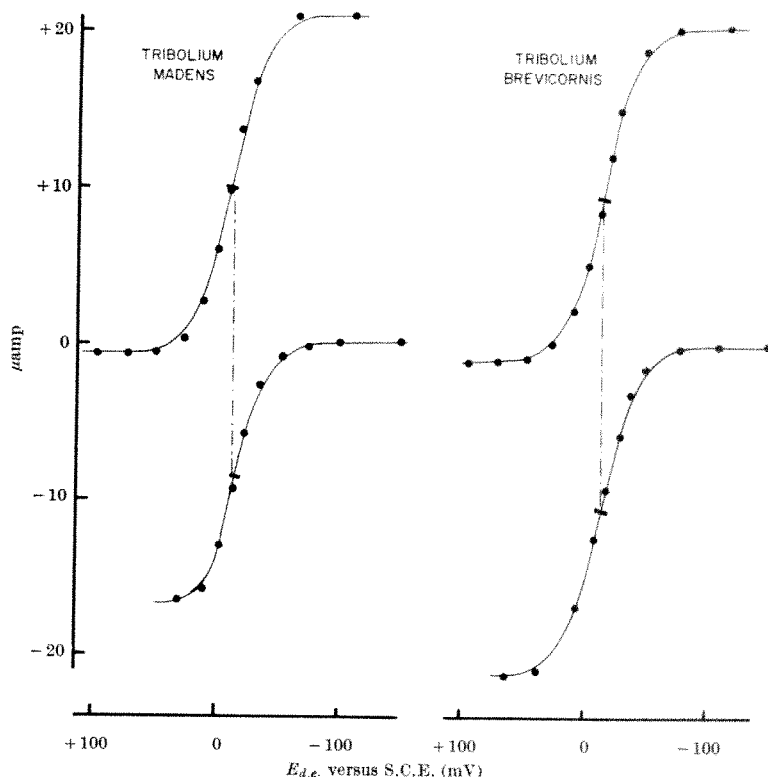


Fig. 1. Polarograms of quinone in insects *Tribolium madens* and *Tribolium brevicornis*. Upper waves, original secretions. Lower waves, samples reduced with  $\text{Pd-H}_2$  (consult text).



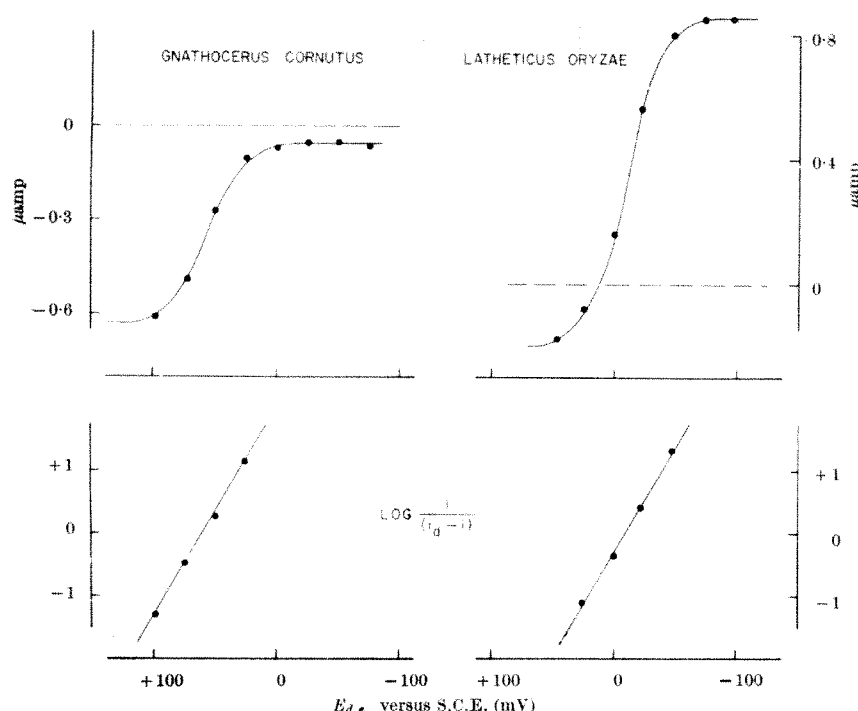


Fig. 2. Polarograms of quinoid secretions in insects *Gnathocerus cornutus* and *Latheticus oryzae*. Log plots  $i/(i_d - i)$  versus potential show reversibility and two electron transfer of the electrode process (consult text).

half-wave potential found for *Gnathocerus cornutus* is +58 mV versus S.C.E., that is, close to that of benzoquinone, the standard oxidation-reduction potential of which is more positive by 0.06 V than that of simple alkyl-parabenzoquinones<sup>4</sup>.

Previous investigations of the chemical composition of quinoid insect secretion, which were based on the isolation of volatile substances from insects by atmospheric pressure or vacuum sublimation, have in no instance produced evidence of the existence of hydroquinone in the glands of these beetles<sup>5-7</sup>. The present method, in contrast, facilitates the detection of hydroquinone, as is shown by the anodic portions of the waves in Fig. 2. This finding is interesting because of the discovery of the melanotic stink gland (*msg*) mutant of *Tribolium confusum*<sup>8</sup>. We have studied the mutant (Table 1), and the data of Engelhardt *et al.*<sup>9</sup> concerning the low quinone content were confirmed, while hydroquinone, reportedly absent, was discovered in some of the beetles as the sole quinoid component of the secretion.

The uniformity of the half-wave potentials of the various insect secretions suggests that most of them are simple alkyl-parabenzoquinones comparable with, or identical with, those previously established in *T. castaneum*<sup>6</sup>. Attention is directed towards large quantitative differences among species, and to the quantitative variability in individuals of any one species. The latter is attributed to the different ages of the specimens, which were taken from random age populations<sup>10</sup>.

Additional species listed in the introductory statement were screened with the method and quinones were found in all of them. The identity of the polarographically detected quinones was in each case further substantiated by a potassium iodide starch test, consisting of crushing a beetle on KI-starch paper that had been moistened with dilute sulphuric acid. Blue spots developed on the paper where it had absorbed the insect secretion, with the intensities of the coloration closely corresponding to the heights of the diffusion currents for any one species of insects.

The results show that quinones, and hydroquinones, are ubiquitous in tenebrionid stored food insects. While

the quantities of these chemicals total only a few  $\mu\text{g}$  to about 0.5 mg/insect, the number of infestants thriving on human food is enormous<sup>7</sup>. A report has appeared in Yugoslavia<sup>11</sup> on infestation of sunflower seeds, which are a principal source of edible oil in eastern countries. The survey of 95,390 tons of stored seeds provides the information that forty thousand million insects contaminated this commodity, eighteen thousand million of which were *Tribolium castaneum*. Computing the amount of quinone on the basis of the supply of 55  $\mu\text{g}$  in this beetle (average of three *Q* values in Table 1), 2,200 lb. of the toxicant were in contact with the food. In addition, 290 lb. of hydroquinone (Table 1) were present.

It has long been known that *Tribolium* infesting wheat flour leave the latter contaminated with coloured toxin-protein conjugates<sup>12,13</sup>. The quinone is taken up avidly. Flour merely exposed to the vapours of the toxin binds 350 p.p.m. of it within 60 min<sup>10</sup>. In the reaction, quinoid protein conjugates plus hydroquinone are formed according to the mechanism first described by Fischer and Schrader<sup>14</sup>. Ladisch has recently published a detailed experimental study on the interaction of the naturally derived toxin with various amino-acids and peptides<sup>15</sup>.

Alkyl-parabenzoquinones are recognized wide-spectrum toxicants<sup>16</sup>. Hydroquinone, likewise considered harmful and deleterious, has not been approved as a food additive. Ladisch<sup>17</sup> and Hueper<sup>18</sup> have enumerated a variety of carcinogenic and potentially carcinogenic quinones, among which are the quinones secreted by common flour beetles. Several more specific reports concerning acutely toxic, allergenic, teratogenic, carcinogenic and anti-fungal activities of these insect secretions have also been published<sup>3,7,17,19-21</sup>. The present results, reflecting the unexpected wide distribution of quinones in food pests, clearly establish the need for further research into the problem.

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## LETTERS TO THE EDITOR

## ASTRONOMY

## Radio Source W49 and Anomalous OH Emission

THE radio source W49 has become of increasing interest recently because of its association with regions of strong anomalous OH emission at radio wavelengths<sup>1-6</sup>. The source is situated in the galactic plane in a direction of high optical obscuration so that information has to be obtained entirely at radio wavelengths. Observations on the continuum have shown the source to be an HII region and in addition spectral line observations at 21 cm wavelength<sup>7</sup> and at the 109 $\mu$  hydrogen recombination line at 6 cm (ref. 8) have indicated that the distance is about 14 kiloparsec. A measure of the electron temperature and of the velocity distribution of the ionized gas has also been obtained from the recombination line observations. This communication presents some new high resolution observations obtained with the recently completed 150 ft radio telescope of the Algonquin Radio Observatory at a wavelength of 2.85 cm, supplemented by observations at 9.45 cm. These results when considered in conjunction with laboratory measurements suggest that the anomalous OH emission observed from the source might be explained as the result of the formation of OH in an electronically excited state by a two-body process.

Fig. 1 shows the results of the observations, presented as a contour diagram for the wavelength of 2.85 cm, and was produced from scans taken across the region, after corrections had been made for atmospheric refraction, for pointing accuracy of the telescope as determined from observations on the source 3C84, and for the change from the epoch of 1966.8 to the standard epoch, 1950.0. The source designated B is the non-thermal source, and the source designated A is the thermal source W49, according to the classification of Mezger and Henderson<sup>9</sup>, whose observations at a wavelength of 6 cm had previously resolved the region into two principal components. With the higher resolution obtainable with the 150 ft. telescope, it is

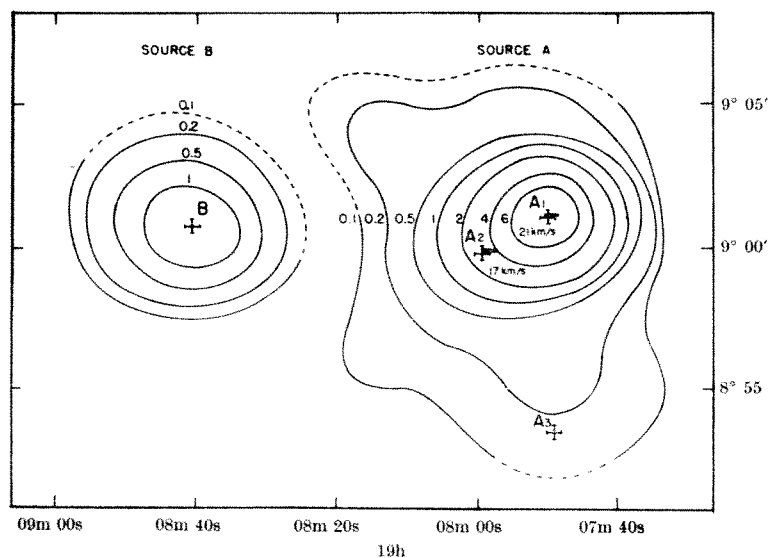


Fig. 1. Contour diagram for the region surrounding source W49. Contour units are antenna temperature. The crosses represent the positions of the individual sources and the black rectangles are the positions of enhanced OH emission. The velocities are those of the chief features of the enhanced line emission. The weak areas of emission to the north of source A are due to a coma lobe of the telescope.

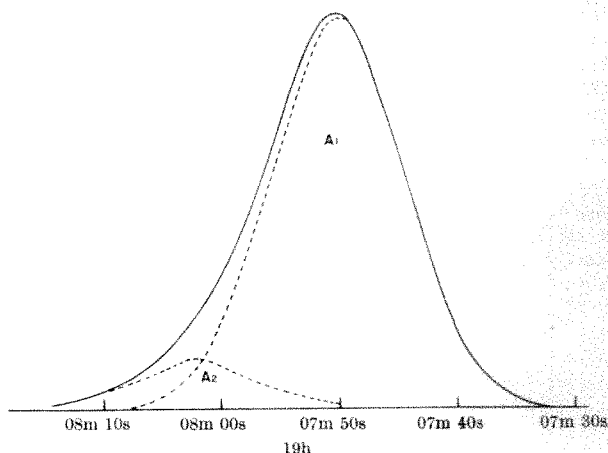


Fig. 2. The full line represents the mean of scans in both directions across the peak A1, at declination  $9^{\circ}01'1$  taken on October 22, 1966. The broken line shows the resolution of the source A into two components, A1 and A2.

obvious that A is complex and can be seen to consist of a main peak with some degree of elongation in the direction of increasing right ascension. The two low level peaks to the north of the main peak result chiefly from a coma lobe of the telescope, but the other additional low level areas of emission are believed to be real. Declination scans across the main peak, at the wavelength of 2.85 cm where the beamwidth is 2.8 min of arc and at 9.45 cm where the beamwidth is 9.2 min of arc, show that the broadening of the half-power beamwidth in each case amounts to 0.5 min of arc. Right ascension scans through the main peak at the wavelength of 2.85 cm show an asymmetry such that, for increasing right ascension, the intensity rises to a peak but then tails off more slowly, as shown in Fig. 2 which is a scan in right ascension at declination  $9^{\circ}01'1$ . If symmetrical curves are fitted to the drift scans at various right ascensions and declinations it is possible to show that the apparent elongation of the chief source is consistent with the presence of the two sources, A1 and A2, in the positions shown in Fig. 1. The sources are separated in angle by 2.8 min of arc—in other words by the half-power beamwidth—in a direction approximately at right angles to the galactic equator. It is obvious that this is not necessarily a unique solution, and the chief source could consist of a ridge of emission, or an elongation, along the direction joining the assumed sources A1 and A2. On the other hand, the fact that the source A1 has small dimension in declination suggests that it might have a similar dimension in right ascension.

Accordingly, a model for this principal feature of the region would consist of two sources each with a minimum dimension of 0.5 min of arc, with corresponding linear dimensions of about 2 pc. Within the limits of experimental error, the continuum spectrum appears to be flat, and this, in conjunction with the presence of hydrogen recombination lines, would indicate that the emission is thermal. The assumption that the optical depth over the whole region is less than unity leads to values for the mean electron densities of less than  $1,230 \text{ cm}^{-3}$  and  $560 \text{ cm}^{-3}$  for A1 and A2 respectively. On the other hand, it is difficult to relate such a simple model to the apparent broadening of the beamwidth at the two wavelengths. The increases in beamwidth by a constant amount indicate an increase of diameter at the longer wavelength. This in turn suggests that if the source is purely



thermal, then it must be optically thick in places, at least for the wavelength of 9.45 cm and possibly also for the wavelength of 2.85 cm. Such considerations lead to electron densities in some areas which must be at least as high as  $10^4 \text{ cm}^{-3}$ . Because  $\text{H II}$  regions will in general be expanding, the density of the neutral gas outside the region will be similar in magnitude to that of the ionized region, and the two sources would then consist of fairly dense  $\text{H II}$  regions, produced by the associated young hot stars, embedded in a larger diameter  $\text{H I}$  region. Evidence for the latter is indicated by the surrounding diffuse area, which would be the weakly ionized gas.

Of added interest is the position of the areas of greatly enhanced OH emission relative to the two principal regions. The more intense components of the 1.665 Mc/s line as determined by Rogers *et al.*<sup>10</sup> are also plotted in Fig. 1, and it is clear that the 21 km/s feature is closely associated with A1 while the 17 km/s feature is either close to A2 for the model assumed, or alternatively along the possible ridge joining the positions A1 and A2. It would appear that these components are associated with the regions of comparatively high density which in addition appear also to be regions of comparatively high internal velocity. But this is probably not unique to the source W49 and in fact the source of OH in Sagittarius is in a correspondingly high density region<sup>11</sup>, as is the source W3C, while the emission peaks of OH in NGC 6334 appear to be associated with the edge of comparatively bright optical emission regions<sup>4</sup>, which probably have high expansion velocities.

The indication that OH emission is associated with areas of  $\text{H II}$  regions of comparatively high density is not inconsistent with previous theories. But on the other hand it also raises the possibility that a further mechanism may be taking place. Recent observations in the laboratory by Spindler *et al.*<sup>11</sup> have shown that OH may be formed in an electronically excited state as a two-body process involving atomic oxygen and hydrogen. Such a reaction could be a competing mechanism in dense regions and is well worth consideration.

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### Mechanism for Anomalous OH Emission from $\text{H II}$ Regions

SEVERAL suggestions have been made to explain the formation of OH in the galaxy, together with possible pumping mechanisms to produce the laser action considered responsible for high anomalous brightness temperatures at least as high as  $10^{10} \text{ }^\circ\text{K}$  (ref. 1). It is usually assumed that OH is produced in the presence of interstellar particles

by sputtering or by a process of chemical exchange on the particles; they also act as the third body and help to conserve energy and momentum. The pumping mechanism suggested by Litvak *et al.*<sup>2</sup> is by continuum ultra-violet radiation which induces selective transitions from the various rotational levels of the ( $X^2\Pi$ ) ground state to the ( $A^2\Sigma$ ) electronic excited state. By this means an inversion of the population of the ground doublet state is obtained, but on the other hand the energies involved are close to the dissociation energy for OH. The analysis by Cook<sup>3</sup> invokes pumping by Lyman- $\alpha$  radiation, but again the energy involved is more than twice the dissociation energy and it is likely that a large percentage of the OH would be dissociated. The two-body process proposed by Symonds<sup>4</sup> of forming excited OH from O- and H+ could possibly also provide the pumping mechanism, but unfortunately it requires the liberation of a large amount of energy and it seems unlikely that this process would be effective. A more interesting mechanism has recently come to light with the observation in the laboratory by Spindler *et al.*<sup>5</sup> of the formation of OH in an electronically excited state, as a two-body process involving atomic oxygen and hydrogen, with the emission of radiation in the 3064 Å band. The rate for the reaction has been determined<sup>6</sup>, and can be expressed by

$$I = K[\text{H}][\text{O}] \text{ photons cm}^{-3} \text{ sec}^{-1}$$

where  $I$ , the intensity of the emission, is assumed equal to the rate of formation of OH molecules,  $[\text{H}]$  and  $[\text{O}]$  are the densities of atomic hydrogen and oxygen respectively, and  $K = 3 \times 10^{-21} \text{ molecules cm}^{-3} \text{ sec}^{-1}$  is the rate constant at room temperature. Because this reaction could well be taking place in the cooler parts surrounding individual  $\text{H II}$  regions it is of interest to assess the possibility that it is also the cause of anomalous OH emission.

A realistic region for OH formation is in the pressure wave immediately ahead of a  $D$ -type ionization front. Kaplan<sup>7</sup> has shown that the density here can rise to about forty times the mean density and the temperature increase to a value of about  $1,000^\circ \text{K}$  in comparison with the  $100^\circ \text{K}$  for the neutral gas. The width of the front is of the order of  $10^{-2} \text{ pc}$ . The  $D$ -type front is the most likely<sup>8</sup>, provided that the gas density is greater than about  $100 \text{ cm}^{-3}$ . Further indications that such a region may be responsible can be seen from observations on NGC 6334 where the OH emission arises at the edge of bright optical emission regions<sup>9</sup>. In general the observed Doppler shifts are consistent with this hypothesis. If we take a previously derived model for W49 (ref. 10) where densities of at least  $10^4 \text{ cm}^{-3}$  can exist in the  $\text{H II}$  regions, the corresponding density of hydrogen in the pressure wave will be about  $4 \times 10^5 \text{ atoms cm}^{-3}$  and, taking the normal cosmic abundance, the density of oxygen will be  $4 \times 10^2 \text{ cm}^{-3}$ . Assuming that the rate constant is proportional to temperature<sup>2</sup>, then the rate of formation of excited OH will be about  $10^{-12} \text{ molecules cm}^{-3} \text{ sec}^{-1}$ . The molecules are formed in a fairly high rotational level of the  $A$ -state, and the way in which the molecule reverts to the ground state requires detailed calculations. Collisions during the lifetime of the  $A$ -state (about  $10^{-6} \text{ sec}$ ) could induce transitions down the vibrational-rotational levels before the transition to the  $X$ -state. Alternatively, the initial transition could be to a high rotational level of the  $X$ -state followed by transitions down to the ground state. In either case it is possible that selection rules could produce an inversion of the population of the relevant ground state  $A$ -doublet level. Transition probabilities have not yet been calculated, but it is possible to obtain the order of magnitude required. The lifetime of the hyperfine  $A$ -doublet states is long in comparison with the lifetime of the other states and amounts to about  $10^{11} \text{ sec}$ . During this time  $10^{-1} \text{ OH molecules cm}^{-3}$  will be formed. Because the required population inversion assumed necessary for laser amplification is about  $10^{-5} \text{ cm}^{-3}$  (ref. 11), if the difference in the resulting transition rates to the upper and lower states amounts to

only  $10^{-4}$ , this would be sufficient. More detailed calculations show that the rates of formation for the proposed mechanism are too small for the intensity to be produced entirely in the pressure wave, but rather that the pressure wave is a region of amplification of the radiation from a source situated behind it. The recent observations<sup>1</sup> that the source of emission can have dimensions as small as  $10^{-4}$  pc., however, supports this suggestion.

The further complication that the observed emission shows very high percentage polarization has not been considered, but it might be mentioned that the parts of an HII region which are most likely to have comparatively large values for the magnetic field are those immediately in front of a moving ionization front. Though the mechanism is still somewhat speculative, it is by no means more so than the other proposals.

The suggested mechanism has the additional attraction that it may be amenable to observation. The transition to the ground state corresponds to emission in the 3064 Å wavelength band, and the brightness of the emission from an average HII region should amount to about  $10^6$ – $10^7$  photons  $\text{cm}^{-2}$ . This should be observable from those HII regions that can be seen optically. Unfortunately, the atmospheric extinction is high at these wavelengths and observations from near the ground may be confused by the presence of atmospheric OH. It should, however, be a suitable observation to make from space vehicles.

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### Critique of the "Clark Effect"

A DIRECT measurement of the magnetic field strengths within the Galaxy would be of the greatest importance for understanding its structure and evolution<sup>1</sup>, and Clark<sup>2</sup> has proposed a method based on the effect of a magnetic field on the anomalous dispersion of 21 cm radiation passing through neutral hydrogen. I wish to point out that Clark's calculation contains a fundamental error, rendering the method much less effective than he claims.

Clark's method makes use of the fact that the right-handed and left-handed circularly polarized components of 21 cm radiation have different indexes of refraction in hydrogen gas embedded in a magnetic field, as a result of the different effects of the 1s triplet excited states on the resonant forward scattering. Feynman diagrams for the two important scattering processes are shown in Fig. 1. Fig. 1A represents scattering from the ground state. Clark's principal error was to neglect the process represented by Fig. 1B—induced scattering from an excited state. Its contribution to the index of refraction is of opposite sign.

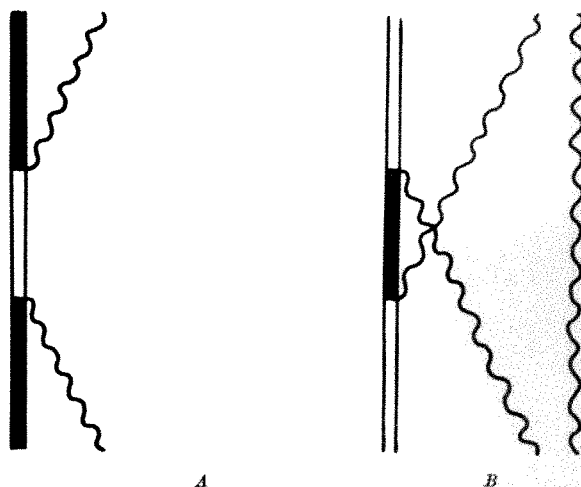


Fig. 1. Feynman diagrams of the two scattering processes which contribute to the index of refraction. The heavy line represents the 1s singlet ground state of hydrogen, while the double line represents one of the 1s triplet excited states. Only the induced emission should be included in B.

Although the correct result may be obtained entirely through the use of the optical theorem<sup>3</sup> modified by the possibility of induced emission, the simplest approach is the use of the Kramers–Kronig dispersion relation<sup>4,5</sup>, which applies quite generally to any scattering process. For each component  $i$  of polarization, this relates the phase shift  $d\varphi_i$  relative to propagation in a vacuum to the optical depth  $d\tau_i$  along a path length  $dl$  at frequency  $\nu$  by

$$d\varphi_i(\nu) = \frac{1}{2\pi} P \int_0^\infty \frac{d\tau_i(\nu')}{\nu' - \nu} d\nu' \quad (1)$$

near resonance, where  $P$  denotes the Cauchy principal value.

In order to relate the Faraday rotation  $\psi$  of the plane of polarization, which is half (Clark used 1) the difference in phase between the right-handed and left-handed circularly polarized components, to the observed total optical depth  $\tau$ , we need the explicit expression for the optical depth for each polarization. This is

$$d\tau_i = \frac{\pi^2 e^2 \hbar \nu dl}{4m^2 c^3} \{ 2 \sin^2 \theta [n_g^{(0)}(\nu) - n_0(\nu)] + (1 \mp \cos \theta)^2 [n_g^{(+)}(\nu) - n_+(\nu)] + (1 \pm \cos \theta)^2 [n_g^{(-)}(\nu) - n_-(\nu)] \} \quad (2)$$

in the limit of vanishing natural widths of the states, which is certainly justified in this case. Here  $\theta$  is the angle between the ambient magnetic field and the propagation direction,  $n_j(\nu) d\nu$  is the number density of hydrogen atoms in excited state  $j$  which can resonantly emit a photon received in the frequency range  $\nu$  to  $\nu + d\nu$ , and  $n_g^{(j)}(\nu) d\nu$  is the same for absorption by a ground state atom into excited state  $j$ . The resonant frequencies are  $\nu_0 = 1.42 \times 10^9$  c/s and  $\nu_{\pm} = \nu_0 \pm 0.5 \Delta\nu$  with  $\Delta\nu = 2.80 \times 10^6 H_0$  c/s, where  $H_0$  is the strength of the magnetic field in gauss. Because

$$\tau_r - \tau_l = \frac{\pi^2 e^2 \hbar \nu}{m^2 c^3} \int \{ [n_g^{(-)}(\nu) - n_-(\nu)] - [n_g^{(+)}(\nu) - n_+(\nu)] \} \cos \theta dl \quad (3)$$

$$\tau \cong \frac{\tau_r + \tau_l}{2} \cong \frac{\pi^2 e^2 \hbar \nu}{m^2 c^3} \int [n_g^{(j)}(\nu) - n_j(\nu)] dl \quad (4)$$

we obtain

$$\tau_r - \tau_l \cong \frac{d\tau}{d\nu} \Delta\nu \cos \theta \quad (5)$$

where we have used the fact that under all astrophysical conditions the four states in question are almost equally populated. Using equations (1) and (5), we obtain for the Faraday rotation

$$\psi(v) = 0.5 (\varphi_r - \varphi_l) = \frac{\Delta v \cos \theta}{4\pi} P \int_0^\infty \left( \frac{1}{v' - v} \right) \frac{d\tau}{dv} (v') dv' \quad (6)$$

$\psi$  is positive in the clockwise direction when looking into the wave.

If one neglects the removal of the derivative to outside the integral in Clark's result, then the ratio of equation (6) to his formula is  $(0.5)(2\pi\hbar v/kT_s) \cos \theta$  (after correction of some numerical errors in Clark's computation), where  $T_s$  is the spin temperature of the hydrogen gas. The second factor,  $0.0681/T_s$ , is just the fractional difference in population of the ground and excited levels, and indicates the reduction in the net phase shift due to the induced scattering. Because typically  $10^3 \leq T_s \leq 10^4$  °K, the effect will be much smaller than Clark claims. In fact, for an absorption profile  $\tau(v) = \tau_0 \exp [-(v - v_m)^2/v_v^2]$ ,

$$\psi(v_m) = - \frac{\tau_0 \cos \theta \Delta v}{2 \sqrt{(\pi) v_v}} \text{ radians} \quad (7)$$

and in general,  $|\psi| \sim 0.3 \tau \Delta v/W$ , where  $W$  is the width of the feature. Because for HI clouds,  $W \sim 10^4$  c/s, the rotation will usually be very small.

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## PLANETARY SCIENCE

### Isolating Lunar Materials

MEMBERS of the Surveyor scientific evaluation and analysis team have shown, by counts of particles on Surveyor I and III photographs of the lunar terrain, that at two places in Oceanus Procellarum the surface rock is particulate with grain sizes down to less than 1 mm in diameter. Laboratory studies recently undertaken by the lunar team of the University of London Observatory point to a new method of investigating the sizes of submillimetric particles on the Moon. Instead of concentrating on only one of the possible physical methods—such as albedo measurements, spectrophotometry and polarization, taken separately—of probing the nature of the surface of the Moon, we integrate these approaches to draw conclusions about the nature of the lunar material only after the requirements of all avenues have been satisfied. In this way, many materials may be eliminated from candidature for the lunar surface and the choice of possible material suffers stricter control. The method may be illustrated by reference to certain physical studies of eleven basic and intermediate rocks ground to various grain sizes.

Eleven Hawaiian rock specimens (listed in Table 1) were prepared in four ranges of grain size centring on 160 $\mu$ ,

83 $\mu$ , 62 $\mu$  and 26 $\mu$ , respectively. Of these, the specimens GF/H/u9 and GF/H/u2 were processed from the original specimens GF/H/9 and GF/H/2 by melting them in a furnace and upwelling them in a vacuum chamber to simulate the formation of lunar lavas<sup>1</sup>. The diffuse normal albedos of the forty-four powdered samples were measured as a function of wavelength in the interval 3750–7200 Å using a Beckmann mark II ratio recording spectrophotometer.

The colour excess,  $E$ , was derived from the records by means of the relation

$$E = 2.5 \log (J_V/J_B) + 0.09 \quad (1)$$

where  $J_V$ ,  $J_B$  are the observed  $V$  and  $B$  fluxes from the powders. It was found that, for all the cases,

$$G \approx CA^{-2} \quad (2)$$

where  $G$  is the grain size in  $\mu$ ,  $A$  is the albedo expressed as a percentage at 5600 Å and  $C$  is a parameter that increases with the mean colour excess of a group of rocks, as indicated in Table 1.

Table 1

Rock powder	Group	Log $C$	Mean $E$
GF/H/u9 Basalt glass	1	3.25	-0.01 (bluer)
u2 Basalt glass			
3 Non-porphyrritic basalt			
9 Basalt	2	3.75	+0.05
10 Basalt			
1 Basalt			
8 Ankaramite			
2 Felspathic olivine basalt	3	4.14	+0.08
11 Hawaiite			
4 Picrite basalt	4	4.62	+0.27 (redder)
7 Trachyte			

This means that, for a given albedo, the redder rocks must in general have larger grains than bluer rocks. For a particular rock, however, the redness increases with decreasing grain size. This is not true for rocks of the higher groups (3 and 4 in Table 1), which have comparatively high albedos for a given particle size; for these rocks the colour excess shows no clear dependence on grain size. This is probably because colour is controlled, in these instances, largely by external reflexion from facets rather than by transmission through and absorption and internal reflexion in grains.

Sytinskaya<sup>2</sup> pointed out the apparently special location of the lunar domain on a plot of colour excess against albedo. Extending this work, Fielder *et al.*<sup>3</sup> found several powders which, in their natural, non-irradiated state, did in fact intrude into the lunar domain. The number of powders matching the Moon in this way may be enlarged further using equation (2) together with the relation (extrapolated where feasible) between grain size and colour excess. (For groups 1 and 2, the relation between  $E$  and  $\log G$  is linear.) Taking  $\log A$  for the Moon to range from 0.7 to 1.23 and  $E$  for the Moon, from +0.09 to +0.45, after Sytinskaya<sup>2</sup>, the relations exclude three of the powders in Table 1 and attribute certain ranges of possible grain sizes,  $G_{A,E}$ , to the others. Thus the range  $G_{A,E}$ , listed in Table 2, states the condition that the named powders are included in the lunar domain of the  $A-E$  diagram.

Dr A. Dollfus is collaborating with us in an extensive polarization research programme in which the same rocks are used. E. L. G. Bowell, who is working with Dollfus, told us that he has shown that the parameters  $G$ ,  $A$  and  $P_m$ —the maximum positive value of the polarization—are mutually dependent. Furthermore, he showed that, by comparison with the lunar data, polarization data on rock powders, including those in Table 1, argued for a lunar grain size of about 20 $\mu$ . Clearly, the 20 $\mu$  particles required to produce the polarizing properties of the Moon must be scattered over, or mixed with, the larger particles recorded by the lunar probes. Taking all these facts

Table 2	
Rock powder	$G_{A.F.}(\mu)$
$GF/H/u9$	15-53
$u2$	<16
3	<19
9	<18
1	16-40
8	19-141
4	44-178
7	>186

into account therefore those of the rocks in Table 2 which are most likely to have the characteristics of the lunar surface are  $GF/H/u9$ , 1 and 8. The trachyte  $GF/H/7$  certainly would not polarize light like the Moon.

It will be seen that this preliminary study—which is intended to be illustrative only—describes a new method of narrowing the choice of lunar surface materials through an examination of the albedo, colour and polarization properties of sub-millimetric particles as a function of grain size. Contrary to earlier findings, certain rocks in their natural, non-irradiated state are evidently possible candidates for a lunar surface model.

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### Serpentine Dehydration Curves and their Bearing on Serpentine Deformation in Orogenesis

SERPENTINITE bodies characteristically occur in regions that have undergone an orogenesis of alpine type<sup>1</sup>. The more recent recognition that significant quantities of serpentinites are associated with the oceanic crust, oceanic trenches and mid-oceanic ridges<sup>2</sup> extends the possible role of serpentinites in tectonic processes which involve the crust and upper mantle. Thus the experimental deformation of serpentinite at high pressures and temperatures<sup>3</sup> has important implications for theories of the tectonic emplacement of serpentinites and principal tectonic processes such as mountain building. Raleigh and Paterson<sup>3</sup> have found that at low temperatures and high pressures serpentine has strength comparable with granite. With an increase in temperature sealed specimens showed marked weakening accompanied by the development of brittleness.

Results<sup>3</sup> for two serpentinites are shown in Fig. 1; the Fidalgo Island serpentinite contains brucite while the Tumut Pond serpentinite does not. Raleigh and Paterson attribute the weakening and embrittlement of the serpentinite to dehydration and suggest that the pore pressure of the liberated water reduced the effective confining pressure. They remark that the temperature at which the serpentinite bearing brucite is weakened does not coincide well with the phase equilibrium data, and that much more detailed phase equilibrium studies may be required.

Bowen and Tuttle<sup>4</sup> have put forward two reactions for the dehydration of serpentine

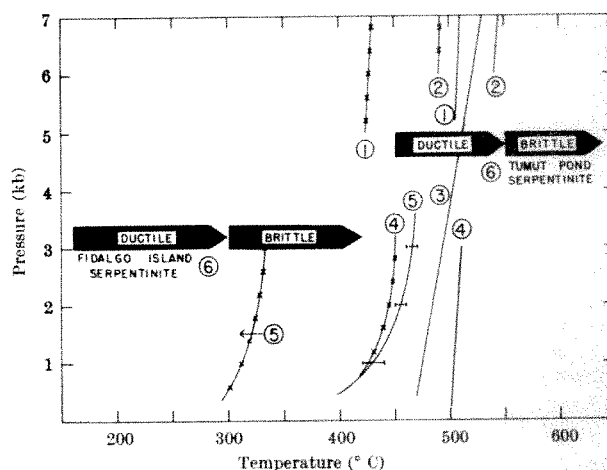
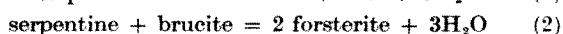
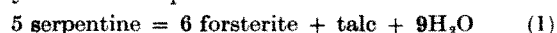


Fig. 1. Reaction curves for the dehydration of serpentine compared with previously reported curves. The conditions of weakening of deformed serpentinites are also shown. Reaction (1):  $\times$ — $\times$ ; reaction (2):  $\bullet$ — $\bullet$ . Numbers refer to: 1, Pistorius; 2, Kitahara *et al.*; 3, Yoder; 4, Bowen and Tuttle; 5, this work; 6, Raleigh and Paterson.

Fig. 1 compares the reaction curves of the two, measured to 3 kbars including the results of more recent experiments<sup>5,6</sup> in the 6–40 kbar pressure range. These show a reasonable fit with the low pressure results of Bowen and Tuttle, although the results of Pistorius<sup>5</sup> for reaction (2) can be extrapolated to somewhat lower temperatures. Yoder's<sup>7</sup> "preliminary unreversed curve" for (1) extends to lower pressures and it lies at slightly lower temperatures than the other determinations. No other detailed studies of these reaction curves below 5 kbars appear to have been published, despite their significance and despite the known problems of metastability in this system<sup>8,9</sup>.

We have undertaken a study of serpentine stability in the system  $\text{MgO}-\text{FeO}-\text{Fe}_2\text{O}_3-\text{SiO}_2-\text{H}_2\text{O}$  in which the oxygen fugacities have been buffered; particular attention being paid to the attainment of equilibrium and to the reversal of reactions<sup>10</sup>. By duplicating experiments, we are confident that the reaction curves presented by Bowen and Tuttle for serpentine free of iron are too high in temperature because metastable products are produced from oxide starting mixtures in short duration runs (3 days). Longer runs, and the use of starting mixtures composed of synthetic minerals involved in the reactions, provide the revised results shown in Fig. 1. Complete reaction was not achieved at temperatures within 50° C of the curves even after runs of 25 days' duration. Each run compared reactions in separate starting mixtures corresponding, respectively, to the phase assemblages stable above and below a reaction curve. The position of the revised curve for reaction (1) is based on recognition of the direction of reaction—hydration or dehydration—in these starting mixtures; the horizontal lines on the curve represent the experimental temperature bracket. A theoretical curve for reaction (1) calculated from new thermochemical data on forsterite and serpentine<sup>11</sup> shows very good agreement with our experimental curve. The arrow on the curve for reaction (2) indicates that its position has been determined so far only on the basis of the reverse hydration reaction. The precise position of the equilibrium curve for reaction (2) is not yet known because partial dehydration occurs in many samples held below the plotted curve for as long as 3 weeks.

Reaction (2) is lowered by about 100° C compared with previous results, bringing it much closer to the temperature of weakening of the Fidalgo Island serpentinite bearing brucite. Reaction (1) is lowered by 40°–70° C compared with the original curve of Bowen and Tuttle, which moves the dehydration to temperatures lower than that of weakening of the Tumut Pond serpentinite free of brucite. The deformation experiments, however, were of short



duration<sup>3</sup> and the previous phase equilibrium results shown in Fig. 1 confirm that with shorter runs the dehydration occurs at higher temperatures than the equilibrium reaction.

The results of these phase equilibrium experiments are consistent with the proposal of Raleigh and Paterson that the weakening of serpentinite is caused by liberation of water during dehydration, which suggests that the presence of brucite is probably responsible for the low temperature weakening of the Fidalgo Island serpentinite. Thus the presence or absence of brucite in serpentinite<sup>12</sup> may provide an important control on the various tectonic processes discussed by Raleigh and Paterson.

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### Potassium-Argon Ages from the Oldest Metamorphic Belt in India

INTENSIVE structural and stratigraphic studies, supplemented by several potassium-argon ages, recently led Sarkar and Saha<sup>1-3</sup> to recognize two distinct orogenic belts in the Pre-Cambrian tract of Singhbhum and adjacent areas<sup>4,5</sup> in Eastern India (Fig. 1, inset). The orogeny which gave rise to the Iron Ore orogenic belt in the south—which includes the Iron Ore Series of low grade metasediments and metavolcanics—had its culmination in the emplacement of the granitic rocks (about  $2,000 \times 10^6$  yr) which constitute the northern part of the Singhbhum granitic complex (Fig. 1). The Singhbhum Orogenic belt in the north, which is thought to have a closing age of about  $900 \times 10^6$  yr and which consists mostly of high grade metasediments and basic rocks, truncates the Iron Ore Orogenic belt along a prominent arcuate thrust zone. Evidence for the existence of an older group—underlying the Iron Ore Series, the Older Metamorphic Series—of moderate to high grade metamorphic argillites, calc-magnesian metasediments and arenites was also recorded<sup>2,3</sup>. Sarkar and Saha<sup>6</sup> also suggested that there was old granitic activity associated with the Older Metamorphic Series about  $3,000 \times 10^6$  yr ago on the basis of a potassium-argon age of  $3,035 \times 10^6$  yr (ref. 7) from a coarse muscovite pegmatite near Joropokhar ( $22^\circ 24' 45''$ :  $85^\circ 45' 10''$ ).

The area of fifty square miles to the west and south of Champua ( $22^\circ 04'$ :  $85^\circ 40'$ ) (Fig. 1) constitutes the type area of the Older Metamorphic Series; there the series is extensively injected and replaced by granite and pegmatite<sup>4,8</sup>. In an attempt to establish the stratigraphic status of the Older Metamorphic Series more firmly the type area

of Older Metamorphics and adjacent Singhbhum granitic complex around Champua (Fig. 1) was selected for intensive structural, petrological<sup>9</sup> and geochronological studies.

An important unit of calc-magnesian metamorphic rocks which outcrops throughout Champua is folded into a megascopic, almost neutral fold plunging steeply E.N.E.; mica schists lie along the core and flanks of this major fold. The prominent bedding foliation ( $S_1$ ) in these rocks and the puckers and mineral lineations ( $L_1$ ) (which are subparallel to the principal fold axis) appear to be continuous and conformable with the gneissose foliation and mineral lineations in the adjacent biotite granodiorite gneiss. In both the metamorphics and the gneiss  $S_1$  has been locally folded megascopically and mesoscopically about moderate to steep S.E. plunging axes and a second foliation ( $S_2$ ) has developed along the axial planes of such folds (Fig. 1). Towards the north, the Older Metamorphics are overlain unconformably by a group of slightly metamorphosed basic lavas (Jagannathpur lavas)<sup>10-12</sup> which dip gently to the west.

The principal mineral assemblages of the Older Metamorphic Series are plagioclase-hornblende-diopside-calcite-quartz-(sphene), plagioclase-hornblende-biotite-calcite-quartz, plagioclase-hornblende-diopside-quartz-epidote, plagioclase-hornblende-chlorite-calcite-(magnetite); quartz-muscovite-biotite-magnetite and quartz-muscovite-(oligoclase). The mineral assemblage suggests a sillimanite almandine sub-facies of the amphibolite facies with partial retrogression to staurolite almandine sub-facies<sup>13</sup>. The small intrusions of non-foliated metabasalt (plagioclase-hornblende-chlorite-biotite-iron ore-quartz-augite) within the calc-magnesian metasediments show a much lower grade of metamorphism (greenschist facies) and appear to have been emplaced subsequent to the chief regional metamorphism (and granitization) of the Older Metamorphics.

The biotite granodiorite gneisses of the area are medium grained, well foliated rocks which consist dominantly of plagioclase ( $An_{24-40}$ ) and quartz, together with fair amounts of biotite, occasional microcline, chlorite, epidote, hornblende sphene and rare muscovite. The average mineral composition (by weight per cent) of these granitic rocks (based on twenty-four analyses) is quartz, 21.6; plagioclase, 65.2; microcline, 3.6; colour index, 9.4 (including 6 per cent biotite). A distinct gradation can be seen from the biotite granodiorite to the calc-magnesian metasediments (through an intermediate stage of hornblende granodiorite) around many inclusions in the metamorphics; clear textural evidence of replacement of the hornblende-epidote aggregate by the later formed plagioclase-quartz is common. These facts, taken together with the evidence of structural unity between the granitic and the metamorphic rocks, suggest a metasomatic transformation of the calc-magnesian metasediments (and meta-argillites) to biotite granodiorite-gneiss, and that  $S_1$  and  $L_1$  of the granodiorite gneiss are relics. Sericitization along the  $S_2$  plane of the gneiss suggests that the granitization occurred after the development of  $S_1$ , but before the second folding.

For the purpose of age determination eight representative samples were collected by A. K. Saha and B. Chakravarti from the Older Metamorphics of Champua area. The mineral samples (two of muscovite from mica schists, three of hornblende from calc-magnesian metasediments and three of biotite from biotite granodiorite gneisses) were prepared from the rocks by grinding and sieving; biotite and hornblende were separated electromagnetically and muscovite by inclined shaking board method. The separated mineral samples were dated by S. N. Sarkar and J. A. Miller, using potassium-argon<sup>14,15</sup>. Potassium oxide content was determined by flame photometry; up to six separate portions of each sample were measured and the mean value and error calculated from the standard deviation of the mean of the measurements (Table 1). To avoid any systematic error in this method, checks against

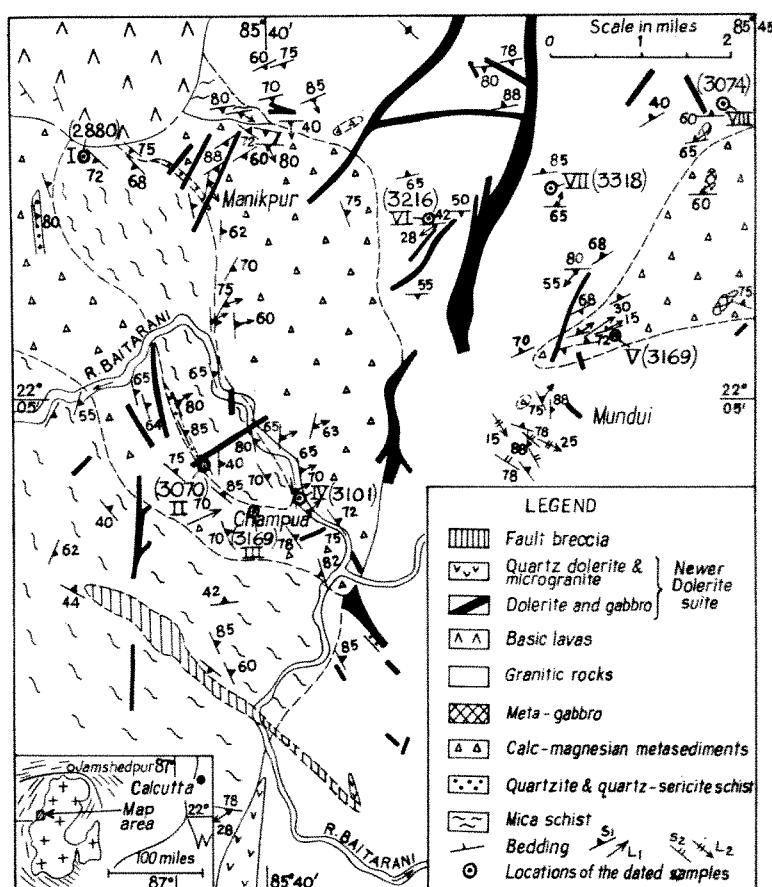


Fig. 1.

standard samples were carried out. The  $^{40}\text{Ar}$  content was determined using the isotope dilution technique. From the  $^{36}\text{Ar}$ ,  $^{38}\text{Ar}$  and  $^{40}\text{Ar}$  ratios (measured with Omegatron type mass spectrometer)<sup>16</sup> the percentage of  $^{40}\text{Ar}$  of atmospheric origin and the volume of radiogenic  $^{40}\text{Ar}$  of sample were calculated. The total error in age determination of each sample was calculated on the basis of observed errors in multiple potassium oxide determinations, the isotopic composition and volume of  $^{38}\text{Ar}$  spike and the isotopic ratios of the final gas mixture. The age data are given in Table 1, and location and petrographic description of the samples in Table 2.

Sericitized muscovites (Table 1 and Fig. 1) from mica schists yielded ages of  $2,880$  and  $3,070 \times 10^6$  yr, three hornblende samples from calc-magnesian metasediments yielded ages of  $3,169$ ,  $3,101$  and  $3,169 \times 10^6$  yr, whereas three biotite samples from biotite granodiorite gneiss gave ages of  $3,216$ ,  $3,318$  and  $3,074 \times 10^6$  yr. Sample 1 (muscovite) is situated about 250 m south of the boundary of the overlying gently dipping younger Jagannathpur lavas, the base of which was less than 50 m above the sample locality before erosion. The relatively low apparent age ( $2,880 \times 10^6$  yr) of the sample may be due to partial

argon loss from the finely divided sericite as a result of heating by the lava.

Thus, with the exception of sample 1, seven samples of three different minerals—muscovite, hornblende and biotite—from parametamorphics and granite gneisses yield fairly concordant potassium-argon ages ranging between  $3,070$  and  $3,318 \times 10^6$  yr. This suggests one chief metamorphic episode in the Champua rocks. As individual dates indicate the time at which metamorphism and/or granitization ended, it may be concluded on the basis of available data that regional metamorphism and granitization of the Older Metamorphic Series must have closed within the period  $3,318$ – $3,070 \times 10^6$  yr. Because these rocks are surrounded on three sides by the younger Singhbhum granite complex, parts of the Older Metamorphics area might have been reheated (probably causing partial argon loss) during the emplacement of the vast volumes of Singhbhum granite magmas in the adjacent areas. Because the average of seven dates is  $3,159 \times 10^6$  yr and there is the possibility of partial argon loss due to later thermal events, the closing date of the Older Metamorphic Cycle may be taken as about  $3,200 \times 10^6$  yr; the actual deposition of the sediments on an older basement

Table 1. POTASSIUM-ARGON DATA FROM THE OLDER METAMORPHIC SERIES

Loc. No.	Indian lab. No.	Cambridge lab. No.	Sample description	K <sub>2</sub> O per cent No. of det. and error	Atmos. contam. $^{40}\text{Ar}$ per cent	Vol. of $^{40}\text{Ar}^*$ Sample wt. (g)	Age and error ( $\times 10^6$ yr)
I	J/10/62	KA/990	Muscovite from muscovite-quartz-plagioclase schist	(5) $9.54 \pm 0.03$	3.9	2.1455	$2,880 \pm 60$
II	AK/28-I	KA/995	Muscovite from muscovite-quartz-plagioclase schist	(6) $1.57 \pm 0.18$	3.6	0.40088	$3,070 \pm 300$
III	AK/75	KA/1,011	Hornblende from hornblende-plagioclase-quartz schist	(6) $0.43 \pm 0.02$	1.55	0.1172	$3,169 \pm 180$
IV	C/1/63	KA/992	Hornblende from hornblende-plagioclase-biotite schist	(6) $0.82 \pm 0.002$	4.07	0.2138	$3,101 \pm 40$
V	AK/102	KA/1,022	Hornblende from hornblende-plagioclase-quartz schist	(6) $0.40 \pm 0.002$	12.06	0.1082	$3,169 \pm 63$
VI	AK/109	KA/1,062	Biotite from biotite granodiorite gneiss	(5) $3.73 \pm 0.075$	2.1	1.047	$3,216 \pm 96$
VII	AK/214	KA/1,150	Biotite from biotite granodiorite gneiss	(3) $6.06 \pm 0.003$	2.4	1.818	$3,318 \pm 99$
VIII	AK/161	KA/1,146	Biotite from biotite granodiorite gneiss	(3) $6.28 \pm 0.003$	4.1	1.607	$3,074 \pm 60$

$\lambda_B = 4.72 \times 10^{-10} \text{ yr}^{-1}$ ;  $\lambda_e = 0.584 \times 10^{-10} \text{ yr}^{-1}$ . \*  $^{40}\text{Ar}$  = radiogenic argon ( $\text{mm}^3$ , N.T.P.).

Table 2. SAMPLE LOCALITIES AND DESCRIPTIONS

A brief description of each mineral sample and the host rock from the Older Metamorphic Series is given in the following order: locality and sample numbers, type of mineral and rock, location in latitudes and longitudes, petrographic description.

I. J/10/62. Partly sericitized muscovite from muscovite-quartz-plagioclase schist ( $22^{\circ} 7' 20''$  N. :  $85^{\circ} 38'$  E.) from stream section west of Daobera, near the boundary of the overlying basic lava. Subidioblastic muscovite flakes (0.5–2.0 mm) with preferred dimensional orientation define foliation. In patches they are altered to aggregates of finely divided sericite showing subparallel arrangement. Inequant plagioclase (oligoclase) and quartz (<1 mm) are often elongated subparallel to foliation. Reddish brown and opaque dusty iron ore as accessories.

II. AK/28-I. Sericitized muscovite from muscovite-quartz-plagioclase (—rutile) schist ( $22^{\circ} 4' 15''$  N. :  $85^{\circ} 39' 15''$  E.) from 1 mile north-west of Champua. Subidioblastic muscovite flakes (0.3–5 mm) occur mostly as relicts in the groundmass of fine acicular aggregates of sericite, individual needles (0.05–0.2 mm). Slightly elongated quartz grains (0.2–1.0 mm) are subparallel to foliation defined by preferred orientation of muscovite and sericite. Coarse quartz (<0.9 mm) occur in pods. Inequant grains of plagioclase (0.2–0.4 mm) are partly altered to sericite aggregates.

III. AK/75. Hornblende from hornblende-plagioclase-quartz schist ( $22^{\circ} 4' 40''$  N. :  $85^{\circ} 40'$  E.), north-west of Champua. Fine grained well foliated rock with fairly elongated subidioblastic hornblende (0.1–1.0 mm), subequant grains of moderately saussuritized plagioclase (0.2–1.0 mm), few xenoblastic quartz (0.1–0.2 mm), sphene and iron ore. Hornblende, 56.2; plagioclase, 38.2; quartz, 5.0; sphene, 0.3 and iron ore, 0.3, weight per cent.

IV. C/1/63. Hornblende from hornblende-plagioclase-quartz schist ( $22^{\circ} 4' 40''$  N. :  $85^{\circ} 40' 20''$  E.), north-east of Champua. A large number of granitic injections occur within 100 yd. of this exposure. Nematoblastic texture, about 70 per cent xenoblastic to subidioblastic elongated hornblende (0.1–1.5 mm), which is pleochroic (pale to dark green) and shows preferred orientation; xenoblastic aggregate of saussuritized plagioclase and quartz (0.1–0.2 mm) with little biotite, calcite, sphene and iron ore.

V. AK/102. Hornblende from hornblende-plagioclase-quartz schist ( $22^{\circ} 5' 45''$  N. :  $85^{\circ} 43' 30''$  E.) from north of Mundui. Coarse granoblastic texture with subidioblastic hornblende (0.2–2.0 mm) (pleochroic from pale green to brownish green), with a little xenoblastic quartz and saussuritized plagioclase (0.2–0.4 mm). Sphene, apatite and dusty ore as accessories. Hornblende, 75.8; plagioclase, 21.3; quartz, 2.6 and sphene, 0.2 weight per cent.

VI. AK/109. Biotite from biotite granodiorite gneiss which is metamorphically granitized hornblende schist ( $22^{\circ} 7' : 85^{\circ} 41' 50''$ ) from north of Pata Joint. Rudely foliated rock with subidioblastic biotite flakes (<2 mm) (pleochroic pale yellow to dark greenish brown and brown), a few flakes partly altered to chlorite along cleavage; hornblende in xenoblastic or skeletal grains (<2 mm) (pleochroic pale yellow to dark green). Xenoblastic plagioclase, quartz, microcline (<5 mm) and myrmekite. Sphene, epidote, apatite and opaque minerals as accessories. Quartz, 22.6; microcline, 2.7; plagioclase, 64.7; biotite, 6.1; hornblende, 0.3; opaque minerals, 1.0 and others, 2.5, weight per cent.

VII. AK/214. Biotite from biotite granodiorite gneiss ( $22^{\circ} 7' 45''$  N. :  $85^{\circ} 43'$  E.) from granitized schist 1.5 miles north-east of Pata Joint. Well foliated rudely banded rock with quartz (0.8–2.5 mm), slightly saussuritized oligoclase-andesine (1.5–2.0 mm), tabular biotite (0.5–1.5 mm), some chlorite (<0.3 mm) in the matrix associated with biotite, sphene (<0.4 mm) and interstitial microcline.

VIII. AK/161. Biotite from biotite granodiorite gneiss ( $22^{\circ} 8' 45''$  N. :  $85^{\circ} 44' 40''$  E.), from granitized schist east of Gangpur. Well foliated, subequigranular rock with quartz (0.4–0.6 mm), moderately saussuritized acid andesine (0.7–1.5 mm), tabular biotite (0.3–1.0 mm), interstitial microcline in small grains, flakes of chlorite (<0.3 mm) intergrown with biotite, sphene and iron ore (<0.2 mm).

and their metamorphism must have taken place much earlier than about  $3,300 \times 10^6$  yr.

Although the actual extent of the rocks representative of this cycle is not fully known at present, the recent field studies by A. K. Saha and his co-workers suggest that a considerable part of the younger Singhbhum granitic complex between longitudes  $22^{\circ} 15'$  E. and  $21^{\circ} 45'$  E. has relicts of rocks belonging to the Older Metamorphic Cycle.

On the basis of all the available age data<sup>6,7,17–19</sup> from the Indian Shield it may be concluded that the Older Metamorphics of Singhbhum-Keonjhar represent relicts of the oldest recognizable orogenic belt in India. This cycle may be correlated with the Katarachean cycle ( $3,500$ – $3,000 \times 10^6$  yr) of the Baltic Shield<sup>20</sup> where Katarachean rocks occur as relicts in the younger Saamian granites and gneisses of the Kola Peninsula. Radiometric ages above  $3,000 \times 10^6$  yr are rather rare and a few reported from Africa<sup>21</sup> are a model age of galena (about  $3,480 \times 10^6$  yr), Bomu Complex, North Congo, a strontium-rubidium age of microcline ( $3,270 \times 10^6$  yr) from Upper Luanyi Cycle pegmatite, South Congo, and a potassium-argon age of biotite ( $3,150 \times 10^6$  yr) from granite at Broderick Falls, Kenya. Two anomalously high potassium-argon ages ( $3,300$  and  $3,700 \times 10^6$  yr) from biotite near the Grenville front in East Canada, where co-existing minerals yielded much younger ages, are thought to reflect<sup>22</sup> the excess argon content of the mineral rather than a relict of an older orogenic belt.

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## Isotopic Ages from the Pegmatite Provinces of Eastern Brazil

EASTERN Brazil is composed mainly of metamorphosed basement rocks<sup>1</sup>, gneisses and paragneisses (Fig. 1), which are found to increase in grade of metamorphism towards the coast, reaching granulite facies around Juiz de Fora and Lima Duarte, but which at the same time decrease in intensity of tectonic deformation. The region has been affected by at least two orogenic episodes each accompanied by characteristic igneous activity. Traces of a third earlier orogeny occur, but the first distinct evidence of movement is that of the Barbacena orogeny with its quartz dioritic type of intrusions. This is followed later by the Paraíba or Algonkian orogeny with its granitic intrusions, rich in alkali feldspar<sup>2</sup>.

The basement gneisses are cut by coarse pegmatite veins which are intruded as part of the late stage igneous activity associated with the orogenies. Samples of micas were collected from the pegmatites by one of us (H. E.) and sent to Cambridge for potassium-argon analysis. The potassium content of the samples was measured on an EEL flame photometer and the argon isotope ratios on an omegatron<sup>3,4</sup> (Table 1).

Six of the nine samples analysed gave ages of around  $550$ – $500 \times 10^6$  yr. Loss of argon, probably resulting from secondary alteration, may account for the younger age of sample SA 164. The high age of the biotite from the Socorro pegmatite may have been caused by incomplete

Table 1

Sample number	Rock type and location	Mineral	Percentage of atmos. contam.	Vol. radiometric $^{40}\text{Ar}$ wt. of samples (g)	Percentage of $\text{K}_2\text{O}$	Age $\pm$ error (yr $\times 10^6$ )
SA 163	Pegmatite from Caculé, Bahia from the Espinhaço Zone	Muscovite	26.1	0.1980	10.09	516 $\pm$ 14
SA 164	Pegmatite from Boqueirão de Parelhas, Rio Grande do Norte	Lepidolite	18.9	0.1861	10.44	490 $\pm$ 20
			3.16	0.1760		451 $\pm$ 10
			11.2	0.1756		450 $\pm$ 8
			14.0	0.179		462 $\pm$ 14
SA 165	Pegmatite from Boqueirão de Parelhas, Rio Grande do Norte	Muscovite	7.8	0.192	9.90	511 $\pm$ 14
SA 166	Pegmatite from Perús (S.P.)	Lepidolite	31.1	0.1848	9.77	493 $\pm$ 10
			4.92	0.2170		574 $\pm$ 14
SA 167	Pegmatite from east of Acari, Rio Grande do Norte	Muscovite	47	0.219	9.66	579 $\pm$ 11
			9.45	0.2015		550 $\pm$ 23
SA 168	Pegmatite from Bicas, Minas Gerais	Muscovite	26.8	0.1896	9.95	516 $\pm$ 25
			29.1	0.1930		511 $\pm$ 14
SA 169	Pegmatite from Manhuassú, Minas Gerais	Muscovite	0.181	0.2140	10.00	483 $\pm$ 12
			25.5	0.1915		556 $\pm$ 16
SA 266	Pegmatite from Socorro, São Paulo	Biotite	72.8	0.250	9.51	505 $\pm$ 10
			73.7	0.232		663 $\pm$ 68
SA 267	Spodumene-pegmatite of Volta Grande, São João del Rei, Minas Gerais	Muscovite	4.44	0.471	9.46	623 $\pm$ 65
			68.6	0.5175		1,105 $\pm$ 39
						1,186 $\pm$ 98

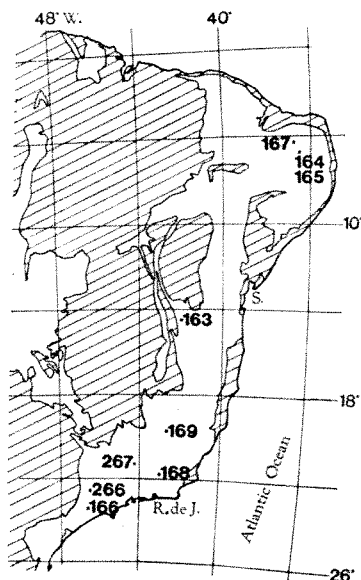


Fig. 1.

out-gassing during the Paraíba orogeny, but is more likely to be because the pegmatite was syntectonic.

These ages may be assumed to correspond to the time of intrusion of the pegmatites, although they do in fact refer to the time when the minerals had cooled sufficiently to become closed systems and trap the radiogenic argon, because the time interval involved is negligible in geological terms<sup>5</sup>. The pegmatites formed a late stage part of the Paraíba activity so the age of the Paraíba orogeny may be taken to be lower to middle Cambrian, and not early Pre-Cambrian as had previously been thought<sup>6</sup>, and the age of the Barbacena orogeny to be around  $1.150 \times 10^6$  yr, from the evidence of the Volta Grande pegmatite.

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## PHYSICS

### Measurement of Ionization Potentials from Contact Charge Transfer Spectra

THE use of charge transfer spectra for the evaluation of ionization potentials is well known. Thus Foster has found an empirical relationship between the ionization potentials of condensed ring aromatic hydrocarbons and the frequencies of maximum absorption of their charge transfer complexes with chloranil in carbon tetrachloride<sup>1</sup>. Foster's formula is limited to the condensed ring aromatic hydrocarbons because the binding of electron donors to acceptors in charge transfer complexes is not caused entirely by charge transfer; intermolecular forces vary between different classes of molecules and influence complex formation. In general, the relationship between the ionization potential and the frequency of maximum absorption of the charge transfer complex is given by

$$h\nu = I_p - E_A - \frac{e^2}{r} - \Delta$$

where  $I_p$  is the ionization potential of the electron donor,  $E_A$  is the electron affinity of the electron acceptor,  $\frac{e^2}{r}$  is the coulombic interaction between the two and  $\Delta$  represents all the other intermolecular forces<sup>2</sup>. There exists a type of charge transfer between molecules which does not produce a bound complex and is termed contact charge transfer<sup>3</sup>. The electron donors and acceptors are randomly disposed in solution but when they come into contact with each other momentary charge transfer takes place which gives rise to an absorption spectrum. One electron acceptor which takes part in contact charge transfer is oxygen<sup>4</sup>. Measurements made of its equilibrium constant with electron donors yield a value of zero<sup>4,5</sup>. Furthermore, the absorption band caused by this type of charge transfer increases to higher frequency without having a maximum absorption peak, the type of behaviour that can be predicted for transitions occurring between energy levels which show no minima, that is two non-bonding levels. Because there is no binding in contact "complexes" it would appear that a very general relationship can be set up between the ionization potentials of electron donors and the onset of absorption of the charge transfer band arising with oxygen.

The absorption spectra of a wide range of electron donors with oxygen dissolved in chloroform under 120 atmospheres has been measured in an apparatus essentially the same as that described by Evans<sup>4</sup>. The absorption spectra of the charge transfer complexes with chloranil in carbon tetrachloride have also been measured. The results are shown plotted as the frequency of maximum absorp-



Table 1. IONIZATION POTENTIALS

Molecule	Predicted ionization potential	Measured ionization potential*	Molecule	Predicted ionization potential	Measured ionization potential*
Methylamine (a)	9.29	8.97	Tetramethyluric acid	7.91	
Trimethylamine	8.48	7.82	Alanine	9.63†	
Ethylamine (g)	8.74	8.86	Proline	9.36†	
Diethylamine (e)	8.36	8.01	Glycine	9.30†	
Triethylamine (f)	8.26	7.50	Tryptophan	8.43†	
n-Butylamine (b)	8.79	8.71	Biphenyl	8.46	8.27
t-Butylamine (c)	8.72	8.64	o-Terphenyl	8.43	
Formamide (M)	9.36	10.25	p-Terphenyl	8.29	
Dimethylformamide (K)	8.92	9.12	Diphenylxalate	7.94	
Dimethylacetamide (L)	8.81	8.81	Benzene (1)	9.21†	9.24
Acetone	9.24	9.69	Naphthalene (2)	7.85†	7.96
Aniline	7.88	7.70	Anthracene (3)	7.37†	
o-Aminobenzoic acid	8.29		Chrysene (7)	7.80†	
Dimethylaniline (D)	8.06		Pyrene (8)	7.55†	
Pyridine (Py)	9.02	9.32	Triphenylene (9)	8.09†	
Quinoline (Q)	7.98				
Acridine (A)	7.39	7.41	1-2-5-6 Dibenzanthracene (10)	7.80†	
Tetrahydrofuran	9.13	9.54 (?)		7.80	
Imipramine	8.03		3-4 Benzpyrene (11)	7.19†	
Reserpine	7.88		1-2-7-8 Dibenzanthracene (12)	7.68†	
Chloroquine (C)	7.84			7.68†	
Indole	8.04		1-2-3-4 Dibenzanthracene (14)	7.61†	
Phenothiazine	8.17				
Caffeine	8.50		1-2-4-5 Dibenzyrene (17)	7.27†	
Ethylbenzene (Et)	9.04	8.76	2-Fluorenylacetylamine	8.34	
$\beta$ -Methylnaphthalene ( $\beta$ )	8.04	7.96	Stilbene	7.60	
Hexamethylbenzene (H)	8.58		Fluorene	7.78	
2-3 Dimethylnaphthalene (N)	8.11		Fluoranthene	7.72	
20-Methylcholanthrene (Mc)	7.66				

\* Ref. 6. † Measured in water. ‡ Obtained from the chloranil spectrum.

tion of the charge transfer band in electron volts against the frequency of onset of oxygen absorption.

It can be seen that the molecules fall into several distinct groups. The condensed ring aromatic hydrocarbons fall on a straight line as do the aliphatic amines and the amides, these lines being at  $45^\circ$  to the axes. A fairly satisfactory fit of the aminoquinoline derivative, chloroquine, can also be obtained to a line of heterocyclic nitrogen compounds. Furthermore, the straight lines are parallel showing that there is a constant difference of interaction between the different groups of molecules. Foster's rule for the condensed ring aromatic hydrocarbons is

$$I_p = 5.12 + 1.13 h\nu_{CT} \text{ (eV)}$$

Using this rule to relate the ionization potential to onset of oxygen absorption gives the equation

$$I_p = 5.24 + 1.13 h\nu_0 \text{ (eV)}$$

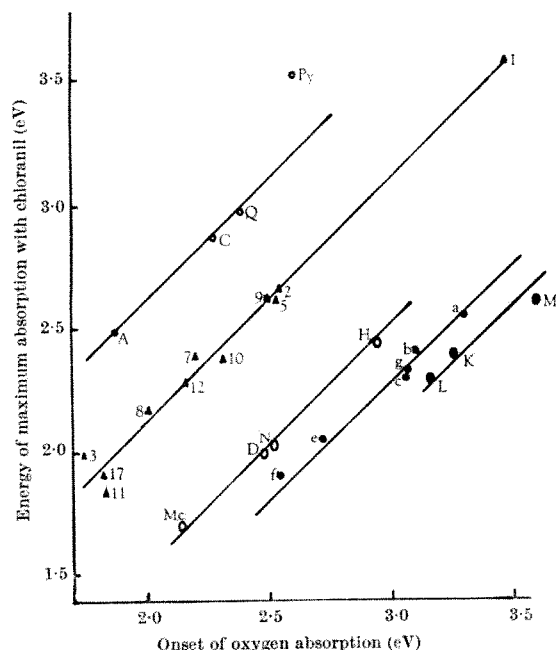


Fig. 1.

Values of ionization potentials derived from the oxygen absorption are given in Table 1 and are contrasted with values obtained from photodissociation studies<sup>6</sup>. The values agree reasonably well and, what is more important, show the same trends. The results are also in satisfactory agreement with polarographic measurements of electron donation<sup>7</sup>. One advantage of this method is that it is more readily applicable than the usual methods of measuring ionization potentials, which depend on obtaining molecules in their vapour phase. Many large organic or biological chemicals have too low a vapour pressure for such measurements to be made. Measurements of charge transfer complexes with oxygen have certain advantages over those with chloranil. The latter reacts with a number of the amines used in this study to produce strongly absorbing products. Under the conditions in the experiments reported here there is no irreversible reaction, as shown by the reversibility of the spectral changes on removal of oxygen. The fact that a wide range of electron donating compounds can readily produce charge transfer complexes, however, may have interesting implications in biology.

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### Scanning Ion Microscopy and Ion Beam Micro-machining

FOR some years it has been possible to focus beams of electrons into fine probes with diameters in the range 10  $\mu\text{m}$ –100  $\text{\AA}$ . These have been used for electron beam machining, electron probe analysis and scanning electron

microscopy. Progress in focusing ion beams has been more limited. Notably, Gabbay *et al.*<sup>1</sup> have used a beam of lithium ions from a coated tungsten hair-pin filament and have reported a resolution of the order of  $3\text{ }\mu\text{m}$  in a scanning ion microscope. The only work in which very fine beams of rare gas ions have been used appears to be that of Saporin *et al.*<sup>2</sup>, who have reported a final beam diameter of  $10\text{--}15\text{ }\mu\text{m}$  at  $30\text{ kV}$ . von Ardenne<sup>3</sup> has described two systems, one using a canal-ray source and the other a capillary source in which ions were focused to about  $5\text{ }\mu\text{m}$ , but in this case the final lens used was of very short focal length and the focus was not readily accessible.

Much larger beams of  $100\text{ }\mu\text{m}$  and above have been used in ion-bombardment sources in mass spectrometers<sup>4-8</sup> and by Kanaya *et al.*<sup>9</sup> for ion beam machining.

One of us has discussed the possibility of obtaining a useful current of rare gas ions in a beam of the order of  $1\text{ }\mu\text{m}$  in diameter<sup>10</sup> and assessed the feasibility of using an ion probe for making localized measurements of isotopic abundance with a spatial resolution comparable with that obtained in electron probe X-ray microanalysis. An ion probe mass spectrometer with a high spatial resolution is being developed in this laboratory and it has been possible to determine the performance of the ion probe-forming system by using it as a scanning ion microscope and for ion beam machining.

At present, the instrument uses a duoplasmatron source<sup>11</sup> similar in design to that described by Moak *et al.*<sup>12</sup> followed by two electrostatic condenser lenses to form a probe of argon ions. The source itself operates at a pressure of approximately  $10^{-2}$  torr and differential pumping across apertures is used to maintain a good vacuum in the specimen chamber. The scanning technique used to form images of the surface is similar to that used in scanning electron microscopy, deflexion of the beam being achieved by electrostatic plates placed after the final lens. This lens is so designed that it may be operated with a clear working distance of  $4\text{ cm}$ . Ions impinging on the specimen cause secondary electrons to be produced by Auger and resonance neutralization and by kinetic emission<sup>13</sup>. These electrons are collected by an accelerating scintillation detector of the type devised by Everhart and Thornley<sup>14</sup>. Contrast in the image is thus

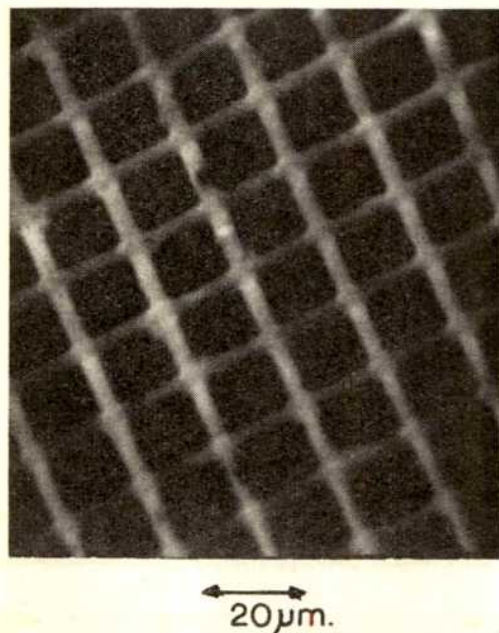


Fig. 1. Scanning ion micrograph showing 1,500 t.p.i. silver mesh ( $20\text{ keV}$  argon ions).

due to variation in the yield of secondary electrons from point to point and may arise from variations in specimen inclination, chemical composition, crystal orientation or electrical potential and from the presence of electrical or magnetic fields. In the examples shown, the contrast is topographic, that is, it arises from varying inclination of the specimen surface. The image of a 1,500 t.p.i. silver mesh inclined at  $45^\circ$  to the incident ion beam is illustrated in Fig. 1. The bars of the mesh are approximately  $4\text{ }\mu\text{m}$  wide and examination of the surface features indicates a resolution of the order of  $0.5\text{ }\mu\text{m}$ .

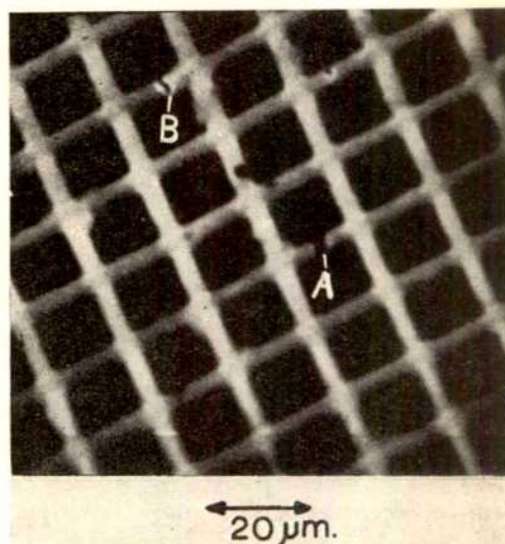


Fig. 2. Scanning ion micrograph showing results of ion beam machining on a 1,500 t.p.i. silver mesh ( $20\text{ keV}$  argon ions).

The sputtering rate for silver bombarded by  $20\text{ keV}$  argon ions can be estimated approximately from the data of Almén and Bruce<sup>15</sup>. For an angle of incidence of  $45^\circ$ , the rate of removal of silver is of the order of  $0.017\text{ }\mu\text{m/sec}$  for a current density of  $1\text{ amp/cm}^2$ . The current density at which the scanning pictures were taken was  $13\text{ amp/cm}^2$ . The same beam was used to cut through two of the grid bars (thickness  $2.5\text{ }\mu\text{m}$ ) shown in Fig. 1. At A (Fig. 2) a slightly defocused beam has been used, while at B the diameter of the beam used was close to the minimum size obtained. The time taken to cut through the whole width of the grid bar in the latter case was about  $2\text{ min}$ , in reasonable agreement with the calculated time on the basis of the rate of removal given here.

It has been found possible to operate the system with a  $1\text{ }\mu\text{m}$  probe and a current density of up to approximately  $0.6\text{ amp/cm}^2$ , and thus fairly rapid machining is possible. The rate of cutting even at this value is considerably slower than that obtainable by electron beam techniques but the use of ions has the advantage that, for reasonable current densities, the process is non-thermal. Furthermore, experience with the present instrument suggests that the beam diameter may be reduced below  $0.5\text{ }\mu\text{m}$ , making even finer machining possible. It should be noted that for fine beams and deep cuts redeposition of sputtered material on the sides of the hole or groove will influence the rate of sputtering. Initially, the outer parts of the bombarded area will be eroded slowly compared with the centre as a result of the Gaussian distribution of current in the beam. As the hole develops, however, the rate of erosion in the peripheral areas should increase because of the increase in angle of incidence. The final hole may therefore be expected to have steep sides rather than a simple Gaussian profile.

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## THE SOLID STATE

### Tensile Strength of Granular Materials

THE contribution by Carr<sup>1</sup> on the tensile strength of granular materials raises two issues of fundamental importance: first, the validity of the equation for tensile strength proposed by Smalley and Smalley<sup>2</sup>

$$\sigma \simeq 4.6 \frac{Bt}{D^3} \quad (1)$$

where  $B$  is the interparticle force per contact,  $D$  is the diameter of the particles and  $t$  is the thickness of the fracture zone; and secondly, the manner in which  $B$  varies with the degree of saturation. Equation 1 was derived on the assumption that fracture occurs on a plane normal to the tensile direction and that the particles intersected by this plane come apart without themselves contributing to the strength. For granular materials, where the bonding is provided by the capillary action of water, rupture of the particles is never possible.

The approach may be used, however, to determine the number of particles involved in the rupture and it may be assumed that these will be equally divided between the two broken surfaces. Hence the number of particles  $P$  in each face is given by

$$P = \frac{3\rho A_a}{\pi D^2} \quad (2)$$

where  $\rho$  is the density of packing and  $A_a$  is the gross area of the section. The mean exposed surface of the particles will be hemispherical, so that if  $k$  is the number of bonds per particle the total number of broken bonds,  $N$ , is  $0.5Pk$ .

The size of each bond site is governed by the size of the particles and, for particles of equal diameter, a site occupies a small circle of diameter  $0.5D$ . If the bonds are uniformly distributed over the surface, equal areas will contain equal numbers of bonds, the tensile component of each bond being  $B \cos \theta$ , where  $\theta$  is the angle between the bond and tensile directions. Hence the mean tensile component  $B_m$  is given by

$$B_m = 0.5B \quad (3)$$

The tensile strength of the entire mass is then

$$\begin{aligned} \sigma A_a &= (0.5Pk)(0.5B) \\ &= \frac{3\rho A_a k B}{4\pi D^2} \end{aligned}$$

or

$$\sigma = \frac{3\rho k B}{4\pi D^2} \quad (4)$$

and accepting the value of  $\rho = 0.6$  given by Scott<sup>3</sup> and  $k = 8.0$  obtained from the experimental data of Bernal and Mason<sup>4</sup>

$$\sigma = \frac{3.6B}{\pi D^2} \quad (5)$$

To obtain the strength of an actual test specimen, this value must be modified by consideration of an appropriate failure criterion. The Mohr-Coulomb criterion (see Skempton and Bishop<sup>5</sup>) gives the tensile strength at failure as

$$\sigma_f = \frac{3.6B}{\pi D^2} \cdot \frac{(1 - \sin \varphi)}{(1 + \sin \varphi)} \quad (6)$$

where  $\varphi$  is the internal angle of friction of the material.

Equation 6 agrees with the corresponding equations by Rumpf<sup>6</sup> and Smalley and Smalley<sup>2</sup> in that the tensile strength is found to be directly proportional to  $B$ . The Haines-Fisher<sup>7</sup> equation for  $B$  is

$$B = \pi DT / (1 + \tan \frac{1}{2} \theta) \quad (7)$$

where  $2\theta$  is the angle subtended at the centre of the particle by the lens of water and  $T$  is the surface tension. Hence  $B$ , and consequently the tensile strength, will increase with reducing moisture content. This conclusion, however, is contrary to the experimental results quoted by Carr, and elsewhere in the literature.

The common factor in the derivation of each of the equations for tensile strength is the concept of a model consisting of particles connected by a finite number of bonds. An examination of moisture characteristics relating the energy of retention of water in the liquid phase to the water content for granular materials with uniform grains indicates that, following the initial air entry, the bulk of the water is removed at a constant energy level and only in the final stages is there an increase in the energy per unit volume. For the present example, energy of retention may be equated to capillary pressure. At any stage, the material can be considered as consisting of two parts: that in which air entry has occurred; and that in which it has not. As each succeeding section drains, it passes from the saturated to the drained state at constant energy level and the effective stress on the sample decreases by a factor of approximately 0.3 while the previously drained sections undergo only a minor stress change. Following this reasoning, Aitchison<sup>8</sup> proposed the following equation

$$\sigma = p.S + 0.3 \int_0^S p dS \quad (8)$$

where  $p$  is the energy of retention and  $S$  is the degree of saturation. For a constant energy of retention, equation 8 reduces to

$$\sigma = p(0.7S + 0.3) \quad (9)$$

from which

$$\sigma_f = p(0.7S + 0.3) \frac{(1 - \sin \varphi)}{(1 + \sin \varphi)} \quad (10)$$

Tensile strengths obtained by Carr<sup>1</sup>, Pearce and Donald<sup>9</sup> and Fowler and Radford<sup>10</sup> are shown in Fig. 1. Pearce and Donald quote their results in terms of  $T/r$  and it has been assumed that they used a value of 50 dynes/cm for  $T$ . Fowler and Radford quote moisture content and net degree of saturation and their results have been converted by the use of compaction data obtained by me.

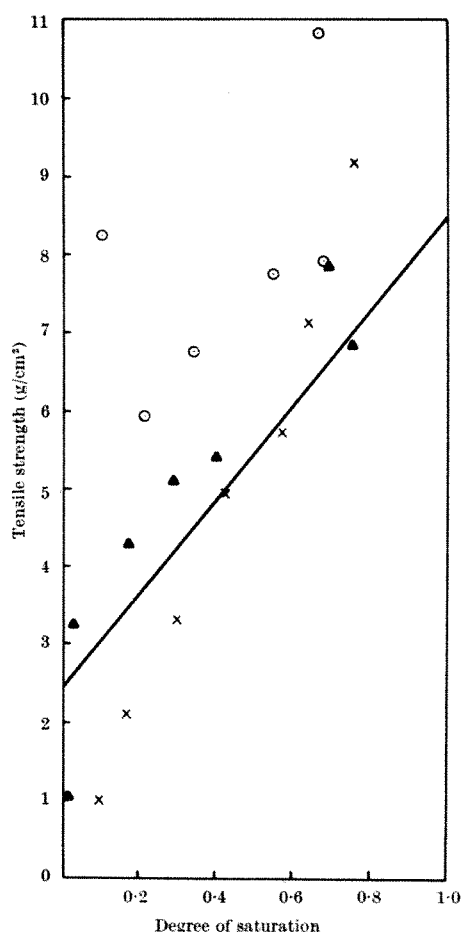


Fig. 1. Tensile strength of granular materials.  $\times$ , Fine coal<sup>1</sup>;  $\blacktriangle$ , 204 $\mu$  (ref. 10);  $\circ$ , 170 $\mu$  (ref. 9). The line is the evaluation of equation 10, assuming the internal angle of friction,  $\phi$ , is 30°.

None of the writers quotes moisture characteristics for their materials but because all particles are of the order of 200 $\mu$ , an air entry value of 30 g/cm<sup>2</sup> will be assumed. If a  $\phi$  value of 30° is also assumed, equation 10 may be evaluated. The evaluation is shown in Fig. 1 and it looks a reasonable approximation. The fit is usually considerably better when the correct energy of retention and  $\phi$  is known for each point. In the final stages of desiccation there should be a transition from equation 8 to equation 5 and it is observed that only the more carefully prepared samples of Pearce and Donald show any indication of this. I have obtained similar results with very fine grained materials by allowing them to come to equilibrium at constant humidity for periods of up to twelve months.

The ultimate value of  $B = \pi DT$ , however, is not approached for two reasons. One is that this value infers an infinite tensile stress in the water but the tensile strength of water is thought to be about 200 kg/cm<sup>2</sup> and is probably much less in practical situations. The other reason is that the changing nature of the material from ductile to brittle must also be taken into account. Carr's notion of "dry joints", for example, is a simple statement of the Griffith<sup>11</sup> crack theory for brittle materials which would predict a maximum tensile strength of

$$\sigma_f = 0.5\sigma \tan \phi \quad (11)$$

and this should replace the Mohr-Coulomb theory in the later stages.

The basic difficulty encountered in this field of research stems from the desire to obtain a simple single failure

theory for a material which adopts a wide range of physical characteristics with variations in moisture content.

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## CHEMISTRY

### Surface Photopolymerization of Vinyl and Diene Monomers

WHITE has shown that gaseous 1,3-butadiene in contact with metallic substrates polymerizes under the influence of ultra-violet light<sup>1</sup>. In contrast to surface photopolymerization induced by electron beams<sup>2-5</sup> or in glow discharges<sup>6-10</sup>, little has been published about the process induced by photons. I have obtained results on deposition from butadiene and on the extension of this method for surface polymerization from the gas to other monomers, with concomitant formation of novel materials in thin film form.

A 700 W medium pressure Hanovia mercury arc provides a convenient source for surface photopolymerization of such vinyl monomers as acrylonitrile, methyl methacrylate, and styrene, and diene monomers such as 1,3-butadiene and 2,4-hexadiene. Effective ultra-violet radiation over the range 2000–3500 Å is transmitted to the substrate area by means of a quartz window. The system is pre-evacuated to a pressure of about 10<sup>-6</sup> torr, chiefly to remove water vapour, and the gaseous monomer is then maintained at a pressure of about 4 torr during the irradiation.

As indicated by White<sup>1</sup>, the growth rates for photopolymerization exhibit a negative dependence on substrate temperature, with activation energies in the neighbourhood of -8 kcal/mole. Similarities in the structure of the films deposited from the various monomers to those of the analogous polymers produced by conventional techniques demonstrate that the low energies of the photon irradiation permit retention of the characteristics of the individual monomer. Multiple infra-red absorption data, however (Fig. 1), show that the films from C<sub>4</sub>H<sub>6</sub> are not simply polybutadiene. They indicate the presence of CH<sub>3</sub> groups (~1,375 cm<sup>-1</sup>), CH<sub>2</sub> groups (~1,450 cm<sup>-1</sup>), and aliphatic CH groups (~2,950 cm<sup>-1</sup>), as well as some small amounts of carbon-carbon triple bonds (~3,300, 2,110 and 697 cm<sup>-1</sup>). Furthermore, at least thirteen gas phase products have been identified from the 1,3-butadiene photopolymerization. Absorption at ~966 cm<sup>-1</sup> suggests that the thermodynamically favoured *trans*-1,4 form of addition may be preferred under these reaction conditions. The films which we deposited from butadiene are unique in that when thicker than about 5000 Å, they are unstable in the presence of air or moisture at high temperatures. They assume a "cracked" appearance, show birefringent behaviour, and may spontaneously peel from metallic substrates.

Hexachlorobutadiene represents an unusual type of "monomer" which is subject to polymerization by this tech-



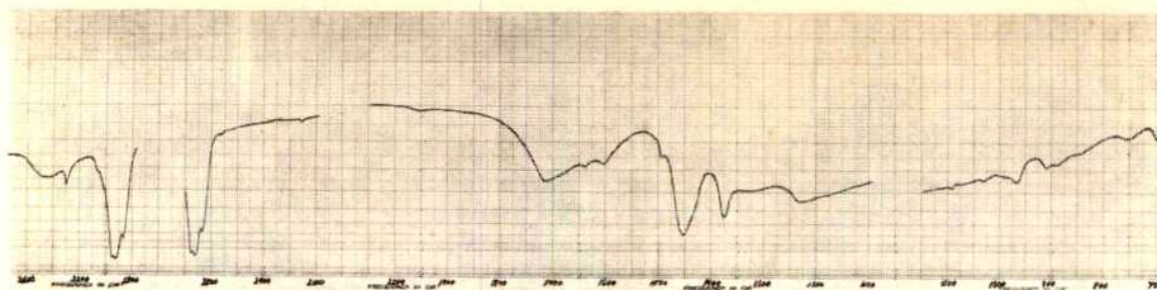


Fig. 1. Infra-red spectrum of film from  $C_6H_6$ , five reflexions from a film about 20,000 Å thick on evaporated aluminium.

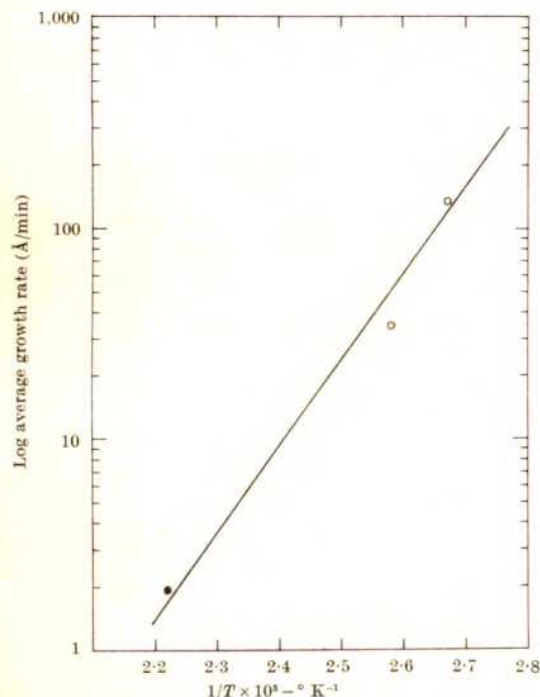


Fig. 2. Log of growth rate of films from  $C_2Cl_4$  on evaporated aluminium against  $1/T$ .

nique. The steric hindrance effect of the halogen atoms makes this molecule quite inert in conventional reaction systems. It does not react with chlorine at temperatures as high as 500° C (refs. 11 and 12), cannot serve as the diene component in the Diels-Alder reaction<sup>13,14</sup> and does not react with sulphuryl chloride in the presence of benzoyl peroxide<sup>15</sup>. It does not undergo any significant free radical copolymerization with styrene<sup>16</sup>. Fig. 2 illustrates growth rate behaviour from the vapour at about 100μ, that is vapour in equilibrium with the liquid monomer source maintained at 18° C in a shaded part of the system.

The solid circle represents growth on an uncooled substrate surface which becomes heated to about 177° C by the mercury arc. The Arrhenius plot indicates a negative activation energy of about -8.5 kcal/mole. The photopolymerized films are essentially transparent in the infra-red. The perchlorinated deposit shows a carbon to chlorine atomic ratio of approximately 2:1, while chlorine has been identified as the principal gas phase product. The surface reaction therefore involves considerable chlorine elimination and is obviously quite complex. The films exhibit high thermal stability. For example, unlike the films from hydrocarbon monomers, no significant carbon monoxide infra-red absorption can be detected on exposure to steam at 100° C. An exothermic reaction, however, does occur in nitrogen at temperatures above 300° C and the films lose weight.

It is also possible to polymerize tetrafluoroethylene by this technique. Fig. 3 illustrates the structure of the continuous transparent films deposited from the gas at a pressure of about 1 torr onto substrates maintained at a temperature of about 30° C. The growth rate is about 30 Å/min under these conditions. Although elemental analysis shows an atomic ratio similar to that in polytetrafluoroethylene, the presence of some  $CF_3$  groups is indicated by absorption at  $\sim 980\text{ cm}^{-1}$ . The absence of crystal-crystal transitions on differential scanning calorimetry, and loss of weight in air beginning at about 220° C, suggest the presence of a considerable amount of low molecular weight material in the as-polymerized films.

The extremely thin films that may be deposited by the ultra-violet surface photopolymerization process are uniquely suited to electron microscopic studies of morphology. Although it has long been evident from the pioneering work of Storks<sup>17</sup> and Till<sup>18</sup> that electron diffraction offers a powerful tool for investigation of crystallinity in polymers, the need for specimens sufficiently thin to permit electron transmission has tended to limit the technique to specimens crystallized from solution or melt. After removal from metallic or other substrates by dissolving the latter in concentrated acids, the inert films produced by photopolymerization may be examined directly in transmission without need for sectioning, carbon support or replication.

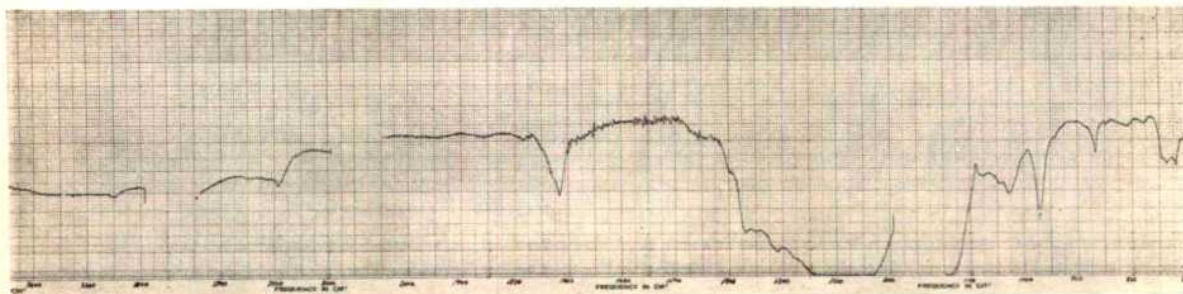


Fig. 3. Infra-red spectrum of film from  $C_2F_4$ , five reflexions from each of two parallel evaporated aluminium slides with polymer films about 2000 Å thick.



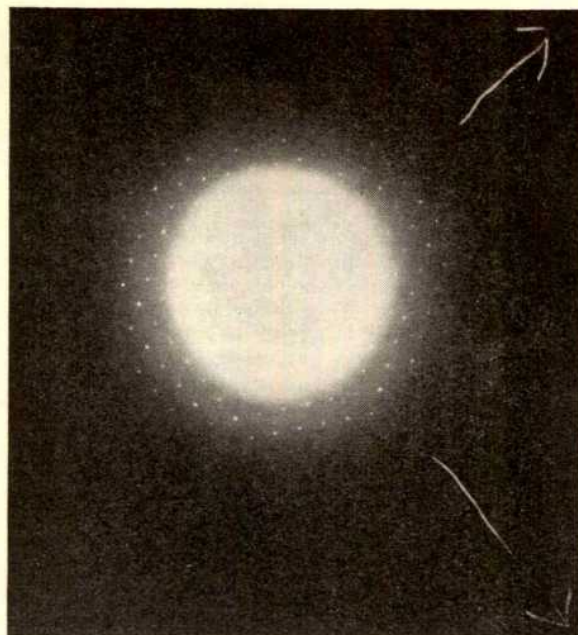


Fig. 4. Electron diffraction pattern from film deposited from  $C_4Cl_6$  obtained with a Siemens 'EMI' electron microscope.

Selected area electron diffraction studies on the as-polymerized films showed evidence for single-crystal structures as illustrated for the films from  $C_4Cl_6$  in Fig. 4. This structure is not representative of the films as a whole and occurs in only a minute fraction of the surface area. Attempts at shadowing indicated the structures to be within the films rather than on either surface. Repeat distances of about 18 and 11 Å for rotations of  $60^\circ$  about the hexagonal—or pseudohexagonal—pattern are remarkably constant for deposition from a wide range of monomers. Similar values for deposition on various metallic and non-metallic substrates indicates that the regularity does not simply correspond to an epitaxial overgrowth on the substrate such as that which appears to occur during gamma irradiation of trioxane in the presence of large single crystals of monomer<sup>19,20</sup>. Perhaps localized crystallization of largely carbonaceous by-products common to the monomers results in minute regions of extreme order. The thickness of the films (a few hundred Å) could permit the long period of the lamellae<sup>21</sup> to lie parallel to the beam of electrons as they pass through the polymeric films within the microscope.

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## Tin Oxide— $Sn_3O_4$

DURING the 170 years since the preparations of stannous and stannic oxides were first reported<sup>1,2</sup>, many authors have claimed to have shown the existence of oxides of tin the compositions of which were intermediate between the two. Included in the most recent of these are the papers by Spandau and Kohlmeier<sup>3</sup> and Spinedi and Gauzzi<sup>4</sup>, who report  $Sn_3O_4$ , and Decroly and Ghodsi<sup>5</sup>, who contend that  $Sn_5O_6$  is formed. No data concerning the crystal structure of the intermediate oxides have so far been given.

In order to verify that an intermediate oxide existed a series of isothermal experiments were carried out in which dry stannous oxide was heated in an atmosphere of nitrogen free from oxygen for times varying from 15 min to 16 h at temperatures between  $700^\circ$  K and  $1,050^\circ$  K. The resultant disproportionation products were analysed non-destructively by the use of electron diffraction, X-ray diffraction and Mössbauer spectroscopy. Electron micrographs were prepared to enable phase identifications to be made.

Besides metallic tin and stannic oxide, a new phase was evident in those samples which were heated for short times or at low temperatures. This material was always in the form of thin single crystal platelets measuring about  $1000 \text{ Å}$  square and  $50 \text{ Å}$  thick. The extremely small size of the crystals made it impossible to separate the platelets from the associated  $\beta$ -tin globules and the very fine stannic oxide crystallites, and so all analyses had to be carried out on material containing all three species. X-ray and electron diffraction analyses showed that the crystal habit of the platelet was triclinic with

$$\begin{aligned} a &= 4.86 \pm 0.16 \text{ Å} \\ b &= 5.88 \pm 0.14 \text{ Å} \\ c &= 8.20 \pm 0.17 \text{ Å} \\ \alpha &= 93^\circ 00' \pm 0.17' \\ \beta &= 93^\circ 21' \pm 21' \\ \gamma &= 91^\circ 00' \pm 24' \end{aligned}$$

The large maximum probable errors are the direct result of using powder data rather than single crystal data for the calculation of the unit cell dimensions.

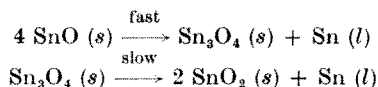
The principal X-ray diffraction lines for the new phase are

<i>hkl</i>	<i>d</i>	<i>I/I<sub>0</sub></i>
001	8.17	15
011	4.84	30
002	4.08	5
101	3.301	25
012	2.916	33
021	2.827	15
112	2.793	30
112	2.778	5
003	2.721	100
021	2.645	50
112		

In all, 110 reflexion lines which are attributable to the new phase were found.

From considerations of the theoretical density of a crystal with the dimensions as given, and of the transformation mechanism necessary to convert stannous oxide to the intermediate material, the most probable composition of the new phase is  $Sn_3O_4$ . Mössbauer spectra obtained using a  $^{119m}\text{Sn}$  source were not inconsistent with a formula  $Sn_2^{II}Sn^{IV}O_4$  for the phase.

The metastable material decomposes on further heating into  $\beta$ -tin and stannic oxide. The kinetics of the decomposition are first order with respect to the intermediate oxide. The two reactions occurring during the disproportionation of stannous oxide are:



Differential thermal analysis measurements show that the disproportionation is indeed a two step process, both reactions being irreversible. This latter point is to be expected because stannous oxide, as a condensed phase, is itself thermodynamically unstable with respect to  $\beta$ -tin and stannic oxide<sup>6</sup>.

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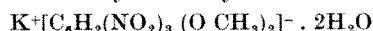
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### Crystal Structure of Meisenheimer Complex

CHEMICAL and spectroscopic evidence exist for the equivalence of the attached substituent groups of Meisenheimer complexes<sup>1</sup>, but the detailed structure of these complexes has not as yet been elucidated. We report here the structure of the 1,1-dimethoxy-2,4,6-trinitrobenzene ion. The crystal analysed was



and was obtained by recrystallization from acetonitrile. The space group and the unit cell dimensions are triclinic,  $PI$  with  $a = 9.36$ ,  $b = 10.84$ ,  $c = 7.40 \pm 0.02$  Å,  $\alpha = 87^\circ 40'$ ,  $\beta = 106^\circ 43'$ ,  $\gamma = 102^\circ 45' \pm 20'$ ,  $Z = 2$ ,  $d$  calc = 1.65, and  $d$  obs = 1.64.

The structure was determined by a three-dimensional Patterson synthesis, and the refinement was carried out by the least squares method with isotropic temperature factors. The  $R$ -value at the present stage is 0.169 for 1,897 reflexions. The bond distances are shown in Fig. 1.

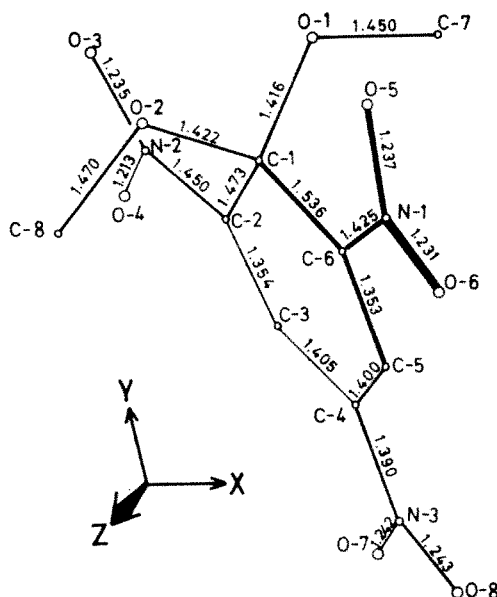


Fig. 1. Structure of Meisenheimer complex.

and the average standard deviation is 0.018 Å. It is remarkable that the two methoxy groups are attached to the benzene ring with the same bond distances. The atoms C-7, O-1, O-2 and C-8 lie almost in a plane, and this plane makes an angle of  $87^\circ 50'$  with the benzene ring plane. Although the angle C-2, C-1, C-6 is  $109^\circ 48'$ , which is nearly equal to the tetrahedral angle, the benzene ring is still nearly planar, the largest deviation at C-2 being 0.03 Å.

Of the three nitro groups, that at the *para*-position and another at the C-1 *ortho*-position are twisted approximately  $6^\circ$ , while the third one at the C-6 *ortho*-position is twisted as much as  $11^\circ 42'$ . The difference in bond distances between C-1, C-6 and C-1, C-2 may be correlated with this difference of twisting. Except for this asymmetry all structures are nearly symmetrical; in particular, the two methoxyl groups are confirmed to be equivalent. All calculations were carried out at the Computation Centre of the University of Tokyo; the least square programme was supplied by the UNICS system.

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### BIOPHYSICS

#### Effect of Gases on the Electrical Conductivity of $\beta$ -Carotene

We have studied the semiconductive and photoconductive properties of single crystals of  $\beta$ -carotene<sup>1</sup>. During these and other studies, changes in the electrical conductivity in the presence of oxygen have been observed<sup>1,2</sup>. The effect of gases on the conductivity of other organic crystals<sup>3-8</sup> has indicated that these changes are caused by donor-acceptor interactions between the gas and the crystal. Studies of this type have been largely carried out with anthracene, which is generally regarded as an electron donor, and chloranil, an electron acceptor.  $\beta$ -Carotene, it has been said, should be both a good electron donor and also a good electron acceptor<sup>9</sup>. These properties of  $\beta$ -carotene are of interest in relation to Platt's theory of the role of  $\beta$ -carotene in the primary photosynthetic process<sup>10</sup>, and may be relevant to the mechanism of olfaction where it has been proposed that interactions between gas molecules and carotenoids may be involved<sup>11-13</sup>. Here we present the results of an initial qualitative survey of the effect of various gases and vapours on the conductivity of single crystals of all *trans*  $\beta$ -carotene.

The methods of obtaining crystals of all *trans*  $\beta$ -carotene and of making conductivity measurements have been previously described<sup>1</sup>. The studies reported here were confined to surface conductivity measurements in the *ab* plane of the crystal. 'Aquadag' was used throughout as the electrode material. The dark and photoconductivity of the crystals were measured *in vacuo* after a small amount of gas or vapour had been admitted to the dewar.

The dark conductivity of  $\beta$ -carotene *in vacuo* is rather low at room temperature ( $\rho \sim 10^{17}$ /ohm cm). To be sure of observing both decreases and increases in conductivity most measurements were made at  $70^\circ\text{C}$ . Some measure-



Table 1. QUALITATIVE EFFECT OF GASES ON THE SURFACE CONDUCTIVITY OF ALL *trans*  $\beta$ -CAROTENE

Gas or vapour	Change in dark conductivity	Change in photoconductivity
O <sub>2</sub>	+	+
SO <sub>2</sub>	+	+
HCl	+	0
C <sub>2</sub> H <sub>5</sub> OH	+	0
(CH <sub>3</sub> ) <sub>2</sub> CO	+	0
CH <sub>2</sub> Cl <sub>2</sub>	+	0
CHCl <sub>3</sub>	+	0
(C <sub>2</sub> H <sub>5</sub> ) <sub>3</sub> N	+	-
C <sub>6</sub> H <sub>6</sub>	0	0
H <sub>2</sub> O	0	0
CH <sub>3</sub> OH	0	0
N <sub>2</sub>	0	0
A	0	0
CO <sub>2</sub>	0	0
NH <sub>3</sub>	(-)	(-)
I <sub>2</sub>	0	(-)

+, Increase in current; -, decrease in current; 0, no observable change in conductivity. All changes were reversible, except for those in brackets.

ments were also made at lower temperatures but these tended to be complicated by changes which were induced by the ambient gases in the insulation resistance of the apparatus. The photocurrents were measured using white light from a tungsten lamp.

The qualitative effects of the various gases and vapours on the dark and photoconductivity of all *trans*  $\beta$ -carotene are listed in Table 1. With the exception of CH<sub>2</sub>Cl<sub>2</sub> and CHCl<sub>3</sub>, these effects were all observed at 70° C. With these gases it was difficult to establish a change in conductivity at this temperature, but at 40° C a clear increase in dark conductivity was observed.

The effects of hydrogen chloride and sulphur dioxide were largely reversed when the dewar was evacuated, but the rate of return of the current to its vacuum level was very slow. For example, in a typical experiment the dark current rose from  $2.0 \times 10^{-12}$  amps *in vacuo* to  $6.5 \times 10^{-11}$  amps in the presence of SO<sub>2</sub>. When the SO<sub>2</sub> was removed and the dewar continuously pumped, the current slowly fell to a value of  $5.8 \times 10^{-12}$  amps during a period of 17 h. (The difference between this and the original current may have been caused by the difficulty of completely outgassing the crystal. The possibility of a small permanent change in the  $\beta$ -carotene crystal cannot, however, be ruled out.) A rather similar result was obtained with hydrogen chloride.

The changes in conductivity range in magnitude from a factor of two to a factor of fifty. In general, the effect of the gases on the photoconductivity was less marked than the effect on the dark conductivity. A definite change in the photoconductivity could be detected for only three gases.

The irreversible changes observed with iodine and ammonia probably indicate that a permanent chemical change has occurred at the surface of the crystal. If we consider only reversible changes the results can be summarized as follows. (a) Both electron donor and acceptor gases increase the surface dark conductivity. (b) The photoconductivity is less affected by ambient gases than is the dark conductivity. (c) There is some suggestion that the photoconductivity is increased by electron acceptors and decreased by electron donors. More evidence is required to establish this conclusion. (d) Inert gases and gases which have only very weak charge transfer properties have no observable effect on the conductivity.

These results are not sufficient for unique determination of the nature of the interactions between the gas molecule and the crystal. It is, however, reasonable to suppose that the increases in dark conductivity which we observe with both donor and acceptor gases are caused by the injection of electrons and holes respectively. This implies that both electrons and holes can contribute effectively to the conductivity of  $\beta$ -carotene. This is confirmed by previous photoconductivity studies with strongly absorbed light which indicate that the hole current is only two to three times greater than the electron current<sup>1</sup>.

The relatively weak effect of ambient gases on the photoconductivity is almost certainly a consequence of

the photoconductivity spectrum of  $\beta$ -carotene. It has been found that charge carriers are most efficiently produced by weakly absorbed light in the bulk of the crystal<sup>1,14</sup>. Thus gases adsorbed on the surface of the crystal might be expected to have little effect on this process.

The increases in photoconductivity observed with oxygen and sulphur dioxide and the decrease observed with tri-ethylamine are in agreement with changes produced by these gases in the photoconductivity of anthracene. In this material it is not clear whether the gases affect the lifetime of the hole or the production of the charge carrier<sup>15</sup>. In  $\beta$ -carotene the effect of tri-ethylamine, which is to decrease the photocurrent while increasing the dark current, is most simply explained by supposing that it is the charge carrier production which is affected in the photoconductivity measurements.

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## Liquid Crystalline Behaviour in Mixtures of Cholesterol with Steroid Hormones

THE liquid crystalline behaviour of mixtures of cholesterol with glycerol and cetyl alcohol has been reported by Młodziejowski<sup>1</sup>, cholesterol molecules being "plasticized" by the molecules of glycerol or cetyl alcohol. Although cholesterol itself is not mesomorphic, all cholesterol esters have characteristic mesophases. It is possible that the hydrogen bonding in pure cholesterol is responsible for the high melting point because the presence of glycerol or cetyl alcohol molecules may provide alternative sites to which hydroxyl groups of the cholesterol molecules hydrogen can bond without imposing a high melting temperature on the crystal lattice but having a sufficiently strong intermolecular attraction to make an anisotropic melt possible.

The molecular interaction of steroid hormones with cholesterol and lecithin<sup>2</sup> spread as surface monolayers on a Langmuir trough suggests that a physiochemical basis may exist which determines the uptake of steroid hormones into cell membranes. Potential binding sites would then be dependent on the phospholipid and cholesterol composition of the membranes<sup>3,4</sup>. The steroid hormones are a group of lipids differing only slightly in chemical structure but having fairly precise target organs and effects. The selective uptake of steroids into cells may be determined by their solubility within the lipid membrane because this solubility is greatly affected by small structural changes.



The present investigation has been of the phase equilibrium behaviour of mixtures of cholesterol with some of the steroid hormones. We have preferred to use a technique of thaw melting to a procedure of thermal analysis because mesomorphic transitions were common and were most easily observed directly or with the use of crossed polarizers. Gray has outlined the limitation of this method of identification<sup>5</sup>. The steroids, cholesterol, progesterone, androstenedione, testosterone, dehydroisoandrosterone, cortisol, oestrone and oestradiol, were used as supplied. They all had melting points in agreement with published ratios.

The solid mixtures were prepared by melting small weighed amounts on watch glasses and allowing them to solidify. Cortisol and oestrone decomposed during this treatment and no phase diagram could be obtained, but in all other cases no variability in the results was experienced.

The mixtures of the steroid hormones with cholesterol all showed mesomorphic transitions that were noted as softening points as the temperature was increased (smectic mesophase) or regions of less viscous liquid (cholesteric mesophase), which again became stiff until finally flowing to form an isotropic liquid state. It was not possible to detect the mesophase transitions on cooling.

Dehydroisoandrosterone with cholesterol (Fig. 1) showed the most interesting liquid crystalline behaviour. Mixtures in the range of composition with 30–40 per cent cholesterol all softened at very low temperatures and individual crystals ran together. Above this temperature the mixture stiffened, forming what appeared to be a stepped smectic liquid crystalline phase which softened and hardened through three other liquid crystalline phases before melting to a clear, free running isotropic liquid. Examination of all these mixtures under cross polaroids was only advantageous in determining the final liquid phase but did not help to distinguish the liquid crystalline phases.

The phase equilibrium curves obtained for mixtures of cholesterol with progesterone, testosterone and androstenedione were all similar. The mixtures showed slight

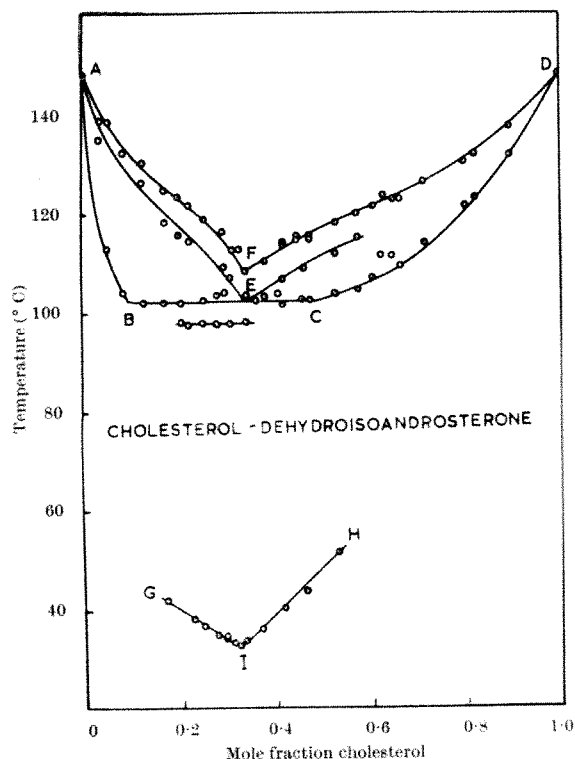


Fig. 1. Phase equilibrium curve for cholesterol-dehydroisoandrosterone mixtures, above *GHI* (smectic mesophase), above *BC* (cholesteric mesophase), above *AE* (cholesteric mesophase), above *AFD* (isotropic liquid).

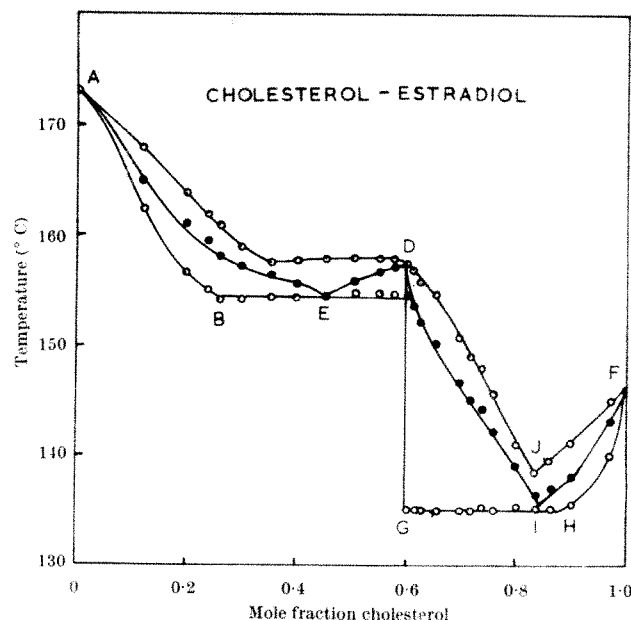


Fig. 2. Phase equilibrium curve for cholesterol-oestradiol mixtures, below *BGH* (smectic mesophase), above *BGH* (cholesteric mesophase), above *AEDIF* (cholesteric mesophase), above *ADF* (isotropic liquid).

softening at the edges at temperatures in the range 40°–80° C as the temperature was increased. A temperature was then reached at which free liquid separated at the edges (cholesteric mesophase), but this later hardened and finally rapid melting was noted. Complete isotropic liquid appeared a few degrees higher.

The mixtures of cholesterol with oestradiol (Fig. 2) behaved differently in that they showed the formation of a complex at a 3:2 molecular ratio although on each side of this complex the transitions noted were similar to those observed with the other steroids.

These results show that the steroid hormones in cholesterol mixtures cause anisotropic melts which give rise to eutectic points as indicated (*E*). In all but one case the point *E* corresponds to a 1:1 mixture. Although the eutectic point does not usually signify complex formation, it is here taken to correspond to the most favourable packing ratio, giving rise to maximum reduction in the melting point. Such a favourable molecular packing ratio is found in other systems<sup>2,3</sup>.

Dehydroisoandrosterone shows the most interesting polymorphism and the eutectic occurs at a 2:1 molecular ratio. The most noticeable difference is that dehydroisoandrosterone has a double bond in the 5:6 position, whereas progesterone, testosterone and androstenedione all have a double bond in the 4:5 position.

Oestradiol is also different in showing the formation of a definite complex with cholesterol under the conditions of preparation corresponding to a 3:2 molecular ratio. Such differences, however, are to be expected and can be compared with the effect of double bond position or variation of  $\alpha$  and  $\beta$  stereo groups in the mesomorphic behaviour of steroid esters<sup>6</sup>.

Such properties may relate to the biological activity and localization of steroid hormones in cell membranes.

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## BIOCHEMISTRY

Glucose-phosphorylating Enzyme with High  $K_m$  in Human Brain

THE recent discovery of an insulin-inducible glucose-phosphorylating enzyme with high  $K_m$  ("glucokinase", ATP-D-glucose 6-phosphotransferase, *E.C.* 2.7.1.2.) in the liver<sup>1,2</sup> and epididymal fat pad<sup>3</sup> of small laboratory animals has added to the current interest in the mechanisms which control glucose metabolism in mammalian tissues. No evidence from kinetic studies<sup>4-6</sup> or electrophoresis<sup>5,7</sup>, however, has yet been obtained for the occurrence of an analogous enzyme with high  $K_m$  in the brains of rats, guinea-pigs or sheep and the view has grown that this type of activity is absent from the brain.

In the tissues where it has been found, activities of glucokinase have been shown to be affected by fasting, post-natal development, insulin and alloxan diabetes<sup>1-3,8</sup>. The report<sup>9</sup> that the human brain is sensitive to insulin, although the response is slower than that of peripheral tissues, led me to investigate the possibility that glucokinase of high  $K_m$  may be present in human brain although it is apparently absent from the brains of the lower mammals (rat, guinea-pig and sheep) which have been studied.

Because the glucokinase in the liver has been reported to be relatively labile<sup>10</sup> and because of the extremely rapid post-mortem changes in the brain of concentrations of relevant chemicals such as glucose<sup>11</sup>, glycogen<sup>12</sup> and ATP<sup>13</sup>, samples should be used immediately after excision, if possible, in this type of investigation. Post-mortem samples are usually difficult to obtain within a few hours after death, when marked autolysis is likely to have occurred. On the other hand, the use of biopsy specimens often involves uncertainty as to the normality of the sample.

The samples which I used were biopsy specimens of cerebral cortex obtained during neurosurgery (Table 1).

The samples were frozen in the operating theatre directly after excision except for sample 4, which was used immediately. All subsequent procedures were performed at 0°-2° C. Adhering dural membranes and blood vessels were removed and the cortex was rapidly snipped off with scissors, weighed and homogenized in 0.32 molar sucrose (adjusted to pH 7.4 with potassium hydroxide) in a 'Teflon'-pestle homogenizer to a final suspension of 10 ml./g fresh tissue. In one case (sample 3), the cortex was divided into two parts and homogenized in two different media: (1) 0.32 molar sucrose, pH 7.4; and (2) 0.25 molar sucrose, 0.05 molar glucose, 0.005 molar cysteine, 0.01 molar KCl, 0.005 molar MgCl<sub>2</sub> and 0.005 molar ATP, pH 7.4. Subcellular fractions were prepared by differential centrifugation; in the work reported here the extracts used were the soluble supernatant fractions obtained by centrifugation at 150,000*g* av. for 60 min.

Glucose-phosphorylating activities were estimated in a final volume of 3 ml. containing 150 mmolar glycylglycine, pH 8.0; 10 mmolar MgCl<sub>2</sub>; 5 mmolar ATP; 0.25 mmolar NADP<sup>+</sup>, 0.2 IU glucose-6-phosphate dehydrogenase and glucose as indicated. The assay solutions were prepared in 1 cm light-path cuvettes in a Unicam 'SP 800' recording spectrophotometer equipped with a constant temperature cell housing maintained at 37° C. After equilibration for 5 min the reactions were started by the addition of 50-100  $\mu$ l. of extract and continued for 15 min at 340 m $\mu$ .

Table 1. SOURCES OF HUMAN CEREBRAL CORTX SAMPLES

No.	Sex	Reason for neurosurgery	Site	Sample Pathology
1	F	Sclerosis of hippocampus	Temporal lobe	Normal tissue
2	F	Hypophysectomy for breast cancer	R. frontal lobe	Normal tissue
3	M	Suspected glioma	Temporal lobe	Normal tissue*
4	M	Suspected glioma	Temporal lobe	Normal tissue

\* The possibility of infiltration of this sample by tumour tissue could not be excluded.

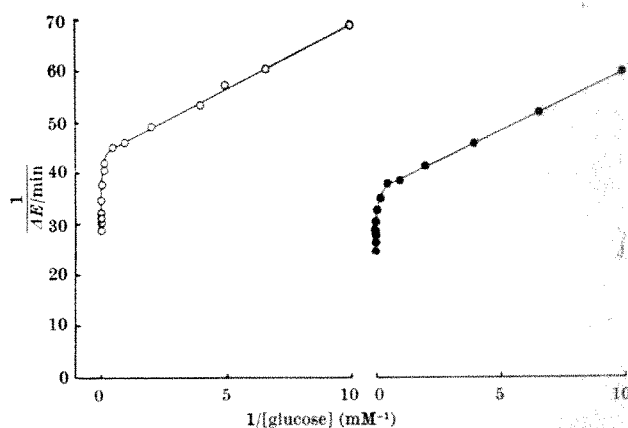


Fig. 1. Reciprocal plot of glucose-phosphorylating activity against glucose concentration (0.1-400 mmolar). ●, Sample 1; ○, sample 4.

using reference cells which contained all the constituents except glucose and ATP.

Reciprocal plots of activity ( $\Delta E/\text{min}$ ) against concentration of glucose were made for concentrations of glucose from 0.1 to 400 mmolar. The extracts from all specimens yielded similar results—two are shown in Fig. 1 (samples 1 and 4).

There was a sharp point of inflexion in the plots at about 2 mmolar glucose. The point of inflexion was not as sharp nor was the increase in activity at higher concentrations of glucose as large as those observed by Walker<sup>1</sup> in liver preparations and no inhibition of the low  $K_m$  hexokinase activity at high concentrations of glucose<sup>1</sup> was detected.

Extrapolation of the plots between 0.1 and 2 mmolar glucose gave  $K_m$  values of 0.05 mmolar (Table 2). The plots (Fig. 1) for concentrations of glucose between 100 mmolar and 400 mmolar have been re-drawn<sup>1</sup> on an expanded scale (Fig. 2). Extrapolation of the plots gave  $K_m$  values of 21 mmolar (Table 2). The presence in the homogenization medium of glucose, ATP, potassium chloride and thiol groups, which might be expected to exert a stabilizing influence on a potentially labile enzyme of this type<sup>10</sup>, did not seem to have much effect (Table 2, samples 3a and b). Because the point of inflexion in the reciprocal plots (Fig. 1) occurred at about 2 mmolar glucose, this concentration was used to calculate the quantitative difference between the activities of the low and high  $K_m$  system. The activity of the low  $K_m$  system was calculated from the rate of formation of NADPH at 2 mmolar and the high  $K_m$  from the difference between the rates at 200 mmolar and 2 mmolar (Table 3).

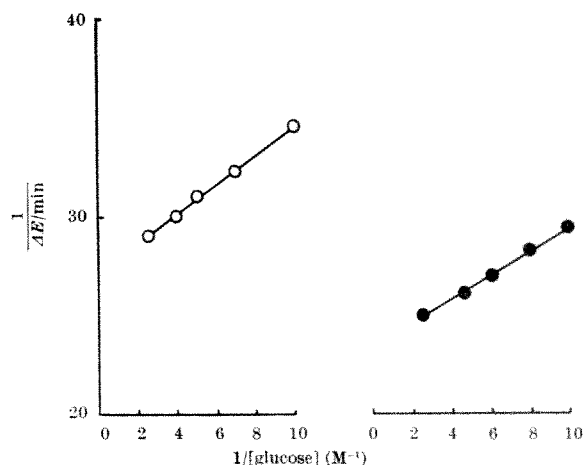


Fig. 2. Reciprocal plot of glucose-phosphorylating activity against glucose concentration at high glucose concentration. The points are the first five from Fig. 1 presented on an expanded abscissa scale. ●, Sample 1; ○, sample 4.

Table 2. *K<sub>m</sub>* VALUES OF GLUCOSE-PHOSPHORYLATING SYSTEMS FROM HUMAN CEREBRAL CORTEX

Sample	Low <i>K<sub>m</sub></i> (mmolar)	High <i>K<sub>m</sub></i> (mmolar)
1	0.053	22
2	0.043	21
3a	0.053	24
3b	—*	22
4	0.057	18

The *K<sub>m</sub>* values were obtained from extrapolations of the reciprocal plots of Figs. 1 and 2.

\* It was impossible to measure this value because the glucose in the brain extract (resulting from the 50 mmolar glucose present in the sucrose used for homogenization) resulted in minimal glucose concentrations in the assay medium of 0.8–1.7 mmoles/l.

Table 3. CALCULATED LOW *K<sub>m</sub>* AND HIGH *K<sub>m</sub>* GLUCOSE-PHOSPHORYLATING ACTIVITIES IN EXTRACTS OF HUMAN CEREBRAL CORTEX

Sample No.	Activity (μmoles NADPH/min/g original tissue)	Low <i>K<sub>m</sub></i> enzyme	High <i>K<sub>m</sub></i> enzyme	Total
1	3.7	1.8	5.5	5.5
2	2.25	1.6	3.85	3.85
3a	1.6	1.2	2.8	2.8
4	3.75	1.7	5.45	5.45
Guinea-pig cerebral cortex*	5.5	0.0	5.5	5.5
Rat liver†	0.6	1.46	2.06	2.06

The low *K<sub>m</sub>* activity was calculated from the rate of formation of NADPH at 2 mmolar glucose and the high *K<sub>m</sub>* activity from the difference in rates between 2 mmolar and 200 mmolar glucose.

\* Non-particulate high-speed supernatant fraction from guinea-pig cerebral cortex\*.

† Salas, Vinuela and Sols\* used in rat liver extracts the rate at 0.5 mmolar glucose and the difference in rates between 0.5 mmolar and 100 mmolar glucose for the activities of hexokinase and glucokinase, respectively.

The results show clear and consistent evidence for the presence of an enzyme of high *K<sub>m</sub>* in human cerebral cortex which may be a "glucokinase" analogous to the insulin-inducible enzyme found in other tissues. Until some correlation between activity and physiological conditions similar to those found<sup>1-3,8,10</sup> for the enzymes in the liver and fat pad has been shown, however, it would be premature to assume that it is a truly analogous enzyme, even though the human brain has been shown to be sensitive to insulin<sup>9</sup>. Because no evidence has been obtained for the presence of an enzyme of high *K<sub>m</sub>* in guinea-pig, rat or sheep brain<sup>4-7</sup>, it provides an interesting and unusual difference between the brains of man and the lower mammalian orders.

The *K<sub>m</sub>* is higher (21 mmolar) than has been reported for rat liver or guinea-pig liver glucokinases<sup>1</sup> and the ratio of high *K<sub>m</sub>* to low *K<sub>m</sub>* activity in the human cerebral cortex is lower than the ratios reported for the liver systems<sup>1,2</sup>. Glucokinase activities in the liver have been shown to fall as a result of fasting<sup>8</sup>, however, and it must be borne in mind that pre-operative care always involves fasting for some hours so the actual levels of the high *K<sub>m</sub>* activity in the human brain may well be higher than those reported here.

The relative consistency of the results for the high *K<sub>m</sub>* activity (Table 3) suggests that the enzyme is stable in the frozen intact tissue, because the result from sample No. 4 (which was fresh when used) was not very different from those obtained for the other samples (which had been stored frozen for varying times before homogenization). On the other hand, the high *K<sub>m</sub>* activity decreased rapidly but inconsistently after dispersion when stored either at 3° C or at -20° C. The assays were routinely performed on the same day as the tissues were homogenized. Inclusion of glucose, ATP, KCl and cysteine in the sucrose used to prepare the extracts had little stabilizing effect—the high *K<sub>m</sub>* activity had disappeared within a few days at -20° C in samples 1–3. The activity lasted longer in sample 4 which was not frozen before use—it remained relatively stable for 5 days at -20° C and then slowly disappeared. The possibility of separating the high *K<sub>m</sub>* activity from the low *K<sub>m</sub>* enzyme will depend on the development of the right conditions for stabilizing the activity.

The presence of two kinetically distinct glucose-phosphorylating enzymes in the human cerebral cortex is of

relevance to the peculiar requirement of the brain for glucose and in view of the suggestion<sup>14</sup> that enzymes of this type provide one of the control points of glycolysis in the brain. It is difficult at this stage to assess the significance of the presence of a glucose-phosphorylating enzyme of such a high *K<sub>m</sub>* without the ability to vary the physiological conditions; however, similar studies (my unpublished work) on monkey brain indicate that it also contains the high *K<sub>m</sub>* activity and so should provide the opportunity for studying its significance in terms of control mechanisms.

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### Growth Hormone and Carbohydrate Metabolism *in vitro*

ONE *in vivo* effect of growth hormone secreted by the anterior pituitary gland is to oppose the action of insulin in promoting the utilization of glucose in muscle (see ref. 1 for earlier references), an effect which is almost certainly of physiological significance<sup>2,3</sup>. Nevertheless, no satisfactorily reproducible effect of growth hormone *in vitro* on carbohydrate metabolism has been described. Rather surprisingly Park *et al.*<sup>4</sup> observed that the addition of growth hormone *in vitro* to diaphragm from the hypophysectomized rat stimulates the uptake of glucose, that is, growth hormone exerts an insulin-like action in this system. This observation has been confirmed<sup>5</sup> but is not reproducible with diaphragm from intact rats (see ref. 6 for further references). The amount of growth hormone added to diaphragm *in vitro* in these experiments was 1 μg/ml. or more.

Using methods similar to those of Manchester and Young<sup>5</sup> we have observed that with diaphragm from hypophysectomized rats the addition *in vitro* of a much smaller amount of growth hormone than has previously been used under such conditions significantly diminishes the glucose uptake (Table 1). The curious biphasic dose-response curve observed in respect of the addition of very small amounts of growth hormone *in vitro* is illustrated in Fig. 1. With the addition of about 4 μg/ml. the previously observed insulin-like action of growth hormone on the uptake of glucose is seen, but with 0.8 per cent of this concentration (32 μg/ml.) a substantial depression of

Table 1. EFFECT OF OX GROWTH HORMONE (76 GH1) ADDED *in vitro* ON GLUCOSE UPTAKE AND ON THE INCORPORATION OF GLYCINE U-<sup>14</sup>C INTO PROTEIN OF THE ISOLATED DIAPHRAGM FROM MALE HYPOPHYSECTOMIZED RATS KILLED 11-13 DAYS AFTER HYPOPHYSECTOMY

Concentration of ox growth hormone in medium (mμg/ml.)	Glucose uptake (% of basal value)	P Control versus experimental	Increase in glycine U- <sup>14</sup> C incorporation (% of basal value)	P Control versus experimental
6.4	-17.8 ± 3.3* (12)	< 0.001	+19.7 ± 4.9 (12)	< 0.01
32.0	-25.0 ± 2.2 (11)	< 0.001	+23.0 ± 2.6 (11)	< 0.001
160.0	-6.8 ± 4.5 (11)	> 0.1	+19.7 ± 4.7 (11)	< 0.01
800.0	+8.8 ± 2.1 (8)	< 0.01	+27.3 ± 4.2 (8)	< 0.001
4,000.0	+33.0 ± 5.0 (6)	< 0.001	+32.0 ± 7.0 (6)	< 0.001

\* Standard error of the mean value. The figures in parentheses indicate the number of rats used to provide the mean value cited.

glucose uptake occurs (Fig. 1), which is abolished by the addition to the medium of 0.6 mU of insulin/ml., a dose which is approximately 32 mμg/ml. of insulin.

The addition to diaphragm from the hypophysectomized rat of similar amounts of growth hormone *in vitro* stimulates the incorporation of radioactive glycine into protein, the dose-response curve being very flat (Fig. 2) and showing no tendency to be biphasic with very small amounts of growth hormone.

When ox growth hormone is added *in vitro* to diaphragm from normal rats, a qualitatively similar effect on the uptake of glucose is seen (Fig. 3), but the variability of the observations is much greater than with diaphragm from hypophysectomized rats and the dose-response curve is very flat indeed. Similar observations have been made with human growth hormone *in vitro*.

These results show that when a very small amount of growth hormone is added to rat diaphragm *in vitro* under suitable conditions, an action can be observed which is

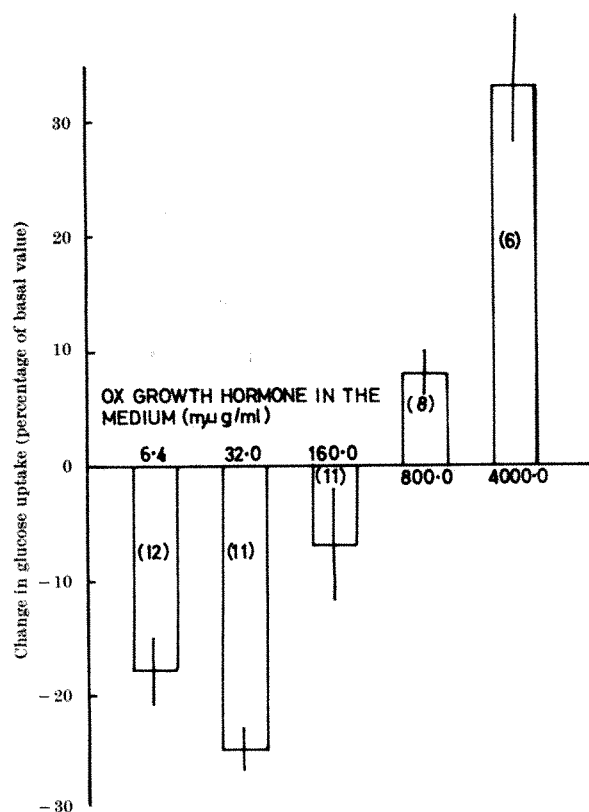


Fig. 1. Effect of addition of ox growth hormone (76 GH1) *in vitro* on the uptake of glucose by isolated diaphragm from hypophysectomized rats killed 11-13 days after hypophysectomy. The heights of the histograms indicate mean values and the lengths of the vertical lines at the top of the histograms indicate twice the standard error of the mean value. The figures in parentheses in the histograms indicate the number of rats used to provide the mean value.

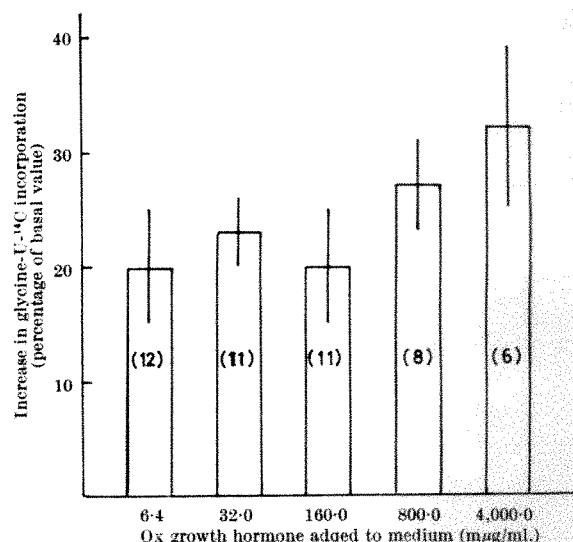


Fig. 2. Effect of addition of ox growth hormone (76 GH1) *in vitro* on the incorporation of glycine-U-<sup>14</sup>C into the protein of isolated diaphragm from hypophysectomized rats killed 11-13 days after hypophysectomy.

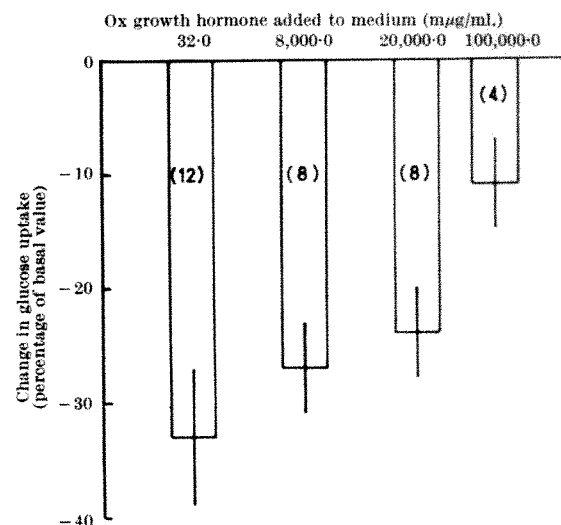


Fig. 3. Effect of addition of ox growth hormone (76 GH1) *in vitro* on the uptake of glucose by isolated diaphragm from normal rats.

consonant with the *in vivo* effect of this hormone in opposing the promotion of the uptake of glucose by insulin.

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### Glucose Metabolism in Fat Cells stimulated by Insulin and dependent on Sodium

SPECIFIC transport of organic and inorganic substances through biological membranes has been well established<sup>1</sup>. Transport of organic substances, such as amino-acids and sugars, may be active and is, as a rule, linked to the



transport of cations<sup>2</sup>, most frequently those of sodium and potassium<sup>3</sup>. Other transport mechanisms may agree rather with the definition of facilitated diffusion which seems to be independent of cations<sup>4</sup>. It is usually thought that in insulin tissues such as muscle and adipose tissue which respond to insulin, this hormone somehow favours the facilitated diffusion of glucose and of certain other sugars<sup>5,6</sup>. Insulin also facilitates, however, the entry of some substrates which pass through by active transport<sup>7</sup>, and, furthermore, it has been shown to increase the resting electrical potential of muscle<sup>8</sup> and of adipose tissue<sup>9</sup>, while stimulating decreasing retention of potassium and sodium ions respectively in these tissues<sup>10,11</sup>. While Hagen<sup>12</sup> has reported that insulin has less effect on glucose metabolism by adipose tissue in the absence of sodium or potassium ions, Rodbell<sup>13</sup> has observed normal responsiveness to insulin of isolated fat cells in the absence of sodium ions. These conflicting views have encouraged us to investigate the effects of changing the concentration of cations in the medium glucose transport and metabolism by isolated fat cells, both in the presence and absence of insulin. A marked sodium dependency of insulin-stimulated glucose metabolism in fat cells on sodium has been found.

Well-fed male mice 6-7 weeks old were used. The fat cells were isolated<sup>14</sup> from epididymal fat pads and incubated in Krebs-Ringer bicarbonate buffer containing 3.5 per cent albumin and 2.5 mmolar glucose-U<sup>14</sup>C (40  $\mu$ C/mmol). After 2 h the incorporation of the carbon of glucose into carbon dioxide<sup>15</sup> and total lipids<sup>16</sup> was measured. These metabolic indices were used as an indirect estimate of glucose uptake, because their sum has been shown to represent 80-90 per cent of total glucose metabolism in these conditions<sup>17,18</sup>. Results are expressed as  $\mu$ atoms of glucose carbon incorporated into carbon dioxide or total lipids, of lipid weight/2 h of incubation. Insulin concentration, when used, was 1 mU/ml. When sodium ions were omitted from the incubation medium, it was replaced by an equivalent amount of another cation.

Table 1. EFFECT OF DEPLETION OF SODIUM IONS ON GLUCOSE-U<sup>14</sup>C METABOLISM BY FAT CELLS

Medium Na <sup>+</sup> (mequiv./l.)	Control	Insulin (1 mU/ml.)	Insulin effect
Glucose- <sup>14</sup> C to CO <sub>2</sub>			
144	2.78 $\pm$ 0.01	12.2 $\pm$ 0.22	9.42
0	3.50 $\pm$ 0.11	7.22 $\pm$ 0.49	3.12
144	1.58 $\pm$ 0.02	11.8 $\pm$ 0.10	10.2
0	3.68 $\pm$ 0.11	8.10 $\pm$ 0.22	4.42
144	2.31 $\pm$ 0.10	9.78 $\pm$ 0.22	7.47
0	3.49 $\pm$ 0.30	5.28 $\pm$ 0.28	1.79
Glucose- <sup>14</sup> C to total lipids			
144	4.55 $\pm$ 0.05	21.9 $\pm$ 0.14	17.4
0	5.15 $\pm$ 0.32	10.4 $\pm$ 0.08	5.25
144	6.47 $\pm$ 0.22	36.8 $\pm$ 0.18	30.3
0	14.2 $\pm$ 0.10	26.6 $\pm$ 0.41	12.4
144	3.73 $\pm$ 0.19	17.4 $\pm$ 0.12	13.7
0	6.16 $\pm$ 0.20	12.1 $\pm$ 0.65	5.94

Results are expressed as  $\mu$ atoms C metabolized/g of lipid/2 h; mean of 6  $\pm$  SEM. Glucose, 2.5 mmolar; sodium was replaced by potassium ions.

Complete replacement of sodium ions by potassium ions in the absence of insulin (Table 1) reproducibly resulted in a moderate stimulation of glucose incorporation into both carbon dioxide and total lipids, showing that this treatment did not result in a non-specific inhibition of metabolic activity of the cells. In the presence of insulin, however, the opposite was the case: overall metabolic activity was decreased by one-third, while the insulin effect (total activity in the presence of insulin minus total activity in the absence of insulin) was decreased by two-thirds. These results might be the consequence of either the depletion of sodium ions or the high concentration of potassium ions, so that replacement of sodium ions by other ions was also investigated, while the concentration of potassium ions was kept constant (6 mequiv./l.). Replacement of sodium ions by *tris* or by choline (Table 2) caused a similar or greater depressive effect of the depletion of sodium ions on glucose metabolism in the presence of insulin. Furthermore, progressive depletion of sodium ions in the presence of insulin (Table 3) resulted in progressive depression of the metabolic indices measured.

Table 2. EFFECT OF REPLACEMENT OF SODIUM IONS BY OTHER CATIONS ON THE METABOLISM OF GLUCOSE-U<sup>14</sup>C BY INSULIN-STIMULATED FAT CELLS

Medium Na <sup>+</sup> (mequiv./l.)	Replaced by	<sup>14</sup> C to CO <sub>2</sub>	<sup>14</sup> C to total lipids
144	—	16.9 $\pm$ 0.33	25.8 $\pm$ 0.46
0	Tris <sup>+</sup>	5.82 $\pm$ 0.10	8.49 $\pm$ 0.20
144	—	16.1 $\pm$ 0.12	26.9 $\pm$ 0.46
0	Choline <sup>+</sup>	6.92 $\pm$ 0.14	10.2 $\pm$ 0.25
144	—	12.2 $\pm$ 0.22	21.9 $\pm$ 0.14
0	K <sup>+</sup>	7.22 $\pm$ 0.49	10.4 $\pm$ 0.68

Results are expressed as in Table 1; insulin 1 mU/ml.

These results agree with those of Hagen<sup>12</sup>, which demonstrated that glucose uptake in adipose tissue induced by insulin was decreased in the absence of sodium ions. This influence of sodium depletion could be the result of action exerted at almost any point of glucose uptake or metabolism on the end-products measured. As has already been pointed out, however (Table 1), depletion

Table 3. EFFECT OF PROGRESSIVE DEPLETION OF SODIUM IONS IN THE PRESENCE OF INSULIN

Medium Na <sup>+</sup> (mequiv./l.)	<sup>14</sup> C to CO <sub>2</sub>	<sup>14</sup> C to total lipids
144	16.9 $\pm$ 0.33	25.8 $\pm$ 0.46
72	10.5 $\pm$ 0.27	15.6 $\pm$ 1.35
50	7.08 $\pm$ 0.26	10.2 $\pm$ 0.17
25	6.55 $\pm$ 0.22	9.07 $\pm$ 0.28
10	8.14 $\pm$ 0.36	8.32 $\pm$ 0.35
0	5.82 $\pm$ 0.10	8.49 $\pm$ 0.20

Results are expressed as in Table 1; insulin 1 mU/ml.

of sodium ions did not apparently decrease glucose metabolism in the absence of insulin. Furthermore, glucose enters adipose tissue cells by a carrier-mediated process of facilitated diffusion<sup>6,19</sup>, which is usually the rate-limiting step of glucose metabolism in this tissue. Insulin enhances the rate of diffusion by this process. The simplest hypothesis to explain dependency of glucose metabolism on sodium in the presence but not in the absence of insulin would be that of insulin activation of an otherwise inactive carrier<sup>20</sup> which would require sodium ions before accepting glucose molecules for transport across the membrane, with subsequent liberation of sodium ions and glucose at the internal surface.

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# Inhibition of True Cholinesterase in TOCP Poisoning with Potentiation by 'Tween 80'

SINCE the work of Earl and Thompson<sup>1,2</sup> on hens, and the brief note by Mendel and Rudney<sup>3</sup> on rats, it has often been accepted<sup>4,5</sup> that in tri-ortho cresyl phosphate (TOCP) intoxication, pseudocholinesterase is markedly inhibited but that there is little or no decrease in true cholinesterase. A decrease in red blood cell cholinesterase has, however, been reported in rabbits<sup>6</sup> and a slight fall in man<sup>7</sup> after treatment with TOCP by mouth, and Taylor<sup>8</sup> has commented on parasympathomimetic effects in cats injected with this compound. I have investigated changes in cholinesterase in baboons after oral doses of TOCP. Monkeys have been found to be very resistant to TOCP by mouth<sup>9,10</sup> and it has been suggested the monkeys do not absorb it from the gut. In addition to pure TOCP therefore a 10 per cent emulsion in 10 per cent 'Tween 80' has also been tried in the present work at the suggestion of Dr J. D. Taylor of the Department of Pharmacology, University of Alberta. He has found this emulsion more active than the pure substance (personal communication from J. D. Taylor). The results to be described show that after administration of either preparation to the baboon, pseudocholinesterase is very readily inhibited, but that marked inhibition of true cholinesterase also occurs. In the reports of TOCP poisoning in man after accidental ingestion, diarrhoea and abdominal pain have often been noted and have sometimes been severe<sup>11</sup>. These effects would be expected to accompany inhibition of true cholinesterase.

The effects of pure TOCP were not uniform. Baboons *F* and *J* developed moderate diarrhoea within 24 h and this persisted for 48–72 h. Baboon *F* had slight limb weakness during this time, but neither it nor baboon *J* showed fasciculation and neither was seriously ill. Baboon *L*, which also showed the most marked changes in cholinesterase, was much more severely affected and was moribund when killed 7 days after poisoning. Diarrhoea was very severe, body weight falling by 20 per cent in the first 24 h and the skeletal muscles showed widespread coarse fasciculation and weakness. Fasciculation ceased after 4 days, but weakness persisted until death.

The effects of emulsified TOCP showed less variation, and were greater than those of the pure compound, despite the fact that the dose was reduced by 60 per cent (see Table 1). Baboons *H* and *K* both developed diarrhoea, severe muscle weakness and fasciculation. Baboon *G* suffered only slight anorexia after the first dose, given at the same time as atropine, but after the second was affected as severely as *H* and *K*. 'Tween 80' alone had no effect on concentrations of cholinesterase even in a large dose. The potentiating effect of 'Tween 80' could be caused by increased intestinal absorption of TOCP. Future toxicity testing of other aryl phosphates used in industry should perhaps take account of this.

The effect of atropine on baboon *I* was unexpected. Atropine was given at the same time as the emulsified TOCP, and 24 h later the animal was well except for a little anorexia. Table 1 shows that true cholinesterase fell much less sharply than in baboons *H* and *K*, and so the well-being of the animal was probably not caused only

Table 1. VALUES FOR CHOLINESTERASE IN RED BLOOD CELLS, MUSCLE, AND PLASMA BEFORE AND AFTER TOCP AND 'TWEEN 80'

Baboon	Weight (kg)	Dose TOCP (ml/kg)	'Tween 80' (ml/kg)	Atropine (mg intra-muscular)	Red blood cell cholinesterase + MCh ( $\mu$ l. CO <sub>2</sub> /ml./min)			Muscle cholinesterase + MCh ( $\mu$ l. CO <sub>2</sub> /g/min)			Plasma cholinesterase + BuCh ( $\mu$ l. CO <sub>2</sub> /ml./min)		
					Normal	Poisoned	Decrease (%)	Normal	Poisoned	Decrease (%)	Normal	Poisoned	Decrease (%)
<i>J</i>	11.4	1	—	—	9.0	4.46	50.4	9.8	3.8	61.2	165	11.3	93.2
<i>L</i>	12.05	1	—	—	13.0	1.39	89.3	4.89	1.94	61.0	218	10.9	95
<i>F</i>	11.1	1	—	—	9.88*	5.43	45.0	—	—	—	198*	2.13	98.9
					SD 1.89						SD 43		
<i>K</i>	8.55	—	22	—	10.1	11.1	9.9 (incr.)	—	—	—	204	199	2.5
<i>K</i>	8.55	0.4†	3.6	—	11.3	1.04	90.8	8.53	1.60	81.2	193	2.67	98.6
<i>H</i>	11.5	0.4	3.6	—	9.5	1.13	88.1	6.87	1.07	84.4	175	5.0	97.1
		0.1	0.9	5	—	3.25	54.4	—	—	—	—	6.93	96.4
<i>G</i>	10.55	0.3‡	2.7	—	7.12	0.325	95.4	5.94	0.24	96.0	193	9.4	95.1
<i>I</i>	11.2	0.4	3.6	5	9.17	4.33	52.8	—	—	—	241	17.9	92.6

\* Normal values not available for this animal. Figures are the mean and standard deviation of the other normal values in the table.

† Given 5 weeks after the dose of 'Tween 80' above.

‡ Given 24 h after the first dose.

The weights of the animals, the dose given to each and the corresponding concentrations of cholinesterase are shown in Table 1. All were sexually mature females, of species *P. papio* and *P. anubis*. The preparations of TOCP provided by Drs H. F. Bondy and K. R. Payne of Coalite and Chemical Products, Ltd., were at least 99 per cent pure; they were made from ortho-cresol, in which phenol, about 0.5 per cent, was the only identifiable impurity. They were given by gastric tube under light phenacyclidine anaesthesia. Cholinesterase was estimated with a conventional Warburg manometric apparatus<sup>2</sup> with butyrylcholine perchlorate (BuCh) and acetyl  $\beta$ -methylcholine chloride (MCh) (Koch-Light) as substrates for pseudo and true cholinesterase respectively. Red blood cells were separated from plasma by centrifuging heparinized blood, washed three times in 0.88 per cent sodium chloride, and finally lysed by adding distilled water up to the volume of the original blood sample. For estimation of muscle cholinesterase, *M. palmaris longus* was washed free of obvious blood in 0.88 per cent sodium chloride, lightly blotted, dissected free of connective tissue, and minced. The mince was then homogenized in 0.25 molar sodium bicarbonate to give 1 g of mince/4 ml. of homogenate. Blood and muscle were taken before and 21–27 h after poisoning, each animal thus providing its own control.

by blocking the parasympathomimetic effects of acetylcholine excess. Possibly atropine, given in large dose at the same time as the poison, delays or reduces intestinal absorption.

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## Uptake of Steroid Hormones into Artificial Phospholipid/Cholesterol Membranes

THE effect of steroid hormones on the movement of ions<sup>1,2</sup> and glucose<sup>3</sup> across artificial lipid membranes has been studied, but their activity in such membranes has not been correlated with their concentration within the membranes. The problem of uptake of steroid hormones into lipid membranes has been the subject of speculation and experiment<sup>4-7</sup>, and the work reported here has been carried out in order to examine this problem further.

The lipids used in this study were egg lecithin, which was chromatographically purified, after which it runs as a single peak in chromatographic systems with a characteristic  $R_F$  value; cholesterol, which was recrystallized from redistilled 'AnalaR' grade petroleum ether (40°–60° C); and chromatographically pure dicetylphosphate. Each of the tritiated steroids (in amounts of 5  $\mu$ c.) was added to 1 l. of water.

Table 1. DISTRIBUTION COEFFICIENTS OF FIVE STEROID HORMONES BETWEEN A LIPID MYELIN SUSPENSION AND WATER

Membrane 1 (90 per cent lecithin, 10 per cent dicetylphosphate)		
Aldosterone	1.125 $\pm$ 0.017	(30)
Cortisol	1.322 $\pm$ 0.041	(78)
Deoxycorticosterone	1.840 $\pm$ 0.060	(200)
Oestrone	3.191 $\pm$ 0.062	(520)
Progesterone	4.080 $\pm$ 0.030	(730)
Membrane 2 (80 per cent lecithin, 10 per cent dicetylphosphate, 10 per cent cholesterol)		
Aldosterone	1.113 $\pm$ 0.024	(28)
Cortisol	1.302 $\pm$ 0.020	(75)
Deoxycorticosterone	1.502 $\pm$ 0.080	(119)
Oestrone	2.978 $\pm$ 0.014	(491)
Progesterone	3.861 $\pm$ 0.068	(710)
Membrane 3 (70 per cent lecithin, 10 per cent dicetylphosphate, 20 per cent cholesterol)		
Aldosterone	1.111 $\pm$ 0.033	(29)
Cortisol	1.277 $\pm$ 0.041	(72)
Deoxycorticosterone	1.390 $\pm$ 0.070	(93)
Oestrone	2.823 $\pm$ 0.049	(477)
Progesterone	3.650 $\pm$ 0.040	(694)
Membrane 4 (60 per cent lecithin, 10 per cent dicetylphosphate, 30 per cent cholesterol)		
Aldosterone	1.133 $\pm$ 0.021	(37)
Cortisol	1.270 $\pm$ 0.014	(75)
Deoxycorticosterone	1.406 $\pm$ 0.080	(96)
Oestrone	2.577 $\pm$ 0.055	(460)
Progesterone	3.320 $\pm$ 0.040	(647)

Values in parentheses are the corresponding lipid : water distribution coefficients.

The membranes were prepared by adding appropriate amounts of the lipids in chloroform solution to each of four 100 ml. flasks, giving a total of 250  $\mu$ moles lipid in the proportions indicated in Table 1. These were evaporated to dryness, 50 ml. of water was added to each and they were shaken for 1 h. The flasks were allowed to stand overnight and reshaken before use. Spherical myelins formed, which were examined by electron microscopy after staining with a 2 per cent solution of phosphotungstic acid and found to provide very good bimolecular structures as described in previous work<sup>3</sup>.

After the final shaking, aliquots of 2 ml. were removed and dialysed against 40 ml. volumes of each steroid solution. These were left to stand overnight—which was found to be a suitable time for equilibrium to be established—after which 1 ml. of the inside and outside solutions were transferred to separate counting vials. The water was removed by standing on a hot plate, and scintillation fluid was then added to each vial and the tubes counted.

It was found that the presence of the small amount of lipid had no quenching effect on a tritium standard, and therefore the distribution coefficients for the steroids between the spherulite suspension and water could be directly obtained by taking the ratio of the counts for the corresponding inside and outside solutions (Table 1). The experiment was repeated and for each steroid with each membrane twelve values were obtained which have been used to determine a mean and a standard deviation.

The values of these distribution coefficients have been converted to a corresponding lipid : water distribution coefficient, which may be useful in the estimation of the distribution of the steroid hormones in the lipoidal environment of binding tissue<sup>8</sup>.

The effect of steroid concentration was investigated and the distribution coefficient found to remain constant up to concentrations of steroid approaching their limiting water solubilities, which have been determined as: cortisol ( $6.2 \times 10^{-4}$  moles/l.); deoxycorticosterone ( $1.6 \times 10^{-4}$  moles/l.); aldosterone ( $1.2 \times 10^{-4}$  moles/l.); oestrone ( $7.6 \times 10^{-5}$  moles/l.); and progesterone ( $2.7 \times 10^{-5}$  moles/l.).

A comparison of these water solubilities with the distribution coefficients suggests that the distribution coefficient reflects a decreased solubility in water rather than an increased solubility in the lipid membranes. The presence of cholesterol in the membrane lowers the membrane solubility of the steroid hormone, the distribution coefficient varying linearly with the cholesterol content. Such observations may be relevant to problems of uptake and localization of the steroid hormones in cells.

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## Production of Ethanol and Succinate by *Moniliformis dubius* (Acanthocephala)

LAURIE<sup>1,2</sup> found that formate, acetate and lactate were the chief excretory products from the carbohydrate metabolism of *Moniliformis dubius*, and we have found lactate and succinate to be the most important excretory products of *Polymorphus minutus*, an acanthocephalan parasite of domestic ducks<sup>3</sup>. Succinate has been reported as an intermediary metabolite of the carbohydrate metabolism of *M. dubius*<sup>4,5</sup>, and so we decided to analyse for excretory products, in particular succinate, the medium in which specimens of *M. dubius* had been incubated.

*M. dubius* was maintained in the laboratory using cockroaches, *Periplaneta americana*, as the intermediate hosts, and Wistar rats as the final hosts. Adult worms 42–57 days old were recovered from the rats and washed thoroughly in Tyrode saline, before being incubated in Tyrode saline containing <sup>14</sup>C-glucose at pH 7.5 and 37° C under commercial nitrogen. Incubation was terminated after a certain time, and the worms were removed and weighed and the medium was analysed for volatile components, non-volatile acids and residual glucose. The results given below apply to an incubation, lasting 2.5 h, of 515 mg wet weight of worms with 5 mg of glucose in 3.4 ml. of saline.

Examination of a sample of the medium using a gas chromatograph with a flame detector revealed that the main excretory product of *M. dubius* was neither formate nor acetate but another very volatile non-acidic substance, and analysis showed that most of the excreted radioactivity was associated with this metabolite. The substance was found to have the same retention time as

ethanol on the gas chromatograph, and support for its identity was obtained on incubation with yeast alcohol dehydrogenase. In conditions of low oxygen tension the production of ethanol was of the order of 200  $\mu\text{g}/100$  mg wet weight of worms/h for the 2.5 h of incubation. A similar quantity of ethanol was produced when another batch of worms was incubated aerobically. By comparison, the amount of acetate excreted by the worms was about 54  $\mu\text{g}/100$  mg wet weight of worms/h, but the radioactivity associated with the acetate was only one-sixtieth of that of the ethanol. This result indicates that most of the acetate had not been produced directly from the absorbed radioactive glucose. No formate could be detected.

Non-volatile organic acids were detected and identified as described before<sup>3</sup>. Radioactivity was associated only with lactic and succinic acids, the former being thirty-three times as active as the latter. It was found that 85 per cent of the original glucose had been metabolized by the worms during the incubation and, of this, about 30 per cent was excreted as ethanol, 2.3 per cent as lactate and succinate, and 0.5 per cent as acetate. Similar results were obtained on two other occasions.

The identification of ethanol among the excretory products of *M. dubius* provides the first record known to us of the formation of this substance by an acanthocephalan worm. It is possible that much of the unidentified, volatile radioactive component found<sup>5</sup> in homogenates of *M. dubius* incubated with <sup>14</sup>C-glucose was also ethanol. Ethanol is known to be a minor excretory product of the cestodes *Echinococcus granulosus*<sup>6</sup> and *Taenia taeniaeformis*<sup>7</sup>. It is clear from our investigation that ethanol is the principal excretory product of *M. dubius*, but succinate has also been found to be excreted.

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### Vinca Alkaloids and the Synthesis of RNA in Mouse Brain

THE dimeric indole alkaloids, vinblastine and vincristine, were isolated during the investigations<sup>1,2</sup> of the reported hypoglycaemic activity<sup>3</sup> of *Vinca rosea* Linn. Although the occurrence of hypoglycaemia could not be substantiated, the alkaloids were found to be potent oncolytic agents in some experimental systems<sup>4</sup>. Clinically, vinblastine has been useful in the treatment of lymphoma, including Hodgkin's disease, carcinomata of the breast and bronchus and certain non-malignant conditions such as Letterer-Siwe disease<sup>5</sup>. Vincristine has been used with varying degrees of success in acute lymphocytic and myelogenous leukaemia, carcinoma of the cervix, malignant lymphomata, neuroblastoma, Wilms' tumour and rhabdomyosarcoma<sup>6</sup>, as well as in certain intracranial gliomata<sup>7</sup>. Both alkaloids may suppress the immune response<sup>8</sup>. Whereas leukopenia has been the limiting manifestation of toxicity in the use of vinblastine<sup>9</sup>, this has

not usually been the case with vincristine, which produces various neuropathies<sup>10</sup> with a picture of polyneuritis running an acute or sub-acute course<sup>11</sup>. In mice, vincristine produces degeneration of the dorsal root cells and of the myelin sheath of the sciatic and median nerves, and inhibits the proliferation of the Schwann cells<sup>12</sup>. The present study was undertaken in an attempt to find a biochemical basis for the neurotoxicity.

White mice (Swiss CD-1) were used; they were given 'Purina' laboratory chow and water freely. Vinca alkaloids, as the sulphate salts, dissolved in isotonic saline, were administered by daily intraperitoneal injections in doses of between 0.1 and 0.5 mg/kg. Single injections of vincristine, or of 4  $\mu\text{c}$ . of either uridine-<sup>3</sup>H (specific activity 2.6 c/mmole) or valine-1-<sup>14</sup>C (specific activity 22 mc./mmole), dissolved in 0.05 ml. of isotonic saline, were given intracranially. These injections were divided so that half was administered to lightly anaesthetized mice into each side of the brain at a point midway between the eye and ear. Intraperitoneal injection of vincristine in doses of 0.3 and 0.5 mg/kg produced signs of neurological toxicity by the seventh day, starting as insensitivity to pain in the tail and digits, and progressing through diminished activity to hind limb palsy. Single intracranial injections of vincristine in doses as small as 0.03 mg/kg produced neurotoxicity by the fourth day after treatment. The manifestations included loss of sensitivity to pain, periods of uncoordinated and frenzied motion, hind limb palsy and almost total paralysis of the limbs leading to death; these signs were not seen in controls treated with saline. Vinblastine given by daily intraperitoneal injections did not produce obvious signs of neurological toxicity, except perhaps a marked depression in activity, but only 10 per cent of the mice survived more than 14 days of treatment.

The synthesis of brain RNA was studied by injecting tritiated uridine intracranially after different periods of intraperitoneal treatment, and killing the animals 30 min later. Brains were removed, washed with cold isotonic saline and homogenized with cold 0.5 molar perchloric acid. After washing five or six times with the same acid, the residues were extracted with absolute ethanol: ether (3:1) to remove lipid, and RNA was hydrolysed by heating with 0.2 normal sodium hydroxide for 30 min at 90°. The solutions were acidified with 0.5 molar perchloric acid, cooled in ice, centrifuged, and the supernatant fractions which contained ribonucleotides derived from RNA were assayed for ultra-violet absorbance at 260 m $\mu$  with a Zeiss 'PMQII' spectrophotometer, and for radioactivity, using a Packard 'Tri-Carb' scintillation spectrometer. Fig. 1 shows that there was a progressive decrease in the specific activity of RNA during treatment, a decrease that was more pronounced initially for vincristine than for vinblastine, as would be expected in view of the greater neurotoxicity produced by the former drug. The results after 21 days are scarcely significant, in view of the low survival rate and moribund condition of the mice. After 7 days of intraperitoneal treatment with vincristine the dose response curve of Fig. 2 was obtained. When RNA was isolated by phenol extraction<sup>13</sup>, rather than by alkaline hydrolysis, and the ribosomal and soluble RNA were separated by precipitation with lithium chloride<sup>14</sup>, the data shown in Table 1 were obtained. Reductions in the specific activity of all RNA fractions were produced by treatment with vincristine, although soluble RNA seemed to be the least sensitive, contrary to the situation in Ehrlich ascites carcinoma cells<sup>14</sup>. This depression in the synthesis of RNA could not be produced by incubating

Table 1. EFFECTS OF VINCRISTINE, AFTER SEVEN DAILY INTRAPERITONEAL INJECTIONS, ON THE SYNTHESIS OF SUBFRACTIONS OF RNA

Fraction	Specific activity (c.p.m./absorbance unit)	
	Control	Vincristine (0.5 mg/kg $\times$ 7)
Interfacial	96.2	14.2
Soluble	14.2	8.9
Ribosomal	42.8	19.8



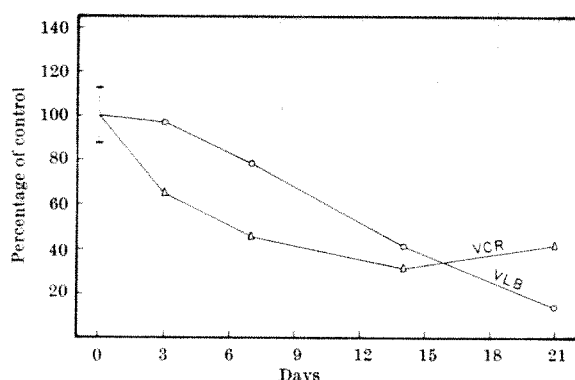


Fig. 1. The depression of RNA synthesis in mouse brain produced by daily intraperitoneal injections of vincristine ( $\Delta$ ) or vinblastine ( $\circ$ ). The drugs were given in a dose of 0.3 mg/kg. Results are normalized to a 100 per cent control shown with the range of its standard error.

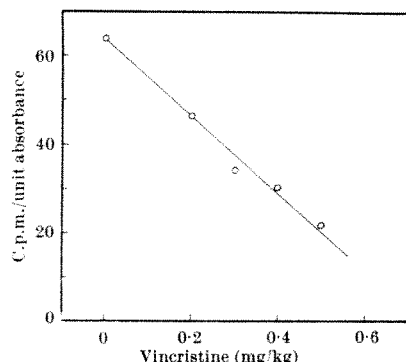


Fig. 2. The dose response curve for the depression of incorporation of tritiated uridine into mouse brain RNA by seven daily intraperitoneal injections of vincristine.

normal brain slices in Eagle's medium in the presence of vincristine and tritiated uridine.

Despite these effects on the synthesis of RNA, that of protein, as measured by incorporation of valine- $1-^{14}\text{C}$ , was not significantly changed after treatment for 7 days with vinblastine or vincristine (0.5 mg/kg).

Further investigations into the effects of the vinca alkaloids on various metabolic pathways in the brain are in progress. The early depression of RNA synthesis during treatment with the relatively more neurotoxic agent vincristine does suggest, however, that this is a factor involved in the development of neuropathy.

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## Synthesis of Ribonucleic Acid and Histone Change during Spermatogenesis in the Grasshopper *Chortophaga viridifasciata*

BLOCH<sup>1</sup> showed that different nuclei of a tissue react differently when the tissue is stained with a mixture of fast green and eosin. He found that fast green has a higher affinity for histones rich in arginine, and eosin for histones rich in lysine, and that in some cell types cessation or slowing of the synthesis of RNA is associated with acquisition of an eosinophilic histone.

We have investigated the correlation between the synthesis of RNA and changes in the reaction to staining with fast green and eosin in spermatids of *Chortophaga viridifasciata*.

Nymphs of grasshoppers collected in Austin, Texas, between October 1965 and April 1966 were injected with 6  $\mu\text{c}$ . of uridine labelled with tritium (specific activity 1.15 c./mmole) and killed 5 or 6 h after injection. The testes were fixed in 10 per cent neutral buffered formalin, dehydrated, embedded in paraffin and sectioned at 10 $\mu$ . The sections were mounted on slides and autoradiographed using Kodak 'AR-10' fine grain scientific plates<sup>2</sup>. The autoradiographs were exposed for 9 days and developed. After development the sections were stained with toluidine blue (0.1 per cent solution buffered at pH 4.1 with acetate buffer) following the method of Prescott and Bender<sup>3</sup>. Grain counts were made of at least twenty individual nuclei in cells in each of eight stages of spermiogenesis taken from cysts in each of four different tubules. We considered only those nuclei the cut surface of which faced the emulsion. The following formula was used to correct for background

$$N = \left( \frac{N_{\text{obs}}}{A} - \frac{N_{\text{bg}}}{A_{\text{bg}}} \right) \bar{A}$$

where  $N_{\text{obs}}$  is the observed number of grains in a nucleus,  $\bar{A}$  is the average area of the nuclei at that stage,  $N_{\text{bg}}/A_{\text{bg}}$  is the average number of grains per unit area of background, and  $N$  is the number of grains in a nucleus, corrected for background. The corrected mean number of grains/area and standard error were calculated for each stage. This value was used as a measure of RNA synthesis. The areas were measured in arbitrary units.

The grasshopper tissue used for staining with fast green and eosin was fixed in 10 per cent neutral buffered formalin, dehydrated, embedded in paraffin and sectioned. The tissue was stained with fast green and eosin according to Bloch<sup>1</sup>, and absorption measurements were made with a microspectrophotometer<sup>4</sup>. Measurements at wavelengths of 640 and 500 m $\mu$  were made of at least twenty cells in each of the designated stages of spermiogenesis. The ratios of extinctions at 500 m $\mu$  and 640 m $\mu$  were determined for each cell, and the mean  $E_{500}/E_{640}$  and the standard error were determined for each stage. A high ratio of  $E_{500}/E_{640}$  is indicative of the presence of the eosinophilic histone (see Fig. 1).

The changes which occur before and during meiosis have been described in detail<sup>5</sup> and were divided into only two comprehensive stages for the purposes of this investigation. The first stage,  $S_a$ , includes spermatocytes in the diffuse prophase stages. The second stage,  $S_b$ , includes spermatocytes in metaphase, anaphase and telophase. The chromatin material in  $S_a$  cells is dispersed, whereas in  $S_b$  cells it is highly condensed. Spermatid development was divided into eight stages on the basis of changes in morphology (Fig. 2).

A striking negative correlation between RNA synthesis and eosinophilia during spermiogenesis is shown in Fig. 2. Intense RNA synthesis, as indicated by grain counts, occurs in the cells which appear to be devoid of the eosinophilic histone (as indicated by a low  $E_{500}/E_{640}$  ratio).

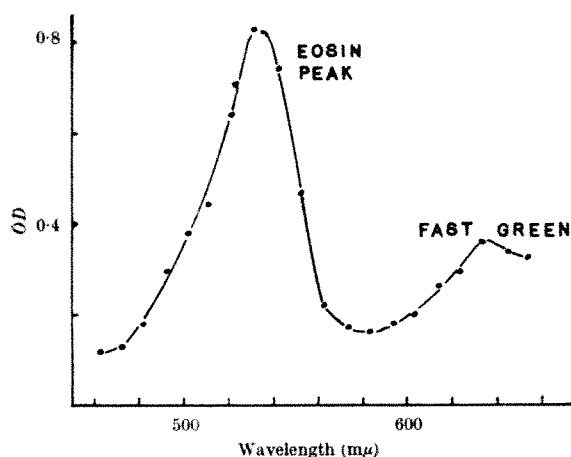


Fig. 1. Absorption spectrum of a nucleus stained with fast green and eosin. The peak to the left is caused primarily by absorption by the eosin, and that on the right, exclusively by fast green.

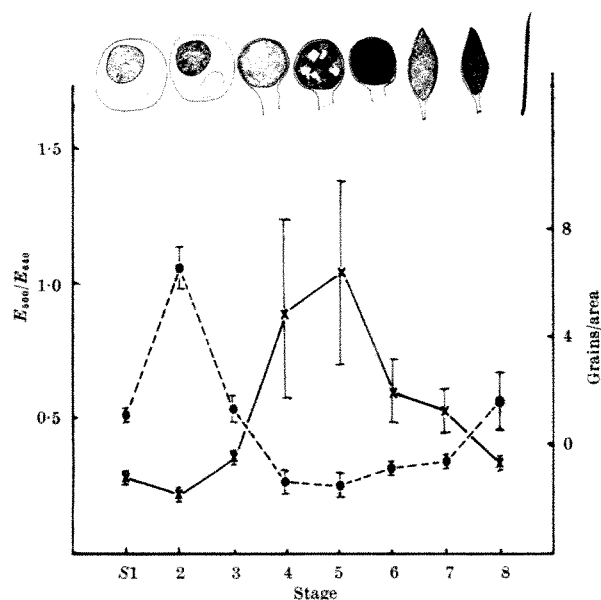


Fig. 2. Comparison of staining and incorporation of uridine into RNA of the nucleus. ●-●, Incorporation; scale at right. x-x, Ratio of  $E_{500}/E_{440}$ , scale at left. The vertical lines show standard errors. The abscissa shows the developmental stages, depicted schematically above the graph.

Decline in RNA synthesis during meiotic chromosome condensation and during the later spermatid stages is accompanied by an increase in eosinophilia and a masking of the fast green staining.

The nature of the eosinophilic protein is not known. It is probably a histone because it is a basic nuclear protein which requires the removal of DNA before staining. In several model systems, it has been shown that eosin successfully competes with fast green in staining proteins that are rich in lysine, and for this reason it is probable that the eosinophilic protein is a histone rich in lysine. Bloch<sup>1</sup> also found that the eosino-

philic histone, when it does occur in cells, does not replace the other histone but merely masks its staining with fast green. The eosinophilic histone may play the part of a non-specific regulator of synthesis of the RNA in the nucleus as a whole.

Such "repression" by histones has been well established in *in vitro* systems<sup>6</sup>, and lysine histones appear to be among the most effective inhibitors of RNA synthesis<sup>7</sup>. These proteins have also been implicated in the control of *in vivo* systems<sup>1,8</sup>. It is perhaps significant that in these two cases control was observed as a nuclear or cellular rather than as a genic phenomenon.

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### Phytohaemagglutinin and Cell Mediated Hypersensitivity Reactions in Rat

PHYTOHAEMAGGLUTININ (PHA) has been used *in vitro* extensively over the past 18 yr<sup>1</sup>. Comparatively few attempts have been made, however, to study its effect *in vivo*<sup>2,3</sup>.

We were impressed by the apparent similarity between the immunological status of patients with Hodgkin's disease, whose lymphocytes show increased DNA synthesis (Crowther, D., personal communication), and the reported *in vitro* action of PHA, which also causes increased DNA synthesis. It was therefore decided to study the effect of *in vivo* PHA on a delayed hypersensitivity reaction in the rat to see if the non-specific stimulation could result in impaired hypersensitivity reactions.

In the present series of experiments, cutaneous hypersensitivity to pertussis vaccine in the rat was used as a test system<sup>4</sup>. Two types of PHA were used in these experiments (Difco<sup>M</sup> and Wellcome<sup>M</sup>); there was no detectable difference in effect between the two types. PHA was administered intravenously in a dose of 0.5 ml. (reconstituted)/200 g body weight. In the first series of experiments, PHA was given 24 h before the primary injection, then daily throughout the 12 day period of sensitization and once after challenge. This procedure led to a 58 per cent suppression of the inflammatory reaction (which consisted of swelling of the foot) 24 h after the challenge and 76 per cent suppression of the reaction 48 h after challenge (Table 1). To investigate the possibility that the PHA might be acting as an anti-inflammatory agent, rats were given a single dose of PHA before the production of a pleurisy induced by turpentine<sup>10</sup>. This treatment failed to affect the development of the exudate. Similarly, rats on a 12 day course of daily

Table 1. CORRELATION OF COLOUR, RELATIVE ABSORPTIONS BY EOSIN AND FAST GREEN, AND URIDINE INCORPORATION BY PRIMARY SPERMATOCYTES IN THE DIFFUSE AND CONDENSED MEIOTIC STAGES

Stage	Colour	$E_{500}/E_{440}$	Incorporation
Diffuse prophase	Green	$0.20 \pm 0.01$	High
Condensed meiotic	Purple	$1.32 \pm 0.02$	None

This pattern of events is similar to that observed in another system, the developing erythrocyte, in which enhancement of eosinophilia also accompanies the depression of RNA synthesis<sup>5</sup>.

Table 1. EFFECT OF VARIOUS TREATMENTS WITH PHA ON CUTANEOUS HYPERSENSITIVITY TO PERTUSSIS VACCINE IN THE RAT AND ITS EFFECT ON ACUTE INFLAMMATION

Treatment with PHA	Per cent suppression	
	24 h	48 h
Day before primary then daily up to challenge dose + 1 day	58	76
For 5 days daily before primary dose, then no more	58	72.8
For 3 days daily before challenge dose	0	0
Turpentine pleurisy	6 h	
Single dose before acute inflammation	0	
Twelve daily injections before acute inflammation	0	

injections of PHA also responded with normal acute inflammatory reactions in response to intrapleural turpentine. It thus appeared that the PHA did not act as a general anti-inflammatory agent.

Groups of rats were then treated for 5 days with daily intravenous injections of PHA before the primary injection of pertussis. Twelve days after the primary injection they were challenged and showed markedly reduced inflammatory responses. At 24 h there was again 58 per cent suppression and at 48 h a 73 per cent suppression of the reaction.

Finally, rats were given three daily injections of PHA immediately before the administration of the challenging dose of pertussis. This procedure failed to cause any suppression of the reaction.

From these observations it would seem that PHA given before the initial exposure to antigen will suppress the delayed hypersensitivity reaction. On the other hand, PHA given before the challenging dose of antigen fails to reduce the reaction. In addition, PHA does not owe its activity to a general anti-inflammatory action.

It seems possible that the PHA acts by causing a stimulation of the non-committed clones of immunologically competent cells, thus rendering them unable to respond for that period to antigenic stimulation of the type administered.

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## Hydroxyproline Indices

WHEN it was known that the excretion of hydroxyproline peptides in the urine was correlated with active growth, and varied in a characteristic way with age<sup>1-3</sup>, Alison *et al.*<sup>4</sup> demonstrated that as a measure of growth the ratio of hydroxyproline : creatinine in a 24 h collection of urine was a great improvement on the hydroxyproline excretion/24 h, because the former eliminated to a great extent the effects of weight, and gave more consistent results for subjects of the same age. They found that the ratio fell in children between 1 and 5 yr, and then remained constant until puberty, after which it fell again to the adult level. Jasin *et al.*<sup>1</sup> had already introduced the factor of surface area to try to eliminate the effects of size, and had found that the results obtained were constant between the ages of 1 and 10 yr.

Whitehead<sup>5,6</sup>, wishing to use random samples of urine for his work rather than 24 h collections, and not knowing the exact ages of the children in which he was interested, (a) satisfied himself of the constancy of the ratio of hydroxyproline : creatinine during 24 h by testing many random samples from the same individuals, and (b) added the parameter of weight to the ratio of hydroxyproline : creatinine, and developed what he called the hydroxyproline "index" as a measure of marginal malnutrition and of a failure to grow. This index had the same advantage that the hydroxyproline excretion/m<sup>2</sup>/24 h would have had in giving a constant figure for the age range in which he was interested. It has subsequently been realized that this "constancy" in a given range of weights is empirical, and may only hold for man. There is no such constancy in rats<sup>7</sup>.

Table 1. HYDROXYPROLINE INDICES BASED ON HEIGHT AND WEIGHT IN 103 NORMAL CHILDREN

No. of children	Age (months)	Hydroxyproline ( $\mu$ m)/creatinine ( $\mu$ m)	Weight index		Height index
			Weight measured in individuals	Weight from tables	
20	1-6	0.59	3.6	3.6	33
23	7-12	0.38	3.1	3.4	26
17	13-24	0.27	2.7	2.8	22
6	25-36	0.22	2.6	2.7	20
21	37-48	0.18	2.7	2.6	18
22	49-72	0.19	3.3	3.3	20

Work now in progress had led us to examine parameters other than weight, which might be incorporated into an index to make it valuable in scientific studies of animal growth as well as in clinical medicine. Height appears to be a satisfactory one. Table 1 shows a comparison of the "index" based on weight, with a corresponding one based on height. The methods used were those described by Howells and Whitehead<sup>8</sup>. The weights of the individual children were all known, and the first figures given for the weight index were obtained by averaging the results for each individual child in the group. The height indices for children up to 3 yr were obtained in the same way. The second figures given for the weight index were obtained from tables<sup>9</sup> and the results were much the same. The heights of children of more than 3 yr were not always known, and the figures given for their height indices were obtained only from the tables. Two points are clear: (a) an index based on height changes with age like the one on weight, and (b) that, if a constant value is desirable between 1 and 6, height has a claim to a place in the index as good as or better than weight.

The use of a height rather than a weight index nullifies one criticism of Whitehead's work made by Anasuya and Narasinga Rao<sup>10</sup>, namely, that the improvement in the index during the treatment of malnutrition was caused by the simultaneous changes in weight, and not by an increase in the excretion of hydroxyproline. In a study of forty-eight children by the index based on height, we have obtained the following results. Index on admission was  $13.5 \pm 6.7$  and index on discharge was  $21.5 \pm 8.0$ .

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## Antimitotic Activity of a Steroid Guanyl-hydrazone

I HAVE previously shown that a guanylhyazone derivative of a known steroid molecule is a powerful antimitotic agent<sup>1</sup>. This compound, the structure of which is shown in Fig. 1, is 3-guanyl-hydrazone-androstan-17-ol; it is listed by the Italian pharmaceutical house Vister as 2052 V (Fig. 1).

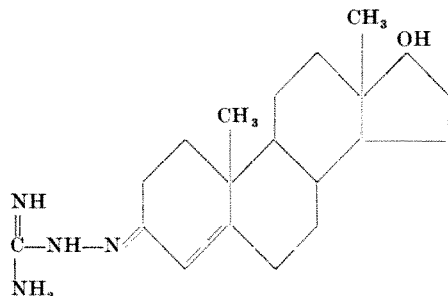


Fig. 1. 2052 V, 3-guanyl-hydrazone-androstan-17-ol

The present investigations were made in order to characterize the antimitotic activity of 2052 V, *in vitro* (in human amnion cell lines and primary kidney cell cultures of pigs and hamsters) and *in vivo* (in mice and hamsters).

Human amnion cell cultures (H. A. Mascoli's line), propagated *in vitro* in Hanks medium, supplemented with lactalbumin hydrolysate and 5 per cent calf serum, were collected by trypsinization from cultures which had been stationary for 36 h, dispersed in the same medium (300,000 cells/ml.) and seeded on coverslips which had been placed on the bottom of Petri dishes; at the same time either 2052 V (1 and 10  $\mu\text{g}/\text{ml.}$ ) or colchicine (1  $\mu\text{g}/\text{ml.}$ ) were added. Untreated cultures were kept as controls. At intervals of 12, 24, 36 and 48 h the coverslips were fixed in methanol and stained with Giemsa. Mitotic figures were registered and the mitotic index calculated.

Table 1. ANTIMITOTIC ACTIVITY OF 2052 V IN COMPARISON WITH THAT OF COLCHICINE ON HUMAN AMNION CELL CULTURES

Percentage of mitotic figures and mitotic index (M.I.) in cell cultures at different times after treatment.

h	No treatment				2052 V (1 $\mu\text{g}/\text{ml.}$ )				Colchicine (1 $\mu\text{g}/\text{ml.}$ )			
	M.I.	P	M	A	M.I.	P	M	A	M.I.	P	M	A
12	2.5	32	40	8	20	14.8	2	98	—	—	—	—
24	2.8	25	34	11	30	46.0	—	100	—	—	—	—
36	2.3	16	49	11	24	12.8	—	98	1	1	27.6	—
48	2.1	15	51	9	25	4.0	—	100	—	—	15.0	—

P, Prophase; M, metaphase; A, anaphase; T, telophase.

This is a typical experiment representative of many other experiments.

2052 V produces an effect like that of colchicine (Table 1); at a concentration of 1  $\mu\text{g}/\text{ml.}$  it produces a complete mitotic block in metaphase, as shown by the disappearance of anaphases and telophases and by the increase of abnormal metaphases. The mitotic index declines faster with 2052 V than with colchicine because metaphases arrested by the former compound degenerate more rapidly, and because a few cells may break through the 2052 V block. At a concentration of 10  $\mu\text{g}/\text{ml.}$  of 2052 V a clear cytotoxic effect is present within 24 h. In cultures treated with 2052 V, cells in inter-kinesis display nuclear budding which leads to polynucleated cells. This process becomes evident after 12 h of treatment and affects a progressively larger number of cells.

In order to evaluate further analogies between 2052 V and colchicine other experiments have been carried out on primary kidney cell cultures of pigs and hamsters. It is known that hamster cells are resistant to the antimitotic activity of colchicine *in vivo*<sup>2,3</sup> and *in vitro*<sup>3</sup>. Either 2052 V or colchicine was added to the culture medium at a concentration of 1  $\mu\text{g}/\text{ml.}$  Twenty-four hours later mitotic figures were registered and the mitotic

index was calculated. Untreated cultures were kept as controls.

As may be seen in Table 2, the kidney cells of pigs are inhibited both by colchicine and by 2052 V while the kidney cells of the hamster are resistant to the antimitotic activity of both compounds.

Table 2. INFLUENCE OF 2052 V AND COLCHICINE ON PRIMARY KIDNEY CELL CULTURES OF PIG AND HAMSTER

Percentage of mitotic figures and mitotic index (M.I.) in cell cultures after 24 h of treatment

Treatment	M.I.	Pig				M.I.	Hamster			
		P	M	A	T		P	M	A	T
No treatment	1.6	31	42	12	15	1.2	28	42	7	23
2052 V (1 $\mu\text{g}/\text{ml.}$ )	2.9	—	100	—	—	1.0	20	50	10	20
Colchicine (1 $\mu\text{g}/\text{ml.}$ )	3.6	2	97	1	—	1.2	17	69	4	10

2052 V was administered intraperitoneally to mice and hamsters in single doses of 10, 25, 50 or 100 mg/kg. Other animals were given colchicine 10 mg/kg intraperitoneally. The animals were killed 8 and 30 h after treatment, bone marrow mitotic stages were registered and mitotic indices calculated.

Table 3. INFLUENCE OF 2052 V (100 MG/KG INTRAPERITONEALLY) AND COLCHICINE (10 MG/KG INTRAPERITONEALLY) ON BONE MARROW MITOSES OF MOUSE AND HAMSTER

Percentage of mitotic figures and mitotic index (M.I.) in bone marrow cells at different times after treatment. Percentage calculated on the basis of at least three animals.

Treatment	h	M.I.	Mouse				M.I.	Hamster			
			P	M	A	T		P	M	A	T
No treatment	—	0.8	20	40	10	30	0.9	9	47	18	26
2052 V	8	2.4	—	95	—	5	0.9	10	45	15	30
2052 V	30	1.6	20	60	5	15	0.7	5	45	20	30
Colchicine	8	6.6	—	100	—	—	1.5	3	63	13	21
Colchicine	30	5.3	—	95	—	5	1.3	5	50	15	30

The most significant results obtained are reported in Table 3. In mice, 2052 V in doses of 100 mg/kg caused a nearly complete disappearance of ana-telophases at 8 h, and also caused a rise of mitotic index from 0.8 to 2.4 per cent. After 30 h these effects regressed. Single doses of 10, 25 and 50 mg/kg were less effective. Colchicine at the dose of 10 mg/kg was more effective than 100 mg/kg of 2052 V and its blockade was more persistent.

In hamsters, unlike mice, both 2052 V and colchicine failed to affect mitoses significantly.

In conclusion, 2052 V is a mitotic poison both *in vitro* and *in vivo*. It shows several analogies with colchicine and griseofulvin. In fact, all three compounds produce a block in metaphase; moreover, like colchicine<sup>2,3</sup> and griseofulvin<sup>7</sup>, 2052 V is ineffective in hamsters both *in vivo* and *in vitro*. The similarity between griseofulvin and 2052 V is more marked because this compound produces nuclear buddings which are identical to those seen with griseofulvin<sup>4-6</sup>. 2052 V is effective *in vitro* at concentrations as low as those of colchicine; however, *in vivo* doses greater than those for colchicine are needed to produce the same effect. Also *in vivo* metaphasic blockade by 2052 V is less persistent than that by colchicine.

2052 V is of great interest because it is the only known steroid molecule endowed with antimitotic activity similar to that of colchicine. The resistance of hamsters to colchicine, griseofulvin and 2052 V suggests that, despite the differences in structure, 2052 V also acts as a mitotic spindle poison.

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### Effect of Azide and Oligomycin on the Transport of Calcium Ions in Corn Mitochondria

INHIBITION of respiration by azide has been known for many years. It is now generally considered that azide not only inhibits electron transfer through the cytochrome chain, presumably by inhibiting cytochrome oxidase<sup>1,2</sup>, but that it also inhibits one of the associated reactions involved in the production of ATP (refs. 3 and 4). The reactions of phosphorylation seem to be more sensitive to azide than the electron transfer reactions because smaller concentrations of azide are required to inhibit oxygen uptake in the presence of ADP and inorganic phosphate than in their absence<sup>5,6</sup>, that is, state 3 respiration is more sensitive to azide than state 4 respiration.

The effect of azide on the partial reactions of phosphorylation, that is, the ATPase, the ATP-Pi and ATP-ADP exchange reactions, appear to be similar to that of oligomycin<sup>7-10</sup>. Wadkins and Lehninger<sup>9</sup> suggested that both azide and oligomycin function by combining with a non-phosphorylated intermediate, thereby preventing formation of any phosphorylated intermediates. Lardy *et al.*<sup>10</sup>, working with rat liver mitochondria, as well as ourselves working with corn shoot mitochondria<sup>11,12</sup>, have suggested that oligomycin blocks after the entry of inorganic phosphate into organic combination. In further comparing the effects of azide and oligomycin on the transport of calcium ions in corn mitochondria we have found that both inhibitors behave similarly, with one important exception, the ADP impairment of substrate-driven transport of calcium ions is alleviated by oligomycin but not by azide. These results indicate separate sites of action of azide and oligomycin on the phosphorylation reactions and are interpreted accordingly.

Corn (*Zea mays*) shoot mitochondria were used in all experiments. The isolation procedure and experimental techniques were essentially as previously described<sup>11</sup>. Uptake of calcium ions was followed using calcium-45. Incubations were carried out in 'Pyrex' centrifuge tubes at 30° C. After incubation the mitochondria were re-isolated by layering the incubation mixture over 2.5 ml. of 0.5 molar sucrose and centrifuging at 0° C. Mitochondrial pellets were suspended in water and counted for radioactivity. Oligomycin was obtained from Sigma Chemical Company, St. Louis, and its molarity was calculated on the basis of 15 per cent oligomycin A and 85 per cent oligomycin B.

Table 1. GENERAL CHARACTERISTICS OF ACCUMULATION OF CALCIUM IONS BY MAIZE MITOCHONDRIA

Experiment No.	Treatment	Accumulation of calcium ions $\mu\text{moles/mg N/2 min}$
1	Blank	0.36
	Substrate	3.10
	Substrate + ADP ( $10^{-3}$ molar)	1.09
	Substrate + oligomycin ( $10^{-6}$ molar)	3.38
	Substrate + azide ( $10^{-5}$ molar)	3.10
2	Blank	$\mu\text{moles/mg N/10 min}$ 0.17
	ATP	0.70
	ATP + oligomycin ( $10^{-6}$ molar)	0.21
	ATP + azide ( $10^{-5}$ molar)	0.37

The assay medium consisted of 650  $\mu\text{moles}$  sucrose, 17  $\mu\text{moles}$  of inorganic phosphate, 0.5  $\mu\text{moles}$  of calcium chloride (250 c.p.m. of calcium-45/ $\mu\text{moles}$ ), 0.2 mg of mitochondrial nitrogen and substrate (10  $\mu\text{moles}$  of potassium pyruvate and 10  $\mu\text{moles}$  of succinate) or 3.25  $\mu\text{moles}$  of ATP where indicated. Experiment 2 also contained 7.5  $\mu\text{moles}$  of magnesium chloride, pH adjusted to 7.5 with *tris*. The final volume was 2.5 ml.

Higher plant mitochondria accumulate calcium and magnesium ions and inorganic phosphate<sup>11</sup> in a fashion very similar to that reported for mammalian mitochondria<sup>13</sup>. Table 1 illustrates that either oxidizable substrate or ATP can serve as an energy source for the transport of calcium ions. Substrate oxidation is, however, considerably more effective than ATP. This is due in part to the competition with magnesium ions as evidenced by the lower blank values. Oligomycin at  $10^{-6}$  molar has little effect on substrate-driven transport but essentially eliminates ATP-driven transport of calcium ions (92.5 per cent inhibition of net absorption of calcium ions). The inclusion of ADP impairs substrate driven transport of calcium ions and this impairment has previously been shown to be partially relieved by oligomycin<sup>11</sup>. These results are similar to those reported for animal mitochondria and are thus consistent with the hypothesis that a common high energy intermediate of oxidative phosphorylation provides the energy for either calcium ion transport or ATP formation in plant, as well as animal, mitochondria. In our experiments with corn mitochondria, a small but reproducible substrate-dependent binding of calcium ions occurs in the absence of inorganic phosphate, but for transport to be significant the phosphate is essential. Other anions such as acetate or arsenate<sup>11,14</sup> will not substitute for inorganic phosphate, and so it appears that a phosphorylated intermediate is involved in the transport of calcium ions.

Table 1 shows that  $10^{-5}$  molar sodium azide does not inhibit substrate-driven transport of calcium ions, but does partially inhibit ATP-driven transport (62.5 per cent

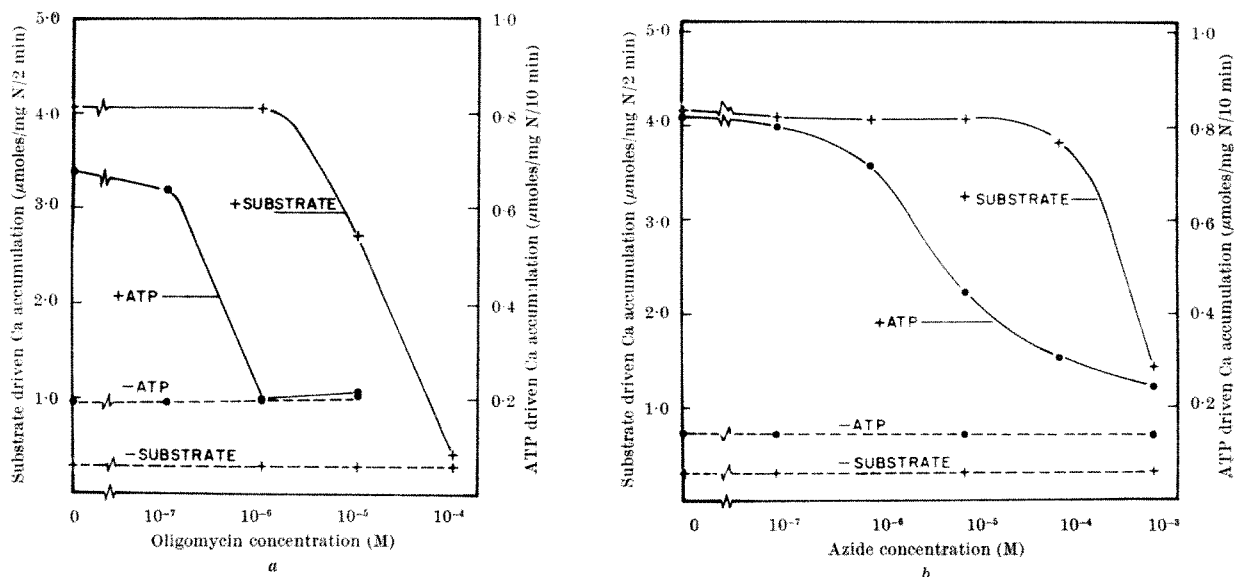


Fig. 1. The effect of oligomycin (a) and azide (b) on substrate- and ATP-driven accumulation of calcium ions in maize mitochondria. Experimental conditions the same as in Table 1 and text.

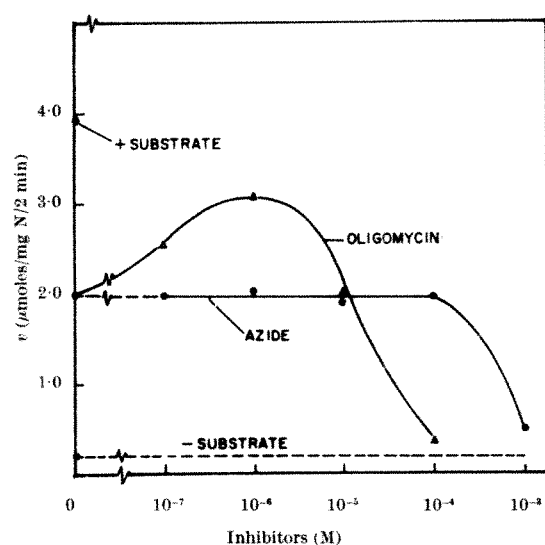


Fig. 2. The effect of azide and oligomycin on uptake of calcium ions in the presence of both substrate and ADP ( $8 \times 10^{-4}$  molar). Experimental conditions same as in Table 1 and text.

inhibition of net absorption of calcium ions). Thus azide and oligomycin seem to have similar effects on the two systems of transport of calcium ions.

The relative effectiveness of azide and oligomycin on both the substrate- and ATP-driven transport systems for calcium ions is shown in Fig. 1a and b. Up to about  $10^{-6}$  molar, oligomycin has no effect on substrate-driven transport (Fig. 1a), but at greater concentrations it becomes a potent inhibitor. Half-maximal inhibition occurs at about 30  $\mu$ molar. As is to be expected from Table 1, the ATP-driven transport system for calcium ions is much more sensitive to oligomycin. Half-maximal inhibition in this case is at about 0.5  $\mu$ molar. The inhibition of the substrate-driven system at high concentrations may indicate that oligomycin has a secondary effect on electron transfer. Azide has practically no effect on substrate-driven transport of calcium ions until concentrations greater than  $10^{-4}$  molar are used (Fig. 1b). On the other hand, the system driven by ATP is quite sensitive to azide. The half-maximal inhibitions for the substrate- and ATP-driven systems occur at approximately 700 and 8  $\mu$ molar, respectively. These results, like those of Lehninger and Gregg<sup>5</sup> and Wilson and Chance<sup>6</sup>, suggest that electron transfer (that is, substrate-driven transport in this case) is much less sensitive to azide than is the formation or utilization of high energy intermediates (that is, ATP-driven transport).

Except for the fact that oligomycin is effective at much smaller concentrations, these results do not indicate any differences in function of azide and oligomycin. The results shown in Fig. 2, however, clearly indicate these inhibitors behave differently. In this experiment, substrate-driven transport of calcium ions was investigated in the presence of ADP. The impairment of transport of calcium ions caused by ADP (see also Table 1) is presumably caused by a competition for a common intermediate involved in both the transport of calcium ions and ATP formation. Previous studies had shown oligomycin to relieve this ADP impairment of calcium ion transport<sup>11</sup> by apparently blocking the transfer of phosphate from a phosphorylated intermediate to ADP. This reversal of the ADP impairment is illustrated in Fig. 2 with the optimal oligomycin concentration being about  $10^{-6}$  molar. The fact that the reversal is not complete may be caused by some binding of exogenous calcium ions by the ADP. In contrast to oligomycin, azide does not alleviate the ADP impairment of substrate-driven transport of calcium ions. This must mean that even in

the presence of azide ADP is still effective in competing for the intermediate involved in the transport. (The inhibitions observed with high concentrations of both inhibitors are similar to those shown in Fig. 1a and b for the substrate-driven system and must be the result of secondary effects. In separate but identical experiments, azide was shown to have no effect on oxygen consumption until concentrations greater than  $10^{-4}$  molar were used.) The different effects of azide and oligomycin may be explained according to the diagram shown in Fig. 3, which is a slight modification of an earlier scheme<sup>11,15</sup>. The various high energy intermediates are denoted as modifications of a single moiety (presumably a protein) with two functional groups, I and X. Details of the initial coupling to electron transport are not shown but it appears to involve at least two non-phosphorylated intermediates<sup>14,16</sup>. The initial phosphorylated intermediate

( $\bar{I} \bar{X} \sim P$ ) is considered to be the reactive form involved in the transport of calcium ions in plant mitochondria (see review by Hanson and Hodges<sup>16</sup>). Assuming this to be the case, then both oligomycin and azide must function beyond  $\bar{I} \bar{X} \sim P$ , because substrate-driven calcium ion transport is not inhibited while ATP-driven transport is blocked. The fact that oligomycin will alleviate the ADP impairment of substrate-driven calcium ion transport must mean that ADP can no longer effectively

compete for  $\bar{I} \bar{X} \sim P$  in these conditions and therefore must be acting before the entry of ADP into the system. Azide does not prevent the ADP impairment of substrate-driven transport of calcium ions, and so this inhibitor must be acting beyond the entry of ADP. This is depicted in Fig. 3 by including a phosphorylated-ADP intermediate. In order for ADP to remain effective in restricting transport of calcium ions in the presence of azide, the ADP must be continually recycled, indicating that the phosphorylated-ADP intermediate must be relatively unstable in the presence of azide. The reason for this instability in the presence of azide is not clear to us but it must pertain only to the ADP linkage to the intermediate (that is, not the phosphorylated part of the intermediate), otherwise azide would act as an uncoupler of oxidative phosphorylation instead of an inhibitor of oxidative phosphorylation<sup>17</sup>.

These findings, which implicate separate sites of action of azide and oligomycin, should be of value in further attempts at elucidating the exact mechanism of oxidative

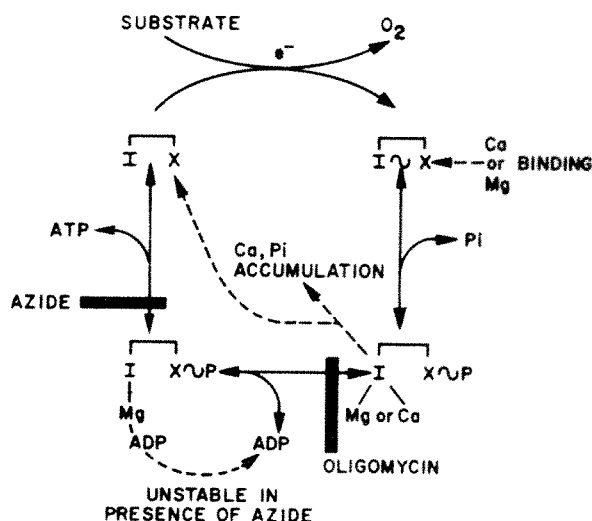


Fig. 3. A scheme depicting possible high energy intermediates of oxidative phosphorylation and suggested sites of action of oligomycin and azide.

phosphorylation and other energy linked functions of mitochondria.

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### Photodynamic Activity of 4-Nitroquinoline-1-oxide and Related Compounds

4-NITROQUINOLINE-1-OXIDE (4-NQO) is a potent water-soluble carcinogen the properties of which include phage induction<sup>1</sup>, mutagenicity<sup>2</sup>, high toxicity to single cell systems<sup>3</sup>, malignant transformation<sup>4</sup> and production of nuclear inclusion bodies and chromosomal aberrations in mammalian cells in tissue culture<sup>5,6</sup>. It also binds to DNA<sup>7,8</sup>. We have now also demonstrated photodynamic activity in 4-NQO and related compounds. Photodynamic activity may be defined as a phenomenon dependent on oxygen, in which a combination of light energy and chemical sensitizer produces effects induced by neither component alone<sup>9</sup>.

Stock solutions of 4-NQO and of thirteen related compounds, nitroquinolines and hydroxyaminoquinolines (Table 1), were prepared freshly for each experiment at 1 mg/ml. concentrations in acetone or 95 per cent ethanol. Serial doubling dilutions of fresh aqueous suspensions were prepared over a range from 100 to 3.125 µg/ml. by standardized procedures<sup>9</sup>. Suspensions of compounds and *Paramecium caudatum*, cultured axenically in semi-defined medium of low protein concentration, were incubated in the dark at 28° C for 1 h in multiple depression plastic trays. As a standard, aqueous suspensions of benzo[a]pyrene, prepared by serial doubling dilutions over a range from 1 to 0.0625 µg/ml., were simultaneously tested, together with three compounds on the same tray. The trays were then irradiated with long wave ultra-violet light with a narrow peak at 3660 Å from two 15 W tubes at an intensity of about 6,500 µW/cm<sup>2</sup> at 15 cm and a 108° beam. The time taken for the radiation to immobilize and kill 90 per cent of the motile cells (*LT*<sub>90</sub>) was determined by observation with a stereoscopic microscope and an arbitrary observational limit of 60 min. Compounds were assayed in duplicate on at least three independent occasions. Under these conditions, high photodynamic activity was manifest by *LT*<sub>90</sub> > 60. By plotting *LT*<sub>90</sub> values against concentrations of the photosensitizing compounds, the concentrations producing *LT*<sub>90</sub> values at 30 min were determined by interpolation. Reciprocals of

these concentrations provide a scale of photodynamic potency<sup>9</sup>.

While under the condition of assay the hydroxyaminoquinolines are devoid of photodynamic activity, activity of nitroquinolines spans a 3-log range. The activity of the nitroquinolines as a class is weak, however, in comparison with benzo[a]pyrene (Table 1). As both hydroxyaminoquinolines and nitroquinolines absorb similarly in the long wave ultra-violet<sup>10</sup>, other factors must account for the marked differences in the photodynamic activity of these compounds.

Table 1. PHOTODYNAMIC ACTIVITY, FREE RADICAL FORMATION AND CARCINOGENICITY OF 4-NITROQUINOLINE-1-OXIDE AND RELATED COMPOUNDS

4-Nitroquinoline-1-oxide

Compounds	Relative photodynamic potency (1/C <sub>50</sub> × 10 <sup>2</sup> )	Free radical formation following photo-irradiation*	Carcinogenicity
<b>Nitroquinolines</b>			
4-Nitroquinoline-1-oxide	11.1	+	+
7-Methyl-4-nitroquinoline-1-oxide	7.1	+	+
2-Methyl-4-nitroquinoline-1-oxide	2.9	+	+
8-Methyl-4-nitroquinoline-1-oxide	2.4	+	( ? )
4-Nitroquinoline	1.7	—	—
6-Methyl-4-nitroquinoline-1-oxide	1.3	+	+
7-Chloro-4-nitroquinoline-1-oxide	1.1	+	+
3-Methyl-4-nitroquinoline-1-oxide	< 1.0	+	( b )
3-Nitroquinoline-1-oxide	< 0.1	—	—
5-Nitroquinoline-1-oxide	< 0.1	+	( b )
<b>Hydroxyaminoquinolines</b>			
4-Hydroxyaminoquinoline-1-oxide-hydrochloride	< 0.1	—	+
7-Chloro-4-hydroxyaminoquinoline-1-oxide-hydrochloride	< 0.1	—	+
6-Nitro-4-hydroxyaminoquinoline-1-oxide-hydrochloride	< 0.1	—	+
6-Methyl-4-hydroxyaminoquinoline-1-oxide-hydrochloride	< 0.1	—	+
Standard Benzo[a]pyrene	2.7 × 10 <sup>3</sup>		

\* Type a, unpaired electron is localized on ring or nitro-group nitrogen; type b, unpaired electron is delocalized on both nitrogens.

Compounds producing free radicals with delocalized unpaired electrons (Table 1, type b free radicals) or no free radicals after photo-irradiation with ultra-violet or visible light<sup>10</sup>, are usually devoid of photodynamic activity; the exceptions are 4-nitroquinoline and possibly 8-methyl-4-nitroquinoline-1-oxide, the free radical type of which has not yet been determined. Conversely, there is an interesting positive association between photodynamic activity and the formation of free radicals with unpaired electrons localized on ring or nitro-group nitrogen (Table 1, type a free radicals). The further positive association between these two parameters—photodynamic activity and type a free radical formation, and carcinogenicity—is of additional interest in view of the strong positive association existing between photodynamic activity and carcinogenicity in polycyclic compounds<sup>9</sup>.

Perhaps photodynamic activity of the nitroquinoline-1-oxides differs from that of benzo[a]pyrene because free radicals have been detected on photo-irradiation of the nitroquinolines at room temperature<sup>10</sup> and also after photo-irradiation of haematoporphyrin (another photosensitizer<sup>11</sup>) but not benzo[a]pyrene, even at -196° C (J. M. Rice and S. S. Epstein, personal communication). Possibly relevant is the apparent failure of various antioxidants, for example, 4-dimethylaminoazobenzene, α-tocopherol or nordihydroguaiaretic acid, to protect against the photodynamic effects of 4-NQO (S. S. Epstein, personal communication) in contrast to their marked protective effects, presumably by scavenging free-radicals, against polycyclic photosensitizers such as benzo[a]pyrene<sup>12</sup>.

The findings here might suggest that some effects of the nitroquinoline-1-oxides, for example, mutagenesis by 4-NQO<sup>2</sup>, are indirect and incidentally mediated through photodynamic mechanisms. This, however, is unlikely because (with the exception of photodynamic activity) 4-NQO and its reduction product and presumed proximate carcinogen, 4-hydroxyaminoquinoline-1-oxide, have similar biological properties<sup>2</sup>.

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## Antigenicity of Mycoplasma Membranes

UNLIKE bacteria, mycoplasmas do not have a rigid cell wall<sup>1,2</sup>, but they are bounded by a triple layered limiting membrane 70–80 Å thick<sup>3</sup>. Immunological reactions with these organisms have been studied by a number of techniques, of which growth inhibition and haemagglutination inhibition are believed to represent reactions with the surface of the organism<sup>4,5</sup>. Whole mycoplasma cells of different species have been chemically fractionated by various workers<sup>4,6–10</sup> and found to contain carbohydrates, proteins and lipids. Studies, particularly on *Mycoplasma pneumoniae*, have shown that most of the antigenic activity is associated with lipids which react with antibody, and when combined with protein stimulate antibody, including growth-inhibiting antibody<sup>4</sup>. It is tempting to believe that these lipids have been extracted from the cell membrane because lipids are an integral part of membranes of many cells. Physical methods have been described for the preparation of isolated membranes from mycoplasmas<sup>11</sup> and we have isolated membranes from the serologically distinct organisms *M. gallisepticum* (S6) and *M. pneumoniae* (FH) and have attempted to stimulate, in particular, growth-inhibiting antibody with them.

The growth medium consisted of Difco PPLO broth containing 20 per cent Burroughs Wellcome No. 6 unheated horse serum, 2.5 per cent D.C.L. yeast extract, 1.0 per cent glucose, 0.002 per cent phenol red, 1:2,000 thallium acetate and 1,000 U of penicillin/ml. To prepare antigens of *M. gallisepticum*, 300 ml. of medium, pH 7.8,

were inoculated with 30 ml. of a culture containing 10<sup>8</sup> or more organisms/ml. and incubated at 36° C for 48 h. At this time the culture medium, pH 6.5, containing 10<sup>9</sup> or more viable organisms/ml., was centrifuged at 10,000g for 10 min; the pellet was resuspended in 10 ml. phosphate-buffered saline (PBS), pH 7.2, and then divided into two equal portions. The first 5 ml. portion was washed once by resuspending in cold, sterile PBS and centrifuging at 10,000g for 10 min. The mycoplasma cells resuspended in 10 ml. of sterile PBS constituted the whole cell antigen. The second 5 ml. portion was diluted to 20 ml. with sterile de-ionized water and this suspension was frozen at –20° C and thawed at room temperature ten times and then centrifuged at 19,000g for 10 min. The supernatant fluid was removed and stored at 4° C and the deposit was resuspended in 20 ml. of water and the freezing-thawing treatment was repeated. This suspension was then centrifuged at 19,000g for 10 min, the small deposit was discarded and the supernatant fluid was pooled with that from the first centrifugation. These pooled supernatant fluids were then centrifuged at 30,000g for 40 min. The deposit resuspended in 10 ml. of PBS constituted the membrane antigen. The 40 ml. of supernatant fluid from the last centrifugation was dialysed at 4° C against polyethylene-glycol until the volume was reduced to 5 ml.; this constituted the cell-content antigen.

In order to prepare antigens of *M. pneumoniae*, this organism was grown according to the method described by Somerson *et al.*<sup>12</sup>. Ten Roux bottles containing 100 ml. of medium were each inoculated with 5 ml. of a culture containing 10<sup>6</sup> or more viable organisms/ml. and incubated at 36° C. After 3 days, when sheets of growth were adherent to the glass, the medium was removed, the sheets washed five times with sterile PBS and scraped into 10 ml. of this saline. Further procedures for antigen preparation were as described.

Samples of cell-membrane and cell-content antigens were stained with 1 per cent phosphotungstic acid and examined by electron microscopy. Whole mycoplasma cells were not observed. In addition, no growth occurred when mycoplasma medium was inoculated with similar samples.

Rabbits were inoculated with the *M. gallisepticum* and *M. pneumoniae* antigens as follows: 1 ml. of antigen homogenized with 1 ml. of complete Freund adjuvant was given intradermally in multiple sites; 3 weeks later 1 ml. of antigen without adjuvant was given intramuscularly in multiple sites. Two weeks after this, that is, at 5 weeks, a sample of serum was taken and 1 week later 1 ml. of antigen was given intravenously. A second sample of serum was taken 1 week later, that is, at 7 weeks.

All the antisera were examined in the metabolic inhibition test<sup>13</sup> with and without the addition of unheated guinea-pig serum at a final concentration of 3 per cent, and, in addition, antisera to *M. gallisepticum* were examined in a micro-haemagglutination-inhibition test<sup>6</sup> with the A5969 strain.

Pre-inoculation rabbit sera at the dilutions tested did not contain antibody. On the other hand, sera from rabbits given whole cell and membrane antigens of *M. gallisepticum* contained antibody measured by both metabolic inhibition and haemagglutination inhibition techniques; the cell-content antigen did not stimulate antibody measurable by either method (Table 1). Com-

Table 1. TITRES OF ANTIBODY AFTER INOCULATION OF RABBITS WITH WHOLE CELL, CELL-MEMBRANE AND CELL-CONTENT ANTIGENS

Antisera prepared against	Titres of antibody to indicated mycoplasma			
	<i>M. gallisepticum</i>		<i>M. pneumoniae</i>	
	Metabolic inhibition test	Haemagglutination inhibition test†	Metabolic inhibition test	Haemagglutination inhibition test
Whole cell	(<20)* 1,280	(<4) 256	(<2) 16	(<2) 16
Membrane	(<20) 320	(<4) 64	(<2) 16	(<2) 16
Cell content	(<4) <4	(4) 4	(<2) <2	(<2) <2

\* Figures in parentheses indicate titres of pre-inoculation sera.  
† 0.5 per cent chicken erythrocytes and four haemagglutinating units of antigen were used.



parable titres were obtained with the 5 and 7 week antisera prepared in two rabbits inoculated with each antigen of *M. gallisepticum*. Furthermore, whole cell and membrane antigens but not cell-content antigen of *M. pneumoniae* stimulated antibody measured by the metabolic inhibition technique. The addition of unheated guinea-pig serum to the metabolic inhibition test did not increase the antibody titres for either *M. gallisepticum* or *M. pneumoniae*. It has been shown previously<sup>13,14</sup> that at the titre end-point in this test there is inhibition of mycoplasma growth. These results agree with the hypothesis that antigens present on the membrane of mycoplasma cells combine at this site with antibody to inhibit growth of the organisms. The exact mechanism of this inhibition is being investigated.

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### Simple Microassay of Protein with Membrane Filter

THE quantity of protein in dilute solution is usually determined by the method of Lowry *et al.*<sup>1</sup>, using phenol reagent. This method is sensitive and simple, but it is applicable only to solutions containing more than 10  $\mu\text{g}/\text{ml}$ . and it suffers from interference by various chemicals. For example, *tris*(hydroxymethyl)aminomethane can react with the reagent to develop an intense colour and in the presence of magnesium ions the phenol reagent forms a precipitate which greatly lessens the strength of the colour. Thus unless freed from these materials the values obtained under these conditions are rather unreliable. Removal of the interfering chemicals cannot be easily performed with dilute protein solutions and is inconvenient if a large number of samples is being analysed. Protein determination by ultra-violet absorbance may also suffer from severe interference by other chemicals. The reading is affected by nucleotides or many

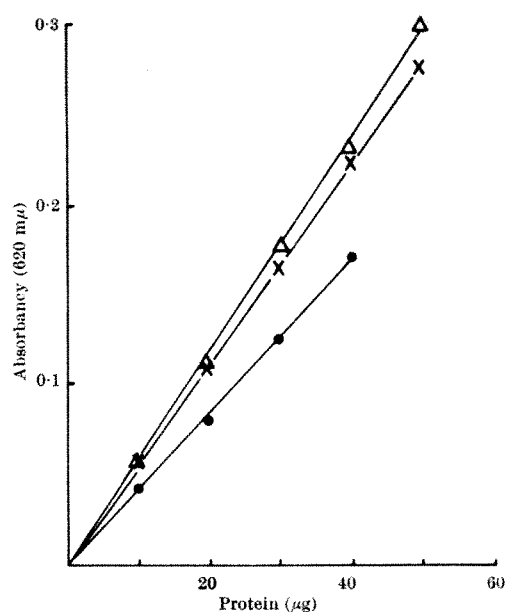


Fig. 1. Quantitative binding of protein on the membrane filter, and quantitative staining with amido black. Samples of 10  $\mu\text{g}/\text{ml}$ . 0.2 molar magnesium chloride solution of each protein were used. The solution was passed through membrane filter which was then washed with 0.2 molar magnesium chloride solution, and protein bound to the membrane filter was stained with amido black. The dye bound to the protein was eluted with 3.5 ml. of 0.01 normal sodium hydroxide and the absorbance of the eluant was determined.  $\Delta$ , Bovine serum albumin;  $\times$ , rat liver soluble fraction;  $\bullet$ , *E. coli* soluble fraction.

other chemicals and the results must be corrected for them<sup>2</sup>.

A previous investigation of purification and classification of RNA phages with the aid of a membrane filter<sup>3,4</sup> (Schleicher B-6 27MM) showed almost quantitative binding of protein to the filter. The extent of this binding varies with salt concentration, and bivalent metal ions like magnesium (II) and calcium (II) are more effective than sodium (I) or potassium (I) ions. On this basis, a simple procedure for microassay of protein was developed, in which the bound protein was stained with amido black and the dye retained on the filter was eluted and measured.

Samples containing 5–50  $\mu\text{g}$  of protein were made 0.2 molar with respect to magnesium chloride. This solution was passed through a membrane filter under reduced pressure. The membrane was washed with 3 ml. of 0.2 molar magnesium chloride and the protein was stained by passing 2 ml. of amido black solution, 4  $\mu\text{g}/\text{ml}$ ., in acetic acid-methanol-water (1:5:4). After washing with 5 ml. of 1 per cent acetic acid solution the bound dye was eluted with 3.5 ml. of 0.01 normal sodium hydroxide. The concentration of the dye in the eluant was measured at 620 m $\mu$ . The quantity of dye bound on the protein is related linearly to the amount of protein applied (see Fig. 1). In this experiment, bovine serum albumin, fraction V (Armour), and the supernatant fractions of rat liver homogenate and *Escherichia coli* B (obtained by ultracentrifugation at 100,000g, 120 min) were used as the protein sample.

To ascertain the completeness of attachment of protein to the membrane filter the filtrate was tested for any unattached protein. Fifty micrograms of albumin in 1.0 ml. of 0.2 molar magnesium chloride were passed through to each membrane filter, and filtrates were combined. The combined filtrate was passed through a new membrane filter and stained for any bound protein as before. The amount of protein which has failed to bind to the initial membrane filter was found to be less than 3 per cent. With this method, solutions of protein with concentration down to 0.3  $\mu\text{g}/\text{ml}$ . gave reliable results providing that the total amount of protein applied to the filter exceeded 10  $\mu\text{g}$ .

Table 1. COMPARISON OF PROTEIN DETERMINATION BY THE PHENOL REAGENT METHOD AND BY THE MEMBRANE FILTER METHOD

Ingredient	Amount of protein observed*	
	By phenol reagent	By membrane filter
None	20.8 ± 0.2	20.0 ± 0.1
Tyrosine 4 µg	20.8* ± 1.2	20.0 ± 0.3
Tris-HCl 0.005 molar	22.1* ± 1.8	19.9 ± 0.1
CMP ‡ 50 µg	21.1 ± 0.5	18.7 ± 0.1

\* Figures are averages of five samples. Each sample contained 20.0 µg of serum albumin.

† Tyrosine and tris-HCl alone gave colour reaction corresponding to 15.8 µg and 21.6 µg of serum albumin respectively. The values shown above were corrected for these figures.

‡ Cytidine monophosphate.

Tests for chemicals which usually interfere in current procedures are summarized in Table 1. The materials which irritated the ultra-violet absorption method and the chemicals which react with phenol reagent showed virtually no interference in our method of assay.

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### Inhibition of ADP Phosphorylation by the Atractyloside of Yeast Respiratory Particles

ATRACTYLOSIDE is known to prevent the phosphorylation of exogenous ADP by intact liver mitochondria<sup>1</sup>, and to block the passage of adenine nucleotides across the mitochondrial membrane<sup>2-7</sup>. The question therefore arises as to whether or not these two effects of atractyloside are independent of each other.

An experimental approach to this problem could be made by using yeast respiratory particles (damaged mitochondria prepared by disruption of yeast cells); these particles, in contrast to liver mitochondria, are able to utilize for the substrate-linked phosphorylation not only  $\alpha$ -ketoglutarate, but also acetyl-carnitine<sup>8,9</sup>.

In contrast to liver mitochondria, it is possible to prepare a soluble extract from these particles which is able, like the particles themselves, to utilize acetyl-carnitine in the presence of succinate for ADP phosphorylation<sup>8</sup> through the last steps of the substrate-linked phosphorylation.

The action of atractyloside has been tested on the particulate and the soluble systems. The results are shown in Table 1.

It can be clearly seen that the acetyl-carnitine dependent phosphorylation of ADP is strongly inhibited by atractyloside when catalysed by respiratory particles, while it is completely unaffected in the soluble enzyme system.

These results suggest that an insoluble structure is required before atractyloside can exert its inhibitory effect, and that any specific inhibition by atractyloside on the enzymes involved in the substrate level ADP phosphorylation described can be ruled out.

It is also interesting that arsenate, unlike atractyloside, prevents synthesis of ATP through substrate level phosphorylation, both in the particulate and in the soluble system.

The requirement of an insoluble structure for the inhibitory effect of atractyloside does not necessarily mean that this inhibitor acts by preventing an enzyme translocation of adenine nucleotides from outside to inside the mitochondrial membrane.

Table 1. EFFECT OF ATRACTYLOSIDE ON ATP SYNTHESIS BY YEAST RESPIRATORY PARTICLES AND BY THEIR SOLUBLE EXTRACT IN PRESENCE OF ACETYL-CARNITINE

	ATP (mµmoles) formed	
	Particles	Soluble extract
Acetate + carnitine + succinate	10	99
Acetyl-carnitine + succinate	126	246
Acetyl-carnitine + succinate + atractyloside	43	250

Yeast particles (1 ml., equivalent to 30 mg of protein) were incubated in 2.7 ml. of a medium containing 100 µmoles of tris-hydrochloric acid buffer pH 7.4, 12 µmoles of magnesium chloride, 1 µmole of phosphate, 20 µmoles of substrate, except succinate (10 µmoles) and 30 µmoles of malonate, 0.6 µmoles of atractyloside when present. No ADP or AMP was added. Incubation was for 10 min at 25° C. Concentration of ATP at zero time was 44 mµmoles. Of the reaction mixture 1.5 ml. contained 1 mg of soluble protein, 75 µmoles of tris-hydrochloric acid buffer, pH 7.4, 10 µmoles of substrate, except succinate (5 µmoles), 20 µmoles of magnesium chloride, 0.4 µmoles of coenzyme A, 20 µmoles of phosphate, 2 µmoles of ADP, 80 µmoles of potassium fluoride, 0.3 µmoles of atractyloside when indicated, 100 Kunitz-MacDonald units of hexokinase, and 50 µmoles of glucose. Incubation was for 10 min at 25° C. The soluble extract was prepared at 0° C by mechanical disruption\* of yeast particles with a Virtis homogenizer for 20 min at 20,000 r.p.m. in the presence of 0.2 molar potassium chloride, 0.5 molar EDTA, 50 mmolar potassium phosphate, pH 7.4. After centrifugation for 15 min at 100,000g, the clear supernatant (10 ml.) was passed through a column of 'Sephadex G-25' (1.8 × 26 cm) previously equilibrated with 10 mmolar phosphate, pH 7.5. Proteins were determined by the biuret method modified by Gornall<sup>10</sup>.

As was previously shown, an insoluble mitochondrial structural protein is able to bind adenine nucleotides in a way which is inhibited and reversed by atractyloside<sup>11</sup>.

This observation suggests that the binding of adenine nucleotides to the structural protein may be the preliminary step required for some processes involving adenine nucleotides in intact mitochondria.

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### Translocation of DNA of Bacterial Origin in *Lycopersicon esculentum* by Ultracentrifugation in Caesium Chloride Gradient

WE have previously reported<sup>1,2</sup> results showing that exogenous tritiated DNA molecules can migrate in the xylem of tomato plant and, after some degradation, be taken up by the meristematic cells. We now report our investigation of the nature of the radioactive molecules recovered after incubation of the plant by bacterial DNA using ultracentrifugation in a caesium chloride gradient.

We used the tomato variety 'Tuckwood' treated in the following way. Apical shoots (5-9 cm) of adult plants with young leaves and without roots were dipped into a solution of *Escherichia coli* or *Bacillus subtilis* tritiated DNA for 6 h. They were then transferred to water for 48 h. The region dipped in the DNA was removed before

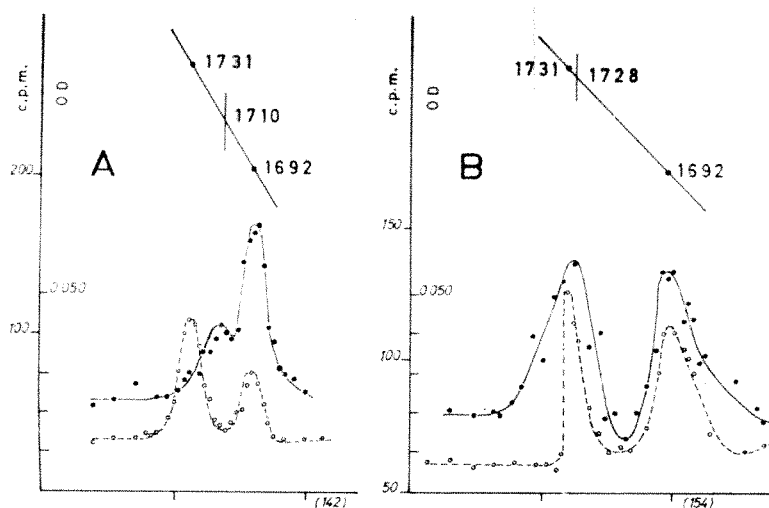


Fig. 1. Caesium chloride ultracentrifugation diagram of the DNA extracted from tomato plants after an incubation of 6 h in presence of bacterial tritiated DNA followed by 48 h in water. A, Plants incubated in presence of *E. coli* tritiated DNA ( $\rho = 1.710$  g/ml.). B, Plants incubated in presence of *B. subtilis*  $D_2O$  tritiated DNA ( $\rho = 1.728$  g/ml.). Broken line: ultra-violet absorption (tomato DNA  $\rho = 1.692$  g/ml.) (*M. lysodeikticus* DNA  $\rho = 1.731$  g/ml.). Continuous line: radioactivity.

homogenization. In order to make sure that no DNA went up the stem by external capillarity, a ring of silicon grease was placed just above the dipped part. When roots were present we obtained results qualitatively similar but quantitatively smaller.

Bacterial DNA was extracted following the method of Marmur<sup>3</sup> from *Escherichia coli* strain CR 34, without thymine and cultured on a medium containing tritiated thymine. The specific activity obtained ranged from  $5 \times 10^5$  d.p.m./ $\mu$ g to  $3 \times 10^6$  d.p.m./ $\mu$ g. We have also used DNA from *Bacillus subtilis*, strain SMYW, type Marburg prototrophic, adapted to  $D_2O$  medium, and cultivated in the presence of tritiated thymidine. In this case the specific activity obtained was about  $2 \times 10^5$  d.p.m./ $\mu$ g. The concentration of the DNA solution used was about 200  $\gamma$ /ml. (similar results are obtained with concentrations ranging from 120  $\gamma$ /ml. to 800  $\gamma$ /ml.).

Plants weighing 0.5 g were cut in small pieces, placed in a 0.025 molar *tris* buffer (pH 7.5), and homogenized for 15 sec in an 'Ultra-Turrax' in the presence of 1 per cent sodium dodecylsulphate (SDS). Two mg/ml. of pronase was added to the solution and it was then incubated for 2 h at 37° C. The mixture was shaken for 15 min with an equal volume of redistilled phenol, saturated with 0.025 molar *tris* solution and adjusted to pH 8. After centrifugation at 1,000g for 10 min the supernatant was precipitated in the cold with 2 vol of ethanol. The 'precipitate' was brought into solution with 2 ml. of diluted saline citrate (0.015 molar sodium chloride and 0.0015 molar sodium citrate). Ribonuclease (100  $\mu$ g) was added for each millilitre of solution, which was incubated for 45 min at 37° C. This step was followed by the addition of 2 mg/ml. of pronase and incubation at 37° C for 1 h. The solution was again treated with 2 vol of alcohol and the insoluble material brought into solution with diluted saline citrate. Caesium chloride was added to the final solution (1.25 g/ml.); 3 ml. of this solution was spun in an 'SW 39' Spinco rotor with 30  $\mu$ g of an unlabelled DNA of *Micrococcus lysodeikticus* ( $\rho = 1.731$  g/ml.) as a reference. After centrifugation (27,000 c.p.m., 62 h, 24° C), the bottom of the tube was punctured and fractions of two drops were collected under constant pressure. One millilitre of water was added to each sample, and the ultra-violet absorption spectrum was recorded on a Beckman 'DK 1' recording spectrometer to determine the position of the *Micrococcus lysodeikticus* reference DNA and the tomato DNA ( $\rho = 1.692$  g/ml.) in the gradient. After hydrolysis with hot 0.1 normal hydrochloric acid the

radioactivity of each sample was measured in a Packard liquid scintillator in presence of a suitable phosphor<sup>4</sup>.

Reference runs were made by adding to the reference samples (*Micrococcus lysodeikticus* DNA and tomato DNA) the bacterial radioactive DNA. Twenty-five experiments were performed with *E. coli* DNA (1.710 g/ml.) or *B. subtilis* deuterated DNA (1.728 g/ml.).

When bacterial DNA is taken up by the plant, the radioactive molecules found in the tomato appear to be distributed into populations having the density of tomato DNA (1.692 g/ml.) and the density of the foreign DNA used (Fig. 1A and B).

In most experiments the amount of radioactivity sedimenting at the level of bacterial DNA is higher than 30 per cent. This indicates that the foreign DNA can be taken up without drastic structural modification. The radioactivity which appears in the position of tomato DNA seems to be that part of the bacterial DNA which has been broken down and reutilized for a synthesis *de novo*.

A previous autoradiographic<sup>2</sup> investigation revealed that when tomato plants were incubated for 6 h with a labelled bacterial DNA and then for 48 h with water, the labelling appeared to be located principally in the nuclei of the cambium cells and of the other meristematic tissues. Our biochemical data imply that part of the labelled molecules found by autoradiography in the cell nuclei are foreign DNA molecules.

Our results show that unlike the situation described in germinating barley seedlings<sup>4,5</sup>, the translocation of DNA is possible not only in the roots but also in the aerial part of the plant. The foreign DNA molecules can migrate in plant tissue and be taken up by cell nuclei without modification of their primary and secondary structures.

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## PATHOLOGY

### Possible Dietary Factors in the Aetiology of Chronic Murine Pneumonia, Nephrosis and Peri-arteritis

MOST stocks of laboratory rats are subject to the chronic murine pneumonia described by Nelson and Gowen<sup>1</sup>. Such rats are also prone to a form of peri-arteritis<sup>2</sup> and to nephrosis<sup>3</sup>. All these are diseases of maturity in the rat;



they increase in frequency with age and may, in some cases, affect most old members of a colony. The pneumonia is known to be much less prevalent in wild rats<sup>1</sup>. The aetiology of these diseases is not clear, though in the case of pneumonia Nelson<sup>4</sup> offered evidence that the condition was viral and initiated the development of "specific-pathogen-free" (SPF) animals for colonies free from pneumonia. SPF animals live longer than "dirty" rats. Paget and Lemon<sup>5</sup>, describing such a colony, reported 19 per cent of SPF animals with lungs classified as other than "healthy normal", 12 per cent with severe renal disease and 9 per cent with polyarteritis, as against 96 per cent, 19 per cent and 16 per cent respectively in "dirty" animals.

It therefore seems possible that factors, other than "specific pathogens", may be concerned in the chronic diseases of laboratory rats. Some authors<sup>2,6,7</sup> have remarked on the relation of diet to these diseases; the only pattern which these reports suggested to us is a somewhat lesser incidence of the disease whenever the rats are given a diet reduced in quantity or limited to specific ingredients. This would necessitate a departure from the standard laboratory feeding practices. Worden<sup>8</sup> has commented on the poorer quality of laboratory animals reared on compressed diets since the late 1930s.

In the course of a 2 yr inhalation study in this laboratory, rats have received intermittent minimal exposures to finely particulate beryllium oxide. Initial studies of the Sprague-Dawley strain in use required macroscopic examination of the lungs of more than 100 rats, aged 5-7 months, and microscopic examination of the lungs of thirty; the colony was then thought to be free of murine pneumonia. The rats were kept to the standards recommended by Innes<sup>9</sup>, in wire-bottomed cages in rooms provided with a partly recirculating air-conditioning system.

They were fed an unrestricted commercial cubed diet containing, *inter alia*, dried milk powder and meatmeal.

Mortality has been low and the control groups healthy, except for the development during the growth period of inflamed and sometimes necrotic tails with thinning of hair over face and neck. A similar description attributed to a deficiency of a dietary factor has been given previously<sup>10</sup>. Rats of all groups have been killed at 20 months. At this age control groups showed a high incidence of murine pneumonia with its characteristic peribronchial lymphoid infiltration. Nephrosis was frequent and peri-arteritis was prominent in microscopic sections of the lungs of all groups. Notable microscopic features in the lungs also included foam cells packed with sub-micron Sudan-positive granules, septal cells containing single bodies exhibiting a polarization cross and thought to be lipid droplets, long birefringent fibres, and intra-alveolar crystals or plates brilliantly birefringent in frozen section and sometimes resistant to the solvents of paraffin sectioning.

Another puzzling feature was the apparent contamination of lung sections by dense intra- or extra-cellular particles in a variety of forms, some being naturally black. This led to painstaking rechecking of histological reagents and techniques, without any indication that the particles were artefacts.

Green's<sup>11</sup> illustrations of mouse lung preparations show quite clearly the large crystalline plates and some of the smaller particles that we have seen in rat lungs. She found them to appear in mouse lungs soon after weaning, and subsequently to become more numerous with increasing age.

We have now found that the whole range of particles, including small and large "lipid" droplets, birefringent objects, fibres, and dense particles can be obtained by

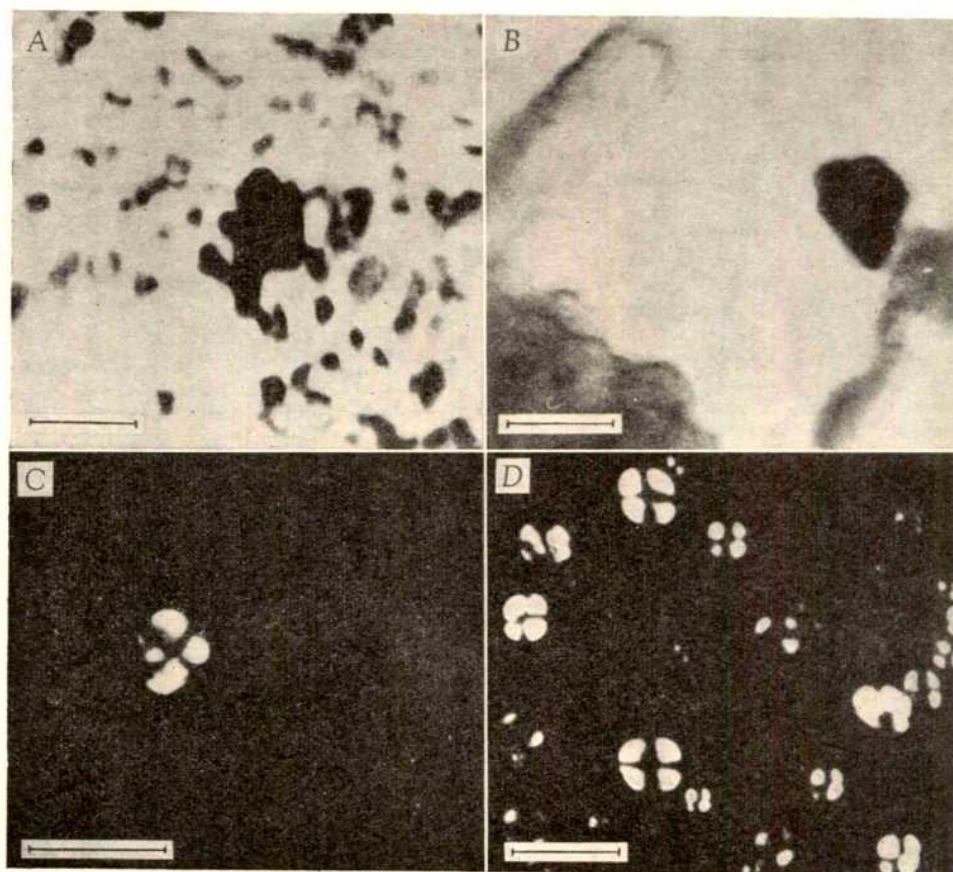


Fig. 1. (A) Naturally black object in peribronchial lymphatic tissue (paraffin section). (B) Naturally black object in powdered rat cube. (C) Polarization cross in a cell of alveolar septum (frozen section). (D) Polarization crosses in powdered rat cube—wheat starch granules. Each segment bears a 50 $\mu$  bar.



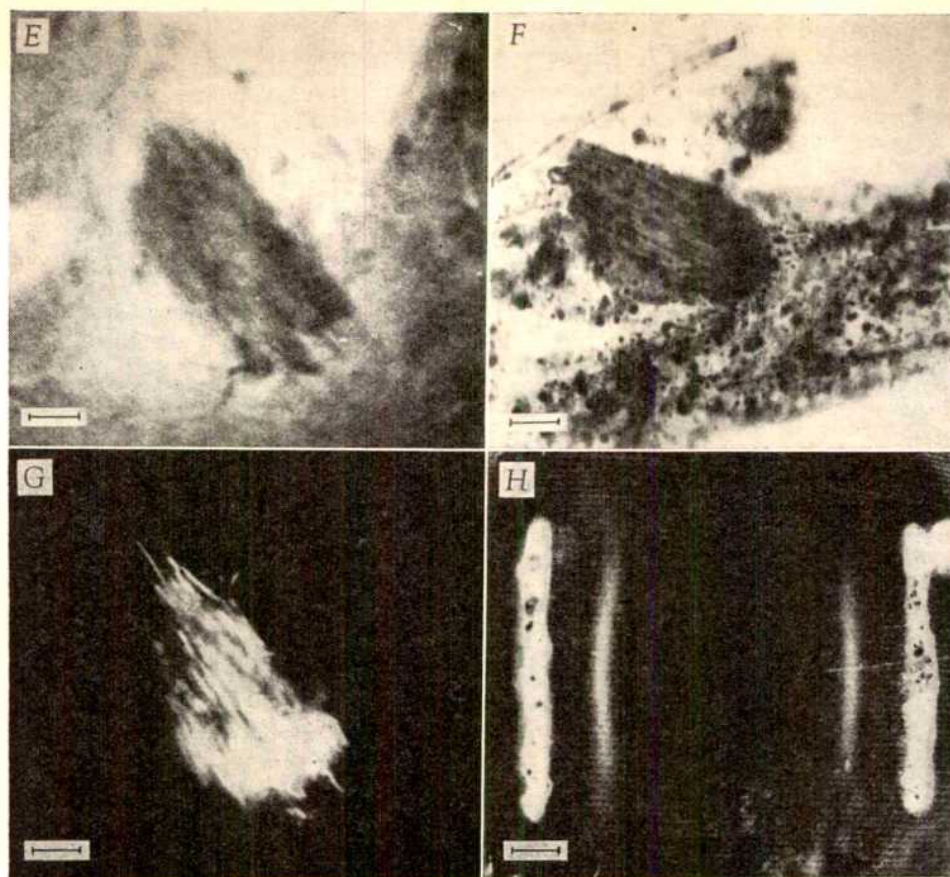


Fig. 2. (E) Angular plate in alveolus (frozen section). (F) Angular plate in powdered rat cube. (G) Angular plate (E) under polarized light. (H) Precipitin bands of rat serum against powdered coconut meal. Each segment bears a 50 $\mu$  bar.

scrapping a food cube with the points of splinter forceps to simulate the action of the rat's incisors, or by preparing a suspension of crushed cubes. The proportion of very small particles, in the range 0.5–10 $\mu$ , is quite surprising. These findings are evidence of the constant, direct inhalation of food dusts by laboratory rats maintained in conventional conditions. Some of these particles are illustrated in the composite photographs of Figs. 1 and 2.

It is evident that irritation from these particles, which include such foreign proteins as casein and meatmeal, cannot be disregarded in an inhalation study, although Innes<sup>12</sup> has dismissed granulomata from such foreign bodies as clinically silent lesions of the respiratory tract of small animals. Evidence that at least one of the constituents of the food cubes is absorbed and is antigenically active is provided by the precipitin line in Fig. 2, between coconut meal and rat serum. All the rat sera tested showed this reaction. We think it probable that the inhaled particles found in the lungs are at least partly responsible for the appearances of murine pneumonia. Unrecognized inhaled particles may well include matter of fungal or bacterial origin. We cannot do more than speculate on how these particles may be harmful, but it is possible that there is an immunological basis to the disease and that it is akin to bagassosis and, perhaps, the pneumoconioses. If an immunological element is accepted, it is also possible that the renal and arterial lesions found in these rats are of similar origin. If these ideas are correct, Nelson's views on the nature of chronic murine pneumonia will need modification and, for long-term experiments of any kind with rats, it will be advisable to devise a more

natural diet which produces less dust than the conventional cubes.

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### Variation of Saccharoid Fraction in Diabetes Mellitus

In diabetes mellitus it is not only the concentration of blood sugar which is altered but also the "saccharoid fraction"—the difference between the total reducing capacity of blood and the true concentration of glucose<sup>1</sup>. The exact nature of the saccharoid fraction is not known, but it would seem to be a non-specific mixture of all the reducing substances present in the blood, including glutathione, creatinine, glucuronic acid and cysteine. The significance of this fraction of the blood in pathological conditions is not well understood.

In blood, a large amount of carbohydrate is also present in the form of mucopolysaccharides. When measured as hexosamine after acid hydrolysis, it is found to be present at about the same concentration as glucose in the blood. The effect of insulin on the disappearance of hexosamines from blood has also been investigated. Gaulden *et al.*<sup>2</sup> have shown that when acetyl glucosamine is injected intravenously into normal subjects the true concentration of glucose in the blood virtually remains unaffected. Weilden and Wood<sup>3</sup> have found that there is no difference between the disappearance of glucosamine from the blood of normal subjects and that from the blood of patients with diabetes. Spiro has compared the biosynthesis of glucosamine with that of liver glycogen in intact alloxan diabetic rats<sup>4</sup> and concludes that there is no impairment in the rate of synthesis of glucosamine despite an almost complete failure in the synthesis of glycogen.

The purpose of the present investigation was to observe the variation of saccharoid and mucopolysaccharide fractions of blood in normal and clinically diagnosed diabetic subjects during sugar tolerance test.

Glucose tolerance test was performed on human subjects who were divided into two groups according to whether they were diabetic or "normal". Ten normal control subjects with no evidence of diabetes mellitus were selected from among the male staff and students of Jinnah Postgraduate Medical Centre aged between 25 and 40 years. The other group consisted of twenty subjects diagnosed diabetics, only three of which were less than 40 years. The youngest member of this group was 14 years old and the oldest was 60. Sixteen of the group were males and four females. Ten patients were classified as moderately diabetic, six as acute and the rest mildly diabetic. The duration of the disease in these

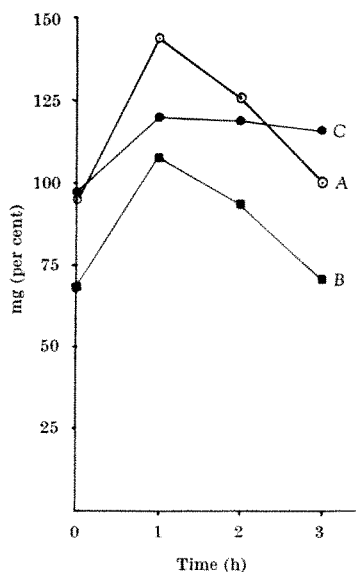


Fig. 1. Concentration of total reducing sugar (A), true glucose (B) and hexosamine (C) during the sugar tolerance tests in normal subjects. Each value is the mean of ten subjects.

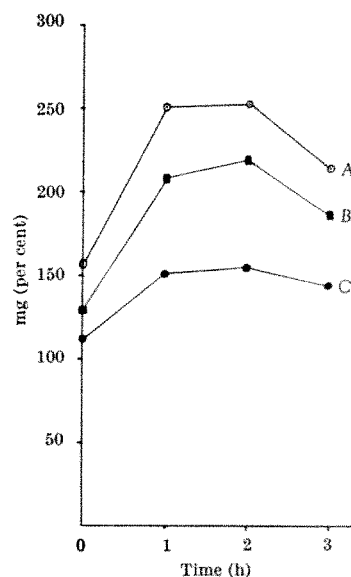


Fig. 2. Concentration of total reducing sugar (A), true glucose (B) and hexosamine (C) during the sugar tolerance tests in diabetic subjects. Each value is the mean of twenty diabetic subjects.

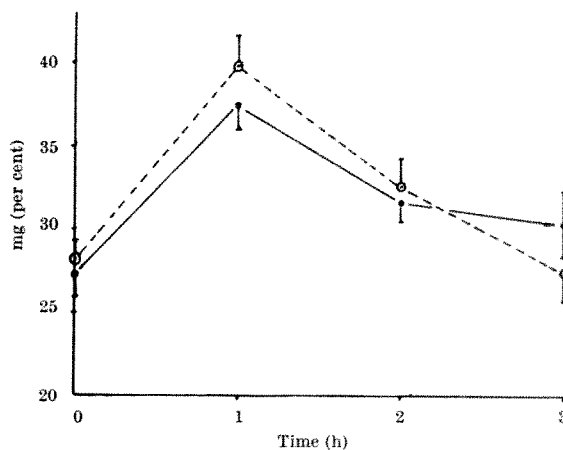


Fig. 3. The variation of the saccharoid fraction during sugar tolerance tests in normal (●—●) and diabetic (○---○) subjects. Each value is the mean of ten normal and twenty diabetic subjects. The vertical bars indicate  $\pm$  S.E.M.

cases varied between 6 months to 14 years. Nine patients had suffered for less than 2 years, seven for 2–6 years and the rest had been diabetic for more than 6 years. At least 48 h before collection of blood samples all antidiabetic drugs were discontinued and the subjects were fasted for at least 12 h before the sugar tolerance test was carried out.

After a sample of fasting blood had been withdrawn from the subjects they were given 50 g of D-glucose in solution to drink. Blood samples were collected in citrated tubes 1, 2 and 3 h later. The concentrations of total reducing sugar, true glucose and hexosamine were estimated for all blood samples. Sugar was determined in whole blood samples while hexosamine was estimated in the plasma. The total reducing sugar was estimated by the Folin-Wu method and the true glucose by the glucose oxidase and peroxidase ("Glucostat") method. Bound hexosamine was estimated by the method of Cessi and Piliego<sup>5</sup> after hydrolysis with 3 normal hydrochloric acid at 106° C overnight. The saccharoid fraction was expressed as mg per cent of the difference of the total reducing sugar and true glucose from individual values.

The results of the glucose tolerance tests are shown in Figs. 1 and 2. Fig. 1 shows the values obtained for normal subjects and it can be seen that the rise and fall

of the true glucose and total sugar in most of the subjects are not accompanied by a similar variation in the concentration of hexosamine. Fig. 2 shows similar values for the diabetic patients and here the variations in the true glucose and total sugar are more inconsistent than the values of hexosamine, which do not vary with the rise and fall of the sugar. The hexosamine content of the plasma, however, remained unchanged during glucose tolerance tests in both normal and diabetic patients.

The variation in the saccharoid fraction during glucose tolerance test is shown in Fig. 3. The increase and decrease in the values of the saccharoid fraction are similar to those for sugar. As the blood sugar rises the saccharoid fraction rises to a similar extent and this is so whether the subjects are normal or diabetic. In diabetes the concentrations of sugar in the blood varies considerably (Fig. 2), but the saccharoid fraction does not change significantly from the normal. As has already been pointed out, the saccharoid fraction represents a variety of non-glucose reducing substances present chiefly in the red cells<sup>6</sup>. The saccharoid fraction of normal human blood can be accounted for almost entirely by glutathione and glucuronic acid<sup>7,8</sup>. The reducing substances in the blood of diabetic subjects are the same as those in normal subjects and the increase in blood sugar is not related to an increase in them. The variation of the saccharoid fraction in the present investigations may result therefore from the conversion of the blood sugar to some fragment or phosphate esters of glucose which are reducing in nature and form a part of the saccharoid fraction which rises and falls with the concentration of sugar in the blood. Hexosamines, however, do not seem to contribute to this fraction.

It seems likely from these investigations that the saccharoid fraction and the circulating mucopolysaccharides are independent of the action of insulin because their behaviour in diabetic subjects is similar to that in normal subjects.

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### Report of a New Site-specific Cleft Palate Teratogen

MORE than ten chemical teratogens are now known to produce cleft palate in various species of Rodentia. All these, however, show their teratogenic potential by a widespread embryopathy of which cleft palate is but one facet of a generalized teratogenic response; little or no site-specificity of action or anatomical localization occurs.

For example, Trasler<sup>1</sup> produced a widespread embryopathy in mice, using acetylsalicylic acid; lesions included cleft palate, short snout, microcephaly, polydactyly and exencephaly. Larsson *et al.*<sup>2</sup> used sodium salicylate to produce cleft palate and other widespread teratogenic effects in mice. In 1963, King<sup>3</sup> used meclozine hydrochloride in Sprague-Dawley rats to produce cleft palate, brachygnathia, microstomia and micromelia, and in 1958 Nishimura and Nakai<sup>4</sup> similarly produced a heterogeneous embryopathy, including cleft palate, using high dosage nicotine.

Fraser and Fainstat<sup>5</sup> used cortisone acetate in genetically susceptible mice to produce cleft palate. Although cleft palate was the predominant lesion, other teratogenic embryopathic features included shortening of the head, mandibular attenuation and spina bifida.

Similar widespread embryopathies which incorporate cleft palate in the teratogenic syndrome have been produced by trypan blue<sup>6</sup>, nitrogen mustard<sup>7</sup> and hyper-vitaminosis A (ref. 8).

It has been shown in this laboratory that a crude extract of *Indigofera spicata* is teratogenic in *Rattus norvegicus* (Sprague-Dawley strain) and that the embryopathy resulting from its oral administration is limited to cleft palate and somatic dwarfism. The discovery of a site-specific cleft palate teratogen in Rodentia is thus significant in that it enables the construction of an experimental model in which local morphopathogenic processes can be manipulated and studied.

*Indigofera spicata* is a palatable, hardy plant of high protein yield which possesses remarkable potential for pasture improvement in infertile tropical countries<sup>9</sup>. The plant is an efficient nitrogen-fixing legume. Its widespread use has been limited because of undesirable side effects, observed clinically in cattle and experimentally in rodents, which have included acute hepatotoxicity and abortion<sup>10-15</sup>. The exact chemical structure of the teratogenic moiety is not yet known. Because the suspected chemical structure of the toxic component of the extract does not correspond with that of the more common substances inducing abortion (ergot alkaloids, quinine, oxytocin, histamine) it was decided to subject the crude plant extract to teratogenic investigation.

Primiparous Sprague-Dawley rats of controlled lineage were used, and mated with stud bucks of the same strain. The spontaneous incidence of cleft palate in this rat colony is less than one in one thousand. No cleft palate occurred in control fetuses. The crude extract from the plant was administered orally at varying stages of gestation, and the fetuses examined following hysterectomy on the twenty-first day.

The embryopathic syndrome produced was limited to somatic dwarfism and cleft palate. No other physical defects were observed in autopsies of more than two hundred exposed embryos, of which 60 per cent had cleft palate. Details of dosage rate and time specificity will be presented in detail when a larger series of investigations has been completed and dose-response relationships calculated.

The cleft palate produced in this way is of the post-alveolar type involving only the secondary palate. Both hard and soft palates are involved.

If gestation is allowed to proceed to term, the fetuses with cleft palates induced by *Indigofera* extract are spontaneously delivered at the usual time; although born alive they do not live for more than a few hours. This is conceivably due to a defect in suckling with inhalation of milk into the lungs, but could also be related to a separate metabolic toxic effect of the teratogenic extract.

Very few chemical teratogens act with sufficient site-specificity to allow chemical and more sophisticated methods of investigation on a localized anatomical area. Thalidomide is probably an exception and its predilection for the left, first metacarpal bone in rabbits exposed to this drug is very striking<sup>16</sup>. In a similar fashion to cleft palate induced by *Indigofera* extract this site-specificity allows one to analyse meaningfully its morphopathogenic action.

Although the *Indigofera* extract produces isolated cleft palate as its only site-specific manifestation, some physical manipulations of the embryonic environment will produce an identical result. For example, puncture of the amniotic sac followed by surgically induced loss of amniotic fluid has been shown to cause this lesion as an isolated defect<sup>17,18</sup>.

Other physical manipulations of the embryonic environment will produce multiple non-specific defects among



which cleft palate is common. Intermittent clamping of the uterine artery and maternal hypobaric hypoxia will produce this state<sup>19,20</sup>. Irradiation of the pregnant uterus produces a similar effect<sup>21</sup>, in which cleft palate is but one abnormality in an embryopathic syndrome comprising more than one hundred features. It seems unlikely that comparative teratological studies using these less specific but intensively investigated research tools will be initially helpful in morphogenetic analysis of cleft palate induced by *Indigofera* extract.

The normal foetal palate closes after the primitive vertically disposed palatine shelves have turned horizontally and grown towards the midline<sup>22</sup>. Several workers have shown that the presence of the tongue interposed between these two shelves can prevent the fusion in certain pathological conditions<sup>23,24</sup>. It is uncertain whether the primary action of *Indigofera spicata* extract is mediated through the physical presence of the interposed tongue. Because of the localized nature of the malformation, however, the interposition of the tongue with failure of shelf apposition and fusion appears to be the most likely hypothesis to explain the observed findings.

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### Concentrations of Pyruvate, Pyruvate Kinase and Lactate Dehydrogenase in the Kidney of the Alloxan-diabetic Rat

INCREASED excretion of pyruvate has been observed in the urine of diabetic humans<sup>1</sup> and rats<sup>2</sup> which could not be correlated with blood pyruvate concentrations. This suggested that the increased excretion of pyruvate in diabetes was caused by altered kidney metabolism which in some way reduced the reabsorption of pyruvate in the kidney tubule. To investigate this possibility the concentrations of pyruvate and the activities of pyruvate kinase and lactate dehydrogenase are here reported in the kidney of the alloxan-diabetic rat and compared with the liver in the same condition.

Diabetes was induced in female Wistar rats, weighing 140–160 g, by an intravenous injection of alloxan monohydrate at a dose of 60 mg/kg of body weight. Diabetic rats had a concentration of blood glucose greater than 300 mg/100 ml. Normal rats of the same age and sex as the diabetic rats were allowed water and food (MRC diet 41B) freely and were killed by cervical dislocation and exsanguination 1–3 weeks after the induction of diabetes. Tissue pyruvate was estimated by a modification of the method of Hohorst *et al.*<sup>3</sup>. Tissues (0.5–1.0 g) were rapidly excised, frozen with tongs precooled in liquid nitrogen, and then pulverized and extracted into 4 ml. of 7 per cent (v/v) perchloric acid. After neutralizing with 5 molar potassium carbonate, estimations were carried out in 3.05 ml. containing 97  $\mu$ mole of  $\text{KH}_2\text{PO}_4$ - $\text{Na}_2\text{HPO}_4$  (pH 7.6), 1.5  $\mu$ mole of EDTA, 0.7  $\mu$ mole of NADH and 1.5 ml. of extract. The decrease in optical density at 340 m $\mu$  was measured 2 min after the addition of 25  $\mu$ g of lactate dehydrogenase. Blood was obtained by cardiac puncture from rats under ether anaesthesia and was used for the estimation of blood pyruvate by an adaptation of the method of Marks<sup>4</sup>. Enzymes were assayed in supernatants obtained by homogenizing tissues in four volumes of 0.154 molar potassium chloride at 4° C with a Potter-Elvehjem type homogenizer and centrifuging at 25,000g and 0° C for 2 h. Pyruvate kinase was assayed by the method of Bücher and Pfeleiderer<sup>5</sup> in 3 ml. of a mixture containing 100  $\mu$ mole of  $\text{KH}_2\text{PO}_4$ - $\text{K}_2\text{HPO}_4$  (pH 7.4), 300  $\mu$ mole of potassium chloride, 25  $\mu$ mole of magnesium sulphate, 25  $\mu$ mole potassium phosphoenolpyruvate, 6  $\mu$ mole of ADP, 2  $\mu$ mole of NADH and 0.1 mg of lactate dehydrogenase. Lactate dehydrogenase was assayed according to the method of Kornberg<sup>6</sup> in 3 ml. of a mixture containing 100  $\mu$ mole of  $\text{KH}_2\text{PO}_4$ - $\text{K}_2\text{HPO}_4$  (pH 7.5), 2  $\mu$ mole of sodium pyruvate and 0.4  $\mu$ mole of NADH. Assays were carried out at room temperature during a 5 min reaction period. Protein was estimated in the supernatant by a Folin-Ciocalteu<sup>7</sup> or biuret method, using a bovine plasma albumin standard.

Table 1. CONCENTRATIONS OF PYRUVATE IN KIDNEY, LIVER AND BLOOD AND OF PYRUVATE KINASE AND LACTATE DEHYDROGENASE IN THE KIDNEY AND LIVER OF ALLOXAN-DIABETIC AND NORMAL RATS

	Pyruvate ( $\mu$ mole/g wet weight)	Pyruvate kinase ( $\mu$ mole NADH oxidized/min/mg of protein in crude supernatant)	Lactate dehydrogenase ( $\mu$ mole NADH oxidized/min/mg of protein in crude supernatant)
Kidney			
Normal	43 $\pm$ 10* (14)†	145 $\pm$ 41 (22)	1,570 $\pm$ 390 (22)
Diabetic	64 $\pm$ 18 (10)	177 $\pm$ 54 (19)	1,880 $\pm$ 290 (19)
Liver			
Normal	73 $\pm$ 33 (8)	345 $\pm$ 114 (11)	2,380 $\pm$ 550 (12)
Diabetic	34 $\pm$ 13 (7)	181 $\pm$ 53 (10)	2,820 $\pm$ 770 (11)
Blood	( $\mu$ mole/ml. of blood)		
Normal	210 $\pm$ 23 (7)		
Diabetic	244 $\pm$ 15 (7)		

\* Mean  $\pm$  1 standard deviation.

† Number of observations is in parentheses.

The results summarized in Table 1 show that in the kidney diabetes caused a 50 per cent increase in the concentration of pyruvate ( $P=0.001$ ), a 22 per cent increase in the activity of pyruvate kinase ( $P<0.05$ ) and a 20 per cent increase in the activity of lactate dehydrogenase ( $P<0.05$ ). In the liver, however, diabetes caused a 53 per cent decrease in the concentration of pyruvate ( $P<0.02$ ), a 53 per cent decrease in the activity of pyruvate kinase ( $P<0.001$ ) and no significant change in the lactate dehydrogenase ( $P<0.1$ ). Concentrations of blood pyruvate were 16 per cent higher in diabetic animals, but this elevation was not significant ( $P<0.3$ ).

With the exception of lactate dehydrogenase, the changes produced in the kidney by diabetes were opposite to those produced in the liver. The present observations in the liver of the diabetic rat agree with those of other authors with regard to pyruvate concentration<sup>8,9</sup> and the activity of pyruvate kinase<sup>10</sup> and lactate dehydrogenase<sup>11</sup>. It seems that the activity of pyruvate kinase in the kidney, unlike that in the liver, is relatively unaffected by alloxan-diabetes and changes in dietary regime<sup>12</sup>.



This is surprising because both kidney and liver possess a similar pyruvate kinase isoenzyme pattern<sup>12</sup> and therefore presumably similar properties. Furthermore, pyruvate kinase is a rate determining enzyme for glycolysis and is involved in the regulation of gluconeogenesis<sup>13</sup>. The results therefore suggest that not all the metabolic changes induced in the kidney by diabetes are necessarily identical to those induced in the liver. The results are also in accord with the recent suggestion<sup>14</sup> that insulin has only an indirect effect on the activity of pyruvate kinase. Lactate dehydrogenase, unlike pyruvate kinase, is a bidirectional non-rate-determining enzyme, and so its control is presumably not of crucial importance to the cell. Finally, the increased concentration of pyruvate in the diabetic kidney, a probable result of altered kidney metabolism, may in part explain the elevated excretion of urinary pyruvate in diabetes<sup>1,2</sup>.

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## MICROBIOLOGY

### Antigenic Relationship between Influenza A Viruses of Human and Avian Origins

INFLUENZA A viruses causing natural infections in man, swine, horses and birds are indistinguishable from one another in the antigenic structure of their internal ribonucleoprotein (S or G antigen) but little or no relationship has been found between the human and animal strains which have been compared so far with respect to antigens present in the viral envelope (V antigens). This is not invariably the case, however, and V antigens common to certain strains of avian and mammalian origins have been observed (ref. 1 and B. Tumova and H. G. Pereira, to be published).

In the course of a comparative study of influenza A viruses isolated from man, pigs, horses and birds, a reciprocal cross reaction between human A2 viruses and a strain of avian origin (A/turkey/Massachusetts/3740/65) was detected in haemagglutination-inhibition (HAI) tests performed with sera from hyperimmunized rats. These cross reactions, although not considerable, were sufficiently striking to prompt us to compare the two strains in question in greater detail.

Initially, a total of fifteen distinct avian influenza A viruses, including all those previously described by Pereira *et al.*<sup>2,3</sup> and a number of additional strains investigated since then, were cross-tested with five human strains

including sub-types A0 (WS, PR8), A1 (FM1) and A2 (A2/Singapore/1/57, A2/Czechoslovakia/9/65) by HAI using hyperimmune rat sera. The only cross reactions observed were between A/turkey/Massachusetts/3740/65 and the two human A2 strains. HAI tests performed with sera from a ferret infected by A2/Singapore/1/57 by intranasal inoculation and from a chicken similarly infected with A/turkey/Massachusetts/3740/65 failed to reveal these cross reactions (Table 1).

Table 1. HAEMAGGLUTINATION-INHIBITION TEST

Strain	Antisera			
	A2/Singapore/1/57 Hyper-immune (rat)	Post infection (ferret)	A/turkey/Mass./3740/65 Hyper-immune (rat)	Post infection (chicken)
A2/Singapore/1/57	1,024	3,840	16	< 10
A2/Czech./9/65	128	NT	32	NT
A/turkey/Mass./65	32	< 10	256	160

Figures represent reciprocals of serum dilutions showing 50 per cent inhibition of 4 haemagglutinating doses of virus.

NT = not tested.

Table 2. STRAIN-SPECIFIC COMPLEMENT FIXATION TEST

Strain	Sera				
	A/turkey/ Mass./ 3740/65	A2/ Singapore/ 1/57	A2/Nether- lands/ 65/63	A2/ England/ 12/64	A2/Lenin- grad/ 29/65
A/turkey/Mass./ 3740/65	640	480	< 10	< 10	< 10
A2/Singapore/1/57	160	> 1,280	60	10	< 10
A2/Netherlands/ 65/63	< 10	240	960	240	320
A2/England/12/64	15	40	480	480	320
A2/Leningrad/29/65	< 10	10	320	80	640

Figures represent reciprocals of serum dilutions showing 50 per cent complement fixation against optimum antigen dilutions.

A number of human A2 strains were then compared with the turkey/Massachusetts virus by strain-specific complement fixation<sup>4</sup> with the results shown in Table 2. A clear antigenic relationship between turkey/Massachusetts and A2/Singapore/1/57 is demonstrated in this test. It may be noted that the degree of cross reactivity between these two strains is greater than between A2/Singapore/1/57 and any of the other A2 strains included in this test. Furthermore, little or no antigenic relationship could be detected between A/turkey/Massachusetts and any of the more recent A2 strains. Similar results were obtained by virus neutralization using sera from hyperimmunized rats (Table 3).

Table 3. VIRUS NEUTRALIZATION TEST IN CULTURES OF *Cercopithecus aethiops* KIDNEY CELLS

Strains (100 TC <sub>50</sub> )	Antisera		
	A2/Singapore/ 1/57	A2/Czech./ 9/65	A/turkey/Mass./ 3740/65
A2/Singapore/1/57	480	< 10	120
A2/Czech./9/65	20	> 640	40
A/turkey/Mass./3740/65	160	80	> 640

The figures represent reciprocals of serum dilutions showing 50 per cent neutralization (expressed in terms of haemadsorption inhibition against 100 haemadsorption units).

The fact that the antigenic relationship between A/turkey/Massachusetts and A2/Singapore/1/57 is revealed so much more clearly by strain-specific complement fixation than by HAI suggests that an antigenic component other than the haemagglutinin may be shared by these two viruses. The possibility that viral neuraminidase might represent this shared component was investigated by testing the capacity of rabbit antisera to human (A0, A1 and A2), porcine (SW) and avian (Fowl Plague and turkey/England/63) strains to neutralize the enzyme activity of homologous and heterologous influenza A viruses. The only outstanding heterologous reaction observed in this series of tests was between an antiserum to A2/Ann Arbor/23/57 and the A/turkey/Massachusetts strain. The antigenic relationship between early strains of human influenza A2 and A/turkey/Massachusetts is seen therefore to be detectable by four different techniques.

So close is the antigenic relationship between these two strains that the possibility that the materials under test contain a mixture of viruses must be taken into account. The possibility that the A/turkey/Massachusetts strain is

contaminated by a human A2 strain was considered first. Attempts to purify the strain by a plaque technique were unsuccessful because this virus, unlike most other avian influenza A strains, fails to produce plaques in chick embryo fibroblasts. The original freeze-dried material received at the World Influenza Centre was therefore titrated in eggs and limit dilution fluids obtained at two successive passages were found to behave in strain-specific complement fixation tests in exactly the same way as the material used for the tests shown in Table 2. Another possible explanation for the cross reactions observed is that the A/turkey/Massachusetts strain might have acquired an A2 antigenic component through genetic recombination resulting from a temporary contamination with an A2 strain. This seems unlikely because the isolation of this virus was performed in a laboratory where no human influenza viruses were being handled at the time. The material received at this centre had been through three allantoic passages before shipment and was tested after a single additional passage carried out under strict precautions against contamination. Finally, it should be mentioned in this connexion that strain A/turkey/Massachusetts, unlike A2/Singapore/1/57, cross reacts by HAI and strain-specific complement fixation with other avian influenza A viruses<sup>3</sup>.

These findings pose a number of interesting questions regarding the possible relationships between human and animal influenza viruses. If the A/turkey/Massachusetts strain had been isolated before 1957 it would have been tempting to suggest at that time that the human influenza A2 sub-type had been of avian origin.

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### Buoyant Density of African Horsesickness Virus

It has been shown that in apparently pure strains of some animal viruses the buoyant density of the infective particles may differ<sup>1</sup>. A range of densities is usually demonstrated with two infectivity peaks which indicate a density difference of about 0.02 g/ml. This heterogeneity is explained by assuming that a varying amount of host lipid or other material is adventitiously incorporated in the virion during the assembly process.

A study of the A501 vaccine strain of African horsesickness virus has revealed a similar situation<sup>2</sup>. After more than two hundred intracerebral passages in suckling mice this virus was shown by caesium chloride density gradient centrifugation to have a density range of 1.05–1.375 g/ml., with infectivity maxima at 1.17 and 1.35 g/ml. (Fig. 1). The sedimentation coefficient of virus obtained from the two principal fractions was determined by method A of Polson and van Regenmortel<sup>3</sup>. It was found that the denser particles had a sedimentation rate of  $S_{20}^{w,0} = 240$  and a calculated particle diameter of 34.6 m $\mu$ ;

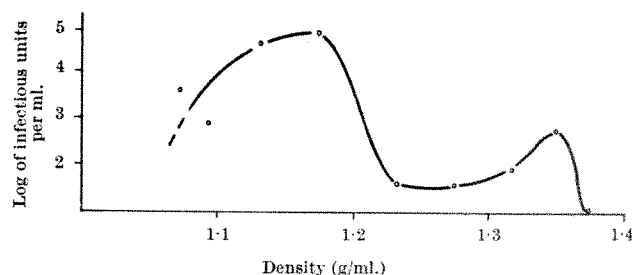


Fig. 1. Buoyant density of African horsesickness virus (Strain A501). The virus is untreated and shows infectivity maxima at 1.17 and 1.35 g/ml.

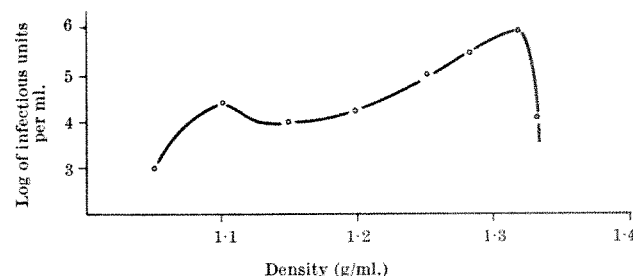


Fig. 2. Buoyant density of African horsesickness virus (Strain A501) after treatment with ether. Infectivity maxima at 1.08 and 1.32 g/ml.

the less dense particles had a rate of  $S_{20}^{w,0} = 561$  and a diameter of 74.9 m $\mu$ . With this virus, heterogeneity with respect to both size and density is apparent.

In order to test for the presence of contaminating loosely bound lipid on the surface of the virus particles, a sample of virus was shaken in 20 per cent, by volume, diethyl ether before caesium chloride density-gradient analysis. No change in the typical heterogeneous nature of the virus density occurred although the density of the two principal fractions was slightly reduced (Fig. 2). Most striking is the increase in the number of particles of high density apparently formed at the expense of those of lower density. There is little reduction in the total number of infectious particles and the change in the density distribution may therefore be ascribed to the removal of lipid material from the low density particles, thus increasing their density. Because the virus was obtained from brain material, which contains many lipid complexes, low density contaminants would not be unexpected.

A second experiment, designed to free the virus from possible contaminating material, was performed by subjecting a sample of the virus to ultrasonic irradiation. The virus was suspended in 0.06 molar phosphate buffer containing 5 per cent by volume rabbit serum (final pH 7.5) and was sonicated with a 60 watt MSE-Mullard disintegrator at 20 Kc/s for 30 min at 0°C. It may be seen from Fig. 3A that this treatment results in an increase in the number of infective particles of density 1.27 g/ml. with a considerable reduction in the number of viruses at both the higher and lower density positions. The virus concentrated at a position where very little infective material was present when untreated virus was used. Prolonged ultrasonic action for 45 and 60 min (Fig. 3B and C) resulted in a progressive change in the density pattern which altered to form a single major peak at a limiting buoyant density of approximately 1.23 g/ml.

The increase in density of the low density particles may be explained by suggesting that lipid material is removed from the virus by ultrasonic treatment resulting in the formation of denser infectious particles. With regard to the virus of higher density the loss of any material, other than nucleic acid, would also result in an



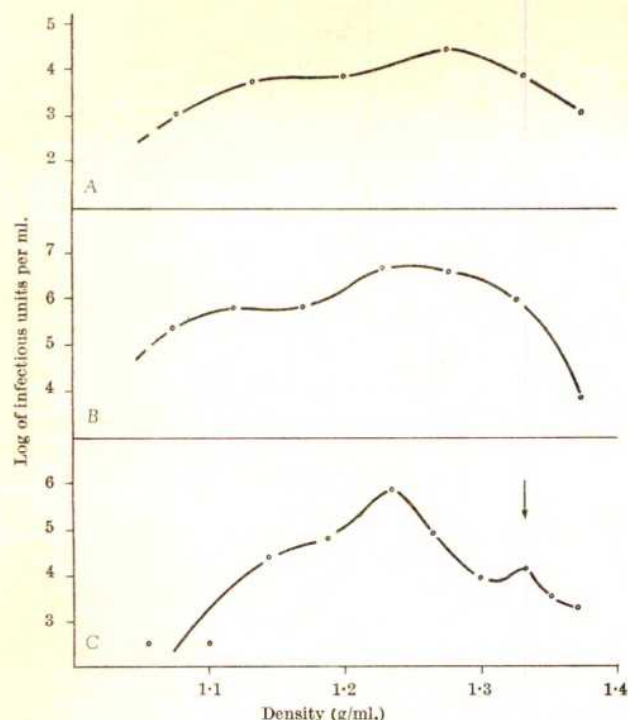


Fig. 3. Buoyant density of African horsesickness virus (Strain A501) showing effect of sonication. A, 30 min sonication. Infectivity maxima 1.15 and 1.275 g/ml.; B, 45 min sonication. Infectivity maxima 1.125 and 1.24 g/ml.; C, 60 min sonication. Infectivity maximum 1.233 g/ml. The arrow in C marks the position of dense opalescent matter in the gradient tube. Adsorption of virus to this matter probably resulted in a false infectivity peak at this position.

increase of density. Ultrasonic treatment reduces the density of these particles but their infectivity remains unimpaired. Nucleic acid essential for infectivity could not therefore have been removed from the virus. To explain the reduction of density of these infectious particles of high density, it must be presumed that there is an addition of lipid material or water or that non-essential nucleic acid, present on the surface of the virus, is removed.

The density pattern of "normal" or untreated infectious particles of this horsesickness virus strain can be reproduced and it seems that this factor may be under some genetic control. There is also the possibility that virus density is not always an unalterable characteristic of the particle but may depend on the presence of contaminating material, the method of purification, storage, etc., and possible interaction of the virus with the material which forms the gradient. The problem of exactly what constitutes "contaminating material" may be difficult to resolve, especially if the substance is incorporated within the body of the virus but released by various treatments. The changeable or mutable property of this horsesickness virus strain may be found to apply to other viruses. If this is the case, and if viral density is to be used to characterize viruses, a standard reproducible measure will have to be found. As shown in Figs. 1 and 3, sonication results in a progressive change towards an apparently constant density. This indicates that it may be advisable, when necessary, to define a kind of intrinsic density for viruses analogous to viscosity and other physical measures.

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### Altered Esterase Zymograms associated with Infection with Leukaemia Virus

THE study of leukaemia viruses was until recently greatly hampered by the lack of cell cultures capable of propagating such viruses, but several cultures have now been found which support the replication of murine leukaemia virus. One of these cultures (*JLS V6*) originated from a mixture of *BALB/c* mouse spleen and thymus cells. Exposure of this culture to a cell free extract of leukaemic spleen from mice infected with Rauscher leukaemia virus (RLV) has resulted in a chronically infected culture (*JLS V5*) which continually propagates Rauscher virus<sup>1</sup>. In our laboratory, gross cytopathic effects are seen in such infected cells<sup>2</sup>. The infected cultures (*JLS V5*) also show a marked increase in mucopolysaccharide and a concomitant loss of contact inhibition, which results in a transformed "criss-cross" growth pattern. Such infected transformed cells have an enhanced tumorigenicity when they are injected into newborn *BALB/c* mice. The resultant tumours are rich in mucopolysaccharide and are classified as myxofibrosarcomata<sup>3</sup>. Tests to detect the presence in the infected cultures of other murine viruses to which the observed alterations might be attributable have been negative<sup>3</sup>. Moreover, newborn mice from mothers immunized with formalized extracts of spleen infected with RLV are resistant to challenge with the transplantable myxofibrosarcoma cells, whereas newborn mice from mothers immunized with extracts of normal spleen tissue show no such resistance<sup>4</sup>. The uninfected control cells (*JLS V6*) do not show gross cytopathic effects or transformation and are much less tumorigenic. Furthermore, the tumours that do result from injection

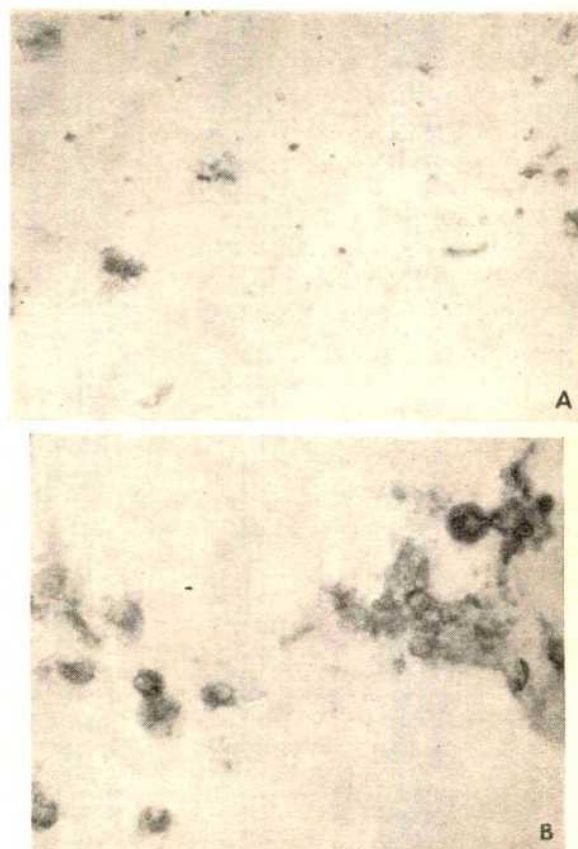


Fig. 1. A, Uninfected *JLS V6* culture showing only slight staining with insoluble fast-blue-RR- $\alpha$ -naphthyl complex, indicative of slight esterase activity. B, *JLS V5* culture infected with the Rauscher leukaemia virus showing marked esterase activity, as judged by increased reactivity with the same stain.



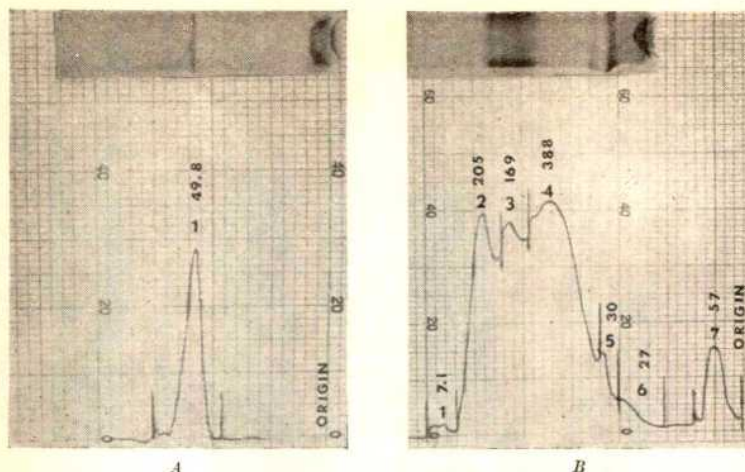


Fig. 2. Esterase zymograms of control cell cultures and cell cultures infected with Rauscher virus, with corresponding densitometric recordings. A, Control cells, 15.1 mg protein extract, showing only one enzyme band and corresponding densitometric recording. B, Cells infected with Rauscher virus, 9.93 mg protein extract, showing seven resolvable bands of esterase activity and their corresponding densitometric recording. Values shown above each numbered peak are units of relative activity of each esterase band determined from the electronically integrated area under each respective peak. The integrated areas are delineated by the vertical lines in the densitometric recording. Activity is corrected to 1 mg of protein extract.

of non-infected control cells are classified as spindle-cell sarcomata<sup>3</sup>.

The metabolic alterations in *JLS V5* cells infected with the Rauscher virus, as indicated by stimulation of mucopolysaccharide formation and neoplastic transformation, prompted us to investigate the effect of the virus on protein and enzyme synthesis in such cells. The first class of enzymes studied was the non-specific esterases. For this purpose, infected *JLS V5* and control *JLS V6* cells were cultured on standard 75 × 35 mm glass slides in Eagle's basal medium with a four-fold concentration of amino-acids and vitamins and 10 per cent calf serum. Before the cell sheet became confluent (24–48 h after sub-culturing), non-specific esterase activity was determined histochemically with  $\alpha$ -naphthyl butyrate as substrate and fast blue RR salt as the diazo-coupling agent, after the method of Markert and Hunter<sup>5</sup>. The slides were washed and then examined microscopically for an insoluble fast blue RR  $\alpha$ -naphthyl complex which would indicate esterase activity. In addition, washed cell pellets of control (*JLS V6*) and infected (*JLS V5*) cultures were homogenized with tissue grinders; the cell free extracts were subjected to electrophoresis on acrylamide gel; and the esterase activity in the gel was determined with  $\alpha$ -naphthyl butyrate as the substrate<sup>6</sup>. The resultant esterase zymograms were quantified densitometrically, as previously described<sup>6,7</sup>. Tissue culture fluid overlying the control and infected cultures was tested in a similar way.

The infected *JLS V5* cells which had been histochemically stained for esterase activity showed a marked increase in staining intensity compared with the uninfected *JLS V6* cells (Fig. 1). The small rounded cells in the infected cultures showed a particularly high esterase activity. Because in cover-slip preparations the cells were not confluent when stained, the increased intensity of staining of the infected cultures should not merely be a reflexion of decreased contact inhibition. The marked increase in esterase activity of the infected cultures is also evident when the zymograms from infected and control cell extracts are compared (Fig. 2). In three separate experiments, the zymograms from the extracts of infected cells showed an increase in total esterase activity by a factor of between fifteen and thirty for each mg of protein, which was attributable to the appearance of at least six additional electrophoretically distinguishable esterases (Fig. 2). Little or no esterase activity was detected in the culture media from either control or infected cultures.

The significance of the alterations in esterase activity associated with RLV infection cannot at present be fully assessed. As previously noted, the infected cultures continuously propagate infectious virus. Titrations in mice of cell free tissue culture fluid from the infected cultures show titres in excess of  $10^3$  ID<sub>50</sub>/ml. of fluid (unpublished data). It is thus of particular interest to explore the part, if any, that the additional esterase bands play in the synthesis of infectious leukaemia virus and to determine whether these bands represent derepressed cellular esterases or "new" esterase coded for by the viral genome. Induced enzyme synthesis after infection with other oncogenic viruses, such as polyoma and SV<sub>40</sub>, is well documented<sup>8,9</sup>. Conversely, the infected cells are highly phagocytic<sup>10</sup> and the altered esterase activity may be related to the phagocytosis and resultant formation of lysosomes in these cells. Studies are also under way to determine whether the alterations in the mucopolysaccharide in *JLS V5* cells are related to the altered esterase patterns in these cells and to determine whether other tissues which propagate the RLV, either *in vitro* or *in vivo*, show similarly altered esterase patterns.

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### Action of Sendai Virus and Neuraminidase on the Alkaline Phosphatase Isoenzymes of HeLa Cells

THE mechanism of virus induced cell fusion is not clear. Among others, Sendai virus<sup>1,2</sup> and Newcastle disease virus<sup>3</sup> have been used to induce cell fusion; both viruses belong to the myxovirus group. In the early stages of fusion the virus is absorbed onto the cell surface and cytoplasmic bridges are formed between adjacent cells<sup>4</sup>. Okada<sup>5</sup> has measured the haemolytic, haemagglutination and sialidase activities of Sendai virus, but found that the ability of the virus to fuse cells together could not be correlated with these activities. Kohn<sup>6,7</sup> has investigated the effect of modified Newcastle disease virus on animal cells and found that fusion was prevented only when the lipid membrane of the virus was hydrolysed. Kohn<sup>6</sup> also found that treatment of the cells with neuraminidase



before the addition of virus also prevented fusion. The presence of sialic acid residues in the cell wall has been described by Kraemer<sup>8</sup> and these residues could be the site of action of neuraminidase. The myxoviruses have a lipoprotein surface with mucopolysaccharide "haemagglutination spikes"<sup>9</sup> and it is thought that the neuraminidase is situated between these spikes, as appears to be the case for influenza virus<sup>10</sup>.

Neuraminidase (*N*-acetyl neuraminidate glycohydrolase *E.C.* 3.2.1.18) has been shown to reduce the mobilities of alkaline phosphatase isoenzymes from human kidney to give a single band of low mobility<sup>11</sup>. This effect was considered to be due to the removal of different amounts of sialic acid bound to the various isoenzymes.

The work reported here compares the effect of neuraminidase and Sendai virus on HeLa cells and on a homogenate of these cells with respect to the alkaline phosphatase isoenzymes.

HeLa cells were obtained from stock cultures and before electrophoresis all samples were grown as monolayer cultures for at least 24 h. The cells were treated with Sendai virus as described by Harris *et al.*<sup>2</sup> using 10<sup>8</sup> HeLa cells and 2,000 HAU of Sendai virus inactivated by ultra-violet light. The action of neuraminidase on monolayer cultures was investigated by incubating a 24 h monolayer (about 2 × 10<sup>6</sup> cells) with 100 units of neuraminidase in 20 ml. of medium for 4 h at 37°C. Longer incubations with neuraminidase caused cell death. Samples were taken for electrophoresis after cultivation in a medium free from neuraminidase after a further 24 and 72 h. A similar experiment was carried out by incubating a HeLa monolayer with 2,000 HAU of Sendai virus for 30 min. The monolayers were trypsinized and the cells homogenized according to Morton's butanol procedure<sup>12</sup>.

To test the action of neuraminidase and Sendai virus on a homogenate of HeLa cells, 1 ml. of butanol extracted homogenate was incubated overnight at 37°C with 50 units of neuraminidase or 3,000 HAU of Sendai virus.

The alkaline phosphatase isoenzymes were separated by acrylamide gel disc electrophoresis according to Davis<sup>13</sup>. A 5 per cent gel was used and the potassium ferriyanide inhibitor was omitted from the gel. After electrophoresis the isoenzyme bands were located by staining the gels in a solution of 12 mg sodium β-naphthyl phosphate and 90 mg fast blue BB in 60 ml. 0.2 molar *tris*-hydrochloric acid buffer (pH 8.1). After 20 min the gels were washed and stored in 7 per cent acetic acid. pH 8.1 was used for staining the gels in order to reduce the background stain caused by decomposition of the diazo coupler.

The isoenzyme patterns obtained are shown in Fig. 1. Gel *a* shows the normal distribution obtained from a monolayer culture. The fastest band is weaker than the others and the slowest band is diffuse and slightly variable in its position.

The patterns for the cells 24 and 72 h after treatment with virus are shown in gels *b* and *c*. They show an almost complete loss of the three fast definite bands and a spread of the slow diffuse band. When the monolayer culture was treated with neuraminidase a similar pattern was obtained (*d* and *e*). In this case cell fusion had not occurred to any appreciable extent as judged from monolayers on coverslips which had been similarly treated and stained<sup>2</sup>. The electrophoretic pattern obtained from the monolayer treated with virus again showed the loss of the discrete bands and an accumulation of the slow diffuse band (*f* and *g*). Coverslip cultures of cells treated with Sendai virus showed that there had been some cell fusion, but the yield of fused cells was much less than that produced by the procedure of Harris *et al.*<sup>2</sup>.

Gels *h* and *i* show the action of neuraminidase and virus on a butanol extracted homogenate of HeLa cells. Neuraminidase has had the same effect on the electrophoretic pattern as before but the virus has caused a negligible alteration.

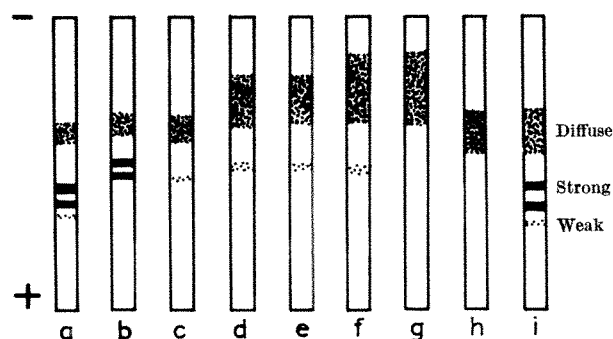


Fig. 1. Alkaline phosphatase isoenzyme patterns on acrylamide gel electrophoresis of: HeLa monolayer control (*a*); HeLa cells treated with virus<sup>2</sup> after a further 24 h (*b*) and 72 h (*c*); HeLa monolayer treated with neuraminidase for 4 h, cultured for a further 24 h (*d*) and 72 h (*e*); monolayer treated with virus for 30 min, cultured for a further 24 h (*f*) and 72 h (*g*); homogenate incubated overnight with neuraminidase (*h*) and homogenate incubated overnight with virus (*i*).

These experiments show that neuraminidase can reduce the mobilities of the fast isoenzyme bands, with an apparent increase in the amount of the slow moving material. The simplest explanation is that of Butterworth and Moss<sup>11</sup> which suggests that the isoenzymes have sialic acid bound to them to various extents and that the sialic acid can be removed by incubation with neuraminidase. The resulting proteins then migrate on electrophoresis with similar mobilities. The presence of sialic acid is not necessary for enzyme activity because the treated material stained as well as the controls. From the poor resolution of the slow band it is not possible to decide whether the only difference between the isoenzymes lies in the amount of bound sialic acid or whether there are also other differences which are not resolved on electrophoresis.

When HeLa cells are incubated with Sendai virus then the change in isoenzyme distribution is identical with that found for neuraminidase-treated cells (*b*, *c*, *f* and *g*). It is probable that this action of the virus results from the presence of neuraminidase in the virus<sup>5,6</sup>. It appears, however, that, before the neuraminidase activity can become effective, it is necessary to alter the structure of the virus particle; when virus is incubated with a cell homogenate no change in the isoenzyme distribution occurs (*i*). The release of viral components when the virus is absorbed on to a cell surface appears to be a rapid process<sup>14</sup>. The presence of alkaline phosphatase in the cell membrane has been described by Lansing *et al.*<sup>15</sup> and this could form part of the receptor sites which are required for viral absorption<sup>6</sup>.

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## PHYSIOLOGY

## Changes in Haemolymph Lipoproteins during Locust Flight

MUCH of the energy for long distance flight in locusts is derived by oxidation of lipids<sup>1</sup>, and the lipid is transported from the fat body (the principal site of lipid storage) to flight muscle through the haemolymph. During flight the lipid content of the haemolymph of *Locusta migratoria* increases to four times the resting value<sup>2</sup> and this is accounted for by an increase in the glyceride fraction. Chino and Gilbert<sup>3</sup> found that diglyceride is released from fat body during *in vitro* incubations with haemolymph, and suggested that diglyceride is important in lipid transport in the several insect species they studied. In mammals lipids are transported chiefly in the form of lipoproteins, and this is also so in a number of insect species<sup>3-5</sup>. We have therefore examined the nature of the increase in glyceride which occurs during locust flight, and have attempted to relate this to changes in haemolymph lipoproteins.

Adult male *Schistocerca gregaria* were used 14-18 days after the final moult, and samples of haemolymph were obtained from the cervical region by puncture with a glass capillary. Electrophoresis of haemolymph was carried out on cellulose acetate strips using 0.05 molar sodium glycinate buffer, pH 9.8; proteins were detected by staining with nigrosine and lipids were detected on separate strips by oxidation with hydrogen peroxide followed by staining with Schiff reagent. Lipids were estimated by direct densitometry of the stained strips.

Electrophoresis of haemolymph from resting locusts showed eight protein components (Fig. 1), only two of which (group A) usually contained lipid. When insects were flown<sup>1</sup> for 2 h the total lipid content of the haemolymph increased to between three and four times the resting value (as estimated by densitometry of electrophoretograms). Part of this increase was accounted for by an increase in the lipid content of the group A lipoproteins to 2.5 times the resting value. In addition lipid appeared in a second pair of proteins (group B, Fig. 1). After a flight lasting 2 h, 70 per cent of the total haemolymph lipid was associated with group A lipoproteins and 30 per cent with group B lipoproteins. Three hours after flight had stopped the haemolymph lipoprotein pattern had returned to the resting state.

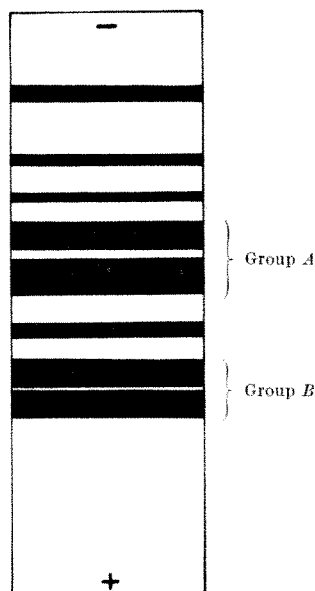


Fig. 1. Electrophoresis of locust haemolymph proteins. (Cellulose acetate; pH 9.8.)

These results were confirmed by the results of other experiments in which locusts were fed from the time of the final moult on a diet containing [ $U-^{14}C$ ] palmitate. The fat bodies of such insects was found to be labelled principally in the lipid fraction; less than 1 per cent of the radioactivity was present as carbohydrate. Electrophoresis of haemolymph from resting animals showed that almost all the radioactivity was present in the group A lipoproteins. After a flight of 2 h the radioactivity of group A lipoproteins had increased, and an additional radioactive area was present coincident with group B lipoproteins. More than 95 per cent of the total radioactivity of the haemolymph was accounted for in these two fractions.

The radioactive lipids were further examined by extraction of haemolymph with chloroform/methanol<sup>6</sup> followed by thin-layer chromatography<sup>7</sup> of the extract.

Table 1. DISTRIBUTION OF RADIOACTIVITY IN HAEMOLYMPH LIPIDS BEFORE AND AFTER FLIGHT

	C.p.m. in 0.01 ml. haemolymph	
	Before flight	After flight
Total haemolymph radioactivity	180	680
Fatty acids	5	6
Diglycerides	26	540
Triglycerides	140	110

Table 1 shows that the main increase in lipid radioactivity after a flight of 2 h was accounted for by an increase in the diglyceride fraction; the triglyceride and free fatty acid fractions remained fairly constant.

The lipids of lipoproteins which had been electrophoretically separated from haemolymph from flown insects were examined by thin-layer chromatography. Group A lipoproteins contained both triglyceride and diglyceride, whereas group B lipoproteins contained chiefly diglyceride.

These results show that the extra demand for lipid substrates during locust flight is reflected by an increased concentration of lipid bound to protein in the haemolymph, and that one group of proteins (group B) carries lipid only when the concentration of lipid is high. Haemolymph from resting locusts at different stages of development has been examined qualitatively. Lipid in the group B fraction was present at two stages: fifth instar hoppers just before moulting and female adults during egg production. At both these stages demand for fat body lipid is likely to be high so the presence of lipid in the group B fraction seems to be related to extensive lipid mobilization from the fat body.

High concentrations of fuels in insect haemolymph are an adaptation to the rapid rates of fuel utilization of active flight muscle<sup>8</sup>. The considerable increase in haemolymph diglyceride during flight may be a response to an increased requirement by flight muscle for lipid in this form. It is noteworthy that flight muscle lipase of the *Cecropia* moth hydrolyses diglycerides at five times the rate of triglycerides<sup>9</sup>. Until the turnover rates of the haemolymph lipids during flight are known, however, it is difficult to assess the relative contributions of the different forms of lipid to flight metabolism.

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### Autoradiography of Decamethonium in Rat Muscle

DECAMETHONIUM and other depolarizing drugs have a pharmacological effect at the endplate and adjacent regions of muscle fibres. Various modes of action have been proposed for these compounds; some are based on attachment to surface receptors while others require penetration into the fibres. In the work reported here the location of labelled decamethonium has been sought by means of autoradiograms prepared from frozen sections. With this method the distribution of the drug within the fibre can be determined and the movements of the water-soluble compound during processing can be prevented.

Decamethonium-( $^3\text{H}$ -methyl) dichloride was injected into rats which had been anaesthetized with sodium pentobarbitone (40 mg/kg). A dose of decamethonium was selected (1.64 mg/kg) which gave a minimal effect on contractions of gastrocnemius muscle which were obtained by stimulation of the sciatic nerve, and respiration continued without assistance. The muscles were removed after known times and plunged into iso-pentane cooled in liquid nitrogen. Frozen sections ( $5\mu$ ) were picked up on slides or coverslips which had been coated with sensitive emulsion, and after exposure at  $-20^\circ\text{C}$  for 3–10 days the silver grains were developed<sup>1</sup>, and the endplates were stained<sup>2</sup>.

Fig. 1 shows a transverse section of diaphragm muscle which was frozen 3 min after injection of labelled drug. Three endplates can be seen in the left section. The microphotographs were taken with dark-field illumination and incident lighting so that the silver grains reflect the light and appear as bright specks on a dark background<sup>3</sup>. The position of the grains indicates that the radioactive compound has penetrated into the fibres. The section on the right was obtained several mm away from the endplate region, and the muscle fibres at this point show few grains. The cluster of grains in the top right-hand corner corresponds to a group of blood vessels which contain radioactive material. Muscles removed 3–90 min after injection gave results which were similar to those shown in Fig. 1.

The distribution of grains along the fibres was studied in longitudinal sections of peroneus muscle. Light was reflected from the grains and collected in a photometer the readings of which were proportional to the grain density<sup>3,4</sup>. Fig. 2 shows measurements obtained from a single fibre. The radioactivity as indicated by the photometer readings was high in the region of the endplate and extended for several hundred microns on either side.

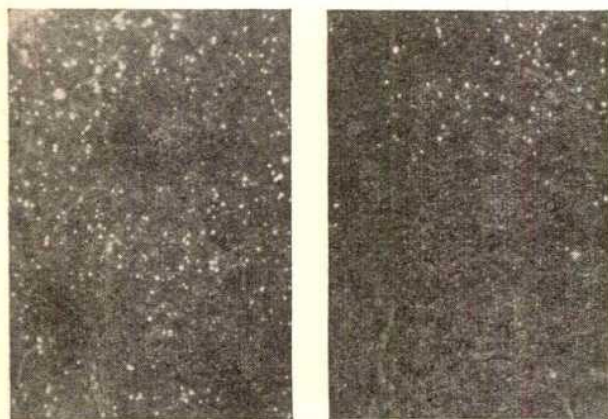


Fig. 1. Autoradiograms obtained from a diaphragm which was frozen 3 min after injection of labelled decamethonium. The transverse sections were photographed with dark-field illumination. The section on the left shows three stained endplates. The white dots show light reflected from the silver grains, and indicate radioactivity within the fibres. The section on the right was taken several mm away from the endplate region, and the fibres show few grains. A group of blood vessels in the top right-hand corner contain radioactive material.

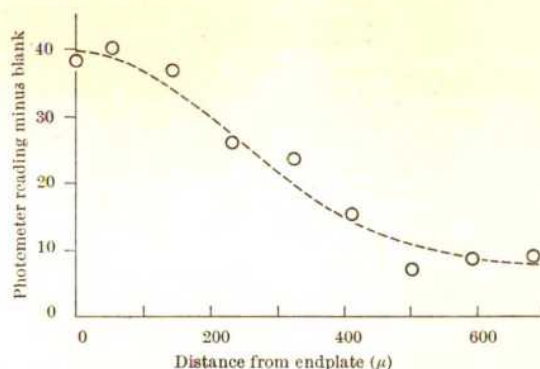


Fig. 2. Photometric estimation of radioactivity in a muscle fibre. An autoradiogram was prepared from a longitudinal section of peroneus longus muscle which was removed 60 min after injection of decamethonium. The light reflected from the grains was recorded at various distances from the endplate. The blank value, obtained from a part of the slide having no grains, has been subtracted, and a Gauss curve has been fitted to the points.

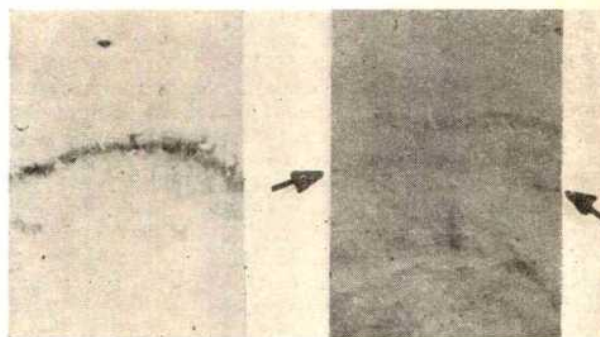


Fig. 3. Autoradiograms of hemi-diaphragms which were removed 15 min after injection of labelled decamethonium. The tissue was arranged with the rib above and the central tendon below. On the left the dark band of silver grains indicates radioactivity in the region of the endplates. The autoradiogram on the right was from a rat which was pretreated with 7-tubocurarine (0.8 mg/kg); the band which corresponds to the position of the endplates (see arrows) is much fainter.

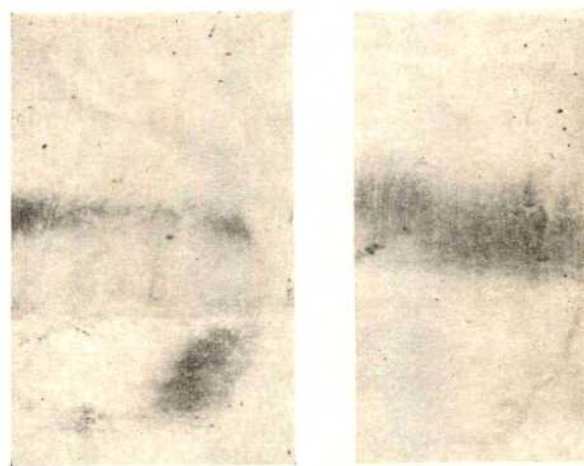


Fig. 4. The left side shows an autoradiogram of a diaphragm which was removed 2 h after injection of labelled drug. The orientation is as Fig. 3. The horizontal band indicates uptake in the region of the endplates. The autoradiogram on the right was obtained from a muscle removed 7 days after injection of the same dose of decamethonium. The width of the band is increased and the radioactivity has spread along the fibres.

A peak of radioactivity in the region of the endplate has also been found in diaphragm muscle which was dissolved and counted by liquid scintillation, and the uptake

of decamethonium can be largely prevented by previous treatment with *d*-tubocurarine<sup>6</sup>. This effect of the antagonist has been confirmed by preparing transverse sections, and also by a different method the results of which are shown in Fig. 3. These autoradiograms were obtained by placing intact hemi-diaphragms in contact with slides which had been coated with emulsion. At the end of the exposure the muscles were removed and the autoradiograms were developed and photographed. The left side of Fig. 3 shows a dark band of silver grains which correspond to the position of the endplates in the stained diaphragm<sup>6</sup>. The right side of Fig. 3 was obtained from a rat which had received *d*-tubocurarine chloride (0.8 mg/kg) 10 min before injection of decamethonium. The density of silver grains in the band at the endplate region was much reduced, and with a larger dose of antagonist the band could be abolished.

Diaphragm muscles which have taken up labelled decamethonium lose their radioactivity at a slow rate. Fig. 4 shows autoradiograms from muscles which were removed 2 h and also 7 days after injection. After 7 days the band of silver grains is considerably wider, and this appearance is consistent with slow diffusion of decamethonium along the fibres.

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### Diffusion of Decamethonium in the Rat

Creese and MacLagan have reported evidence that labelled decamethonium enters the fibres of striated muscles when the drug is injected into rats (preceding communication). It was found that this radioactivity is lost slowly from the muscles and can still be detected after days or weeks.

The upper histogram in Fig. 1 shows the distribution of radioactivity in a diaphragm which was removed 2 h after injection of decamethonium-<sup>3</sup>H-methyl) dichloride into the tail vein of a rat. The dose (1.64 mg/kg) produced a transient weakness in some animals and no obvious effect in others. The left diaphragm was removed, frozen, sectioned into strips 1 mm wide and the radioactivity was expressed as counts min<sup>-1</sup> mg<sup>-1</sup>. The counts showed a peak in the region of the endplate, as found<sup>1</sup> previously, and a Gauss curve could be fitted to the results.

The lower histogram in Fig. 1 was obtained from a rat which was injected with the same dose of decamethonium and kept for 10 days before the diaphragm was removed. Considerable radioactivity remained in these muscles and it appeared that less than half had been lost. The Gauss curve is more dispersed, and this would be expected if there had also been some degree of diffusion along the fibres<sup>2</sup>. This interpretation is supported by the spread of radioactivity among the muscle which was shown in autoradiograms (preceding communication).

Curves can be fitted to the histograms of the form

$$y = d + A \exp \left\{ -\frac{(x - \mu)^2}{2\sigma^2} \right\} \quad (1)$$

where  $y$  represents counts min<sup>-1</sup> mg<sup>-1</sup> and  $x$  is the distance in millimetres from the tendon. The Gauss curves were found by a method to be described separately in which the parameters  $d$ ,  $A$ ,  $\mu$  and  $\sigma$  were computed to give a fit with least squares. In twelve muscles removed after 2 h the mean standard deviation  $\sigma$ , was 0.802 mm (range 0.614–1.17), while for twelve muscles removed after 10 days  $\sigma$  was 1.64 mm (range 1.33–2.03). Intermediate values were obtained which were consistent with these results. The lower curve of Fig. 1 is still diminishing at the ends, and with longer times the effects of reflexion may become significant<sup>3</sup>.

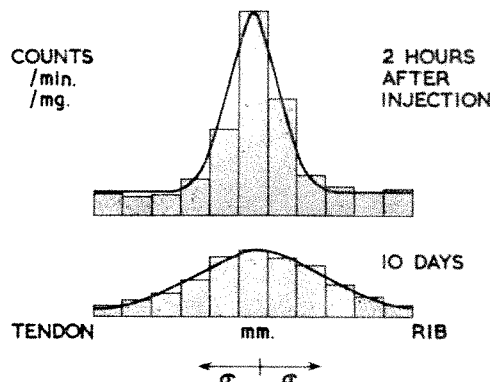


Fig. 1. Upper histogram shows radioactivity of rat diaphragm which was removed 2 h after injection of labelled decamethonium. The muscle was cut into strips 1 mm wide, and a Gauss curve has been fitted to the counts. The lower histogram was obtained from a rat which was injected and kept for 10 days before removal of the diaphragm. The standard deviation  $\sigma$  of the lower curve is indicated.

For longitudinal diffusion

$$\sigma_2^2 - \sigma_1^2 = 2D't \quad (2)$$

where  $D'$  is the apparent diffusion coefficient and  $t$  is the time. Thus  $D'$  is  $1.2 \times 10^{-8}$  cm<sup>2</sup> sec<sup>-1</sup>. This rate is very slow, and differs from the unidirectional transport which has been demonstrated in nerve axons<sup>4</sup>. The diffusion coefficient of labelled decamethonium in the interspaces of isolated rat diaphragm was found to be  $2.4 \times 10^{-6}$  cm<sup>2</sup> sec<sup>-1</sup>—the method being similar to that used previously<sup>5</sup>. The figure for internal diffusion is 200 times smaller than that for the interspaces, which in turn is less than in free solution<sup>6</sup>.

Decamethonium enters the fibres of rat muscle in the region of the endplate, and then migrates towards the ends of the fibres and is slowly lost. These results apply to the labelled compound which remains after injection, and the early distribution of the drug has not been explored.

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## Effect of Calcium Ions on the Release of Acetylcholine from the Cerebral Cortex

THE presence of calcium ions ( $\text{Ca}^{2+}$ ) in the external medium is essential for the release of acetylcholine (ACh) at peripheral synapses (superior cervical ganglion, neuromuscular junction)<sup>1-6</sup>. Evidence now suggests that ACh is a transmitter in the cerebral cortex, and several investigators have shown that this substance is continuously released from the cerebral cortex in amounts which can be directly related to the level of cortical activity<sup>7-12</sup>. This communication describes how changes in local  $\text{Ca}^{2+}$  affect the spontaneous and evoked release of ACh from the cat cerebral cortex.

*d*-tubocurarine chloride ( $10^{-5}$  g/ml., California Biochemical Corporation). This procedure abolished the response to standard solutions of ACh, as well as to the active principle present in the samples. The results were analysed statistically by using Student's *t* test.

When the normal Ringer-Locke solution was changed for one containing no calcium chloride, there was a significant fall (of 34 per cent on average) in the amount of ACh collected from the surface of the cerebral cortex in the absence of peripheral stimulation (Table 1). The depressant effect of the lack of  $\text{Ca}^{2+}$  was readily reversed by restoring  $\text{Ca}^{2+}$  to the collection fluid. It is interesting that the action of  $\text{Ca}^{2+}$  is exerted only on a portion of the spontaneous release of ACh, while the remaining fraction

Table 1. MEAN RATE OF ACETYLCHOLINE RELEASE (ng/min/cm<sup>2</sup>) FROM UNSTIMULATED CEREBRAL CORTEX INTO RINGER-LOCKE SOLUTION CONTAINING DIFFERENT CONCENTRATIONS OF  $\text{Ca}^{2+}$

Region	No. of cats	Rate in 2 mmolar	Rate in 1 mmolar	Rate in 0.2 mmolar	Rate in 0 mmolar	P
Sensory-motor and parietal cortex	12	0.74 ± 0.09 (S.E.)	—	—	0.49 ± 0.06	< 0.05
	2	0.90 ± 0.20	0.81 ± 0.19	—	—	*
Sensory-motor cortex	4	0.48 ± 0.12	—	0.36 ± 0.07	—	*

\* These data are of a preliminary nature, but they suggest that there is no effect of low- $\text{Ca}^{2+}$  solution (0.2 mmolar and 1.0 mmolar) on the rate of release of ACh as compared with the control values.

Experiments were performed on cats anaesthetized with a mixture of diallyl-barbituric acid and urethane ('Dial', Ciba, Ltd.) given intraperitoneally (0.7–0.8 ml/kg). ACh was collected from the exposed surface (1 cm<sup>2</sup>) of the pericruciate and suprasylvian cortex, using the 'Perspex' chamber technique described before<sup>7</sup>. The chambers were filled with 1 ml. of Ringer-Locke solution containing eserine sulphate ( $1 \times 10^{-4}$  g/ml., British Drug Houses) and aerated with 5 per cent carbon dioxide in oxygen. The Ringer-Locke solution used had the following ionic composition: 137.0 mmolar sodium chloride; 2.5 mmolar potassium chloride; 2.0 mmolar calcium chloride; 1.0 mmolar magnesium chloride; 1 mmolar sodium phosphate; 12.0 mmolar sodium bicarbonate; and 11.0 mmolar glucose.

was unaffected by the absence of calcium. This result is qualitatively in agreement with the data obtained at mammalian motor nerve terminals<sup>4-6</sup>.

Mitchell<sup>9</sup> has reported that electrical stimulation of a peripheral sensory nerve produced an increase in the rate at which ACh was liberated from the surface of the contralateral sensory cortex. Using this procedure we have found that with 0.2 mmolar  $\text{Ca}^{2+}$  and with  $\text{Ca}^{2+}$ -free solutions there is no increase in the output of ACh during peripheral stimulation (Table 2). These results are of interest, because in all synapses so far examined the release of the chemical transmitter by the nerve impulse requires the presence of  $\text{Ca}^{2+}$  in the external medium. It seems that the cerebral cortex is not an exception to this. These observations also provide supporting evidence for the

Table 2. MEAN RATE OF SPONTANEOUS AND EVOKED ACETYLCHOLINE RELEASE (ng/min/cm<sup>2</sup>) FROM CEREBRAL CORTEX INTO RINGER-LOCKE SOLUTION CONTAINING DIFFERENT CONCENTRATIONS OF  $\text{Ca}^{2+}$

Region	No. of cats	[ $\text{Ca}^{2+}$ ]	Resting rate	Rate on contralateral forepaw stimulation	P
Sensory-motor cortex	8	2 mmolar	0.54 ± 0.09 (S.E.)	0.81 ± 0.09	< 0.05
	8	0.2 ± 0 mmolar	0.39 ± 0.05	0.42 ± 0.05	Not significant

The concentration of  $\text{Ca}^{2+}$  was varied in different experiments ( $\text{Ca}^{2+}$ -free, 0.2, 1.0 and 2.0 mmolar) by omitting calcium chloride; the other ions and the concentration of eserine remained constant. The small changes in osmolarity were not corrected for. The stimulating pulses were usually rectangular pulses lasting 1 msec, delivered at a frequency of 0.5/sec to the contralateral forepaw, through a radio-frequency isolation unit of the Grass 'S8' stimulator. Efficiency of stimulation was checked by recording the evoked potential with a cathode-ray oscilloscope. ACh was sampled from the surface of the cerebral cortex unilaterally for between fifteen and eighteen collection periods, each of 15 min duration. The usual procedure was as follows: two resting samples alternated with a stimulated one, first with normal (2.0 mmolar  $\text{Ca}^{2+}$ ) Ringer-Locke and then the sequence was repeated with low- $\text{Ca}^{2+}$  or  $\text{Ca}^{2+}$ -free solution. In this way, we were able to detect a significant change in the release of ACh, which was reproducible. Immediately after removal, samples were frozen at  $-15^{\circ}\text{C}$  and stored at that temperature.

Bio-assays were performed within 48 h of collection on the dorsal muscle of the leech (*Hirudo medicinalis*). As a control, muscle was soaked for 10 min in a solution of

involvement of ACh as a synaptic transmitter in the cerebral cortex.

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## Electrical and Mechanical Responses of the Skeletal Muscle of a Primitive Insect

INNERVATION of arthropod excitatory muscle is of two types, distinguished by mechanical effect; these are "fast" (twitch) and "slow" (tonic), and only in the fast type is there a significant mechanical response to a single nerve impulse. The classes Insecta and Crustacea are the only arthropods the neuromuscular mechanisms of which have been studied in any depth, and considerable differences have been found between them, both in the anatomy and in the form of electrical events which accompany the fast and slow mechanical responses. Nearly all insect muscle fibres are fast-innervated and the electrical response is large, with a non-propagated spike component (active membrane response). Some fibres are also slow-innervated, and in these the electrical response is much smaller, but summates and facilitates greatly to repetitive stimulation, producing large depolarizations<sup>1-5</sup>. In crustaceans there are relatively fewer fast-innervated fibres, but all are slow-innervated. The two types of response are often difficult to distinguish electrically, and the fast response has no spike component; summation is incomplete although facilitation can be significant, and large depolarizations to either fast or slow stimulation are very rare<sup>6,7</sup>. Investigations of some of the more primitive insects could possibly show some responses which do not have the large spike activity of the higher insects, and some interesting neuromuscular patterns have been discovered in dragonfly larvae (species of *Aeschna*) which are thought to be primitive for several other reasons.

Excised preparations of the extensor tibia muscle and of the coxal depressor of trochanter muscle (here referred to as the coxal muscle) of the mesothoracic leg of late-instar *Aeschna* nymphs were mounted on the wax bed of a 'Perspex' chamber. They were covered with a saline of the following composition in mmoles/l.: 136 of sodium ions, 6 of magnesium ions, 10 of calcium ions, 156 of chloride ions, 16 of bicarbonate ions and 160 of sucrose. The distal apodeme was attached to the plate of an RCA '5734' mechano-electric transducer valve, and the innervating nerve trunk, which was left attached to the excised ganglion, was stimulated by a suction electrode. Electrical responses of the muscle fibres were recorded intracellularly.

The coxal muscle is innervated by a single excitatory nerve fibre, which is of the slow type. This block is thus unique among known insects, although muscles are known in which a few fibres are only slow-innervated<sup>8</sup> and muscle blocks receiving only one excitatory axon (but of the fast type) are also known<sup>9</sup>. Solely slow-innervated muscles are common in crustaceans. The extensor tibia muscle is innervated by one fast and one slow fibre, and, in the typical insect manner, the slow fibre innervates less than half of the muscle fibres.

Electrical responses of the extensor tibia are shown in Figs. 1A and B. It can be seen that the fast responses (B) are much larger than the slow responses (A), and are simple junction potentials with no spike components. They are thus comparable with the fast crustacean responses. The sizes of both fast and slow electrical responses are very variable from fibre to fibre in the same muscle block. The coxal muscles show electrical responses similar to the slow responses of the extensor tibia muscle, varying in size from fibre to fibre in the range 0.5–25 mV. The larger among these responses are much larger than those recorded from other insects, where most single slow responses can scarcely be seen, and few exceed 10 mV. The fast electrical responses of the extensor tibia may reach 50 mV, so both fast and slow responses are very comparable with those of crusta-

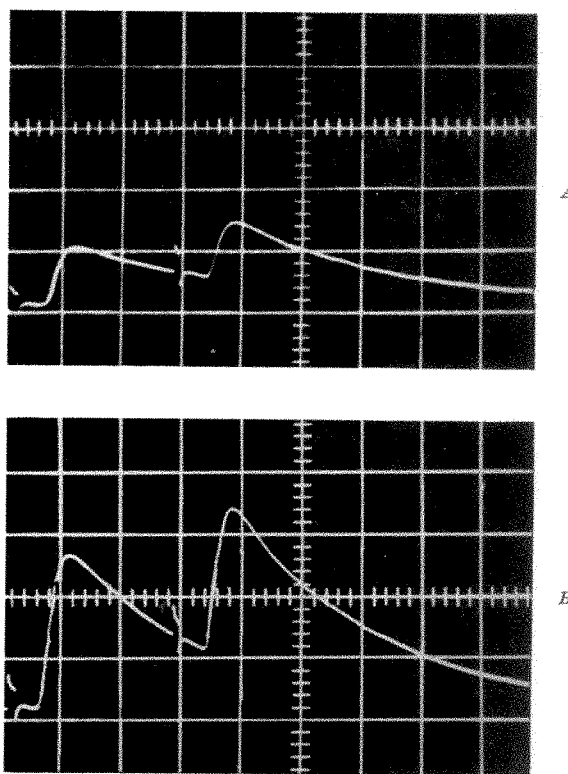


Fig. 1. The electrical response of the extensor tibia muscle of the mesothoracic leg of *Aeschna* to paired stimulation of the slow axon (A) and of the fast axon (B). There is no facilitation of the second response. Grid: horizontal, 5 msec, and vertical, 10 mV.

ceans. At any one place on a fibre successively evoked action potentials may vary greatly in size, by  $\pm 15$  per cent of the mean value; for a long series the sizes are normally distributed. Similar variation in size of slow junction potentials has been noted in the crayfish<sup>9</sup>.

The mechanical response of the extensor tibia to stimulation of the slow axon together with the simultaneous electrical activity of a single fibre is shown in Fig. 2. Very slight response to the first stimulus of the train (effectively a single stimulus) can just be detected in this figure. In other preparations a rather larger response was seen, but in all cases it was at least three orders of magnitude smaller than the corresponding twitch response to single fast stimulation. A striking feature of the mechanical response to repetitive slow stimulation is that there is a distinct response to each stimulus, almost invisible at the start of stimulation, but rapidly facilitating to give a distinctly pulsed tetanus. This can clearly be seen in

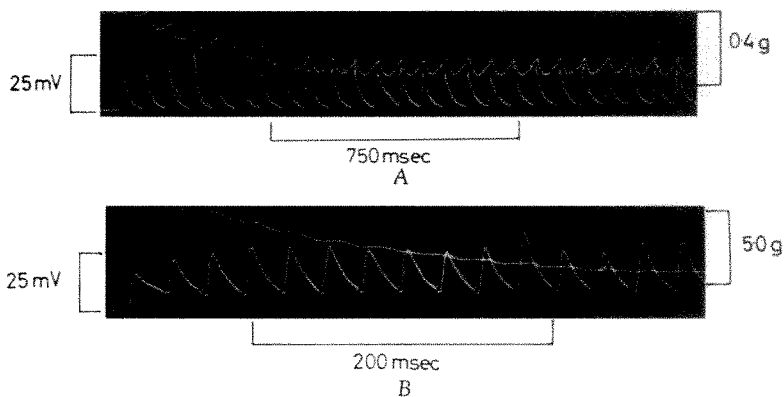


Fig. 2. Mechanical response of the coxal depressor of trochanter muscle of the mesothoracic leg of *Aeschna* to repetitive neural stimulation at 14/sec (A) and 25/sec (B) (upper traces) and the simultaneous electrical activity recorded intracellularly from one muscle fibre (lower traces).

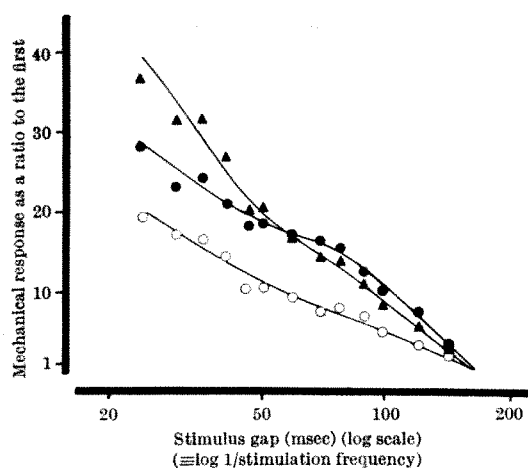


Fig. 3. The relationship between the frequency of stimulation and the size of the second (○), third (▲) and fifth (●) individual mechanical responses evoked by a stimulus train. The size of the fourth response followed a pattern identical to the third, and all subsequent responses resemble the fifth. Mechanical response is expressed as a ratio to the first, very small response, to eliminate effects due to mechanical fatigue.

Fig. 2 in which the electrical activity of a fibre is recorded simultaneously. The degree of facilitation is dependent on two factors: the frequency of stimulation (that is how closely one stimulus follows another) and the size of the preceding response. This is illustrated in Fig. 3 which shows that any one response increases relative to the preceding one by a factor related approximately to the frequency. The individual mechanical responses, however, swiftly reach a maximum, so that after the third or fourth response there is little difference in size though summation continues. This is clearly illustrated by Fig. 2A.

This unusual mechanical response may well be a separate development among these insects which fulfils the function of a fast twitch system; this seems to be borne out by the fact that the slow-only innervated coxal muscle is much more accentuated than the extensor tibia, which is also innervated by a fast axon. The response, however, resembles that of crustaceans in one important respect. A "paradox phenomenon" has been reported<sup>10</sup> in certain decapod crustacea, where large slow electrical responses can be seen while there is very little mechanical activity. Fig. 2 shows that in dragonflies very different mechanical activity can accompany apparently identical action potentials. This is particularly clear in Fig. 2A. There is no electrical facilitation, and there is no continuous relationship between membrane potential and tension.

The mode of summation of the electrical responses, as seen in Fig. 2B, is comparable with the summation of both

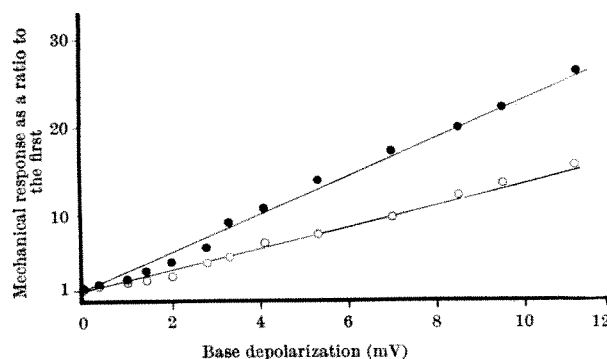


Fig. 4. The relationship between the base depolarization evoked by neural stimulation at various frequencies in one fibre of the coxal muscle and the corresponding mechanical response of the whole muscle. The relationship between mechanical response and the base depolarizations of other fibres of the muscle gives similar patterns, but with greatly variable gradients. ○, Second response; ●, third response.

fast and slow crustacean responses<sup>7</sup>, in that within two or three potentials summation levels off, and thereafter action potentials originate from approximately the same membrane potential level. The difference between this level and the resting potential may be called the base depolarization. The principal difference between this and the crustacean response is the absence here of facilitation.

The base depolarization varies from fibre to fibre, in a way not dependent on the size of the action potential, although this must have some effect; it is partly dependent on the rate of decay of the action potential, which is also variable. The base depolarization of any fibre of a muscle block is directly and linearly related to the tension developed in the block, and also to the size of any one mechanical response in the chain (Fig. 4). This relationship holds however small or great the base depolarization, so again there is no one-to-one relationship between electrical and mechanical events. This relationship must be a pointer to some deeper membrane event, perhaps permeability changes to ions not responsible for the electrical action potential.

The dragonfly neuromuscular system is thus rather more like that of crustaceans than it is of other known insects, although it seems to have some specializations of its own. It is tempting to speculate that the system is more primitive than those of other insects in that the fast innervation and response are not present in some muscle blocks, and also that there is no active membrane response. As in crustaceans, the mechanical response to either fast or slow stimulation bears little obvious relationship to the action potential, because fast and slow responses are so alike and repetitive stimulation shows different mechanical responses responding to apparently similar slow action potentials. The relationship between slow mechanical responses and the greatly variable base depolarization may offer a pointer to some secondary membrane phenomenon, not directly reflected in the action potential, being at work. Further elucidation of these observations is being undertaken, and may throw light on both the paradoxical mechanical responses of some crustaceans, and on the connexion between excitation and contraction in arthropods generally.

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### Stimulation of the Sodium Pump in Frog Bladder by Oxytocin

THE stimulating effect of the neurohypophyseal hormones on the transport of sodium ions across amphibian epithelial layers *in vitro* has been established for frog skin<sup>1</sup> as well as for toad bladder<sup>2</sup>. The site of action of the hormone is uncertain, however. Because the hormone increases the sodium pool in the bladder it has been suggested that the entry of sodium into the cells is facilitated by the hormone

acting at the mucosal membrane<sup>3</sup>. Similarly, with frog skin, it was inferred from the kinetics of the exchange of labelled sodium that the neurohypophyseal hormone enhanced the entry of sodium across the outer membrane into the epidermal cells<sup>4</sup>. In neither case was it suggested that the hormone stimulated the sodium pump directly, as the sodium pump is localized at the opposite face of the epithelial layer and expels sodium from the cells. Rather, this pump was supposed to become more saturated by the increase in concentration of intracellular sodium. Other workers<sup>5</sup>, however, have found that neurohypophyseal hormones recover the normal direction of the short-circuit current in frog skins bathed from the epidermal side by very dilute sodium sulphate solution. They have thus shown that an active step, whether localized at the outer or the inner border of the cells, must be directly stimulated by the hormone.

The aim of the present investigation is to show that whatever the character of sodium transport across the mucosal border and the action of the neurohypophyseal hormone on this transport, it is certain that the hormone directly stimulates the expulsion of sodium ions from the epithelial cells. In these experiments, the transport of sodium ions across the mucosal surface of epithelial cells of frog urinary bladder was prevented by covering the surface with liquid paraffin. Thus it was possible to separate the behaviour of the non-mucosal parts of the cell membranes for study and to examine the effects of oxytocin on this behaviour.

The frogs (*Rana temporaria*) were kept in the cold before the experiment, and killed by cutting the spine and pithing. The bladders, emptied by massaging the abdominal wall, were filled with liquid paraffin through a tube which was inserted into the cloaca. Two halves of the bladder were tied off separately, giving a control bag and an experimental bag. These bags were then incubated separately in aerated frog Ringer solution (m.equiv./l.: sodium, 114.5; potassium, 5.0; chlorine, 119.0; bicarbonate, 2.5; calcium, 1.0), the experimental medium containing 50 mU of synthetic oxytocin ('Spofa')/ml. After incubation for 25–30 min at room temperature (20–30° C), the bags were cut, the tissue thoroughly blotted on filter paper and weighed on a torsion balance. The tissue was then dried at 95° C overnight, weighed and its water content estimated as kg of water/kg of dry solids. The solids were extracted in 0.1 molar sulphuric acid for 5 days, and the sodium and potassium contents were determined by flame photometry of the extract using the EEL apparatus. Chloride content was determined by potentiometric titration of the extract according to Sanderson<sup>6</sup>. The results were expressed/kg of dry solids and are summarized in Table 1. Because the results were obtained in pairs, the standard deviation of the mean of the differences between the paired values was used in the *t* significance test.

It can be seen that oxytocin produces a significant loss of chloride anions and highly significant ( $P < 0.001$ ) losses of water and of sodium and potassium cations. The loss of cations is on average greater than that of chloride, but the difference is not significant, being  $20.5 \pm 11.4$  ( $n = 25$ ),  $0.05 < P < 0.1$ .

Other experiments were carried out to estimate the proportion of the observed changes that were respectively intracellular and extracellular. For this estimation, the inulin space was measured for the preparation with oil on the mucosal side, and an estimate made of the effect

Table 2. EFFECT OF OXYTOCIN ON THE INULIN ACCESSIBLE AND INULIN INACCESSIBLE WATER CONTENT IN FROG BLADDERS FILLED WITH LIQUID PARAFFIN

	Controls	Oxytocin (50 mU/ml.)	Significance level
Water accessible to inulin (kg/kg DS)	$1.65 \pm 0.08$	$1.47 \pm 0.08$	$0.1 < P < 0.2$
Water inaccessible to inulin (kg/kg DS)	$1.63 \pm 0.10$	$1.30 \pm 0.09$	$P < 0.02$

DS, Dry solids.

of oxytocin on this space. The inulin spaces, calculated as a per cent of wet weight of bladder tissue, were  $38.47 \pm 1.75$  ( $n = 28$ ) for the control and  $39.09 \pm 2.00$  ( $n = 28$ ) for the half bladder treated with oxytocin. The amounts of inulin accessible to water and inulin inaccessible to water were calculated (Table 2) from the estimates of the inulin space and those of the wet weight/kg of dry solids. The latter were determined in the first series of experiments ( $n = 25$ ) as  $4.28 \pm 0.097$  in the controls and  $3.77 \pm 0.089$  in the half bladders treated with oxytocin. The standard errors of the means were combined according to the expression

$$\sigma_{xy} = \sqrt{(\bar{y}^2\sigma_x^2 + \bar{x}^2\sigma_y^2)}$$

where  $\bar{x}$  and  $\bar{y}$  are the mean values and  $\sigma_x$  and  $\sigma_y$  the corresponding standard errors. It will be seen that the change in the "extracellular" water is not significant, whereas the change in the "intracellular" water is probably significant,  $P < 0.02$ . The hormone seems to lower the concentration of sodium from 30 m.equiv./l. to 19 m.equiv./l. and raise that of potassium from 133 m.equiv./l. to 150 m.equiv./l. These changes can be seen if the intracellular concentrations of sodium and of potassium in control bladders and bladders treated with oxytocin are calculated from the mean values given in the two tables.

These results suggest that the expulsion of sodium is stimulated by the neurohypophyseal hormone and the loss of sodium chloride is followed by a loss of osmotically driven water. Thus potassium chloride is concentrated and also diffuses out of the cells to some extent.

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### Changes in Plasmalogen Content of Human Heart and Skeletal Muscle with Age and Development

The proportion of plasmalogen to total phospholipid in mammalian heart muscle seems to depend on both the age and species of the animal, with the choline plasmalogen (glycerol-2-acyl-1-alkenyl-3-phosphorylcholine) showing the most marked variations<sup>1-3</sup>. Species differences in the plasmalogen content of skeletal muscle also seem to be considerable and on the basis of analyses of monkey muscle, primate skeletal muscle has been suggested to be considerably richer in plasmalogens than tissue from other species, such as the rabbit<sup>4</sup>. Some of our analyses of mature and immature human muscle have shown that maturational changes, somewhat similar to those occurring in heart muscle, also take place in skeletal muscle. In addition they suggest that the plasmalogen content of

Table 1. EFFECT OF OXYTOCIN (50 mU/ml.) ON WATER AND ION CONTENTS IN FROG BLADDERS FILLED WITH LIQUID PARAFFIN (25 PAIRS)

	Controls	Oxytocin (50 mU/ml.)	Significance level
Water kg/kg DS	3.28	2.77	$P < 0.001$
Sodium molar equivalent/kg DS	237.4	192.7	$P < 0.001$
Potassium molar equivalent/kg DS	224.7	202.6	$P < 0.005$
Chlorine molar equivalent/kg DS	296.0	249.6	$P < 0.001$

DS, Dry solids.



mature human skeletal muscle is similar to that of monkey muscle.

Specimens of mature and foetal human heart and skeletal muscle were frozen in liquid nitrogen and crushed into a pellet which was then extracted by homogenizing with chloroform-methanol (2:1). The resultant extract after treatment with chloroform-methanol (2:1) containing 4 per cent water to precipitate protein<sup>5</sup> was washed with 0.1 molar potassium chloride<sup>6</sup>, evaporated to dryness and redissolved in a suitable volume of dry chloroform-methanol (2:1) for chromatography. The extracts were stored at -25° C until analysis. Thin-layer chromatography on silica gel was carried out by the two dimensional technique of Owens<sup>7</sup> and the phosphorus in the separated zones was estimated by a slight modification of Bartlett's method<sup>8</sup>.

Data for choline and ethanolamine phospholipid (excluding sphingomyelin) are presented in Tables 1 and 2. Two things are apparent: (a) human heart muscle contains rather less total choline phospholipid at all stages of development than does skeletal muscle; and (b) despite the differences between the two tissues the same trend of increasing choline plasmalogen can be seen. The data also suggest that there is an appreciable increase with development in the ethanolamine plasmalogen of skeletal muscle, but at the earliest foetal age studied, about 10 weeks, nearly half the ethanolamine fraction exists in the plasmalogen form. In heart muscle, on the other hand, the proportion of ethanolamine plasmalogen does not show any consistent change with development.

Table 1. PLASMALOGENS IN HUMAN HEART MUSCLE

Tissue	Age	Choline phospholipid		Ethanolamine phospholipid	
		Plasma- log-en	CP % total lipid phosphorus	Plasma- log-en	EP % total lipid phosphorus
Foetal	c. 10 weeks	n.d.	38	51	22
Foetal	c. 13 weeks	n.d.	43	52	30
Foetal	c. 15 weeks*	7, 4	47, 45	52, 54	30, 27
Stillbirth	—	14	40	60	25
PM	22 months	25	37	45	32
PM	38 yr	36	40	49	27
PM	65 yr	48	40	59	27

EP, ethanolamine phospholipid phosphorus; CP, choline phospholipid phosphorus (excluding sphingomyelin); n.d., not detected ( $\leq 5$  per cent) possibly because of a small amount of material available; PM, post-mortem.

\* Estimations made on two foetuses.

Table 2. PLASMALOGENS IN HUMAN SKELETAL MUSCLE

Tissue	Age	Choline phospholipid		Ethanolamine phospholipid	
		Plasma- log-en	CP % total lipid phosphorus	Plasma- log-en	EP % total lipid phosphorus
Foetal	c. 10 weeks	2	48	45	23
Foetal	c. 15 weeks*	3, 4, 4	48, 53, 50	47, 50, 51	27, 27, 29
Foetal	c. 20 weeks	5	49	46	26
Stillbirth	—	12	51	60	28
PM	5 months†	11	46	56	30
PM	13 months†	17	47	65	29
PM	22 months	15	48	73	25
Biopsy	27 years	19	51	63	24

Abbreviations are as in Table 1.

\* Estimations of three foetuses.

† Psoas muscle.

The choline plasmalogen content of human heart muscle increases after birth and possibly even in the period after maturity, as the result for the 65 yr old individual suggests. In the case of skeletal muscle, however, the present results are inconclusive because the analyses were not all carried out on the same muscle, and we have some evidence that mature human muscles may differ in their content both of choline and ethanolamine plasmalogen (unpublished analyses of biopsy material); Masoro *et al.*<sup>4</sup> found considerable differences between monkey gastrocnemius and soleus muscle in this respect. On the other hand the tissue derived after death from 5 month old and 13 month old infants was psoas muscle in each case and here there does appear to be appreciably more plasmalogen in the psoas muscle of the older child.

It is interesting that whereas choline plasmalogen as a proportion of choline phospholipid increases during foetal development in heart muscle at least for some time after birth, total choline phospholipid phosphorus as a percentage of tissue phospholipid phosphorus remains fairly constant. Kiyasu and Kennedy<sup>9</sup> suggested that the enzyme system which synthesizes plasmalogens from cytidine diphosphate choline and plasmalogenic diglyceride may be the same as that responsible for the formation of the corresponding diacyl phospholipids from cytidine diphosphate choline and the diacyl glyceride. If this is the case then the observed increase in plasmalogen may reflect increased synthesis of plasmalogenic diglyceride as development proceeds, leading to the formation of more plasmalogen by virtue of the increased precursor concentration.

In the case of heart muscle a direct relation has been reported between tissue plasmalogen and body surface area for various species<sup>1</sup>. If this is correct then the changes occurring during development may simply reflect an increase in size. These data<sup>1</sup>, however, are in conflict with other data<sup>2</sup> which indicate considerably larger plasmalogen concentrations in the heart muscle of smaller mammals, for example, mouse and cat. Furthermore, the greatest plasmalogen concentrations were found in the heart of a small mammal, the guinea-pig. Our own analyses of mouse heart gave for plasmalogen expressed as moles per cent of total phospholipid, values of 10 per cent and 13 per cent respectively on two batches of pooled tissue; a result which is in good agreement with that of Spanner<sup>3</sup> and which conflicts with the value of 4.4 per cent found by Thiele *et al.*<sup>1</sup>. Consequently there seem to be other factors, apart from body surface area, which influence the concentration of plasmalogen.

Our results for human skeletal muscle confirm the observation of Masoro *et al.*<sup>4</sup> that primate muscle is rich in plasmalogen. Whether, as these authors suggest, it is remarkable in this respect may be questionable, because, for example, ox muscle seems to contain at least as much plasmalogen as human and monkey muscle<sup>10</sup>. Furthermore, Masoro *et al.*<sup>4</sup> compared their total molar ratio of plasmalogen : lipid phosphorus for monkey muscle of 0.25 with a figure of 0.11 for rabbit muscle<sup>11</sup>, although this latter figure refers only to choline plasmalogen. Consequently, the total ratio of plasmalogen : lipid phosphorus rabbit muscle is likely to be considerably greater than this. Nevertheless, compared with some species, such as sheep (molar ratio 0.10 (ref. 12)), and the mouse (molar ratio 0.08, calculated from the results of Hughes<sup>13</sup> and Owens<sup>7</sup>), the plasmalogen content of mature human and monkey muscle is large.

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### The Salt Gland in a Sea Snake (*Laticauda*)

ONE marine lizard and several marine turtles possess salt glands capable of secreting highly concentrated solutions of sodium chloride<sup>1</sup>. The salt gland appears to be of adaptive advantage to reptiles exposed to electrolyte loads, because the kidney is unable to excrete urine hyperosmotic to the blood. A sea snake (*Laticauda semifasciata*) follows the general pattern of ionic regulation found in marine turtles and a marine lizard<sup>2</sup>. The work reported here was designed to identify the salt glands of *Laticauda semifasciata* and compare it with the homologous gland of another sea snake, *Pelamis platurus*. *Laticauda* excretes a relatively dilute urine (7–148 mmolar sodium) from the kidney and a concentrated sodium chloride solution (380–625 mmolar) orally<sup>2</sup>. The rate of excretion of extrarenal sodium chloride increases to as much as 72  $\mu$ moles/100 g/h after injection of a sodium chloride or sucrose load<sup>2</sup>. This response must be due to a salt gland. In all lizards the salt gland is the nasal gland; secretions pass from the nasal passage out through the external nares<sup>1,3,4</sup>. In turtles, salt gland secretion elaborated by the Harderian gland is discharged under the eyelid<sup>1</sup>. Crocodiles may have a similar route of extrarenal electrolyte excretion<sup>2</sup>.

The genus *Laticauda* is usually regarded as less specialized for marine life than *Pelamis*<sup>5</sup>. It is coastal in distribution and partly terrestrial in habitat<sup>5</sup>. Thus *Laticauda* is less restricted to the sea than *Pelamis*, a pelagic form. This difference in habitat might be reflected in the relative size of the salt gland and the ability of the two genera to excrete salt loads extrarenally.

One *Laticauda* head was preserved in Bouin's solution, decalcified in sodium citrate and formic acid, dehydrated, and cleared in cedar wood oil for two weeks. After embedding in paraffin, serial sections of the whole head were cut at 10 $\mu$ . The sections were stained with Masson's trichrome. The body weight of this snake was 300 g. The tissue believed to be the salt gland was removed from another freshly killed snake and preserved in cold buffered formalin. After embedding and sectioning at 5 $\mu$ , the

sections were stained with toluidine blue (pH 2.0), haematoxylin and eosin, and periodic acid-Schiff (PAS) reagent before and after ptyalin (salivary) digestion. A gross morphological comparison was made between the cephalic region of *Laticauda* and that of *Pelamis platurus*. Dr J. Peters kindly provided the specimen of *Pelamis*. Other specimens are in the collection of Dr A. M. Taub.

Because fluid from the salt gland had been collected from the tip of the mouth between the rostral and mental scales<sup>2,6,7</sup>, attention naturally focused on the glands draining into this region. Taub<sup>6</sup> has recently reviewed the distribution of the cephalic glands in snakes, which can be divided into oral and non-oral types. The latter category consists solely of the nasal and Harderian glands and the venom gland is reported to be the only serous oral gland in sea snakes. The histology and distribution of the sublingual and supralingual glands are unknown in sea snakes. Thus the only major serous glands draining directly into the oral cavity of snakes are the Harderian glands found in the post-orbital region, and the venom glands. The nasal glands have only a very indirect connexion with the oral cavity via the nasal passage. After dissection of the muscles of the head (see Haas<sup>9,10</sup> for details of the anatomy of head muscles in the sea snake), a very small Harderian gland was found anterior and deep to the m. levator pterygoidei (Table 1). This gland lies almost in the mid-line. Comparison with a specimen of *Pelamis platurus* revealed that *Pelamis* did not have a grossly discernible Harderian gland. No grossly distinguishable nasal gland was found in the nasal region of *Pelamis* (Table 1). This confirmed the results of Kathener<sup>11,12</sup>. *Laticauda* was found to have a well developed nasal gland (Fig. 1). We were unable to collect any fluid from the nasal vestibule of *Laticauda* after salt loading. The absence of nasal and Harderian glands in *Pelamis*, the pelagic Hydrophiid, supported the conclusion that in sea snakes these glands are not the salt glands.

Table 1. THE PRINCIPAL NON-SALIVARY GLANDS OF *Laticauda* AND *Pelamis*

	Coastal <i>Laticauda</i>	Pelagic <i>Pelamis</i>
Harderian	Yes	No
Nasal	Yes	No
Natrial	Yes	Yes

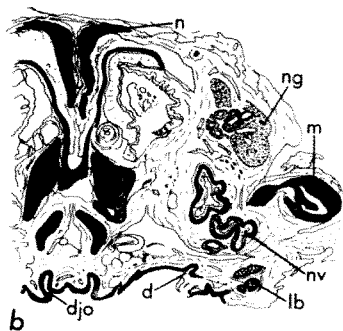
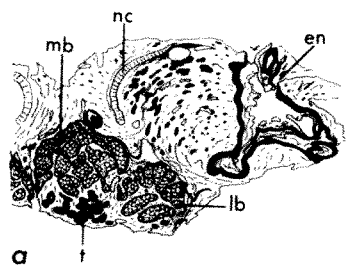


Fig. 1. Cross section of the left side of the head of *Laticauda semifasciata* ( $\times 8.5$ ). a, Anterior to the premaxilla showing the main body (mb), lateral branch (lb), tubules of the natrial gland (t), nasal capsule (nc) and external nares (en). b, Anterior to Jacobson's organ showing the duct of Jacobson's organ (djo), duct of the natrial gland opening into the mouth (d), end of lateral branch of natrial gland (lb), posterior nasal vestibule (nv), maxilla (m), nasal gland (ng), and nasal bone (n). (Drawn by R. D. Chambers.)

In an attempt to find additional oral glands a median incision was made, bisecting the interpalatine groove of a preserved *Pelamis* head. An azygous, pear-shaped gland was revealed anterior to the choanae. This new gland was heavily encapsulated in connective tissue, and about 5 mm long (Fig. 2). Dissection of *Laticauda* revealed a

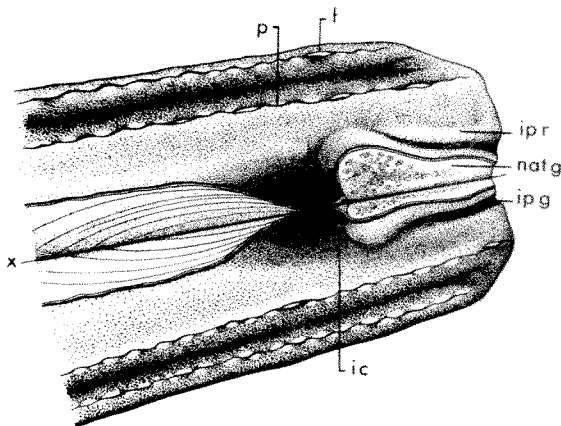


Fig. 2. Palatal view of the nasal region (rostral on right) of *Pelamis platurus* showing the fang (f), palatine tooth row (p), natrial gland (natg), interpalatine groove (ipg), interpalatine ridge (ipr), and internal choanae (ic). There is an incision in the midline (x) bisecting the palate and natrial gland. (Drawn by R. D. Chambers.)

much smaller gland in the same region (Fig. 3). Serial sections of a skinned head of *Laticauda* were prepared. A scale model of the gland was reconstructed by tracing low power projections of every fifth section from a viewing screen on an 'AO' trinocular microscope. The tracings were pasted on mounting board of appropriate thickness and glued together. The *Laticauda* gland has an unusual shape (Fig. 3). It apparently is secondarily fused as shown by the bilobed anterior tip, and the paired lateral ducts. There is a main body about 0.6 mm long, two posterior lateral branches about 1 mm long, and paired ducts emptying into the interpalatine groove. There are six apparently blind tubules running posteriorly in the midline from the main, medial portion of the gland. The main body of the gland underlies the premaxilla; the lateral branches extend to the level of the medial edge of the maxilla. The ducts run medially for about 0.5 mm before turning posteriorly again to empty into the connective tissue which makes up the posterior boundary of the interpalatine ridge. The ducts empty anterior to the openings of the vomeronasal organ. Secretory fluid could easily be pushed down the interpalatine groove to the outside by the tongue. Salt gland fluid was collected from the tip of the mouth when the tongue was extended<sup>2</sup>. The gland is apparently a multilobulate tubuloacinous gland. Because of the complexity of the convolutions it was not possible to trace every lobule to the duct. The glandular tissue is fairly diffuse, and the majority of the tubules are clearly separated by connective tissue. The gland is well innervated, but the nerves have not been traced to their origin. The gland cells are PAS positive, and remain so after ptyalin digestion. They show metachromasia with toluidine blue, indicating that polysaccharides other than glycogen are present.

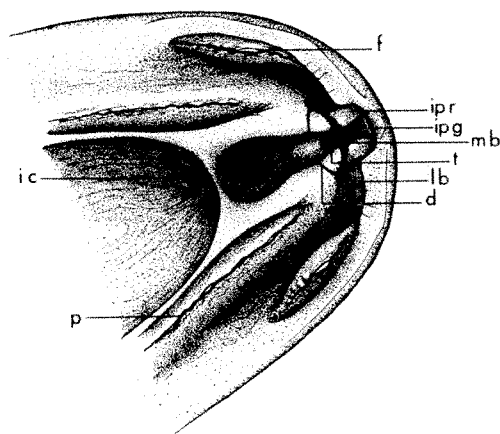


Fig. 3. Palatal view of the nasal region (rostral on right) of *Laticauda semifasciata* with the superimposed outline of the natrial gland. Label as in Figs. 1 and 2. (Drawn by R. D. Chambers.)

The oral epithelium of *Laticauda* is a stratified squamous epithelium. This is in sharp contrast to the simple mucous epithelium of other snakes which have been studied<sup>8</sup>. The stratified squamous epithelium in *Laticauda* is thickest on the medial surface of the interpalatine ridge, and becomes progressively thinner towards the oesophagus. The presence of a thickened, stratified, squamous epithelium near the ducts of this new oral gland may result from the presence of hyperosmotic fluid in this region.

No gland in this position has previously been described in snakes. Because this may be an extrarenal salt gland, we have named it the natrial gland (*natrium* is Latin for sodium). The large size of the natrial gland in *Pelamis* indicates that secretory activity may be greater than in *Laticauda*. There is no evidence that sodium rather than

chloride is actively transported by the gland. The homology of the gland is uncertain, but it seems likely that it is derived from the premaxillary gland. Alternatively, the vomerine gland may be involved, but it is found in this region only in lizards<sup>13</sup>. Without more adult and embryonic material it is impossible to assign a definite homology.

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### Maturity at Birth and Adult Protein Bound Iodine

Few adult biophysiological measurements can be traced back to the neonatal state. In a series of twenty-three pairs of monozygotic twins studied at the Clinical Center at the National Institutes of Health, Bethesda, adult protein bound iodine (PBI) was found to be directly related to weight at birth ( $r = 0.58$ ,  $P < 0.001$ ) (Fig. 1).

The most widely used measure of foetal maturity is the weight at birth<sup>1</sup>. Measurements of PBI and of the iodine

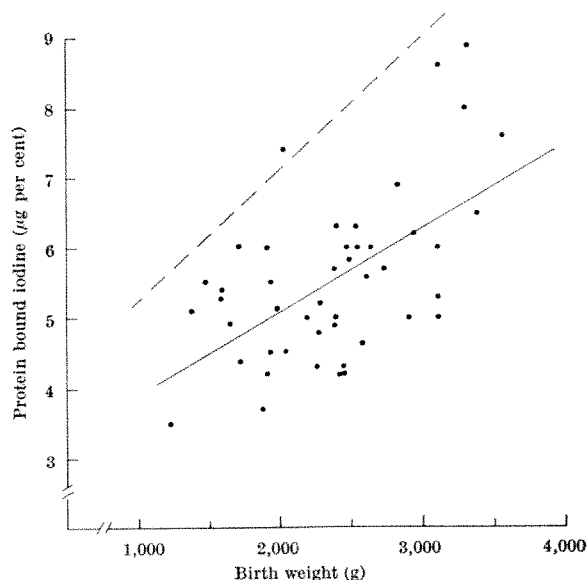


Fig. 1. The relationship between adult protein bound iodine (PBI) and weight at birth for 46 monozygotic twins. The solid line represents the least squares linear fit.  $PBI (\mu g \text{ per cent}) = 2.7424 + 1.1805 \text{ birth weight (kg)}$  ( $N = 46$ ). The correlation coefficient is  $+0.58$ ,  $P < 0.001$ . The broken line represents the least squares linear fit for 129 neonates studied by Marks and Man<sup>2,4</sup>.  $BEI (\mu g \text{ per cent}) = 3.3217 + 1.8963 \text{ birth weight (kg)}$ . The correlation coefficient is  $+0.65$ ,  $P < 0.001$ .

that can be extracted with butanol (BEI) are higher in infants heavier in weight at birth and lower in infants of low birth weight even though PBI or BEI in the neonate undergo rapid changes during the first year of life<sup>2,3</sup>.

A re-analysis of the neonatal data of Marks and Man<sup>2</sup> and Man<sup>4</sup> has demonstrated that BEI in the neonate is directly correlated with birth weight ( $r = +0.65$ ,  $P < 0.001$ ,  $N = 129$ ).

Protein bound iodine has long been used as a clinical indication of current thyroid function and as an indirect measure of the current metabolic state of the body<sup>5</sup>. While the range of PBI for large samples of normal individuals extends from 3.1 to 7.7  $\mu\text{g}$  per cent (95 per cent limits of confidence)<sup>6</sup>, test/re-test variance over time for individuals has been noted to be less than variance between individuals<sup>7</sup>.

The normal range of PBI and BEI in the neonate is higher than in the adult<sup>8</sup>. Provided that artefacts are eliminated, PBI and BEI can be used as equivalent measures of thyroid activity<sup>9</sup>. As can be seen in Fig. 1, the similarity of the lines of least squares linear fit for each study—that of PBI in adults and that of BEI in the neonates—suggests a biological constancy between the degree of maturity as estimated by weight at birth and thyrometabolic function in the neonate and adult as measured by protein bound iodine.

The series of twins are part of an investigation of schizophrenia now being carried out at the National Institutes of Health<sup>10,11</sup>. Zygosity was estimated by serological methods. In twelve of the fourteen pairs where one twin had been diagnosed as schizophrenic, the twin of lower birth weight was the twin who subsequently developed schizophrenic symptoms<sup>12</sup>. The direct relation between PBI and birth weight is significant with ( $r = +0.58$ ) or without ( $r = +0.65$ ) the schizophrenic subjects. This indicates the relation in the adult is not dependent on the presence of schizophrenia.

All determinations were made as coded blind samples by Bio Science Laboratories, Los Angeles, California. Duplicate determinations for the last twelve individuals studied had a mean difference of  $0.17 \mu\text{g}$  per cent  $\pm 0.03$ . Test/re-test for PBI for seven subjects gave similar values (mean difference  $0.47 \mu\text{g}$  per cent  $\pm 0.10$ ) and the same intrapair relation was found. Correlations were obtained by Pearson product-moment method. Possible sources of error due to inaccuracies in the reported birth weights, variance of PBI as a result of diurnal and daily cyclical fluctuation, changes arising from increased binding related to the menstrual cycle or contraceptive drugs, effects of anquillizers, artefactual effects of organic and inorganic acids and variations due to differences in environmental temperature have been evaluated and do not appear to terminate the foregoing correlation.

In the adult series of monozygotic twins, when each twin was compared with his co-twin, the within pair variance for PBI was  $0.81 \mu\text{g}$  per cent, while estimates of variability between pairs was  $0.66 \mu\text{g}$  per cent. Because variance within the pairs is greater than between pairs, the factor of genetic similarity does not seem as important determinant for PBI as individual non-genetic differences. One source of the large variance in PBI values between individuals may therefore result from intrauterine factors, perhaps the intrauterine nutritive experience which may have determined in part the size of the foetus. It seems, however, that it is not just the size of the foetus at birth, but the size of the foetus at birth that is important in establishing adult concentrations of bound thyroid hormone.

If PBI does reflect the level of free metabolically active hormone, it will be necessary to re-explore adult differences in somatic growth and central nervous system development in relation to the intrauterine experience, fetal maturity and neonatal thyrometabolic state.

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## CYTOLOGY

### Elimination of Ribosomes during Meiotic Prophase

At the conclusion of the meiotic divisions in the angiosperm anther, the cytoplasm of each mother cell is partitioned more or less equally between the four daughter spores, which then become isolated from each other by the formation of the callose tetrad wall. As a possible determinant of the pattern of gene expression in the gametophyte, the state of the meiocyte cytoplasm at the time of cleavage is of some significance. Is the cytoplasm bequeathed simply a sample of that of the original diploid archesporial cell, or does some metamorphosis occur concurrently with meiosis? An answer to this question could cast light on the control of the sporophyte-gametophyte alternation, and be relevant also to the problem of cytoplasmic inheritance.

Changes in RNA content and composition and in ribosome populations have been studied in the meiocytes of two liliaceous species, *Lilium henryi* and *Trillium erectum*. For the direct determination of the quantity and base composition of meiocyte RNA, the ultramicrochemical methods of Edström were used<sup>1</sup>. Individual meiocytes were isolated from sections in a de Fonbrune oil chamber, using a micromanipulator<sup>2</sup>. The callose meiocyte walls were removed, and RNA was extracted with ribonuclease. Total amounts of RNA were estimated by redissolving the extracts and photographing the droplets obtained, together with a reference system, in ultra-violet light at 265 m $\mu$ . For base analysis, the RNA extracts were hydrolysed in 4 normal hydrochloric acid, applied to a cellulose fibre, and the bases separated by microphoresis. After separation, the bases were measured by means of photometry in ultra-violet light at 265 m $\mu$ . Quantities as small as 150  $\mu\text{g}$  of RNA can be analysed in this way, so that determinations could be made on as few as three isolated meiocytes. Contamination with tapetal or other extraneous cells is wholly excluded by the method.

Material of *L. henryi* for electron microscopy was fixed in 1.5 per cent glutaraldehyde, post-fixed in 1 per cent buffered osmium tetroxide and embedded in 'Araldite'. Because apparent ribosome density in electron micrographs is related to section thickness, sections uniformly showing silver/gold interference colours were used. A grid of 2 cm squares was placed over each ( $\times 46,000$ ) print, and



ten squares were selected using a table of random numbers, passing over squares happening to fall over organelles or the nucleus. The ribosomes in each square were then counted. Three micrographs from each meiotic stage were surveyed, giving 30 square-counts/stage. The means obtained refer to the number of ribosomes present in standard volumes of cytoplasm, and to provide a more precise indication of variations in the population/cell correction for volume change would be required. In *Lilium*, as in *Zea mays*<sup>3</sup>, however, there is little growth of the meiocytes from mid-prophase to the close of the meiotic divisions.

Meiocyte RNA contents are shown in Figs. 1 and 2. The curves for the two species agree in showing a dramatic drop in total acetic-alcohol fixed RNA in the interval pachytene-diplotene-diakinesis, followed by a rise to

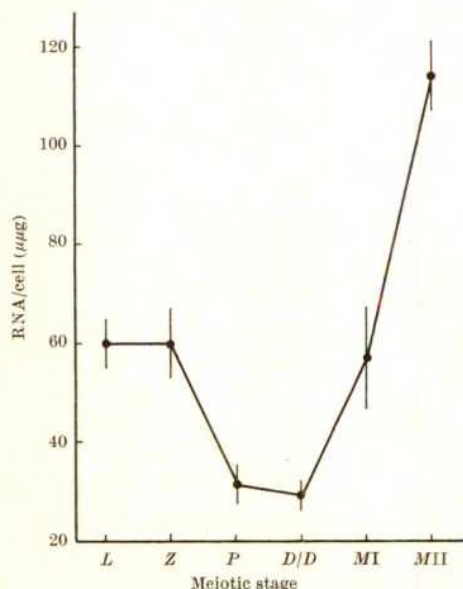


Fig. 1. *Lilium henryi*. Total RNA/meiocyte or dyad at various times during meiosis. Six to ten replicate samples of three cells estimated for stages leptotene to metaphase I; ten replicate samples of six dyads for metaphase II. L, leptotene; Z, zygotene; P, pachytene; D/D, diplotene-diakinesis; MI, metaphase I; MII, metaphase II. Vertical lines indicate  $\pm$  standard error.

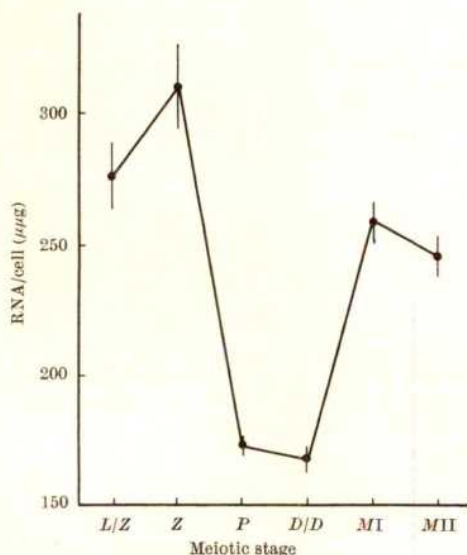


Fig. 2. *Trillium erectum*. Total RNA/meiocyte or dyad at various times during meiosis. Ten replicate samples of three cells estimated for leptotene-zygotene and metaphase I; nine of three cells for zygotene; ten of six cells or dyads for pachytene, diplotene-diakinesis and metaphase II. Stages as in Fig. 1, except that L/Z indicates cells transitional between leptotene and zygotene. Vertical lines indicate  $\pm$  standard error.

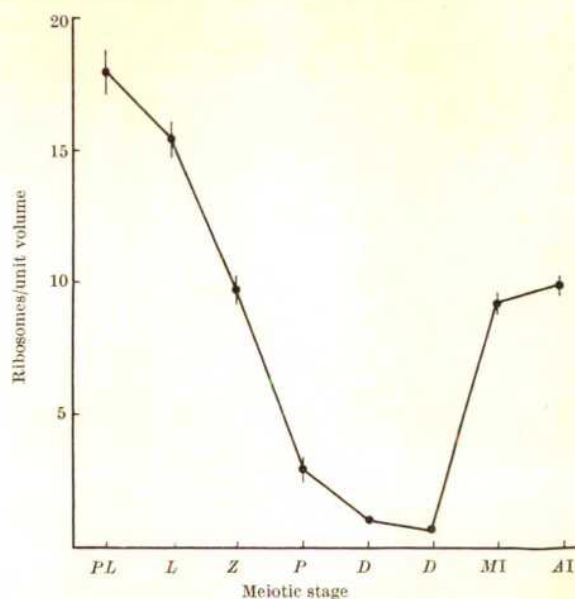


Fig. 3. Mean numbers of ribosomes/unit volume of cytoplasm estimated from electron micrographs in the way indicated in the text. Meiotic stages as in Fig. 1 with the addition of PL, pre-leptotene, the separation of diplotene and diakinesis, and the inclusion of AI, anaphase I. Vertical lines indicate  $\pm$  standard error.

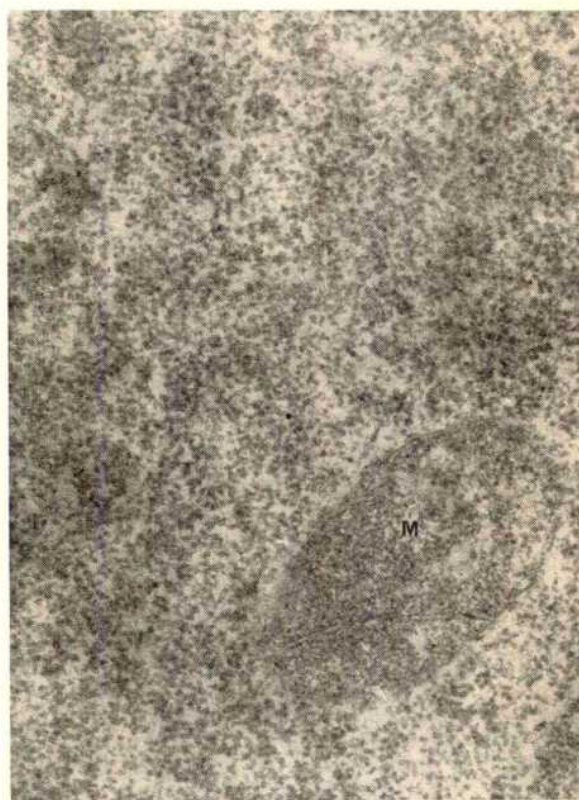


Fig. 4. Pre-leptotene cytoplasm, showing numerous ribosomes. M, mitochondrion.

metaphase I. Total RNA content is higher in the large *T. erectum* cells than in those of *L. henryi*, but the proportionate fall from zygotene to diplotene-diakinesis is similar.

The electron micrographic evidence indicates that the decline in RNA in mid-prophase is associated with reduction in the ribosome population. Fig. 3 shows striking fall in numbers in each unit volume to a minimum again in the interval pachytene-diplotene-diakinesis followed by a rise to anaphase I, the latest stage for which



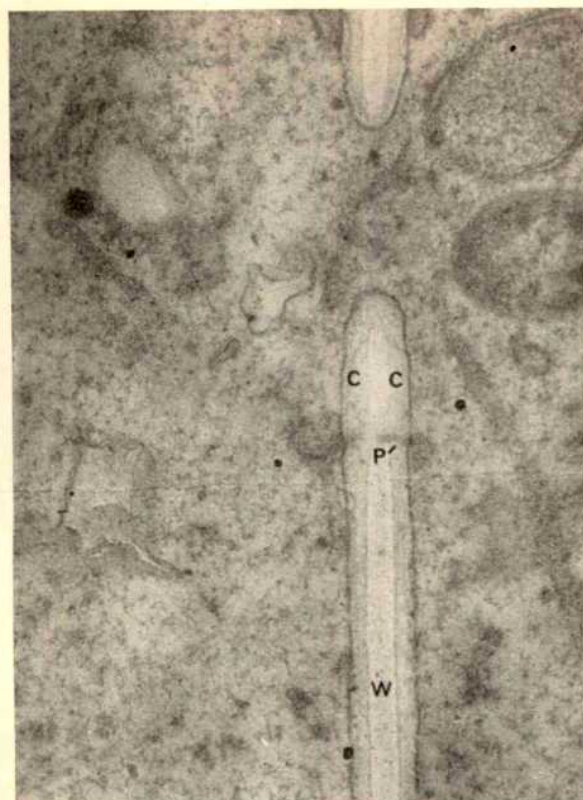


Fig. 5. Pachytene cytoplasm, denuded of ribosomes. The photograph shows two adjacent meiocytes connected by a broad cytoplasmic channel. W, Primary meiocyte wall (cellulose); C, special meiocyte wall (callose); P, site of a severed plasmodesma.

observations were made. The remarkable contrast between the pre-leptotene and the diplotene cytoplasm is brought out by Figs. 4 and 5. Few ribosomes were discernible at all in the cytoplasm of diplotene and diakinesis cells, but small aggregates were usually apparent within the multi-membraned bodies which replace the plastids during this part of the meiotic prophase.

Adenine/guanine ratios for total cell RNA at different meiotic stages are set out in Table 1 for *L. henryi* and *T. erectum* with the corresponding ratios for the DNA of the two species. In each species there is a significant increase in the A/G ratio during the period of least RNA content. The ratio shifts towards that characteristic of the DNA, and in *L. henryi*, where the A/G ratio of the DNA is greater than in *T. erectum*, the upward shift is proportionately greater.

Table 1. ADENINE/GUANINE RATIOS FOR TOTAL CELL RNA OF *Lilium henryi* AND *Trillium erectum* DURING MEIOSIS

<i>L. henryi</i>			
Meiotic stage	Adenine/Guanine	Units/estimation	Replicates
Zygotene	0.99 ± 0.02	3 cells	7
Pachytene	1.24 ± 0.04	6 cells	10
Diplotene-diakinesis	1.67 ± 0.10	6 cells	7
Metaphase II–telophase II	0.95 ± 0.04	6 dyads	8
DNA	2.04		120
<i>T. erectum</i>			
Meiotic stage	Adenine/Guanine	Units/estimation	Replicates
Leptotene–zygotene	0.85 ± 0.03	3 cells	17
Diplotene	0.96 ± 0.02	6 cells	7
Metaphase II–telophase II	0.87 ± 0.04	6 dyads	13
DNA	1.48		60

These observations answer the question posed in the introductory paragraph: the cytoplasm received by the spores is not merely a portion of that present in the mother cells, for a drastic reorganization occurs immediately before the cleavage. Speculation on the significance of this fact is perhaps premature, but it may be noted that

wholesale elimination of the ribosomes of the diploid cell might be understandable if it were a requirement for the initiation of the haploid phase that a new protein-synthesis system should be established. This could be a device ridding the cell of long-lived messengers, or for restoring to a norm whatever controls of gene action transmissible through mitotic cycles may be effective in the diploid cell lineages<sup>4</sup>.

Among the questions raised by these findings are the location and nature of the residual RNA present at the time of the elimination of ribosomes. That its base composition is closer to that of the DNA of the species concerned than to that of the ribosomes may indicate a nuclear site; but the seeming persistence of small clusters of ribosome-like bodies in the modified organelles may be significant.

A further problem is the source of the ribosomes that populate the cytoplasm anew by metaphase I. The timing of the principal increase appears to be post-diakinesis—at a period, that is, when no nucleolus is present. Incorporation experiments have not given evidence of any massive RNA synthesis during the interval in question<sup>5,6</sup>. This is, however, perhaps to be expected, because the meiocytes and later the spores in the tetrad form closed systems after the sealing of the callose wall<sup>7,8</sup>, and presumably the new synthesis is largely at the expense of the pool of break-down products released by the degeneration of the ribosomes originally present.

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## BIOLOGY

### Effect of Temperature on the Selective Value of Genotypes of the Copepod *Tisbe reticulata*

IN the Lagoon of Venice, the harpacticoid copepod *Tisbe reticulata* shows a striking polychromatism which is controlled by three alleles at the same locus, *VV*, *VM* and *v*. The first two, dominant over the common recessive *v*, are responsible for the forms violacea and maculata, respectively, whereas the homozygote *vv* is responsible for the form trifasciata. The alleles *VV* and *VM* are co-dominant and therefore the heterozygote *VV VM* is phenotypically recognizable. It has been shown that this polymorphism is adaptive and balanced, and, in most cases, maintained

Table 1. PHENOTYPE DISTRIBUTIONS IN SPRING AND SUMMER SAMPLES OF THE COPEPOD *Tisbe reticulata*

Population			Phenotypes				Total	$\chi^2$ and $P$
			Maculata	Violacea	Maculata-Violacea	Trifasciata		
Spring	$P$	Observed	36	34	5	41	116	$\chi^2 = 2.54$ $P > 0.3$
		Expected	31.2	30.4	7.8	46.6		
	$F$	Observed	1,370	1,292	508	2,156	5,326	$\chi^2 = 123$ $P < 0.001$
		Expected	1,421	1,339.5	329.7	2,235.8		
Summer	$P$	Observed	27	52	8	68	155	$\chi^2 = 0.46$ $P > 0.9$
		Expected	29.1	49.1	8.3	68.5		
	$F$	Observed	1,693	1,931	597	3,729	7,950	$\chi^2 = 150.6$ $P < 0.001$
		Expected	1,746.6	1,991.5	367.3	3,844.6		

by the greater survival value of the heterozygotes compared with the homozygotes<sup>1</sup>.

There is experimental evidence that salinity may be a selective environmental factor. In fact, the various forms show different relative abilities to tolerate fluctuations in salinity<sup>2,3</sup>.

Recent observations suggest that water temperature may also act as a selective factor to an even greater extent than salinity. The experiments reported here have been carried out, both in natural and laboratory populations, to provide more detailed information on the action of this important ecological parameter.

In the Lagoon of Venice the species can be found only in late spring and in summer, and practically disappears in autumn and winter. Only on a few occasions have we been able to collect samples of *Tisbe* sufficiently large to permit estimates of gene frequencies. A first sample of 116 females was collected in May, when the water temperature was about 18°C. Very few males have been collected, and as they are not easily classifiable they were not considered.

The females of this sample, which we shall refer to as the parental population ( $P$ ), were individually transferred into single culture vessels in a constant temperature room at 18°C, according to the method usually followed in our laboratory for rearing copepods<sup>2</sup>. They were left in the vessels until they had produced two egg sacs each, in order to get a sufficient number of offspring for genetic analysis.

The first generation, or  $F$  population, consisted of 5,326 individuals of both sexes. By analysis of this first generation—and, where necessary, of the next two generations—it was possible to identify the genotypes of each mother as well as the type of cross. This permitted us to infer also the genotypes of the male parents although not necessarily their actual frequency in nature. The gene frequencies in the  $P$  population were thus counted directly, whereas in the  $F$  population they were calculated by applying the Hardy-Weinberg formula.

A second sample of 155 females was collected in the same area in July, when the water temperature was about 24°C. Their first generation in the laboratory consisted of 7,950 individuals of both sexes.

The distributions of phenotypes in the spring and summer population samples are given in Table 1. The data show that in the  $P$  populations of both samples there is quite good agreement between the observed distributions of the various phenotypes and those expected on the basis of the Hardy-Weinberg. In the  $F$  populations there is, instead, a strong disagreement between the observed and the expected distributions, chiefly caused by the excess of the heterozygotes violacea-maculata.

The results of previous experiments demonstrated that the excess of heterozygotes increases with the degree of crowding in the cultures. Actually, the  $F$  populations have developed in very crowded conditions, principally because they derive from females collected in the wild, which usually lay more eggs.

The gene frequencies of spring and summer samples are shown in Table 2. A comparison between the gene frequencies of the two parental populations indicates a

slight increase of  $VV$  and  $v$ , and a strong decrease of  $VM$  in the hot season. The difference is statistically significant ( $\chi^2 = 4.114$ ,  $P < 0.05$ ) only so far as  $VM$  is concerned.

Table 2. GENE FREQUENCIES IN SPRING AND SUMMER SAMPLES OF THE COPEPOD *Tisbe reticulata*

Population		Gene frequencies (per cent)		
		$VM$	$VV$	$v$
Spring (18°C)	$P$	18.5	18.1	63.4
	$F$	18.1	17.1	64.8
Summer (24°C)	$P$	12.9	20.6	66.5
	$F$	14.3	16.1	69.5

The environmental factor which shows the greatest change from spring to summer in the waters of the Lagoon of Venice is temperature. Salinity, nutrients and other factors, although fluctuating, do not undergo consistent changes in the same period. We therefore assumed that the observed changes in gene frequencies might be caused by a change in the temperature of the water.

A further suggestion that temperature could be important in this respect comes from the fact that the gene frequencies of the  $P$  and  $F$  populations do not seem to differ from each other in the spring sample, whereas they do differ in the summer sample. In the spring sample the  $F$  population developed in the laboratory at the same temperature (18°C) as the parental population in the wild; however, in the summer sample the parental population developed at 24°C and the offspring at 18°C.

Of the three genes considered,  $VM$ , which shows the greatest difference in frequency between the spring and the summer samples, seems to be more affected by temperature. Its selective value seems to be greater at lower temperatures, and vice versa.

The validity of this working hypothesis is supported by the results of experiments carried out with laboratory populations, maintained by a method the details of which have been given before<sup>4</sup>. The experiments have been made with populations in which only the genes  $VV$  and  $VM$  were present. One population was started with 80 per cent  $VV$  and 20 per cent  $VM$  and another one with 80 per cent  $VM$  and 20 per cent  $VV$ . Both populations were kept at 18°C and sampled from time to time, with an average interval of 20 days, during a period of 250 days, corresponding to about seventeen generations<sup>5</sup>.

Two other populations, with the same initial composition as the first two, have been kept at 23°–24°C and sampled at slightly shorter intervals for a period of 230 days which, at this temperature, corresponds to about twenty-four generations<sup>6</sup>. The populations maintained at 18°C reached equilibrium after about fifteen generations, at a frequency of the gene  $VM$  of about 60 per cent. The populations kept at 23°–24°C also reached equilibrium after fifteen generations, but in this case the equilibrium frequency of  $VM$  was of about 40 per cent.

These experiments show that the adaptive value of the gene  $VM$  can be influenced to a large extent, both in natural and experimental populations, by temperature. This is a result similar to those obtained in the study of chromosomal polymorphism of certain species of *Drosophila*.



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## Food-chain Toxicity of Systemic Acaricides to Predaceous Mites

It is considered that one of the theoretical advantages of systemic materials applied to roots or stems is the selective action against phytophagous pests without toxicity to their predators. This ecological selectivity was demonstrated by Ripper<sup>1</sup>, who found that bean plants dipped in a solution of dimefox were toxic to both aphids and predators, but when dimefox was applied as a root drench, aphids were killed and predators survived.

Organophosphate materials have proved to be highly toxic to the predaceous mites *Typhlodromus fallacis* (Garman) and *Phytoseiulus persimilis* Athias-Henriot<sup>2</sup> and, in detailed toxicological studies, parathion was found to be three times more toxic to *P. persimilis* than to the prey mites, *Tetranychus urticae* Koch, at the  $LD_{50}$  level<sup>3</sup>. The use of ecological selectivity by applying root drenches of systemic acaricides seemed to be a reasonable approach to an integrated control programme for the two-spotted spider mite on cucumbers. Because several materials had been found to be effective as root drenches against the two-spotted mite<sup>4</sup>, the effect of similar treatments on *P. persimilis* was examined.

'Burpee Hybrid' cucumbers were grown in sand with a complete nutrient solution. The systemics were dissolved in 1 ml. acetone and 99 ml. water were added to give solutions 5 ml. of which were applied to the base of each small cucumber plant. After 24 h the first true leaf was excised and placed with the bottom surface up on a wet cheesecloth pad. Thirty adult female two-spotted mites and five adult female *P. persimilis* were placed on a leaf and the temperature was held at 23° C. Predator mortality was evaluated after 24 h. The toxicity of similarly treated leaves to *Tetranychus urticae* alone was determined after exposure for 48 h.

The differential effect on predators and prey is shown in Table 1.

From previous work<sup>4</sup> the  $LD_{50}$  values (in  $\mu$ g/plant) for the same type of treatment against *T. urticae* were: dimethoate 138, phorate 93, 'Temik' 154 and thionazin 106. These systemics were all of the same order of toxicity to the two-spotted mite. If there were complete ecological

selectivity, predators should not be killed by this type of treatment because they do not feed on the plants. It is obvious that there is a food-chain toxicity when predators fed on prey mites which were feeding on toxic plant juices. There were significant differences in the predator mortality caused by the various systemics and the order of decreasing toxicity was dimethoate, thionazin, phorate and 'Temik'.

The only material which consistently favoured *P. persimilis* was 'Temik'. This material is a carbamoyl oxime while the other three are phosphorothioates or phosphorodithioates. Perhaps the predator mites can metabolize 'Temik', so that it is not accumulated. The predators fed on many prey in a period of 24 h, and if they are not able to metabolize the phosphate materials there would be a "biological magnification" of the residues comparable with that found in other ecosystems<sup>5</sup>.

Thus the application of systemic acaricides to the root zone does not provide an ecological selectivity in all cases, and materials will have to be evaluated to find those that do not show a food-chain toxicity to the predators.

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<sup>1</sup> Ripper, W. E., Geenslade, R. M., and Hartley, G. S., *J. Econ. Entomol.*, **44**, 448 (1951).

<sup>2</sup> Smith, F. F., Henneberry, T. J., and Boswell, A. L., *J. Econ. Entomol.*, **56**, 274 (1963).

<sup>3</sup> Herne, D. C., and Chant, D. A., *Canad. Entomol.*, **97**, 172 (1965).

<sup>4</sup> McClanahan, R. J., *Proc. Entomol. Soc. Ont.*, **97** (in the press).

<sup>5</sup> Hunt, E. G., in *Scientific Aspects of Pest Control*, 251 (Nat. Acad. Sci. Publ. 1402, Washington, D.C., 1966).

## Regulation of Egg Output of Populations of *Ostertagia ostertagi*

THE concentration of eggs or larvae of parasitic nematodes in the faeces of host animals has long been regarded as an indication of the number of mature parasites present. Several causes of variation in the relationship between the number of worms and the faecal egg count have been considered, among them changes in the quantity of faeces passed and in its consistency, and diurnal fluctuations in count have been much studied. Moreover, it is recognized that the ovulation of parasites in a resistant host may be inhibited.

Recent work with *O. ostertagi* in cattle has contributed to an understanding of how the egg output of populations of this parasite is regulated. Groups of calves were fed infective larvae daily at different rates with the consequence that their worm burdens were maintained at different levels. The pattern of their faecal egg counts, shown in Fig. 1, was identical, rising to the same peak and declining in the same way. The mean faecal egg counts of groups of calves, carrying different numbers of worms, were the same at any given time, and, by slaughtering calves from each group at intervals and examining their worm burdens, it was found that on each occasion the total number of ova contained in the uteri of all the worms from each calf was very similar.

In groups of calves infected with different numbers of larvae on one occasion only, the faecal egg counts were also very similar although the number of worms present was different. Calves from each group were slaughtered at intervals and it was found that the numbers of eggs contained in the uteri of all the female worms in each calf was about the same. These findings suggest that there is a limit to the number of eggs which can be produced by the population as a whole and that, except in very small populations, the worms produce eggs at a rate considerably lower than that of which they are potentially capable.

Such a limitation might occur if the production of each egg required a fixed quantity of some factor which was present in a limited amount. There is evidence, however,

Table 1. EFFECT OF SYSTEMIC ROOT DRENCHES ON TWO-SPOTTED SPIDER MITES AND THE PREDACEOUS MITE *Phytoseiulus persimilis*

Treatment	Dosage ( $\mu$ g per plant)	Mortality (%)	
		Two-spotted mite	<i>P. persimilis</i>
Dimethoate	150	36	96 (25)*
	50	44	85 (13)
	35	29	93 (15)
Phorate	150	88	84 (25)
	150	86	100 (13)
	100	75	44 (16)
'Temik'	225	100	25 (16)
	200	94	30 (10)
	100	70	0 (25)
Thionazin	150	90	100 (25)
	80	89	100 (25)
	35	72	50 (16)

\* Number of predators in each test.



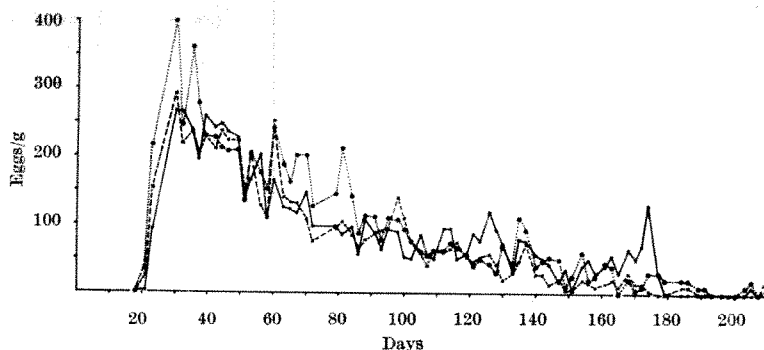


Fig. 1. Mean faecal egg counts of three groups of calves infected daily with 500 (●—●), 1,000 (×---×) and 1,500 (○....○) larvae, respectively, and carrying appropriately different burdens of worms.

that the immunity of the host is involved. Treatment of the host with cortisone derivatives largely eliminates the restriction of egg output and egg counts of calves given cortisone may be many times higher than those of control calves carrying a comparable number of worms (Fig. 2). The egg counts of groups of calves receiving similar cortisone treatment, but infected at different rates, were the same, which indicates that the effect of cortisone had been to raise the limit to egg production and not to stimulate the worms directly. Abnormally high egg counts may also occur in calves which appear unable to respond normally to infection and in calves clinically affected by severe ostertagiasis in which the absence of the usual signs of resistance suggests that immunity has broken down.

Faecal egg counts of calves receiving regular daily doses of infective larvae, of calves infected on one occasion only and of naturally infected calves all follow the same pattern, rising to an early peak from which they decrease logarithmically (Fig. 3). This pattern appears to be quite independent of whether the numbers of worms are increasing or decreasing. In calves infected once, egg counts begin to decline before the number of worms and although egg counts decrease logarithmically the curve of decreasing worm numbers resembles a rectangular hyperbola. It seems that the regulation of egg output is a self-contained mechanism. The logarithmic form of decrease implies

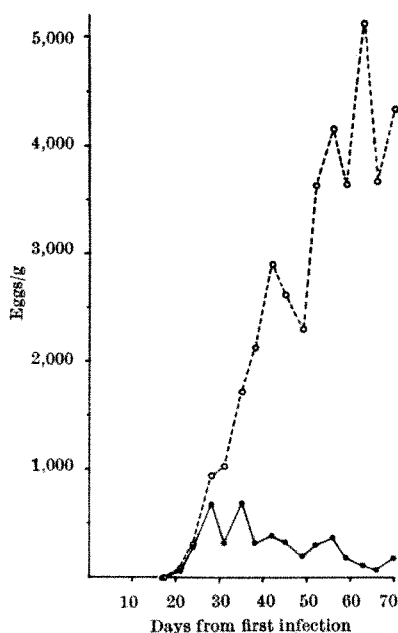


Fig. 2. Mean faecal egg counts of two groups of infected calves. ●—●, Control group; ○---○, group treated with cortisone.

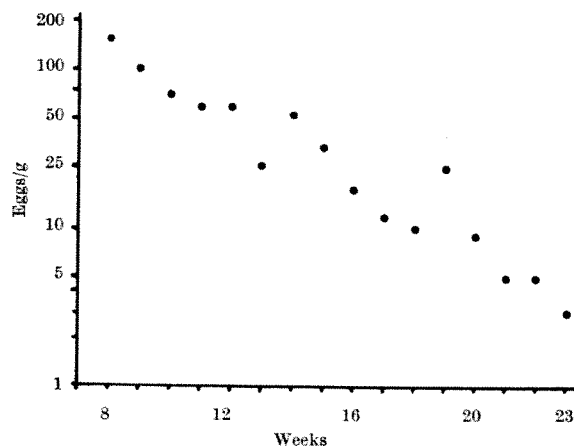


Fig. 3. Faecal egg counts (on a logarithmic scale) of a group of calves which became infected on pasture.

that the egg count at any one point in time bears a constant relation to that at another point in time which precedes it by a fixed interval. This could mean that the limit to egg production depends on the experience that the host has had of the egg laying activity of the worms and not on any other aspect of the infection.

Logarithmic decreases in egg counts have been seen in infections of many species of nematodes in a great variety of hosts. When the characteristics of the egg count curve have been noted, as by Sarles<sup>1</sup>, who fitted curves of the form  $y = a(b)^{-x}$  to the egg counts of dogs infected with *Ancylostoma caninum*, it has been assumed that the curve reflects a loss of worms proceeding at a constant rate.

The finding that faecal egg counts are likely to run a stereotyped course which bears little relation to the worm burden has two important practical implications. First, within wide limits, the extent to which a pasture will be contaminated when grazed by cattle infected with *O. ostertagi* will not be influenced by how heavily the calves are infected. Secondly, no conclusions regarding the worm burdens of calves can be drawn from faecal egg counts except in two situations where these differ greatly from the expected pattern. Very low counts in the second month after first exposure to infection are likely to mean that few worms are present and high counts in calves that have been exposed to infection for some months and in which they would normally have fallen to low levels, may indicate severe helminthiasis.

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<sup>1</sup> Sarles, M. P., *Amer. J. Hyg.*, **10**, 667 (1929).

## BOOK REVIEWS

### ATOMIC ENERGY AND AGRICULTURE

#### Isotopes in Plant Nutrition and Physiology

(Proceedings of a Symposium on the Use of Isotopes in Plant Nutrition and Physiology jointly organized by the IAEA and the FAO of the UN, and held in Vienna, 5-9 September 1966. Proceedings Series.) Pp. 596. (Vienna: International Atomic Energy Agency; London: H.M.S.O., 1967.) 323 schillings; 84s. 4d.; \$12.50.

THE publication of the proceedings of agricultural symposia by the International Atomic Energy Agency in association with the Food and Agriculture Organization of the United Nations has nearly become an annual event. The first meeting took place in Bombay in 1962; Brunswick and Ankara were visited in 1963 and 1965. In September 1966 a meeting on "Isotopes in Plant Nutrition and Physiology" was held in Vienna. The forty-five papers presented at that meeting are reproduced, together with the resulting discussion, in this volume.

The joint FAO/IAEA Division on atomic energy in agriculture is located in Vienna. Its objectives are not explicitly set out in this volume, but they are perhaps implied in the foreword: "Shortage of food is the world problem of our time, and there is little doubt that the adequate and correct use of fertilizers provides the most certain and rapid means of increasing food supplies. Studies such as are reported here are of great value to the proper understanding of the basic factors affecting plant nutrition and crop fertilization. For many such research problems in plant physiology and nutrition the use of isotopes has become indispensable, and the scope for isotope techniques is great." Similarly, at the beginning of the previous volume it was stated that "The early application of radioisotopes to studies of soil fertility and plant nutrition revealed new approaches and gave rise to new research procedures which are yielding valuable additional information on improved methods of crop production", and that the symposium had been arranged to discuss recent progress in this field. Thus it seems that the organizers of this enterprise wish their publications and other activities to be judged by their potential contribution towards raising the standard of agricultural production.

The reader who seeks news of such achievements is likely to turn first to the opening group of papers in this volume which are concerned with the nutrition of plants grown in soil and the concluding sections on genetic aspects of nutrition. The decision of a library committee on whether to purchase the book is also likely to be determined by the content of these sections. The remainder of the book, over half the total, is concerned with a wide range of topics in plant physiology, mostly relating to inorganic plant nutrition, though the movement of growth substances and photosynthesis receive some mention. The main link connecting these varied topics appears to be the use of tracer methods. A few of the contributions are by well known authors in Europe or the United States, whose work is familiar from scientific journals.

Representatives of the European Association for Research in Plant Breeding contributed to the discussions on genetic aspects of nutrition and F. G. H. Lupton (Cambridge) summarized lucidly the importance of this subject in plant breeding, noting important questions on which information is meagre. Among the subjects reviewed were the adaptability of species to nutritional

factors (R. G. Turner, J. Antonovics and their associates, Aberystwyth) and intervarietal differences in response especially to minor nutrients (J. C. Brown, Beltsville). Many of the contributions were of much narrower interest, attention being confined to the quantities of ions absorbed by different varieties in a single set of environmental conditions. In discussion G. G. J. Bange (Netherlands) aptly indicated the limitation of this approach; changes in environmental factors can induce similar effects on the uptake of nutrients by a single species. Unfortunately, his comments did not stimulate general discussion on the types of physiological observations which are meaningful and it is surprising that relationships between nutrient uptake and the growth and morphology of plants, especially their root systems, received so little mention. Considerable differences in the total extent and branching of root systems can occur between closely related varieties, and may markedly affect the intimacy of contact between roots and the soil. Thus the comparative study of root form would appear to be one of the most obvious starting points (though not necessarily the only one) in work on varietal differences in absorption of nutrients from the soil. The relevance of this aspect is evident from the results presented by Baker and his colleagues (Pennsylvania). Some intervarietal differences observed in field conditions were not reproduced in plants grown on a homogeneous substrate in the greenhouse; intervarietal differences in the depth of rooting were suggested but not examined. This is a field in which the "whole-plant" physiologist, interested in nutrition, could make a considerable and essential contribution.

Because of the complexity of factors which affect the availability to plants of nutrients in the soil and the consequent slow progress of research, it would be unreasonable to criticize the papers on this subject because no important new concepts were discussed. The types of tracer procedure used in most of the experiments, however, have now been used for a sufficient period for it to be reasonable—and because of the cost involved most desirable—to consider their likely long-term contribution to the solution of agricultural problems.

There is, of course, no doubt that tracer methods can be of considerable value—indeed often indispensable—in work on the physical chemistry of ions in the soil or the absorption mechanisms of plants. But the same is true of many other modern research tools, and it does not establish that special place for tracers in the armament of the research worker which symposia of the present type may suggest. This volume provides no clear evidence to refute the view that in field studies of the use of fertilizers, as opposed to basic research, conventional chemical methods may yield information of equal practical value.

The use of "labelled" fertilizers in large field experiments has been encouraged in a number of parts of the world, and so the description by Cho and his colleagues (Joint FAO/IAEA Division of Atomic Energy in Agriculture) of an experiment in the placement of ammonium sulphate as a fertilizer for maize, has topical interest. Eight methods of placement were compared but none affected the yield and nitrogen content of the crop. The use of nitrogen-15 as a tracer, however, enabled uptake from a fertilizer zone to be compared with that from the remainder of the soil and appreciable differences between different methods of placement were evident early in the season. These findings are valuable only if it is valid to assume that the pattern of uptake from different parts of the soil would have been exactly the same if the experiment had been carried out on a soil where fertilizers are beneficial. There seems to be no basis for believing this—especially when no direct evidence is provided—because gradients of nutrients can markedly affect root development and performance. Would it not be wise to limit experiments on fertilizer placement to areas where crops respond to fertilizers?

In the early stages of the evolution of a technique which demands new expertise, there may be some justification

for special arrangements to stimulate attention to the new opportunities it may provide. Once a procedure has become generally accepted and widely used, as tracers now are, such arrangements may become redundant, or even a disadvantage; they may encourage undue emphasis on one type of approach relative to others—and also inflate an already distended literature. The present volume suggests that it may now be timely to consider whether this situation has been reached in the Vienna agricultural enterprise. It would be of much interest if the Food and Agriculture Organization were to review this subject. With its wide responsibility for agricultural development throughout the world that organization is well placed to assign tracer methods to their appropriate place side by side with other modern methods for studying the field problems of agriculture. If the volume should stimulate such discussion, its publication would be most welcome.

R. SCOTT RUSSELL

## KEEPING FOOD FRESH

### Radiation Preservation of Foods

A Symposium. (Advances in Chemistry Series, No. 65.) Pp. viii + 184. (Washington, D.C.: American Chemical Society, 1967.) \$7.

ALTHOUGH attractively turned out, this volume represents a classic example of a symposium publication which might have served a useful purpose had it been available within a reasonable time after the meeting it reports (September 16–17, 1965). As it is, it is limited to work done in the United States and Great Britain, and much of the material which it contains has been superseded by the rapid publication of the proceedings of the International Symposium on Radiation Preservation of Food sponsored jointly by the FAO and the International Atomic Energy Agency, which was held in Karlsruhe in 1966 (*Proceedings of an FAO/IAEA Symposium on Food Irradiation*, Karlsruhe, 1966, STI/PUB/127, Vienna, IAEA, 1966, H.M. Stationery Office). Indeed, much of the blame for the delay in publication of the present volume can be laid squarely on the editor, as two papers by one author bear a “date received” which is one full year later than that on the remaining papers.

If papers given at specially organized symposia are to be of value, they must appear promptly, and the discussion which develops from them is often of greater importance than the papers themselves. As it does not include discussion, the present volume fails on both grounds; however, the quality of the individual papers is good. Of particular interest to me was the voluminous (but data-packed) contribution on *Radioactivity Criteria for Radiation Processing of Foods* by H. W. Koch and E. H. Eisenhower. They point out that a useful criterion for induced radioactivity is its non-measurability—by limiting the maximum energy of the radiation used to less than 10 MeV, radioactivity induced by irradiation of foodstuffs is unmeasurable. T. R. Benn, in a report on shallow irradiation of oranges to delay or prevent mould infestations, shows the technical feasibility of the process but underlines the lack of a commercial market for this process in the United States, while A. F. Novak *et al.*, in a report on the radiation pasteurization of fish and shellfish make the cogent point that radiation processing offers no panacea to replace quality control in the selection of wholesome products for preservation. Radiation processing will not improve the quality of poor food, but it will retain the attributes of good food.

In the preface to this volume, the symposium chairmen point out that in a world where more than half the human race is ill-fed, more than one-third of all food grown or raised is spoilt. The application of ionizing radiation for food preservation has great potential, but because it is not yet a commercially competitive process in the well

developed countries and is thus little used, the developing world views irradiated foods with suspicion—“if you don’t eat it, why should we?” This very real problem seems to have received little attention in the present volume, and indeed, despite the wide ranging topics of the fifteen papers it contains, this book cannot be regarded as a comprehensive view of its subject. ROGER J. BERRY

## LIFE UNDER CONTROL

### Regulation and Control in Living Systems

Edited by H. Kalmus. Pp. viii + 468. (London, New York and Sydney: John Wiley and Sons, Ltd., 1966.) 90s.

THE most characteristic feature of living organisms is their capacity to adapt themselves, by means of regulatory responses, to a wide range of different circumstances. Until a generation ago many biologists ascribed this regulatory capacity to “vital” forces, not amenable to a mechanistic analysis. This attitude has radically changed during the last two decades. The gap between “mechanistic” and “vitalistic” systems has been greatly narrowed by developments from both sides of the gap. Mechanistic systems, thanks to progress in engineering, mathematics and computer science, can closely simulate the regulatory responses of living systems, and the analysis, by the methods of biochemistry and molecular biology, of biological controls has provided an extensive understanding on the basis of the physical and chemical properties of the molecules concerned.

In this book, Dr Kalmus and twelve other experts (mostly from University College, London) survey various aspects of biological control mechanisms. The emphasis is on the conventional “biological” type of control based on hormonal rather than molecular analysis. Brown-Grant discusses extensively regulation and control in the endocrine system. Other chapters deal with the regulation of plant growth, by P. R. Bell, of animal development, by D. R. Newth, and with circadian regulation, by H. Kalmus. Three chapters review the control of populations. These are by J. B. Free, on seasonal regulations in the honey bee colony, A. D. Blest, on relationships between insects and their predators, and V. C. Wynne-Edwards, on regulation in animal societies and populations.

The molecular aspects of regulation are discussed by D. Lewis. There are also chapters on the basic mathematics of control and on control in engineering, by B. R. Wilkins, and on the early history of biological regulation, by J. S. Wilkie.

This book will be of great value to those who are interested in a broad survey of the subject.

H. A. KREBS

## MAN, TREES AND HISTORY

### Forestry in the English Landscape

By Roger Miles. Pp. 303 + 39 plates. (London: Faber and Faber, Ltd., 1967.) 105s. net

MULTIPLE land-use is a much discussed topic nowadays but implementation of planning is frequently difficult to achieve. Often forestry has a part and sometimes an important part to play in land-use because it can provide utility, amenity, recreation and sport. Roger Miles combines the professions of forester and landscape architect and in his book he traces the importance and the neglect that have been given to trees in the English landscape. This historical account is not only interesting but essential to a proper understanding of the subject because it does show the background to the present philosophy of landscape design. Evelyn’s concern at the devastation of England’s woodlands and the likelihood of a shortage of oak for the navy brought about a short-lived campaign of

planting. But it was largely due to the eighteenth and nineteenth century landowners that an English landscape was created by large-scale planning and planting of broad-leaf-trees for aesthetic and material reasons. With the industrial revolution came blocks of conifers and the rise of pressure groups which tried, without any unity, to adjust a balance between the utilitarian and the aesthetic. The next phase was dominated by war and the Forestry Commission and its modifications in policy and by lethargy and then enthusiasm in non-State forestry. Now we have arrived at the stage where multiple land-use is accepted, certainly in theory, but not yet sufficiently in practice.

Roger Miles uses his experience on Exmoor as "a contribution to the study of techniques for expanding the forest industry so that harmony between use and beauty can be assured". He shows how his system of survey, analysis and deduction can be applied to make positive recommendations and his sketches amply demonstrate his text. This book will attract the attention of those interested in the countryside whether they be professionals or amateurs. It is factual (except for a confusion between the Universities of Aberdeen and Edinburgh on page 129), well presented and bears the stamp of the unbiased expert.

C. J. TAYLOR

## DO NOT EAT

### Deadly Harvest

A Guide to Common Poisonous Plants. By John M. Kingsbury. Pp. 128. (London: George Allen and Unwin, Ltd., 1967.) 21s. net

THIS small book, intended primarily as a guide to common poisonous plants of the United States for the non-specialist, is written in clear, intelligible language. The subject matter is concerned chiefly with the higher plants but there are brief accounts of a few toxic plants belonging to other groups such as the fungi and algae.

The author defines a poisonous plant as one which contains a specific substance, often still unidentified, which produces a deleterious reaction in the body of man or animals when taken in a small or moderate amount. Plants which provoke allergic reactions such as "hay fever" and dermatitis are therefore excluded from consideration, with the exception of the very useful account of the allergic reaction caused by poison ivy (*Toxicodendron*) and a few other well known plants which are commonly, if mistakenly, regarded as poisonous by the public.

The historical background to our knowledge of poisonous plants of the United States in relation to both man and animals is well covered and interesting as is the discussion of such plants grouped according to the poisonous principles involved (for example alkaloids, glycosides, saponins and oxalates). A particularly useful part of the book is that devoted to plants which everyone should recognize as dangerous, with emphasis on common garden plants and weeds. This is followed by advice on treatment of cases of poisoning which may be summed up as "Send for the doctor, quick!"

Although primarily written for the American public there is a considerable amount of data relating to European plants commonly planted in the New World. Transatlantic visitors to the United States will undoubtedly find this book helpful especially families with young children. The majority of the plants encountered are illustrated by small sketches, but while these give a rough idea of what the species look like, I doubt if they are always sufficient to enable anyone totally unfamiliar with the flora of North America to recognize them.

Although useful as a book of reference this can also be enjoyed by being read at random. In this way one may read an account of the death of Socrates after drinking his cup of hemlock (*Conium maculatum*), learn of the gruesome symptoms of poisoning by water

hemlock or cowbane (*Cicuta virosa*) which led to the death of one poor unfortunate Jacob Maeder in 1670, or find that such familiar plants as lily-of-the-valley (*Convallaria majalis*) and wisteria are toxic. It may also come as a surprise to those who, like me, consume apples down to and including the core and pips, that the latter are decidedly poisonous. A case is cited of a man who considered apple pips such a delicacy that he saved them until he had a cup full and then ate them with fatal results. One can but hope the poison is not cumulative!

British readers should be warned that while the author refers to the plants by their popular non-scientific names, these do not always refer to the same plant so-called in this country. For example the term cowslip in the United States is used as an alternative popular name for the marsh marigold (*Caltha palustris*). An index is provided however, in which latin generic names are indicated against each popular name. One further criticism is that because this work is published in Britain it is a great pity the text was not suitably modified to include accounts of some of our more common toxic plants such as deadly nightshade (*Atropa belladonna*), henbane (*Hyoscyamus niger*), and laburnum, to name but a few.

This attractively produced little book, however, succeeds well as a guide to the common poisonous plants of the United States and the author is to be congratulated for presenting his data in both readily understandable language and an interesting manner.

DEREK A. REID

## CANCER RESEARCH GOES ON

### Methods in Cancer Research

Vol. 1. Edited by Harris Busch. Pp. xvi + 612. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1967.) 224s.

THE first volume of this series is divided into three sections, "Morphology", "Transplantation and Metastasis", and "Carcinogenesis". The ten chapters have been written by different authors. The first three chapters on electron microscopic cytology, autoradiographic methods, and karyological methods have no specific bearing on oncology and deal with methods for the analysis of normal as well as cancer cells. These chapters offer concise practical information for applying the techniques and are sufficiently detailed for the beginner. Some of the individual sections possibly are too brief, but in general adequate reference to more detailed accounts is given.

The chapter on tumour transplantation contains sections on inherited susceptibility to transplanted cancer, histocompatibility genes, actively acquired immunity and exceptions to the laws of transplantation. The section on transplantation techniques is well written and illustrated by helpful diagrams. The remainder of the chapter describes tumour-host relationships, tumour progression, metastasis, and factors affecting tumour growth.

The chapter on tumour metastasis has sufficient scope and practical detail to be of interest to a wide range of readers. The section on carcinogenesis contains chapters on epidemiology, tests for chemical carcinogens, aminoazo carcinogenesis, viral oncogenesis and the identification of viruses by electron microscopy.

Adequate testing of compounds for carcinogenic activity, for co-carcinogenic activity or for other synergistic effects has become more important as it has become more obvious that many human cancers are produced by environmental carcinogens. This comprehensive critical account is therefore timely. There are one or two statements, however, concerning the carcinogens themselves which are unjustifiably presumptive, in particular "that compounds which cause tumours at the point of application are the actual (carcinogenic) agents", because there is now considerable



evidence to implicate metabolites of the carcinogenic hydrocarbons, for example, as the proximate carcinogens. The following section in which enzyme activations are discussed is weakened by the almost total absence of literature citations.

The chapter on aminoazo carcinogenesis is heavily weighted in favour of the importance of protein binding in carcinogenesis and the section describing nucleic acid interactions is too sketchy and inadequate by comparison. A statement such as "except for a few papers, the binding of the carcinogenic aminoazo dyes to rat liver nucleic acids has not yet been confirmed" obviously requires further amplification and a list of the references the author has in mind.

The long chapter on viral oncogenesis contains a wealth of information, although the factual, sometimes uncritical manner in which the material is presented tends to make reading tedious. The final chapter describing viral identification is very technical and might have been better placed before the preceding chapter.

This volume should prove useful to those wanting to acquire a general background knowledge of cancer research and the specific techniques employed in it; it is relatively free from errors and is adequately illustrated.

G. P. WARWICK

## LOOKING DOWN

### Air Survey in Economic Development

By Rolt Hammond. Pp. viii + 246. (London: Frederick Muller, Ltd., 1967.) 45s.

It would be very difficult to guess what topics are included in this book, for the subject could be approached in a number of ways according to the author's specialist interest. In general, the book covers some aspects of photogrammetry, air photo interpretation with special reference to geology, airborne geophysics, survey aircraft and navigational aids, and a review of techniques, and examples, of selected aspects of air surveys which have been carried out. The two main defects of the book very soon become evident. The first is the way in which the material is presented; the second is in the breadth, balance and content of the material selected.

The chapter titles suggest an orderly and systematic approach which is far from evident in the text. For example, chapter four, "Cameras and Plotting Equipment", includes detailed information about colour film processing, stereoscopy, and a comparative study of colour and black and white film in a photogeology study of Hong Kong. On the other hand, there are lengthy descriptions of plotting equipment under the heading of "Air Survey Techniques" in chapter two. Nowhere is there a comprehensive review of the types of aerial photographs in current use. Information on this topic is scattered throughout the book, often in the most unsuitable places; for example, an explanation of Trimetrogon photography occurs in the middle of a section on air survey for geological purposes.

Most of the information in the book is either a summary, or a direct copy, of an article or paper. Unfortunately, the choice of material seems to have been quite random. In many instances, instead of a general review of the topic under consideration the information presented deals either with only a single, and often non-representative, example or it is a review or copy of an entire paper, much of which is quite irrelevant to the topic under consideration. This results in considerable repetition; photogeology is dealt with in three different chapters in spite of the index giving only one.

At the end of most chapters the references are listed, although there are a number of instances where papers are referred to quite extensively in the text, but which are not listed. The author's obvious lack of specialist

knowledge in the topics he deals with is evident in the apparently haphazard choice of material, much of which is not really suitable, and most of which is out of date. A typical example is the way in which he has dealt with the measurement of distance using electronic instruments. These instruments use either micro radio waves, light waves or lasers, but only one make—the 'Tellurometer'—a radio-wave instrument, is dealt with. Furthermore, the bulk of this deals with the early 10 cm wavelength models (*MRA* 1 and 2), and, except for the general discussion on the theory of measurement, most of what is written is now only of historical interest. The lengthy details of field operation and accuracy tests of these early models are of little value now that newer and more improved models are available. The whole range of light wave instruments are disposed of in a single paragraph, and lasers are not even mentioned.

The much shorter reference to a more recent model of the 'Tellurometer' range (model *MRA* 101) is taken from a paper presented at a symposium on electromagnetic distance measurement at Oxford in 1965. While the author refers to this paper, he does not indicate that it is almost a direct copy of it. Furthermore, he fails to mention some very important differences between the *MRA* 1 and 2, which he discusses fully, with this newer model. One of the main differences is that the *MRA* 101 has an entirely different method of read out—a dial which gives direct readings in metres and centimetres. No mention is made of the other 'Tellurometer' models—the *MRA* 3, and the recently developed *MRA* 4, which has a direct digital metric read out and for which is claimed an accuracy of  $\pm 3$  m.m.  $\pm 3$  p.p.m.

The first chapter, "The Scope of Air Survey", is concerned primarily with the use of aerial photographs in geological mapping, and to a lesser extent with airborne techniques in geophysical surveying. Apart from a short paragraph on the application of air survey to forestry, the vital considerations of such important topics as agriculture, hydrology, soil surveys and urban and rural land use studies are not even mentioned.

The second chapter, on air survey techniques, is particularly mixed up, and only a small part of it is concerned with the actual techniques of air survey. It contains subject matter relating to almost every other chapter in the book, much of it merely a repetition of what has been dealt with elsewhere. Two photogrammetric plotting instruments are discussed: the 'Wild A8' briefly, and the Thompson-Watts plotter in considerable detail. Many technical terms are used but not explained, so unless the reader already has some knowledge of photogrammetry most of the descriptions and comment will have little meaning. An interesting review of an example of an air survey which was carried out in 1947 by Huntings Surveys for the British Railways is discussed. This involved the production of a 1:480 plan of several miles of railway track, and is an early example of very large scale plan production using air survey methods, though nowadays the compilation of 1:500 plans with 1 ft. contours is quite a common air survey task.

The third chapter, "Instruments for Air Survey", deals with the historical evolution of gyros and the gyro-pilot, the Doppler and Decca systems of air navigation, and the Airborne Profile Recorder. The Decca system is considered at great length; the Decca Navigator Company having compiled a number of well illustrated and informative publications, including one entitled "The Decca Navigator as an Aid to Surveying".

In the fourth chapter, "Cameras and Plotting Equipment", only four out of the sixty or more air survey cameras available are dealt with, and of the plotting equipment only the 'Wild A8' is dealt with in any detail.

Unfortunately, the author has misunderstood some of the information which he has copied from the Wild

Company's pamphlet on the A8. He states that "In addition, a mobile unit has been developed which can be readily mounted, dismounted, and transported to any required site". This implies that a second type of A8 unit has been developed, whereas the pamphlet is merely pointing out the improvements in portability of the A8 over its predecessor, the A6.

Less than one quarter of the fifth chapter, "Aircraft for Survey", deals specifically with survey aircraft. Most of the chapter is concerned with two examples of survey projects in which aircraft were used. The first example is the air lift organized in the construction of the mid-Canada Line, an air detection unit, and the second, which takes up more than half the chapter, is a very general review of the work carried out by the Falklands Islands Dependencies Aerial Survey Expedition of 1955-57.

In the final chapter, "Examples of Air Survey", the author states that he refers "to some typical examples carried out in recent years". He then proceeds to review a paper, written twelve years ago, which discusses three non-typical "border line cases in which air survey was used". This is followed by a discussion on aerial survey for cadastral mapping and includes both techniques and a number of examples of work carried out. The author states, "Probably the most valuable and objective advice given to countries since the war on economic development has been contained in the reports prepared by the technical missions organized by the International Bank for Reconstruction and Development". Several references are made to these most valuable and useful reports, which deal particularly with the underdeveloped territories of the world. In almost every case they recommend the taking of aerial photographs for both map compilation, and as a source of information in the conservation and development of the natural resources. This is followed by some very useful information on photogeology taken from a paper by J. A. E. Allum. This paper is quoted as a reference though no mention is made of a very well written and informative book, *Photogeology and Regional Mapping*, by the same author. Finally, there is another review dealing with airborne geophysics in geological surveys.

Almost at the end of the book (the author's nineteenth) he reveals his reason for writing it when he comments: "The technical advances which have taken place in air survey over the past twenty years or so have received less publicity than was their due, with the result that there still exists widespread ignorance of the methods now employed in this field, and this book is an attempt to try and improve matters".

It is doubtful whether this attempt has been successful.

W. GORDON COLLINS

## FIND A WORD

### Russian-English Translators Dictionary

A Guide to Scientific and Technical Usage. By Mikhail J. Zimmerman. Pp. 294. (New York: Plenum Press, 1967.) \$12.

THIS dictionary calls itself a translators' dictionary and the translators envisaged are those working on scientific and technical texts. What is ideally demanded of such translators is a good working knowledge of the Russian language together with a working knowledge of the subject of the book or article to be translated. This, in the majority of cases, is an impossible demand. If a specialist in a subject is also fluent in Russian he is more likely to devote his time to original work than to translation. Translators, then, are usually more familiar with ordinary Russian than with the scientific or technological terms of a particular discipline. It was to help such people that Dr Zimmerman conceived and executed his book. Has he succeeded in his very laudable purpose?

His method has been to take Russian words, terms or phrases, printed in Russian and arranged alphabetically. Each is followed by one or more example in English, drawn from technological or scientific writings, in which the words which could be translated by the Russian word or phrase are in heavy print. Where desirable there are cross references. It is an ingenious idea, but one which is extremely difficult to realize in practice. The present dictionary will be far more useful to technologists than to scientists, for only a small proportion of the terms is of interest to scientists. When these terms are spread over the numerous sciences the coverage for any one science is very thin indeed.

Another criticism concerns the inclusion of a large number of everyday prepositions, adjectives and adverbs, the use of which in a scientific or technological context differs little from their use in ordinary literary Russian. About nine pages (that is eighteen columns) deal with the use of *B* (in) in various phrases. Quite a number of these are very obvious uses of the preposition in its generally accepted meaning. On page 101 seven quotations are given in connexion with the Russian word *Krome* (besides). The seventh quotation is: "There was little doubt about the good process performance of all the functional elements with the exception of the fluidized bed itself". It is difficult to see "with the exception of" as a synonym of "besides".

Examples such as these only emphasize the difficulties which beset an author who is "blazing a trail". With the ever growing volume of translations from Russian technological works, the need for a dictionary of this kind is clear and although, as Dr Zimmerman himself points out, this one is selective rather than exhaustive, it should, if used with discretion and common sense, prove a useful tool for the harassed translator.

S. I. TOMKEIEFF

## SOCIOLOGY FOR STUDENTS

### Sociology

An Introduction. Edited by Neil J. Smelser. Pp. xx+744. (New York and London: John Wiley and Sons, Inc., 1967.) 68s.

THIS volume is intended as an introductory text for students of sociology at university level. It consists of eleven chapters by well known authorities, which cover the main areas of specialization within modern sociology. There is an introduction and a concluding chapter by the editor, who has been successful in ensuring a high degree of consistency in both the matter and style of his collaborators' work. Unlike most of the other multi-author texts in the field, the book thus achieves a definite unity; the advantages of specialist contributions are not cancelled out by a lack of overall coherence. In view of this and the general quality of the writing, it is probable that during the next few years this text will become one of the most widely used on both sides of the Atlantic. For this reason it is perhaps all the more important that certain shortcomings—at least from the ideal—should be noted.

First, it must be said that despite the editor's express aim of providing an introduction to sociology on a rather higher intellectual level than is usual, the treatment of basic methodological and theoretical issues is often superficial. Many of the questions the intelligent newcomer to sociology is likely to ask about the nature of the subject as a science or discipline are not discussed in a way that will be found satisfying. Secondly, very little is said directly about the procedures through which sociological investigation is carried out. To regard this as a matter which can be left entirely to a separate text on "research techniques" now appears as somewhat old-fashioned. Thirdly, the overall view of sociology that the book presents carries a considerable American bias. Even accepting American pre-eminence in the field and the fact that the text is

intended primarily for American students, the almost total neglect of the rather divergent perspectives of much present-day European sociology is to be regretted.

JOHN H. GOLDTHORPE

## ELECTRONIC DEVICES

### Low Noise Electronics

By W. P. Jolly. (Introductory Science Texts.) Pp. vii + 149. (London: The English Universities Press, Ltd., 1967). 25s. net.

THIS is the first of a new series of introductory texts. Professor Jolly, who edits the series, and who is also the author of this book, writes in the foreword: "The books in this series are intended to be short and simply written so that they may be read in a few days by anyone with the general background of a second year student".

Although the book is called *Low Noise Electronics*, only one of the nine chapters and two of the six appendices are concerned particularly with electrical noise. Many topics have been squashed into this small book, and a superficial treatment is inevitable. The structure of the book and the selection of the material seem to have been motivated by an attempt to include references to as many modern electronic devices as possible. To devote four pages to applications of lasers (including punching a hole in a razor blade) seems to be an extreme example of seeking for topicality at the expense of neglecting the main theme of the book.

The treatment is almost entirely descriptive. When the occasional mathematical expression is given, the reader is usually referred to another book for the derivation. Throughout the text, the reader is confronted with strange new words and expressions. If he turns up the index, he will be referred back to the page on which he first saw them. For example, he meets for the first time on page 13: "elliptical orbits", "electron spin", "nuclear spin", "quantized"; on page 20: "crystal lattice", "electrons of opposite spin"; and on page 29: "effective mass", "hot electrons". Without previous knowledge of many of the terms, the reader must surely find much of the text incomprehensible. The scant illustration makes his task more difficult.

The book is well indexed, and it is possible that some busy engineers will find it useful for reference. They can, for example, turn up "Gunn effect", and be referred to original papers. Ironically, it is this all-embracing character of the book which is largely contributory to its failure to fulfil the professed aim of the author. I believe that this book falls well short of being a good introductory text.

J. G. THOMAS

## YIELD AND FRACTURE

### Physical Basis of Yield and Fracture

Conference Proceedings, Oxford, Sept. 1966. (Conference Series, No. 1). Pp. vii + 303 + 21 plates. (London: Institute of Physics and Physical Society, 1966.) 90s.

THIS volume contains collected papers on various aspects of fracture presented at a conference organized by the Stress Analysis Group of the Institute of Physics and the Physical Society. Although the meeting was originally billed as a national meeting, papers from the United States, Soviet Union, Australia and Japan were presented indicating the genuine interest in this field. During the past eight years, however, no fewer than four international conferences on this now over-exposed subject have been held (Swampscott, USA, 1959; Maple Valley, USA, 1962; Melbourne, Australia, 1963; and Sendai, Japan, 1965) all published in either book or journal form, and inevitably containing the main ideas on the subject. The Oxford

meeting comes at the end of the line of meetings and too soon after Sendai for any substantially new ideas on fracture to have been produced. As a consequence, the book turns out to be a fairly insignificant volume, but with the editorial staff working valiantly in an attempt to give good value for money by including thirty-eight papers either in full or in summary together with reported discussion.

The main papers are grouped together in four sections under the chapter headings "Theory", "Metals", "Polymers" and "Miscellaneous Materials", respectively. The first small chapter deals with certain aspects of stress distributions associated with cracks in photo-elastic materials and with the dislocation approach to fracture nucleation and propagation. The second chapter contains several papers dealing with yield and fracture in mild steel or iron-based solid solutions, some interesting observations of ductile fracture in metals containing hard second-phase particles, and an electron metallographic study of stress-corrosion cracking in austenitic steels and titanium alloys. The third and longest chapter of the volume is concerned with fracture of polymers from both the microscopical and continuum viewpoints. Particularly interesting papers include fatigue fracture behaviour in both rubber and plastics, and a fracture mechanics approach to corrosion stress cracking in plastics. The fourth chapter contains a mixed collection of papers on various ceramics such as magnesium oxide, lithium fluoride and graphite, and one, rather out of place, on cleavage surface energy experiments in zinc; no discussion was offered in response to this paper even though the surface energy values obtained were extremely low and less than reliably derived values for the stacking fault energy.

Possibly the most useful feature of this book is that several experimental and theoretical approaches can be compared for a wide range of materials. R. SMALLMAN

## LANGUAGE BY COMPUTER

### Introduction to Computational Linguistics

By David G. Hays. Pp. xvi + 231. (London: Macdonald and Co. (Publishers) Ltd., 1967.) 70s. net

IN his preface Mr Hays defines computational linguistics as "a body of techniques that make the computer an effective, workable tool for language processing"; and towards the end of his book, on the subject of automatic translation, he writes, "The problem is clearly one of engineering". Indeed it is clear throughout that when Mr Hays is faced with a new and intractable linguistic puzzle, his first reaction is to send a man out to hire a faster computer. It is not surprising then that although he devotes a whole chapter, for instance, to techniques for representing and handling grammars in a machine, he nowhere considers whether the types of grammar in question are adequate to describe the syntax of a natural language. In sum, the book is scarcely concerned at all with the linguistic side of computational linguistics, but only with the realization of the linguist's ideas in terms of tapes and disks and core store.

Moreover, it is notable that Mr Hays describes in detail virtually every aspect of the programmes with which he is concerned, with the sole exception that he neither compares their capabilities nor provides any examples of their results. Are there nowadays parsing algorithms capable of producing an output which is recognizably a sentence plus an assigned syntactic structure? Are there any examples of automatic translation fit to print? My own answers to these two questions would be "Yes" and "No" respectively, but it is curious that Mr Hays does not give any account at all of how well different systems work in practice.

Leaving aside the lack of linguistic theory, someone with a previous knowledge of computers entering the field

of computational linguistics for the first time would find this book a pleasant and useful introduction to some of the tricks of the trade. Simple accounts are given of the more straightforward parsing algorithms at present in use, of suitable techniques for establishing and maintaining large computer dictionaries, and also of such loosely related aspects of language data-processing as concordance-making and on-line indexing and abstracting. Mr Hays's RAND Corporation background occasionally obtrudes (does any other group of linguists really care, for instance, that line-printers can't usually produce italic or bold-face letters?) but on the whole, and always with the limitations already mentioned, his book is a very fair introduction to some main areas of present day work.

PAUL BRATLEY

## EARLY ASTRONOMER

John Kepler

By Angus Armitage. Pp. 194+4 plates. (London: Faber and Faber Ltd., 1966.) 21s. net

KEPLER's life and work present the most difficult problems of narrative and interpretation for a modern biographer. The work he did was strongly shaped by his personal and professional affairs, which were conducted in a provincial corner of Central Europe, then torn by religious dissension and eventually by the Thirty Years' War. Also, his personal style of work is extremely difficult for us to grasp; the intimate combination of a speculative mathematical-religious mysticism with painstaking and self-critical research is foreign to our experience of either science or "mysticism".

In this brief and elementary biography, Dr Armitage succeeds in overcoming most of these difficulties. The political and religious background to Kepler's environment is sketched in just sufficient detail to make it comprehensible; and the "neo-Platonic" aspect of Kepler's thought is presented clearly and with a minimum ofology. The technical side of his astronomical and mathematical work is popularized well, although the description of the classic study of the orbit of Mars relies rather heavily on older sources, and ignores the recent studies which exhibit Kepler's "seventy trials" of a circular orbit as an iteration procedure intended to yield approximation solution. For the assessment of Kepler's place in the history of science, the safe course is to stick to the later use of the "Three Laws" up to their re-statement in Newton's grand synthesis. This is Dr Armitage's approach. But from this we achieve no perspective on the tradition out of which Kepler's peculiar style and choice of problems rose, nor of the milieu in which his speculations were sober and moderate compared with those of such influential figures as Robert Fludd. Both of these problems have been but scantily explored by historians; but Armitage's incidental descriptions of these matters may at least inform the modern reader that they are there to be studied.

J. R. RAVETZ

## OBITUARIES

Professor H. T. H. Piaggio

June 25 there disappeared from the Nottingham scene a remarkable man who had served the University College the University of Nottingham from his appointment in 1908 until his retirement in 1950. When I first met him he was professor of mathematics and in his teaching covered the whole range of the subject to the standard of the University of London honours degree. He was 83 when he died.

Piaggio organized his life somewhat rigidly in compartments: academic activities, chess at the Mechanics Institute, and social service in connexion with his sister's work for the PDSA. At our first meeting he made it clear that he never accepted social engagements. He was unmarried and lived with his sister, a highly cultured and intelligent woman, until her death a few years ago.

On the research side he was interested in the algebra of invariants, the theory of relativity and the mathematics of psychology. But he will best be remembered for his book, *Differential Equations*, and his great teaching ability. He told me that as a young lecturer he was very disappointed with his teaching, and that while watching the antics of a cheapjack at Nottingham Goose Fair it suddenly occurred to him that the cheapjack's techniques might be applied to the serious teaching of mathematics, and accordingly he applied them with great success.

He took keen interest in the welfare of his students. Any unlikely to obtain honours in mathematics were firmly, if gently, shunted into another department; but his good students were carefully tended. Once, when an able woman student was having a poor time in her home, he asked my wife if she would arrange through a women's organization for him to pay the student's fees for residence in a hostel, without disclosing that he was involved. When she agreed, he further asked her to suggest a suitable dress allowance for the student, and this also was secretly passed on.

During the Second World War I saw much of him on the firewatching teams and learned much about him. He bitterly regretted the time which in his youth, for scholarship reasons, he had been forced to spend on the study of classics—particularly Greek—to which he attributed his defective eyesight. He followed to the end of his life a strict diet prescribed for him by a doctor in about 1910. He was very keen on chess and organized an international conference in Nottingham in 1938. But, perhaps, even more than chess or mathematical research, he enjoyed reviewing books for *Nature*, and I am sorry that we shall no longer hear his characteristic half-chuckle.

L. F. BATES

Dr. Carl Kenty

CARL KENTY, who died at his home in Cleveland Heights, Ohio, on June 10, was a research physicist with the General Electric Company for more than 30 years.

With a B.Sc. from Dalhousie and a Ph.D. from Princeton he first worked at Princeton under K. T. Compton on electron-positive ion recombination in what would now be called a decaying plasma. In 1929 he joined the General Electric Company, where his technical work was devoted to the development of gaseous discharge light sources—lamps of the "illuminated sign" type. He obtained more than fifteen patents in this field. Other more purely scientific work consisted of a series of investigations in gaseous electronics, many centred on the "active nitrogen" problem. Other investigations were a sequel to his work at Princeton, and there was also a group of experiments in which he pioneered into the medium pressure region, from the low pressure area, using inert gases, mercury, nitrogen and various metal vapours, particularly thallium. He was not content with merely recording the beautiful and complex effects observed, but used them to obtain data such as mobilities, and to search for new high-lying metastable levels of molecular nitrogen. Towards the end of his life he returned, among other work, to investigate the persistence of fine dust in plasmas.

Kenty's demonstrations of basic processes of light production were for many years a highlight of the annual conference on gaseous electronics held at various cities in the United States. He was also a fellow of the American Physical Society, past president of the Cleveland Physics Society and a member of the Ohio Academy of Sciences.



## University News:

### Alberta

PROFESSOR J. A. JACOBS, at present head of the Department of Geophysics and director of the Institute of Earth Sciences in the University of British Columbia, has been appointed Killam Memorial professor of Science.

### Hull

DR S. A. RAMSDEN has been appointed to the chair of applied physics.

### Southampton

THE Department of Education and Science and the Ministry of Health have agreed to set up a new medical school to start work early in 1971. To begin with, Southampton will take 130 medical students a year. The University Grants Committee is to be given an additional allocation of capital funds to finance the rebuilding of the Southampton General Hospital and the construction of the medical school buildings. The Government is proud to boast that annual intake to the medical schools has increased from 2,020 in 1960 to more than 2,500 last year and also that proposals for other developments in medical education will be considered after the Todd Commission on Medical Education reports early in 1968. The University of Keele has taken the unusual step of making a general statement of its willingness to set up a medical school which can begin to take students two years earlier than Southampton.

### Massachusetts Institute of Technology

MR J. F. COLLINS, at present mayor of Boston, has been appointed visiting professor of urban affairs.

## Appointments

COMMANDER JOHN O. BOYER has been appointed chief of the US Coast and Geodetic Survey's Marine Chart Division.

CAPTAIN NORMAN E. TAYLOR has been appointed director of the Pacific Marine Center of the US Coast and Geodetic Survey in Seattle, Washington, in succession to Rear-Admiral Harold J. Seaborg.

MR ROBERT W. RAMSEY, jun., has been appointed the US Atomic Energy Commission's scientific representative in Chalk River, Canada. Mr Ramsey is at present chief of the Technical Policy Branch of the Division of Operations Analysis and Forecasting at the commission's headquarters in Germantown, Maryland.

PROFESSOR A. G. WARD has been re-appointed chairman of the Food Standards Committee for a further period of three years. Mr C. S. Dence and Dr R. Passmore have been re-appointed members of the committee and Mr C. C. E. Sopp has been appointed a member, all for a period of three years.

THE following appointments have been made to the American Society of Plant Physiologists: *President*, Professor A. T. Jagendorf of Cornell University; *President-Elect*, Professor L. Bogorad of Harvard University; *Secretary*, Dr H. W. Siegelman of the Brookhaven National Laboratory.

## Announcements

PROFESSOR EUGENE I. RABINOWITCH of the University of Illinois has been awarded the 1967 Kettering Award by the American Society of Plant Physiologists in recognition of his scientific contributions on photosynthesis, and Professor Paul Kramer of Duke University is the 1967 recipient of the Barnes Life Membership Award of the society, conferred in recognition of his contributions to science through research and teaching.

THE Seismological Society of the South West Pacific is being formed in order to devote attention to the earthquakes of South East Asia, Australasia and Oceania. Further information can be obtained from the Convener Seismological Society of the S-W Pacific, Box 8005 Wellington, New Zealand.

THE John A. Hartford Foundation, Inc., of New York City, has awarded \$268,000 to the Massachusetts Institute of Technology for a three-year programme to continue development and evaluation, prepare manufacturer's drawings and specifications and build twenty production models of a high-speed English to Braille embossing system, being studied at MIT. This machine would enable an ordinary typist with no knowledge of the raised dot Braille code to produce immediate Grade 2 Braille the most widely used form of the touch system of reading for the blind.

## Meetings

LECTURES on Radioisotope Work in School, September 4-15, Harwell (Post-Graduate Education Centre, Building 445, AERE, Harwell, Didcot, Berks.).

LECTURES in Engineering, September 11-15, Harwell (Post-Graduate Education Centre, Building 455, AERE Harwell, Didcot, Berks.).

COLLAGEN Discussion Group, September 25, University of Manchester (Professor D. S. Jackson, Department of Medical Biochemistry, Burlington Street, Manchester 13).

EXPERIMENTAL Medicine and Surgery in Primate, September 27-30, New York City (Executive Director: The New York Academy of Sciences, 2 East 63 Street, New York, NY).

GONDWANA Stratigraphy, September 26-October 1, Buenos Aires (Dr E. P. Plumstead, Honorary Secretary, Subcommission on Gondwana Stratigraphy, Bernard Price Institute for Paleontological Research, University of Witwatersrand, Johannesburg).

CREATING Scientists and Technologists for Industry, September 28, Imperial College (The Secretary, The Research and Development Society, 51 The Mall, Ealing, London, W5).

BUSINESS Decision 67, September 28-29, University Southampton (Conference Information, The British Computer Society, 23 Dorset Square, London, NW1).

METAL Ions in Biological Systems, September 28-29, Inorganic Chemistry Laboratory, Oxford (Dr H. A. Hill, Inorganic Chemistry Laboratory, South Parks Road, Oxford).

INFRA-RED Spectra of Gas Chromatographic Fractions, September 29, University of Technology, Loughborough (Miss J. Healey, Institute of Petroleum, 61 New Cavendish Street, London, W1).

ERRATUM. In Table 1 of the article "Free Amino-acids in Blood Plasma and Erythrocytes of Normal Ducks and Ducks infected with Malarial Parasite, *Plasmodium lophurae*" by Wasim Ahmad Siddiqui and William Traub (Nature, 214, 1046; 1967) the value for cystine for erythrocytes (parasitized, 80 per cent) should have been 2.0, 50.0.

ERRATUM. In the leading article "Molecular Mode" (Nature, 215, 802; 1967) the name Hodgkin was, of course misspelt and should have been Hodgson.

CORRIGENDUM. In the communication "Concentration of Immunglobulins in Lethally X-irradiated Mice" (Nature, 215, 742; 1967) the names H. Bazin and H. S. Mick were unfortunately printed in the wrong order.

## FORTHCOMING EVENTS

Meetings marked with an asterisk are open to the public

Wednesday, August 30—Wednesday, September 6

BRITISH ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE (at Leeds)—29th Annual Meeting.

Wednesday, August 30

At 7.30 p.m.—Lord Jackson of Burnley, F.R.S.: "Science, Technology and Society" (Presidential Address).

Thursday, August 31

At 10 a.m.—Prof. C. E. H. Bawn, F.R.S.: "Horizons for Plastics" (Presidential Address, Section B).

At 10 a.m.—Dr. R. F. Parrington, F.R.S.: "The Origins of Mammals" (Presidential Address, Section D).

At 10 a.m.—Prof. A. R. Prest: "Sense and Nonsense in Budgetary Policy" (Presidential Address, Section F).

At 10 a.m.—Dr. R. Week: "Co-operative Research in a Competitive Industry" (Presidential Address, Section G).

At 10 a.m.—Miss Freda Gwilliam: "New Ventures in Education" (Presidential Address, Section L).

At 10 a.m.—Mr. F. R. Horne, C.B.E.: "The Potentiality of Plants for Improving Food Supplies" (Presidential Address, Section M).

At 10 a.m.—Dr. J. Needham, F.R.S.: "The Roles of Europe and China in the Evolution of Occumenical Science" (Presidential Address, Section X).

At 11.15 a.m.—Prof. E. A. Shils: "The Profession of Science" (Presidential Address, Section N).

At 11.30 a.m.—Prof. M. B. Wilkins: "Biological Clocks" (Darwin Lecture).

At 2.30 p.m.—Prof. E. R. Laithwaite: "Propulsion Without Wheels" (Young People's Lecture).

Friday, September 1

At 10 a.m.—Prof. R. F. Peel: "Geomorphology—Trends and Problems" (Presidential Address, Section E).

At 10 a.m.—Dr. R. D. Keynes, F.R.S.: "The Predetermination of Sex" (Presidential Address, Section I).

At 10 a.m.—Mr. T. A. Oxley: "A Scientific Policy for the Better Use of Food" (Presidential Address, Section K\*).

At 11.20 a.m.—Dr. M. J. Henchman: "What Happens During a Collision When Two Molecules Collide and React" (Kelvin Lecture).

At 2.30 p.m.—Dr. B. D. Shaw: "Explosives" (Young People's Lecture).

At 8 p.m.—Dr. B. J. Mason, F.R.S.: "Weather Forecasting by Computer" (Evening Discourse).

Friday, September 3

At 10.30 a.m.—Official Service in Leeds Parish Church. Preacher: The Most Reverend and Right Honourable F. D. Coggan, P.C., D.D., Lord Archbishop of York.

Friday, September 4

At 10 a.m.—Dame Kathleen Lonsdale, F.R.S.: "Physics and Ageing" (Presidential Address, Section A).

At 10 a.m.—Mr. N. L. Falcon, F.R.S.: "The Geology of the North-East Margin of the Arabian Shield" (Presidential Address, Section C).

At 10 a.m.—Dr. A. T. Lucas: "Felling and Napping in Early Ireland" (Presidential Address, Section H).

At 11.30 a.m.—Dr. D. E. Broadbent: "Aspects of Human Decision Making" (Presidential Address, Section J).

At 11.30 a.m.—Prof. T. A. Bennet-Clark, C.B.E., F.R.S.: "The Role of the Senses in the Modern World" (Presidential Address, Section K).

At 2.30 p.m.—Dr. B. J. Mason, F.R.S.: "Thunderstorms and Lightning" (Young People's Lecture).

At 8 p.m.—Mr. D. R. Pilbeam: "Human Origins" (Evening Discourse).

Saturday, September 5

At 11.30 a.m.—Dr. J. A. Gray: "The Physiological Basis of Personality" (Young People's Lecture).

At 2.30 p.m.—Prof. C. S. Whewell: "The World of Fibres" (Young People's Lecture).

## APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

SCIENTIFIC OFFICER or SENIOR SCIENTIFIC OFFICER (with a first- or upper second-class honours degree in botany, agricultural botany or agriculture, and at least three years' postgraduate experience) in the SYSTEMS SYNTHESIS SECTION of the DEPARTMENT of ECOLOGY, to work on theoretical aspects of plant and crop growth—The Secretary, Grassland Research Institute, Hurley, Maidenhead, Berkshire (August 31).

ASSISTANT LECTURER (preferably wishing to develop an interest in either bacterial microbiology, electron microscopy, microbial genetics, or protozoology) in MICROBIOLOGY in the DEPARTMENT of BIOLOGICAL SCIENCES, to assist in the teaching of students preparing for the B.Sc. Honours Degree in Microbiology, and to participate in the research work of the Department—Academic Registrar (LFG), University of Surrey, Battersea Park Road, London, S.W.11 (September 4).

LECTURER (with some experience of diagnostic morbid anatomy and of histopathology to medical undergraduates) in the DEPARTMENT of ANATOMY—The Secretary, The University, Aberdeen, Scotland (September 6).

LECTURERS (2) (registered medical practitioners, preferably with some experience of medical bacteriology) in the DEPARTMENT of BACTERIOLOGY—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh (September 8).

ASSISTANT LIBRARIAN (graduate)—The Secretary, The University, Dundee, Scotland (September 9).

ASSISTANT LECTURER in ORAL ANATOMY and PHYSIOLOGY—The Registrar, The University of Sheffield, Sheffield (September 9).

TECHNICAL OFFICER (graduate, or equivalent, with some experience in crystallography or electro-mechanical equipment) in the DEPARTMENT of CHEMISTRY to assist with the use of automatic X-ray crystal diffractometers—The Secretary, The University, Dundee, Scotland (September 9).

LECTURER (registered medical or science graduate preferably with previous teaching experience) in ANATOMY—The Registrar, University College, Cathays Park, Cardiff (September 11).

ASSISTANT LECTURER or LECTURER in PHARMACOLOGY in the DEPARTMENT of PHARMACY—The Registrar, The University, Nottingham (September 15).

JUNIOR RESEARCH FELLOW (with a good honours degree in physics and research interests in solid state physics) in PHYSICS—The Registrar, The University of Sheffield, Sheffield (September 15).

LECTURER or ASSISTANT LECTURER (registered member of the veterinary profession) in VETERINARY PATHOLOGY, for duties which will include the teaching of morbid anatomy and histopathology—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh (September 16).

LECTURER (with a medical qualification) in the DEPARTMENT of ANATOMY—The Registrar, The University, Liverpool, quoting Ref. RV/142/N (September 16).

LECTURER or ASSISTANT LECTURER (qualified in an appropriate science or in medicine) in PHARMACOLOGY—The Registrar and Secretary, The University, Leeds, 2 (September 18).

RADIOGRAPHER/TECHNOLOGIST in the DEPARTMENT of RADIATION BIOLOGY and RADIOTHERAPY, College of Medicine of the University of Lagos—The Inter-University Council, 33 Bedford Place, London, W.C.1 (September 20).

SENIOR TUTOR-DEMONSTRATOR in GEOGRAPHY at the University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 22).

POSTDOCTORAL FELLOW in ORGANIC CHEMISTRY to join a group working on the chemistry of ortho-quinones—Professor J. M. Tedder, Chemistry Department, The University, Dundee, Scotland (September 23).

LECTURER (with at least a Ph.D. or equivalent, and preferably some teaching experience in biochemistry) in the SCHOOL of BIOCHEMISTRY, University of Melbourne, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 30).

BASIC GRADE SCIENCE GRADUATE (with some basic knowledge and interest in human physiology) for research into the oxygen-binding properties of red-blood cells—Dr. T. L. Dormandy, Whittington Hospital, Highgate Hill, London, N.19.

BOTANIST/PLANT PHYSIOLOGIST (with a good honours degree, sound experience in physiological and biochemical techniques, and adequate research achievement) for studies on the biological changes accompanying maturation and senescence of fruit under selected storage regimes, with particular reference to the physiological disorders arising during long storage and to the application of the results of basic research—The Secretary, East Malling Research Station, Maidstone, Kent.

LECTURER in CIVIL ENGINEERING; a LECTURER in GEOLOGY; and LECTURERS (2) in ZOOLOGY at University College, Nairobi, University of East Africa—The Inter-University Council, 33 Bedford Place, London, W.C.1.

LECTURER in AGRICULTURAL BIOCHEMISTRY—Grade III; and an ASSISTANT ADVISORY NUTRITION CHEMIST—Grade IV (applicants should possess an honours degree in biochemistry, agricultural chemistry or equivalent)—The Secretary, The Edinburgh School of Agriculture, West Mains Road, Edinburgh, 9.

MASTER to teach PHYSICS; and a MASTER to teach MATHEMATICS—The Headmaster, Whitgift School, Haling Park, South Croydon, CR2 6YT.

MICROANALYST to take charge of the MICROANALYTICAL LABORATORY—The Laboratory Superintendent, Chemical Laboratory, The University of Sussex, Falmer, Brighton, Sussex, quoting Ref. 940/2.

RESEARCH ASSISTANT in the EXPERIMENTAL PSYCHOLOGY LABORATORY for a research project on long- and short-term memory—The Assistant Registrar (Establishment), The University of Sussex, Essex House, Falmer, Brighton, Sussex, quoting Ref. 937/1.

RESEARCH STUDENTS in CHEMISTRY for work in the following fields: solid state chemistry; "hot atom" chemistry; nuclear chemistry—The Registrar, The University, Canterbury, Kent, quoting Ref. A71.

SCIENCE GRADUATE or SENIOR TECHNICIAN for specialized work on blood groups and electrophoresis of haemoglobin, serum proteins and enzymes—The Director, Serological Population Genetics Laboratory, c/o St. Bartholomew's Hospital, West Smithfield, London, E.C.1.

SENIOR LECTURER in the SCHOOL of MATERIALS SCIENCE—The Registrar (S), The University, Bath, Somerset, quoting Ref. 67/93.

SENIOR MATHEMATICS MASTER (graduate with experience) at Markham College, Lima, Peru—The Appointments Branch, The British Council, 65 Davies Street, London, W.1, quoting Ref. 7/WS 298.

TECHNICIAN (at least 21 years of age, preferably with City and Guilds or I.S.T. qualifications) to assist in the teaching and research laboratories of the CHEMISTRY DEPARTMENT—The Staff Officer, University of Surrey, Battersea Park Road, London, S.W.11.

## REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

## Great Britain and Ireland

Proceedings of the Royal Society. Series A: Mathematical and Physical Sciences. Vol. 299, No. 1456 (13 June 1967): A Discussion on Nonlinear Theory of Wave Propagation in Dispersive Systems. Organized by M. J. Lighthill. Pp. 145+1 plate. (London: The Royal Society, 1967.) 22s. 6d.; \$3.35. [47]

Ministry of Agriculture, Fisheries and Food. Bulletin No. 136: Watercress Growing. Pp. iv+35+4 plates. (London: H.M. Stationery Office, 1967.) 4s. 6d. net. [47]

The British Steel Castings Research Association. 14th Annual Report, 1967. Pp. 42. (Sheffield: The British Steel Castings Research Association, 1967.) [47]

International Nickel. Mechanical and Physical Properties of the Austenitic Chromium-Nickel Stainless Steels at Ambient Temperatures. Pp. 44. (London: International Nickel, Ltd., 1967.) [47]

Annual Report of the Meteorological Office, 1966. Pp. xii+71+8 plates. (Met. O.791). (London: H.M. Stationery Office, 1967.) 8s. [57]

The University of Liverpool. Annual Report of the Tidal Institute and Observatory 1966. Pp. 24. (Birkenhead: University of Liverpool, Tidal Institute and Observatory, 1967.) [57]

Johnson Matthey and Co., Ltd. Chairman's Review, Director's Report and Statement of Accounts for the year ended 31st March 1967. Pp. 24. (London: Johnson Matthey and Co., Ltd., 1967.) [57]

The University of Aston in Birmingham. Development Plan Report. Pp. 96. (Gosta Green, Birmingham: The University of Aston in Birmingham, 1967.) 80s. [67]

Philosophical Transactions of the Royal Society of London. Series A: Mathematical and Physical Sciences. No. 1123, Vol. 261 (6 July 1967): A Discussion on Advanced Methods of Energy Conversion—Magnetohydrodynamic Power Generation. Organized by L. Rotherham, F.R.S. Pp. 345-582 + plates 18-27. (London: The Royal Society, 1967.) 100s.; \$15. [77]

Building Research Station. Digest 84 (Second Series): Accuracy in Building: Where, When, How Much? Pp. 8. (London: H. M. Stationery Office, 1967.) 4d. [117]

Humane Killing of Animals. Pp. 24. (London: The Universities Federation for Animal Welfare, 1967.) [117]

White Fish Authority. Research and Development—Progress Report 1967. Pp. 16. (London: The White Fish Authority, 1967.) [117]

The Institute of Physics and the Physical Society. Seventh Annual Report of the Council, 1966. Pp. 19. (London: The Institute of Physics and the Physical Society, 1967.) [117]

Ambassade de France, Service de Presse et d'Information. Inland Navigation in France. Pp. 21. (London: Ambassade de France, Service de Presse et d'Information, 1967.) [127]

The Committee of Vice-Chancellors and Principals of the Universities of the United Kingdom. A Compendium of University Entrance Requirements for First Degree Courses in the United Kingdom in 1968-69, excluding Part Time and External Degree Courses. Pp. 201. (London: The Association of Commonwealth Universities, 1967.) Obtainable from Percy Lund, Humphries and Co., Ltd., the Country Press, Priestman Street, Bradford, Yorkshire.) 14s. 6d. [127]

### Other Countries

CERN—European Organization for Nuclear Research. Sites for the Proposed CERN 300 GeV Proton Synchrotron. Vol. 1: General information and Comparative Tables on the Various Sites by the CERN Study Group. Pp. 36 + 39 figures. Addendum to the Report on the Design Study of a 300 GeV Proton Synchrotron. Pp. iii + 85 + 20 figures. Annual Report 1966. Pp. 167. (Geneva: CERN—European Organization for Nuclear Research, 1967.) [196]

Canada. Physics in Canada: Survey and Outlook. Prepared by a Study Group of the Canadian Association of Physicists, headed by D. C. Rose. (Special Study No. 2, May 1967.) Pp. xii + 385. (Ottawa: Queen's Printer, 1967.) \$2.50. [196]

Smithsonian Miscellaneous Collections. Vol. 151, No. 5: Precipitation in Five Continents. By Dr C. G. Abbot. (Publication 4694.) Pp. 32. Vol. 152, No. 5: Supplement to a Long-Range Forecast of United States Precipitation (Smithsonian Publication 4390). By Dr C. G. Abbot and Mrs Lena Hill. (Publication 4711.) Pp. 8. (Washington, D.C.: Smithsonian Institution Press, 1967.) [196]

United States Department of the Interior. Fish and Wildlife Service: Bureau of Sport Fisheries and Wildlife. Fish Distribution Report No. 1: Propagation and Distribution of Food Fishes for the calendar years 1963 and 1964. Pp. ii + 50. (Washington, D.C.: Government Printing Office, 1967.) [196]

Applied Science and Technological Progress: a Report to the Committee on Science and Astronautics, U.S. House of Representatives, by the National Academy of Sciences. Pp. iv + 497. (Washington, D.C.: National Academy of Sciences—National Research Council, 1967. Available from Government Printing Office, Washington, D.C.) [196]

United States Department of the Interior: Geological Survey. Bulletin 1199-N: Geology and Bauxite Deposits of the Rock Run and Goshen Valle Areas, Northeast Alabama. By Preston E. Cloud, Jr. Pp. iv + 74 + plates 1-4. Bulletin 1230-G: Mineral Resources of the Devil Canyon-Bear Canyon Primitive Area, California. By Dwight F. Crowder. Pp. v + 19 + plates and 2. \$0.55. Water-Supply Paper 1839-B: Water Resources of Pinal and Saline Counties, Arkansas. By Raymond O. Plebuch and Marion Hines. Pp. iii + 25 + plate 1. Water-Supply Paper 1839-H: Development of Ground Water Supplies at Mississippi Test Facility, Hancock County, Mississippi. By Roy Newcombe, Jr. Pp. iv + 28 + plate 1. \$0.50. Water-Supply Paper 1834: Geology and Ground-Water Resources of Laram County, Wyoming. By Marlin E. Lowry and Marvin A. Crist. With section on Chemical Quality of Ground Water and of Surface Water by John R. Tilstra. Pp. iv + 71 + plates 1 and 2. Professional Paper 303-H: Geology of the Umat-Maybe Creek Region, Alaska. By William P. Brosge and Charles L. Whittington. With Heavy-Mineral Studies of the Umat-May Creek Region by Robert H. Morris. (Exploration of Naval Petroleum Reserve No. 4 and Adjacent Areas, Northern Alaska, 1944-53. Part 1: Areal Geology.) Pp. v + 501-638 + plates 52-58. (Washington, D.C. Government Printing Office, 1966 and 1967.) [19]

Companhia de Diamantes de Angola (DIAMANG). Servicos Culturais Dundo-Lunda-Angola. Museu do Dondo. Subsidios para a Historia, Arqueologia e Etnografia dos povos da Lunda. Publicacoes Culturais, No. 70: C Akixi (Mascarados) do Nordeste de Angola. Por Mesquita Lima. Pp. 34 (75 plates). (Lisboa: Companhia de Diamantes de Angola, 1967.) [20]

Journal of Communication Disorders, Vol. 1, No. 1 (May 1967). Edited by R. W. Rieber, Austin Fowler, R. S. Brubaker and Leo Van Gelde. Pp. vi + 1-107. Subscription price of a volume of about 400 pages is \$1.10; 54 guilders; 60 D.M. (Amsterdam: North-Holland Publishing Company, 1967.) [20]

Canada: Department of Energy, Mines and Resources. Geological Survey of Canada. Bulletin 138: Reconnaissance of the Surficial Geology of Northeastern Ellesmere Island, Arctic Archipelago. By R. L. Christie. Pp. 5 (7 plates). \$1.65. Bulletin 146: The Devonian Cedared and Harroga Formations in the Beaverfoot, Brisco, and Stanford Ranges, Southeastern British Columbia. By H. R. Belyea and B. S. Norford. Pp. 64 (19 plates). \$2. Bulletin 148: Granite and Pegmatite Studies at Northern Indian Lake, Manitoba. By R. Kretz. Pp. 42 (18 plates). \$1.50. Memoir 34: Geology of Mingo Lake-Macdonald Island Map-Area, Baffin Island, District of Franklin. By R. G. Blackadar. Pp. 54 (5 plates). \$2. (Ottawa: Queen's Printer, 1967.) [2]

Bulletin of the American Museum of Natural History. Vol. 136, Article A Review of *Simulium* (*Pternaspatha*) Enderlein (*Simuliidae*, *Diptera*). Pedro Wygodzinsky and Sixto Coscarón. Pp. 47-116 + plate 2. (New York: American Museum of Natural History, 1967.) \$2. [2]

Ontario: Department of Economics and Development. Ontario Report No. 102. (Toronto: Department of Economics and Development, Government of Ontario, 1967.) [2]

The Rockefeller Foundation. The President's Review from the Annual Report, 1966. Pp. 137. (New York: The Rockefeller Foundation, 1967.) [2]

The Diffused Electrical Contacts and Their Applications. By G. Matsumoto and Shigeo Fukase. Pp. 33. (Monograph Series of the Research Institute of Applied Electricity, No. 15.) (Sapporo, Japan: The Research Institute of Applied Electricity, Hokkaido University, 1967.) [3]

Fonds National de la Recherche Scientifique. Trente-neuvième Rapport Annuel, 1965-1966, Vol. 1. Pp. 317. (Bruxelles: Fonds National de Recherche Scientifique, 1967.) [3]

Institut for Atomenergi, Kjeller Research Establishment. Kjeller Report No. 117: The Second NPV International Advanced Summer School on Reactor Physics, Norway, August 1966. Pp. 235. (Kjeller: Institut for Atomenergi, Kjeller Research Establishment, 1967.) [3]

New Zealand: Department of Civil Aviation. New Zealand Meteorological Service. Miscellaneous Publications. Meteorological Observations for 1965—Stations in New Zealand and Outlying Islands, including the Cook Group. Pp. 106. Meteorological Observations for 1965—Stations in New Zealand and Outlying Islands, including the Cook Group. Pp. 109. (Wellington: Government Printer, 1966 and 1967.) [3]

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Further particulars may be obtained from The Secretary to the University, University of Edinburgh, Old College, South Bridge, Edinburgh, with whom applications (six copies), giving the names of two referees, should be lodged not later than September 16, 1967. (644)

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Further details should be obtained from the Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1. Applications close in Australia and London on September 29, 1967. (697)

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RC 213/19/02

Duties: To carry out, control and advise on a programme of range management investigations at two existing pasture research stations in different ecological zones; and to carry out ecological and vegetation surveys as part of a programme of assessing the natural resources of Botswana.

Qualifications and terms: A degree in Agriculture, preferably with experience of pasture research in arid areas. Salary £1,284-£2,556 a year plus a non-taxable gratuity of 25%. Two-three year contract.

## THE GAMBIA

### Agricultural Officer

RC 213/68/02

Duties: To carry out general extension work, including particularly oil palm nursery and plantation practices, to supervise mixed farming centres and to administer and supervise main Agricultural Station. Limited touring.

Qualifications and terms: A degree in agriculture with experience in general extension and mixed farming. Salary £1,140-£2,224 a year plus 25% terminal gratuity. 18-24 months contract.

## KENYA

### Adviser on Farm Management

RC 213/95/01

Duties: To conduct studies and to advise the Agricultural Development Corporation on the most economical systems of farming, and also to advise farmers connected with the Corporation.

Qualifications and terms: A degree in Agricultural Economics with experience in farm management studies, surveys and techniques. Salary range £2,500 to £3,000 a year subject to British income tax plus a variable tax-free overseas allowance currently payable at rates from £270 to £915 a year depending on marital status. Two year contract.

## TANZANIA

### Agricultural Officers (Animal Husbandry)

RC 213/173/025

Duties: To study the distribution of livestock breeds and to advise farmers on the improvement of animal husbandry.

Qualifications and terms: A university degree, with animal husbandry as a major subject, and an aptitude for work involving animal production and nutrition. A knowledge of the distribution and feeding values of different grasses; and an ability to speak Swahili is desirable. Salary £1,329-£2,757 a year plus 25% terminal gratuity. 21-27 month contract.

A contributory pension scheme is available in certain circumstances.

## Entomologist

RC 213/173/023

Duties: To be in charge of the entomological section of a regional research centre, to study the distribution of and damage done by field insect pests and to advise on their control.

Qualifications and terms: A university degree in natural science with entomology as a major subject. Post graduate training leading to a higher qualification is desirable. Salary £1,329-£2,757 a year plus 25% gratuity. 21-27 months contract.

## UGANDA

### Principal/Senior Research Officer

RC 213/183/05

Duties: To take charge of a small section of scientists investigating the breeding and selection of food crops.

Qualifications and terms: Candidates must have at least a second class honours degree in Botany or Agricultural Botany with post graduate training in plant breeding, and overseas experience. Basic salary from £1,839 to £2,175 a year, liable to Uganda income tax. In addition an allowance, normally tax-free, ranging from £888 to £936 a year will be paid by the British Government on behalf of the Uganda Government direct to an officer's bank account in Uganda. 25% terminal gratuity. Contract 21-27 months in the first instance.

## Agronomist

RC 213/183/01

Duties: To carry out an experimental programme on tea with the object of improving production methods and quality.

Qualifications and terms: A second class honours degree in Agriculture with at least three years additional experience of tropical agriculture, preferably specializing in tea. Basic salary from £798-£1,791, liable to Uganda income tax. In addition an allowance, normally tax-free, ranging from £636 to £888 a year will be paid by the British Government on behalf of the Uganda Government direct to an officer's bank account in Uganda. 25% terminal gratuity. Contract 2 years.

## ZAMBIA

### Lecturers (Grade I and II) in Agricultural Economics

RC 213/132/011

Duties: To lecture in basic and applied economics to two year diploma students taking agriculture and allied subjects, and possibly to take part in extra-mural activities. The Grade I lecturer will be responsible for the distribution of duties in the Agricultural Economics Section.

Qualifications and terms: A degree in economics or agricultural economics. A knowledge of co-operatives and agricultural marketing is desirable. For the Grade I post graduate teaching experience is essential. Salary Grade I—£1,930 to £2,275 a year plus 25% gratuity. Grade II—£1,645 to £1,955 a year plus 25% gratuity. A supplement of from £200 to £250 a year is also payable direct to an officer's home bank account. Both the supplement and gratuity are normally tax-free. 3 year contract.

For further particulars of these vacancies and an application form please write giving your full name, age and brief details of professional qualifications and experience, and quoting appropriate reference number, to the Appointments Officer, Room 324 N/8

**MINISTRY OF OVERSEAS DEVELOPMENT**

Eland House, Stag Place, London SW1.

## CSIRO PHYSICIST

### AUSTRALIA DIVISION OF CHEMICAL PHYSICS

**GENERAL.** The Division of Chemical Physics is a component of the Organization's Chemical Research Laboratories and is located in new laboratory premises adjacent to Monash University at Clayton, Melbourne, Victoria. The Division has facilities for research in the fields of optical spectroscopy; mass spectroscopy; electron diffraction and electron microscopy; X-ray diffraction; solid state chemistry, including NMR and ESR investigations; and theoretical chemistry. In addition the Division has well-equipped instrument laboratories for the design and construction of mechanical, optical and electronic equipment.

**DUTIES.** A Physicist is required for the mass spectroscopy group to conduct research, initially into the development of high efficiency ion sources, and to study the ion optics of mass analysing devices (magnetic, monopole, or quadrupole).

The research interests of this group lie in the study of the energy levels of molecular ions, of the fragmentation of molecules by electron or photon bombardment, the use of mass spectra in the identification of organic molecules and elucidation of their structure, in the development of ion sources and ultraviolet light sources, and in the application of mass spectrometers to chemical problems.

The group has three magnetic deflection spectrometers, one used for electron impact work, one coupled to an ultraviolet monochromator for photon impact work, one coupled to a vapour-phase chromatograph for organic chemical identification, and is pursuing an active programme in the development of monopole and quadrupole mass spectrometers.

**QUALIFICATIONS.** Applicants should possess a Ph.D. degree, preferably in Physics, although a chemist with physical experience would be acceptable, or have had postgraduate research experience of equivalent standard and duration supported by satisfactory evidence of research ability.

**SALARY.** Depending upon qualifications and experience, the appointment will be made within the ranges of Research Scientist \$A5,250–\$A6,622 p.a., or Senior Research Scientist \$A6,892–\$A7,974 p.a. Salary rates for women are \$A428 p.a. less than the corresponding rates for men. Promotion within CSIRO to a higher classification is determined by merit.

**CONDITIONS.** Fares paid for the appointee and his dependent family. Further particulars supplied on application.

Applications, quoting reference number 582/6 and stating full name, place, date and year of birth, nationality, marital status, present employment, details of qualifications and experience, together with the names of not more than four persons acquainted with the applicant's academic and professional standing, should reach:

Chief Scientific Liaison Officer,  
Australian Scientific Liaison Office,  
Africa House, Kingsway,  
London, W.C.2.

by the 30th September, 1967.

(685)

## GRADUATE IN AGRICULTURE OR SCIENCE

Required by An Foras Taluntais (The Agricultural Institute) for a challenging post on the development of analytical methods on feed-stuffs and biological materials in the Animal Sciences Division. Salary up to £2,465 depending on qualifications and experience. Marriage and children's allowances. Superannuation scheme including special arrangement for F.S.S.U. member. **ESSENTIAL:** Honours University degree in Agriculture or Science (majoring in Chemistry, Biochemistry or Agricultural Chemistry.) **DESIRABLE:** Experience of analytical work and staff supervision.

Application forms from The Director, An Foras Taluntais, 33 Merrion Road, Ballsbridge, Dublin 4. Latest date for receipt of application forms September 25, 1967. (658)

## UNIVERSITY OF MANCHESTER

### MANCHESTER, 13 DEPARTMENT OF PHYSICS

A Research Assistant required to run a PDP7 computer connected on line to nuclear physics experiments on a 6 MeV Van de Graaff generator. Successful candidate should have research experience in nuclear physics. Knowledge of computer programming desirable, but not essential. He will be expected to initiate and develop programmes for the on-line handling of data, and to participate in the research programme of the group. Initial salary according to qualifications and experience in the range: £1,470 to £1,830 per annum.

Particulars and application forms (returnable by September 23) from the Registrar. (667)

## AGRICULTURAL RESEARCH COUNCIL

Assistant Experimental Officer required for research on the muscular activity of large and small animals. The work will utilize a wide range of physiological and biochemical techniques, for which a basic training in Zoology or General Biology is desirable with some Chemistry.

The post exists at the new Meat Research Institute due to be completed at Langford, nr. Bristol in October/November. Basic qualifications: Pass degree, H.N.C. or equivalent in appropriate subject for candidates aged 22 or over, under 22 years—G.C.E. in five subjects, to include two 'A' level scientific subjects and English language. Salary: according to age on A.E.O. scale: £803 at age 22, £1,017 at age 26 or over; rising to £1,243, with promotion prospects. Optional contributory pension scheme: five-day week.

Application forms: Meat Research Institute, Low Temperature Research Station, Downing Street, Cambridge, quoting BP 9. (704)

## UNIVERSITY OF NOTTINGHAM

### DEPARTMENT OF PHARMACY

Applications are invited for an ASSISTANT LECTURESHIP or LECTURESHIP IN PHARMACOGNOSY. The appointee will be required to participate in teaching undergraduate students in Pharmacognosy up to Honours degree level and to participate in the supervision of post-graduate work. The appointment will be effective from January 1, 1968 and the salary will be within the range £1,105 to £1,340 (Assistant Lecturer) or £1,470 to £2,630 (Lecturer).

Further particulars and forms of application, returnable not later than September 15, from the Registrar. (671)

## UNIVERSITY OF EDINBURGH

### DEPARTMENT OF GENETICS

**POST DOCTORAL RESEARCH WORKER** required to work in association with Dr. R. A. Beatty on problems of animal gamete genetics. Current problems include the study of the genetics, immuno-genetics and physical and chemical variation of spermatozoa. Initial appointment will be for a period of two years. Salary comparable with University Lecturer's Scale.

Applications, by letter, giving the names of two referees, should be lodged with the Secretary to the University, University of Edinburgh, Old College, South Bridge, Edinburgh, 8. (655)

## UNIVERSITY OF LEEDS

### RESEARCH ASSISTANTS IN AGRICULTURAL CHEMISTRY

Applications are invited for appointment as Research Assistant in the Agricultural Chemistry section of the School of Agricultural Sciences at a salary of £825 by £50 to £925 a year. Appointment will be initially for one year and renewable up to a maximum of three years.

There are two appointments available. One is for research into the effect of the plane of energy and protein nutrition on the yield and composition of milk in the sow. The second is for research into the effect of food additives on food utilization and growth in sheep and calves. Persons appointed may be able to register for a higher degree.

Applications (three copies) stating age, qualifications and experience and naming three referees, should reach The Registrar and Secretary, The University, Leeds 2, (from whom further particulars may be obtained) not later than September 4, 1967. (664)

## SPECIAL LIBRARIAN

The rapidly growing Library of the CENTRAL ELECTRICITY RESEARCH LABORATORIES, Leatherhead, Surrey, covers a wide range of subjects including chemistry and biology, physics, metallurgy, electrical, mechanical and civil engineering. Some 450 technical and scientific journals are currently taken.

The Librarian will be responsible for maintaining close liaison with National and other Special libraries. In addition, he will be responsible for the efficient administration of the Library, the ordering, cataloguing, and classification using U.D.C.

Applicants must be professional librarians with experience in a scientific or technical library.

Salary within the range £1,555 to £1,845 per annum.

Applications, stating full relevant details and present salary to the Personnel Officer, Central Electricity Research Laboratories, Cleve Road, Leatherhead, Surrey, by September 6, 1967. Quote Ref. N/303. (680)

## BRIGHTON EDUCATION COMMITTEE

### BRIGHTON COLLEGE OF TECHNOLOGY

Required as soon as possible:

- SENIOR LECTURERS in (a) BUILDING PROCESSES AND ADMINISTRATION
- (b) MATERIALS AND ENVIRONMENTAL SCIENCE

Honours graduates, with research, industrial and/or teaching experience, to teach at all levels of Honours degree courses required. Salary (under review) £2,140 to £2,380 per annum. Increments within the scales allowed for approved experience.

Further particulars and application forms, returnable by September 4, 1967, from Registrar, Brighton College of Technology, MOULSE-COOMB, Brighton 7. (675)

## MERRY-GO-ROUND

THE changes in the composition of the British Government made public on August 28 are unlikely to have much direct effect on the interests of science and technology in the months ahead. On the whole, the gains seem nicely balanced by the losses. The most worrying change is the departure of Mr Anthony Crosland from the Department of Education and Science and his replacement by Mr Patrick Gordon Walker, if only because it introduces the biggest uncertainty in the new pattern. Since he went to the Department of Education and Science nearly three years ago, Mr Crosland has won for himself a reputation which engendered something akin to a sense of security among those associated with the department. Almost the only blot on his copybook—but a big one—is the pigheaded decision that university fees would be increased (by a factor of roughly three) for students from overseas. By way of compensation, Mr Crosland seems to have put up a stalwart fight in the past few months to prevent too great an erosion of the educational budget for the financial year ahead, and everybody will be grateful to him for that. He has been an able minister, and his friends will not be the only ones who will be disappointed that he is only being moved sideways, to the Board of Trade, and not upwards to the Department of Economic Affairs. (If Mr Crosland should choose to reconsider his predecessor's decision to build an airfield at Stansted, that will be an uncovenanted benefit of enormous value.)

Mr Gordon Walker is by comparison an unknown quantity. He is scholarly (which should help), donnish (which is if anything a disadvantage), and has such a reputation for loyalty to the Government as a whole that people will naturally fear that he will compromise too readily with other departments. To say this does not imply that compromise is necessarily bad, but merely that in the two years ahead there are bound to be great pressures on the management of resources for expenditure on research in the universities, and that the most reasonable courses of action will not necessarily be the best. The issue of whether the responsibilities of Mr Gordon Walker's department should be transferred to the Ministry of Technology is an obvious snare. There is some logic in it, and obviously there is a great and continuing need of better liaison between the two departments, yet any change in this direction should be vigorously resisted until the Ministry of Technology has shown that it would be able sympathetically to absorb university research without giving in to the temptation to spend the money on something else.

The loss occasioned by the departure of Mr Crosland will to some extent be cancelled out by the simultaneous departure of Mr. Goronwy Roberts, who has for several months been concerned with the admini-

stration of the research councils. He has not always inspired confidence on scientific matters. He will be succeeded either by Mrs Shirley Williams or by Miss Alice Bacon, either of whom would be a distinct improvement. Mrs Williams would be a particular asset. She is, after all, the best candidate in sight for the somewhat distant goal of the first woman to be Prime Minister.

For the rest, the reconstruction of the British Government may raise important issues by the changes it will bring about in the balance of power between the economic ministries and the Ministry of Technology. For several months now, and for the best of reasons, the edges between the Ministry of Technology and the Department of Economic Affairs have been confused. Both ministries, for example, are concerned with the economic health of large sections of British industry, the Ministry of Technology by its terms of reference and the Department of Economic Affairs through the working parties which are studying, with varying degrees of success, the functioning of particular sectors of industry—the "little Neddies" as some wag has christened them. Then both ministries claim credit for devising the Industrial Reconstruction Corporation, and the Ministry of Technology has moved steadily, in the three years of its existence, towards the view that the solution to a great many industrial problems is to be found in economics and not in technology in the old-fashioned sense. Yet there are tensions between the ministries as well as common purposes. Should the Ministry of Technology continue to support expensive developments such as the Concord supersonic aircraft, for example? The DEA is likely often to disagree with what the Ministry has to say. In the days when Mr Michael Stewart was in charge at the DEA, the Ministry of Technology may have had its own way too easily. Whether that state of affairs will continue now that Mr Harold Wilson has decided to take charge of the DEA himself will depend on how much time the Prime Minister can spare for paying attention to the details of his new department's work. His mere presence there, however, could create a more productive relationship between the two departments. With luck, that could be a benefit.

## WHAT IS SCIENCE POLICY?

LORD JACKSON has done a public service in his presidential address to the British Association (see page 1023) by drawing attention to the underlying complexity of what people are usually pleased to call science policy. For several years now, but particularly since the governments of several countries have been seek-



ing ways of keeping the growth—and the cost—of science and technology within what seem to be reasonable bounds, there has been a danger that many essential issues would be grossly over-simplified. For example, there has been a tendency for governments to judge the success of their policies entirely by the size of the fraction of the Gross National Product being spent on research and development. Thus in Britain, it has frequently been held that there is no great need to change the scale of support for science and technology because the magic ratio is not very much greater in the United States. In France, by contrast, where the cost of research and development was a mere 1.7 per cent of the GNP three years ago, comparisons with the United States were one of the principal reasons why the French Government embarked deliberately on a programme to increase the amount of money being spent on science and technology. The danger is that, in their preoccupation with these and other yardsticks, those responsible for public administration will overlook the more enduring criteria for deciding what to do and how much to spend.

There is already some evidence in Britain of unhealthy tendencies in directions like these. Lord Jackson has quite properly raised the question of how people should determine the sums of money to be spent on the support of fundamental research, chiefly through the research councils, and this is very much a field in which rules of thumb have recently become fashionable. Recent history is a sufficient explanation of why the administrators are rushing to embrace convenient but possibly misleading yardsticks. Between them the research councils and the University Grants Committee are now spending the best part of £100 million on the support of basic research, two-thirds of it in the universities. Expenditure under these headings has been growing more quickly than most other forms of public expenditure—by 13 per cent or so in the first half of this decade. It did not take long for people to point out that things could not go on like that. In two decades or so, pure research would be costing as much as the National Health Service—that is how the argument used to go. By the end of the century, scientific research would be consuming the resources of the whole of manufacturing industry. So it was concluded that there must at some stage be a break in the growth curve. And why not sooner rather than later? For is this not a time when Britain should be spending more on technology (which makes money) and not basic science (which consumes it)? By these and other arguments, it seems to have been agreed that there should now be a deliberate slackening in the pace of growth of spending on science. The trouble is that there is no way of knowing whether this decision is of the kind that would have done justice to Solomon or whether, on the other hand, it is just a guess.

The objective, of course, is that decisions about the amounts of money to be spent on university research should ideally be made in the light of a hard-headed appreciation of what university research is for. This is where the numbers are not much help. There is no

way of escaping the question of why university teachers should be allowed, even encouraged, to devote much of their time to activities which are not strictly pedagogical. Lord Jackson is right in saying that it is not good enough to say that research is always inestimably valuable because nobody can predict what benefits may emerge from it. That is a counsel of unreason.

The real defence of university research must ultimately lie in the old dogma that research and teaching are inseparable. If there is anything at all in that assertion, the rapid growth of expenditure on university research in the early sixties is not nearly as outrageous as it sometimes seems to the administrators. The numbers of people teaching science at the universities increased by roughly 9 per cent a year between 1961 and 1965. Is it not reasonable that the expenditure on academic research should have increased by something like 13 per cent a year, especially when account is taken of the inevitable increases of costs from one year to another—the sophistication factor as it is called—now reckoned to account for a steady increase of 4 per cent a year? And what is to happen now that there are polytechnics as well as universities providing higher education?

Two important conclusions emerge from this. First, the question is not how much the country can afford to spend on academic research, but how much it can afford to spend on higher education. Second, if the policy makers really want to make the best use of public money—as they should—it would be prudent of them to carry out a sensitive field study of the relationship between teaching and research at universities so as to devise meaningful yardsticks for deciding what costs are reasonable.

Similar pitfalls attend the making of policy for the support of industrial research. Here, too, the simpler numbers spell success. Lord Jackson points out, with all due pleasure, that industrial organizations now spend about two-thirds of all the money allocated in Britain to research and development, and that only a third of what they have to spend comes from contracts for defence research. That picture is much more cheerful than it used to be even five years ago. But how much of what is being spent is valuable? What, in any case, should be the objectives of industrial research and development? Should the policy makers have it in mind that industrial research is a kind of investment and should they seek to distinguish between successful projects and the failures by calculating some kind of economic return on every investment? By that test it seems fairly clear that many popular technological causes, the Concord supersonic aircraft among them, would be counted not triumphs but a waste of money and, what is more important, a misinvestment of men as well. In other words, in technology much more than in basic science, the sum of money being spent may be a measure of how many people are occupied on research and development but may say nothing at all about the effectiveness with which they are deployed.

But if the Government and its committees cannot

take decisions on matters like these, who is to take the risk? Luckily, the experience of the two straitened years just past has helped the British Government to a more realistic appreciation of events than had previously been possible. For one thing, it now seems to be acknowledged that industrial research and development is not really an end in itself, but one of several means by which economic prosperity may be attained, or at least sought.

From this, alas, it follows that decisions about the scale on which research and development should be supported must be made, in the last resort, by people able to calculate the potential rewards and to take the risk of deciding whether to embark on something new. For the most part, the policy makers can only cheer from the sidelines, providing encouragement and some basic help—decent information services and reproducible standards, for example. In the long run, if they really want to encourage the greater use of research and development in industry, they must find some way of helping enterprises of all kinds to take adventurous decisions. In this context, of course, it does not matter whether an industrial enterprise is nationalized or privately owned—what matters is merely that assessments about the potential value of research and development should rest with the people responsible for day-to-day survival. This is the strongest argument for asking that the Ministry of Technology should now practise what Professor Blackett has been preaching, and take effective steps to channel the talents of the public laboratories into industry.

But what if industry chooses to spend more on research and development than the existing stock of trained men and women can successfully carry out? This is a reasonable anxiety, if only because it is but a decade since Britain was acutely short of people. In this sense, it is entirely right that Lord Jackson should have devoted much of his attention to manpower. In the late sixties, however, the old problems have been transformed if not quite melted away. There is now a good deal of slack in the system. Of the 211,000 scientists and engineers at work in Britain in 1965, for example, only 40,000 or so seem to have been employed on industrial research and development. It is hard to think that industry would suddenly run short of people to carry out development if companies collectively decided to increase the scale of their research and development by, say, 50 per cent. It is true that there would be some difficulties, and that companies would have to pay their technical people less meanly, but that is a secondary consideration. It is also true that the men recruited into industry would usually leave some other field of activity bereft, for, even though the system may now be fairly flexible, there is on balance a shortage of trained people. Yet there is also a greater capacity within the system for responding to the changing pressure of demand.

In other words, manpower has almost ceased to be a cause of anxiety in its own right. Instead, it is a field in which the policy makers should be ready to seize the opportunities which may offer themselves.

It is, in particular, a crying need that something more real than lip service should be paid to the principle that the modern world needs an educational system qualitatively different from that which the Victorians invented. The most urgent need is to arrange that all of those who leave schools should know what science and technology are about, that those who leave universities trained as scientists should also know who Keynes was—and Samuelson as well—and that nobody should be required at some over-tender age to declare himself for science, medicine or the humanities without much chance of going back. If there is anything which can be dignified as science policy, it should be the province of men whose real aim is a thorough revolution of the educational system.

In this and a host of other ways, the pursuit of objectives in science policy ends up in fields as different from each other as education and the management of the economy. That is entirely as it should be, for science is no longer a separable part of a nation's activity. It follows, however, that attempts to talk about science policy in isolation from other kinds of policies run the risk of seeming nonsense. But it is also plain that the proper management of scientific resources—a term which is almost entirely synonymous with people—requires that decisions should be made on a small scale and not usually at the centre. Certainly where the fuller exploitation of technology is concerned, the Government must act remotely; there will be trouble if it intervenes too directly on its own accord. In the field of science and technology as a whole, the Government can do much to encourage a more efficient use of facilities for research. Lord Jackson was right to emphasize that in the last resort, a sensible appreciation by the public at large of what science and technology can provide is the only guarantee of good sense. He could well have added that it is equally important that there should be a general appreciation of how science and technology are not almost magical entities in their own right, but, rather, integral parts of national activities of all kinds.

How is the British Government performing by these tests? That is the question which Lord Jackson raised but did not answer. It is fair, however, to acknowledge that in the past two years a great deal has been done to create some useful machinery by means of which sound decisions can be reached. Bodies like the Council for Scientific Policy are working well. It remains to be seen how effectively the Central Advisory Council on Science and Technology will function, but there is at least a chance that good works will be done. Yet it is also plain that the contexts within which these otherwise admirable bodies function are often far too circumscribed. The Council for Scientific Policy, which has a good record for tackling general questions such as the need of computers in universities and the relationship between universities and government laboratories, has nevertheless fought shy of attempts to influence the quality of teaching in the schools and universities. That may be easier after the Dainton Committee has reported (whenever

that may be), yet there is no reason why this central issue should not have been tackled long ago. And there remain, of course, outstanding the thorny questions of the scale and the manner in which public funds should be used to support research. In other words, there has been some progress in the past three years and some of it is valuable, but there is a danger that the British Government and its committees will interpret the term science policy too literally, and thus attempt to make too many decisions at the centre. What Lord Jackson has been saying may help to push things in the other direction.

## ONE STEP ON

THE announcement in Geneva a week ago that the Russian and United States Governments have been able to put forward a draft treaty intended to limit the proliferation of nuclear weapons is a hopeful if small step forward. The Disarmament Committee has been in session at Geneva for so long now that its credibility is much in danger of erosion. If it now becomes the forum for a realistic debate on the extent to which an agreement on the proliferation of nuclear weapons can be successfully controlled by international inspection or by some other means, much will have been done to restore its reputation—and the hopes of those outside the conference that some progress on disarmament may eventually be possible.

That said, however, it is inevitable that the weeks ahead will not be comfortable for the delegations represented at Geneva. After several months of private talks, the United States and the Soviet Union have been forced to admit failure in their attempts to win agreement behind the scenes for the incorporation of safeguards provisions in the treaty they have tabled. It is no surprise that things have turned out that way (see *Nature*, **214**, 753; 1967). The United States has plainly failed to win the agreement of the member nations of Euratom that responsibility for inspection and control should be placed with the International Atomic Energy Authority at Vienna, and the Soviet Union is entirely justified in its insistence that it would be improper to delegate this task to Euratom itself.

But this, of course, is only skirmishing. The difficulties ahead are much more serious. The outstanding uncertainty is whether the nations which are not nuclear powers, and which have no intention of making nuclear weapons for themselves, will stomach a treaty which permanently confirms the nuclear powers in their present grandeur and throws the rest of the world open to international inspection designed to stifle imitative ambitions. The nuclear powers have hitherto been unreasonably hopeful of what the smaller nations would be happy to accept. For all the cheerfulness which has accompanied the tabling of the treaty at Geneva, it remains unlikely that the nuclear powers will be able to win agreement from the smaller nations without making substantial concessions on their own

account. But there are technical as well as political problems still to be resolved. How feasible will it be to design cast-iron inspection systems? How soon could a safeguards system operate? In any case, what value is there in a treaty for the non-proliferation of nuclear explosives which does not include China and France as signatories?

In the circumstances it would be entirely sensible if the nations now negotiating at Geneva were to go back almost to the beginning of the discussions about the non-proliferation treaty. To begin with, some years ago, people seemed entirely happy with the notion that a non-proliferation treaty should not include a rigid safeguards system, at least at the beginning. Only when bellicose noises from West Germany had made the Russians take fright, a year or so ago, did the issue of safeguards become a central issue and a stumbling block. But things have changed a great deal in the past twelve months. European nations have clearly become much less concerned about the advantages or otherwise of manufacturing nuclear explosives for themselves. So may it not be prudent to think now of a non-proliferation treaty in which the attempt to design a safeguards system is replaced by something less ponderous? This would be something well worth trying for. One possible line of compromise would be an international agreement that all nations would make an honest public declaration at regular intervals of all activities connected with the exploitation of nuclear materials. Nuclear powers, for example, would be required every so often to say how much uranium they had converted into a fissile form, and how much of this they had committed to the manufacture of explosives. By itself, this would do a lot to salve the injured pride of smaller nations. But there is good reason to expect that such a system, operated by the non-nuclear powers and the rest, would be a good assurance that the manufacture of nuclear explosives would not spread. After all, in the climate of mutual curiosity that would be certain to follow the signing of a non-proliferation treaty, it is extremely improbable that a nation forced to make public declarations of its activities in potentially important fields would be able successfully to keep secret the clandestine manufacture of nuclear explosives. At the same time, there would be good cause to hope that a treaty drawn on comparatively flexible lines like these would serve much more effectively than the cumbersome instrument now being negotiated as a platform from which further forays into disarmament might be attempted. Certainly it would be a great misfortune if there were so much bickering about safeguards in the months ahead at Geneva that the members of the Disarmament Committee would be persuaded to give up once more the hope of signing a treaty to bring the production of nuclear explosives by the nuclear powers under some kind of control. In other words, compromising on a system less rigid than the safeguards now in prospect would be not merely an immediate benefit but an insurance for the future. Will the negotiators seize this opportunity?

## NEWS AND VIEWS

### Aluminium at Home

SEVERAL aluminium companies have made independent proposals to the British Government about the construction of an aluminium smelting plant in Britain. Last year, 259,000 tons of unwrought and 80,000 tons of wrought aluminium were imported at a cost of more than £65 million, while the home production of aluminium ingots was only 35,000 tons. The government is doubtless aware of the savings to be made by setting up smelting works; the problem is one of cheap and adequate power supplies for these hungry users of electricity. The small Scottish plants, running on hydro-electricity and which produce most of the British aluminium, were built well before the last war. The sudden renewal of interest in the possibilities of smelting in Britain derives partly from the fear of import duty on ingots if Britain is to enter the European Economic Community and partly from the prospects of cheaper fuel.

Most of the companies are more interested in the cheapness of the electricity for their projected plants than in its source. Rio Tinto Zinc Corporation, however, has submitted a plan whereby its aluminium plant would share the entire output of a nuclear power station with the Atomic Energy Authority's gaseous diffusion plant at Capenhurst. This is at present supplied with enormous quantities of electricity by the national grid. There may indeed be logic in making large and constant users of electricity independent of the public supply, though less than 10 per cent of the electricity produced in Britain last year was generated outside the public electricity boards. The cost of the plan, both to the aluminium company and, through the effect on the national grid, to the ordinary consumer, is at present being studied by the government. There have been suggestions that the government may be able to participate in these ventures by means of a bill, soon to be introduced in the House of Commons, that would enable it to take up shares in private industry. In any case, there is always the Industrial Reconstruction Corporation. The implications of the proposals are many, and a statement from the government, when it comes, may affect a good deal more than just the aluminium industry.

### Engineering at Cambridge

At the recommendation of the Faculty Board of Engineering, the Mechanical Sciences Tripos at Cambridge is to be replaced by an Engineering Tripos. Courses for this new tripos will begin in 1968 and the first examination for Part One will be in 1970 and for Part Two in 1971. The importance of this change

lies in the fact that Part One of the new tripos will take only two years and will not be considered sufficient for a degree. All engineering students will be required to take Part Two. This change brings the engineering faculty into line with the others at Cambridge which have one by one decided to insist on a Part Two. The existing Mechanical Sciences Tripos is now the only one that still considers Part One to be a three year degree course. At present, exceptional students have been completing Part One in only two years and then going on to take Part Two, which in many ways is the equivalent of a postgraduate course.

One of the important effects of the change is that the new Part One courses will be less specialized—it must, or should, be impossible to teach in two years what previously took three. This in turn should make it easier for students to move between faculties, from natural sciences or mathematics to engineering for example, and, after all, this sort of flexibility is supposed to be a great virtue of the Cambridge Tripos system.

The new Part Two will offer a much greater variety of courses. The new tripos includes several more general courses including economics, statistics, operation research, sociology and psychology. The faculty hopes to cater not only for the outstanding engineer who wants nothing else, but also for the numerous students who would benefit from a more general outlook on how engineering fits into society and the economy. At present large numbers of engineering graduates go directly into management rather than spending some time in design, and for them the general courses now being offered are virtually vocational training.

Another change is that the results of the examination for Part Two of the new engineering tripos, like those of all other faculties, will be classified. At present Part Two of the Mechanical Sciences Tripos is a pass or fail affair. This decision to give classes is not universally welcome. There is a strong case for arguing, especially in such a broad field as engineering, that it leads to the very antithesis of education, with too much emphasis on subjects likely to turn up in examination papers rather than those that really interest the students, and, of course, supervisions becoming coaching periods. All in all, however, the new engineering tripos should prove to be a distinct improvement on the one it replaces.

### Drugs in Britain

WHEN the Dangerous Drugs Bill becomes law, two important changes will be made in the treatment of drug addicts in Britain. All addicts will have to be registered and drugs such as morphine and heroin will not be available except through special centres. The Ministry of Health has announced that there are shortly to be ten such centres for in-patients in London and thirteen centres, less closely associated with hospitals, for out-patients. Both types will cater only for heroin and morphine addicts, whose number is estimated at between 1,250 and 2,000, mostly in London. The measure was prompted by the conclusion of the Brain Committee that a few doctors were prescribing too many drugs to registered addicts, and thus supplying a black market.

The World Health Organization has now published a report on Services for the Prevention and Treatment



of Dependence on Alcohol and Other Drugs which recommends a method of treating addiction that differs in many respects from that which will be established in Britain. Rather than restrict the capacity to treat addicts to a small number of centres, the WHO committee considers that "treatment of high quality should be made available through an adequate variety of facilities to all who suffer from alcohol or drug dependence . . . The general practitioner can also be involved in treatment and rehabilitation." The WHO committee puts a great deal of emphasis on the problems of psychological and social rehabilitation. But it is difficult to see how an effective "network of facilities" concerned with helping the addict back into society can be maintained in Britain if addicts are required to register with the Ministry of Health and to move from their family doctors to impersonal clinics half way across a city. Some doctors and social workers doubt whether this kind of discipline will appeal to the drug addict, but even if the majority of addicts do attend the centres, very little attention seems to have been paid to psychological rather than physiological cure.

How far addicts can or should be compelled to undertake cures is a vexed question. Dr M. M. Glatt, the only British member of the WHO committee, endorses the view that compulsory treatment of addicts is often successful and should certainly be given to criminal addicts. He points out that it is sanguine to hope that many drug addicts would come for treatment voluntarily. On the other hand, at the homes run by the Simon Community, addicts are allowed to continue to take drugs until they may decide to be cured. The director of the Community, describing the bill as a panic measure, said that it is absurd to isolate "hard drug" addicts by subjecting them to a unique treatment. What is needed is an understanding of the "addict personality", which will become impossible if addicts are caused to reject authority further.

At this stage there is no way of knowing whether the bill will succeed in its main aim, the restriction of the supply of drugs, or whether by stimulating a black market it will make the association between addiction and crime closer still. In any case, the bill is certainly something of a makeshift: no specific sum has been allocated to the new centres and no training programme for the doctors working in them has been instituted, despite the recommendation of the WHO committee that specialists of this kind should undergo extensive training. If doctors are not trained, as Dr Glatt points out, there is a danger that the out-patient clinics will degenerate into drug dispensaries.

It is also difficult to appreciate the need to change the whole system of treatment of drug addicts in Britain in order to curb the irresponsible activities of those few doctors who prescribe excessive doses of drugs. The Swedish Drug Addiction Control Committee, faced with the same problem, has proposed that a computer record should be kept of all prescriptions. Doctors who supplied the black market could then be disciplined immediately, and the system would also prevent addicts from going from doctor to doctor in search of drugs. Could not the British Government have introduced legislation to deal with irresponsible doctors separately, and then have paid close attention to the far more serious questions of discouraging drug addiction and curing addicts both in body and mind?

## BA President

THE Council of the British Association has announced that Sir Peter Medawar will be the President of the British Association for the Advancement of Science for 1968-69 in succession to Dame Kathleen Lonsdale. Sir Peter has been the Director of the National Institute for Medical Research since 1962. He was educated at Marlborough, and Magdalen College, Oxford. While at Oxford he won the Christopher Welch Scholarship in Zoology, and became a Fellow of Magdalen by examination in 1938. He remained at Magdalen as Fellow and University Demonstrator until 1944, when he took up a fellowship at St John's College. In 1946 he returned for a year to Magdalen before becoming Mason Professor of Zoology at Birmingham University.

In 1951 he was appointed to the Jodrell Chair of Zoology and Comparative Anatomy at University College, London. It was his researches here that won him the Royal Medal of the Royal Society, of which he became a Fellow in 1949, and, in 1960, the Nobel Prize for Medicine. This latter award he received jointly with Sir MacFarlane Burnet, Director of the Walter and Eliza Hall Institute for Medical Research, Melbourne. Burnet had predicted that if an animal was exposed to an antigen at an early enough stage in development, "immunological tolerance" to that antigen would result. Medawar proved this by injecting spleen cells into newborn mice; skin grafts made on the mice showed that the mouse was tolerant of what would normally be a foreign body.

Sir Peter gave the Croonian Lecture of the Royal Society in 1958 and the BBC Reith Lectures in 1959. Before assuming the direction of the National Institute for Medical Research, he had been a member of the Agricultural Research Council and of the University Grants Committee. He is a foreign member of the New York Academy of Science and the US National Academy of Science. He was knighted in 1965.





## Tribology Catches On

IN March 1966 a committee of the Department of Education published a report on "Tribology", which is the name it coined for the science of lubrication. The committee estimated that £500 million is being wasted each year by industry through lack of knowledge of the problems of lubrication in the design of machinery, and recommended the setting up of institutes of tribology to provide a "specialist tribological service to industry". The project was widely criticized: there seemed little point in setting up grandiose and specialized institutions when the need was to spread an awareness of the problems of lubrication through industry. Even so, Britain is to get its first Centre of Tribology, according to the Ministry of Technology. The site will be announced in September.

A more immediate effect of the committee's report was to stimulate interest in lubrication science at universities and colleges. Imperial College, which last year began one week courses in lubrication, was one of the first institutions off the mark. The lecturers came from industrial companies particularly concerned with lubricants and bearings; their students have been designer draughtsmen, production engineers and technical representatives, as well as more senior staff. So diverse were the problems, from the lubrication of zoom lenses to tin dredgers, that the Department of Mechanical Engineering has decided to introduce four specialized courses into its programme for next year. These will deal with the problems of lubrication in steadily loaded, and reciprocating bearings, in car, train, ship and aircraft machinery and in machinery working in hostile environments. The courses, which are open to thirty-two people at a time, are too short for experimental work. Demonstrations do, however, follow the lectures, and the department has a flourishing mortuary of failed ball bearings. There will be two courses next term, five at Easter and three the following summer. The cost is twenty-five guineas.

Bradford College of Technology also began courses in lubrication after the committee issued its report. The college at present offers two sets of courses, one as an optional part of the examination for mechanical engineering technicians. The other is intended for semi-skilled workers, and lasts for three weeks. The college is planning to introduce a third course, to make designers and managers aware of the problems of lubrication.

The forthcoming journal *Tribology* (Hiffe Science and Technology Publications) will fulfil the most valuable of the functions of the proposed institutes, that of disseminating knowledge without arrogating a special importance to tribology. And not even the most ardent well-wishers of the new science can be altogether happy at what it has been christened. For *tribos* means "rub off", not "rub". Not "friction", but "deletion".

## Television on Tape

ICI Ltd, Ciba Ltd of Switzerland and the Columbia Broadcasting System of America have announced the formation of a partnership to manufacture and market in Great Britain and Europe a new audio-visual system known as Electronic Video Recording and Reproduction, EVR for short, which has been described as a

momentous technological achievement and an entirely new medium of communication likely to cause a revolution in the educational use of television. It is claimed that EVR will be the long playing record of television. Indeed the research and development that has led to it was done under the direction of Dr P. C. Goldmark of CBS, who earlier developed the long playing record.

The outstanding feature of EVR is that it allows for the first time the showing of pre-recorded programmes on conventional television sets. Pre-recorded film in small cassettes, only seven inches in diameter and half an inch wide, is placed in a reproduction unit, a player, which in turn is linked up to a commercial television set. Each cassette has enough film for an hour long programme in black-and-white and half an hour in colour. Furthermore, each frame can be held on the screen, and this flexibility is one of the great classroom advantages of the EVR system. A teacher can stop a programme at any time to emphasize a point.

Perhaps the most notable technological advance of the entire EVR system is the film used for pre-recording. It was developed by CBS and Ilford Ltd, a jointly owned subsidiary of ICI and Ciba, to overcome the two major problems of miniaturization, namely, the difficulty of projecting sufficient light through a very small frame to give a readable image on a viewing screen, and preventing the seemingly inevitable flickering and shakiness that occur when small frames are quickly passed through a projector. Their success in solving the problems can be judged from the fact that the film, unperforated and 8.75 mm wide, in each seven-inch cassette contains no fewer than 180,000 frames, and when projected it moves at the rate of five inches a second.

There seem to be endless possibilities for the EVR system. At present there are 20,000 television sets in English schools, not to mention the universities and technical colleges, and these could be much more efficiently used if teachers were freed from the restrictions of national programmes. Moreover, EVR may find an immediate market in libraries, relieving them of some of the problems of storage and retrieval of information. There is also home entertainment.

No doubt the manufacturers look forward to the day when every school and every home has its library of cassettes. They predict a sale of one million cassettes a year by 1971, and since the system is cheap they will probably achieve this. They estimate that the player will cost £100 and each cassette £5, which seems good value, given that the whole *Encyclopaedia Britannica* would only occupy one and a half cassettes.

EVR will bring comfort to the officials of NASA, who have long been predicting the values of spin off from the American space programme. The work of Dr Goldmark and his collaborators at CBS was initiated under a contract from NASA to develop the miniaturization of television for space research.

## Development and Aid

In the annual report of the Overseas Development Institute, the director, Mr William Clark, describes 1967 as a difficult year for those who care about development. The economic difficulties that caused Britain to cut the aid budget also affected the institute's

sponsor companies. The institute was founded in 1960 as a study centre for problems of overseas development, and as an independent advisory body on governmental and other aid. The subsequent establishment of the Ministry of Overseas Development has not, apparently, reduced the need for the institute as a propagandist. Today, more than ever, according to the director, the institute is swimming against the tide of political disenchantment.

The year has seen publications for research works on the French aid programme and on volunteers in development, as well as the first of a series of annual reports on British development policies. The institute seems to pay little attention to the particular problems of scientific aid, despite its all important role in helping developing countries. Its report gives the impression that what makes aid ineffective is inefficient administration in the programmes of donor countries, combined with lack of money. Giving, it would seem, has become a science, and how aid is given seems at least as important as what is given.

## Two Radiotelescopes

THE Dicke panel, set up by the National Science Foundation to decide what new radiotelescopes should be built in the United States, has decided that only two of the six proposals put before it should be accepted, and these two are the simplest and least expensive (see *Nature*, 215, 340; 1967). The panel's decisions, however, seem to be based on sound technical grounds and not because the Johnson administration had put pressure on it to economize in the interests of the war in Vietnam. One of the proposals accepted is for improving, at a cost of about \$3 million, the surface of the hemispherical 300 metre antenna at Arecibo, Puerto Rico, which is administered by Cornell University. This will increase the optimum frequency of the telescope from 68 cm (440 megacycles) to 10 cm (3,000 megacycles).

The other acceptable proposal is for the addition of seven 40 metre dish antennae to the one being built at Owens Valley, California, by California Institute of Technology. The total cost of this is estimated at \$15 million.

For the time being, the panel has suggested that the proposal for a fully steerable parabolic dish 135 metres in diameter put forward by the consortium of twelve universities in the north-east of the United States should be postponed for the time being, chiefly so as to allow an opportunity for experience of radiotelescopes fully enclosed in plastic radomes.

## Much about Meteorites

AN informative and attractive pamphlet *Meteorites—a concise account* by Dr A. A. Moss has just been published by the British Museum (Natural History) at 3s. There are, of course, several authoritative and expensive books on meteorites available to the specialist, but Dr Moss's pamphlet, giving as it does a succinct and very well illustrated account, is designed for the general reader. What is known of the origins, distribution, composition and structure of meteorites is clearly stated and no doubt it will surprise many readers to find that only twenty-one meteorites have fallen on the British Isles. Nothing remains of three of these, which

fell in the seventeenth and eighteenth centuries, but with one exception the British Museum has material from all the others, including a rare iron meteorite which fell in 1876.

## The Sutton Hoo Ship



A photograph of the British Museum archaeologists re-excavating the Sutton Hoo Ship earlier this summer (see *Nature*, 215, 914; 1967).

## Butterflies Fluctuate

THERE has been some alarm about the declining populations of British butterflies such as the meadow brown, which in some places is reduced to one-third of its normal population. All grassland butterflies seem to be rarer this year than previously, but it is not yet certain whether this is more than a temporary decline, for the sizes of populations are often subject to considerable fluctuation from year to year. The adonis blue and common blue butterflies in a particular area may decline to two or three pairs in one season, and numbers may later increase again to two or three thousand. The adonis blue was almost completely removed from some places during the Second World War when tank tracks passed across its breeding ground, but after the tracks were taken away the butterflies recolonized the area and have continued to flourish. Another reason for diminished numbers in a particular area could be that like the silver studded butterfly the species concerned has suddenly moved to another habitat, perhaps between a quarter and half a mile away, after two or three years in one place.

Those species which are now being driven from their habitats when houses are built on breeding grounds may well be able to survive an enforced move to another habitat. This may also apply to butterflies which are being deprived of their food as a result of the use of weedkillers. Most butterflies feed on common weeds, and so they are being driven from areas in which weedkillers are used extensively. Many species are being poisoned by these chemicals, and also by insecticides, which are harmful to a very wide range of insects. The development of more specific insecticides, which kill only particular insect pests, may cut down losses among butterflies. Like all insects, butterflies are able to build up resistance to insecticides, although the speed with which this occurs is dependent on many factors and can vary considerably from insect to insect. The cabbage white butterfly has developed immunity to DDT, and other species may well develop similar properties.

So far, the declining populations of British butterflies are not necessarily more spectacular than would be expected from the normal fluctuations from year to year. Even the decline of two-thirds in the numbers of the meadow brown may not be particularly ominous.

## Put it Down to Sunspots

IN the July issue of the *Canadian Journal of Botany*, Dr Bruce Cumming, of the University of Western Ontario, claims that there is a correlation between periodicities in the germination of seeds and variations in the solar radio flux, which is an indicator of sunspot activity. Seeds of *Chenopodium botrys* were stored in darkness under controlled conditions of heat, humidity and pressure. Each week a certain number were then removed for germination tests, and the percentage germinating was recorded. There was no correlation, apparently, between germination and humidity, pressure, magnetic intensity or the phases of the Moon. There was, however, a close relation between germination frequency and the level of sunspot activity one week before the wetting of the seeds, although linear trends in solar flux and germination were opposite. At the same time, correlation between germination frequency and sunspot activity is expressed in the form of somewhat compressed diagrams—more than a hundred pairs of values have had to be represented on a horizontal scale only 12.5 cm across. Statistical analysis of periodic functions is necessarily complex, and many workers have emphasized the difficulties of comparing periodicities. It is therefore with caution as well as great interest that further details of Dr Cumming's work will be awaited.

## Chinese Fry

THE white amur or grass carp occurs naturally in the rivers of Siberia and China, but it has been introduced into most of Asia where, raised in stews, it is an important commercial fish and source of protein. Unfortunately the fish do not reproduce naturally in southern latitudes. Much of south-east Asia depends for a supply of fry on China, where there is a considerable industry devoted to catching, sorting and exporting the young fish. Naturally enough, countries like Malaya and India would prefer—and may be obliged

to have—a native supply of young fish instead of depending on Chinese sources. Moreover, the Chinese themselves are worried by existing arrangements, if only because they are leading to over-fishing of the rivers, notably the Yangtze Kiang and Si Kiang. Several attempts have been made, both in China and the importing countries, to induce successful spawning of pond reared fish by injecting female carp with fish pituitary gland extract. Apparently it is always the females that are infertile; the males seem to produce normal sperm no matter where they are. In northern latitudes, Japan, Formosa, Manchuria and Northern India, the experiments were successful and in Cuttack in northern India, for example, hundreds of thousands of young grass carp are raised annually.

In more southern countries, however, the outlook is grimmer. Some recent work done in Malacca, at a latitude of 2° 17' N., by Hickling (*Proc. Roy. Soc. Edin., B*, **70**, 62; 1967) shows that it is impossible to induce spawning of fertile eggs. In Malacca, grass carp grow about three times faster than in natural habitats—Hickling's fish gained between 8 and 10 grammes a day, growing from 2 g to between 6 and 7 kg, compared with a gain of 2.8 g a day by river carp in Siberia. This, of course, is what makes the grass carp such a valuable commercial fish. Furthermore, in Malacca the carp mature earlier and at a smaller size and, being so near the Equator in a climate without marked seasonal changes, they do not have a spawning cycle; some fish are ripe at all times of the year. The males shed active sperm, but the females fail to spawn. The ovaries fill with partially developed eggs which eventually atrophy. When Hickling injected female carp with pituitary extract from another cyprinid fish, *Puntius gonionatus*, the ovaries increased in size as the number of eggs increased, but the eggs themselves were still incompletely developed. When shed, none of these defective eggs was successfully fertilized.

Hickling believes that it is the lack of a climatic stimulus—a spring season with rising temperatures and increasing day length—that prevents egg development in Malacca. The synchrony of the breeding season of mammals with periods of increasing or decreasing light intensity is, of course, well documented. It would be interesting to see if, by artificially altering temperature and day length, the carp could be induced to spawn in Malacca. But even if such experiments proved successful the cost would probably prohibit them as a commercial proposition. It seems that the unfortunate Malaysians must remain dependent on an imported supply of carp fry.

## Sedimentology Congress

from a Correspondent

THE seventh International Sedimentological Congress was held in the Universities of Reading and Edinburgh from August 11 to 15 under the general chairmanship of Professor J. H. Taylor, president of the International Association of Sedimentologists; nearly 450 delegates from 41 countries attended. The choice of two conference centres, several hundred miles apart, was justified on the grounds that both Reading and Edinburgh had excellent claims, the former because of its internationally renowned sedimentology laboratory, and the



latter by its illustrious associations and strong traditions in the earth sciences. Both centres were well placed, moreover, for field excursions in the southern and northern parts of Great Britain.

At the congress attention was focused particularly on modern work involving experiments on the hydraulics of sedimentary processes, which should lead eventually to a fuller knowledge of the silting of harbours and estuaries, among other things. The congress was also concerned with the application of sophisticated statistical techniques to the production of simulation models, for example, and to the more accurate prediction of oil, gas and metal-bearing deposits. There was discussion of the geotechnical properties of clays, which have important economic applications in civil engineering. In addition, biological and chemical aspects of sedimentation received full attention, while the more traditional fields of research were not neglected.

There were two major innovations in the organization of the congress which may be of interest to other scientists. First, the proceedings will not be published. Privately, many cynical remarks are expressed about the somewhat gratuitous proliferation of the literature by "symposium volumes", much of which consists of papers summarizing work already published or which is shortly to appear in print in the established scientific journals. Nevertheless, there is widespread resignation at the collusion of publishers and scientists anxious to promote their subject, while the organizers of the Sedimentological Congress have endeavoured to resist. An added advantage is that contributors to public discussions should feel less inhibited if they know that their remarks are not being recorded for posterity. However, to permit as much time as possible for free discussion, contributors had their short papers cyclostyled as preprints for members of the congress, so that their verbal presentation was restricted to a few minutes. These preprints also served the function of "travel vouchers" for those delegates unfortunate enough not to be able to acquire their expenses in other ways.

Secondly, an attempt was made to counteract the frustration that arises at so many conferences when the chairman, operating to a strict time schedule, is obliged to terminate a discussion just when it is becoming lively. Each day of the congress was therefore so organized that, following general lectures by distinguished scientists and formal discussion groups in the various specialized research fields, at which short papers were read and commented on, delegates had the opportunity to convene informal discussion groups at the end of the afternoon session, to pursue further topics that had provoked a widespread interest. There seemed to be general consent that these informal sessions tended to be among the most stimulating of the whole congress.

## New Looks at Disulphide Bonds

from our Correspondent in Molecular Biology

DISULPHIDE bonds are known to play an important part in maintaining the conformation of those proteins—the great majority—in which they occur. When the disulphide bonds of a protein are in the reduced (broken) state, they must be reoxidized before the native

conformation can be regained. It is also generally true that in the native protein they display a considerable, though variable, resistance to cleavage.

Thus in a survey of a large number of proteins, Davidson and Hird (*Biochem. J.*, **104**, 473; 1967) find that the majority cannot be appreciably reduced by glutathione and a few others react to a small extent. When the native structure is weakened or disrupted by heat or limited proteolysis, the disulphide bonds become available for reaction. Now in ribonuclease, which has four disulphide bonds, Davidson and Hird (*ibid.*, 480) find that reduction begins at a temperature of 50° C, but it is observed that the enzyme then becomes a mixture of the fully intact and completely reduced forms. Thus it appears that the reduction of the first disulphide bond is rate-limiting, in that it makes the remainder instantly accessible by (presumably) provoking a conformational collapse. It has not been possible to establish that it is one in particular of the four cystines which acts as a hinge-point for the conformation.

A remarkable case in which differential reactivity of different disulphide bonds is strikingly apparent is reported by Seon (*J. Biochem., Tokyo*, **61**, 606; 1967). This concerns the Japanese enzyme (as one may call it), Taka-amylase A. This molecule has four disulphide bonds and a cysteine residue, which is unavailable to reagents in the native state. When incubated with sodium borohydride as the reducing agent at 80° C, the appearance of titratable sulphhydryl groups with time occurs in a stepwise manner. Although there is some variation between different preparations, the best results show three perfectly developed plateaux. The first corresponds to two titratable groups, deriving from reduction of a single labile disulphide bond; one further group is titrated at the second plateau, and is identified by labelling and peptic digestion experiments as the single hidden cysteine residue of the native protein, which thus becomes available after scission of the first disulphide bond. One implication is that the conformational relaxation is slow compared with the disulphide reduction. The reaction then continues, with at least one further plateau. One of the most curious aspects of this remarkable reaction is the substantial conservation of enzyme activity up to the second plateau. Thus, although a conformational change must occur to liberate the hidden cysteine residue, the enzymatically important parts of the conformation are preserved (or reform in the presence of substrate).

Also in a current article (Pontremoli *et al.*, *Proc. US Nat. Acad. Sci.*, **58**, 286; 1967), a case is described in which the formation of an extraneous disulphide bond by an enzyme may act as a metabolic control mechanism. The enzyme, fructose diphosphatase, is activated by some, though by no means all, disulphide reagents, notably by cystamine which occurs physiologically. The cystamine reacts with the protein via available sulphhydryl groups, and although three cysteamine residues are incorporated, two of these can be removed again, without loss of the newly gained activity. All cysteamine residues are removed, and the enzyme deactivated, by exposure to reduced glutathione, and the authors suggest a coupled feedback scheme, whereby this new method of regulation can function. It is supposed to operate independently of the established process of inhibition by AMP.

# Science, Technology and Society

by

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In this extract from his presidential address to the British Association, delivered on August 30, Lord Jackson discusses the impact of the progress of science on society, the kinds of benefit which the public derives from this progress, and the kinds of problem which it creates for the community, for government, and for the scientists themselves.

## The Scope and Problems of Scientific and Technological Policy

THE progress of science and technology is affecting increasingly, if not in large measure determining, the trend of national and international affairs. It is affecting the life and the policies of the highly developed and the developing countries alike and each, in its own context, is seeking urgently to take advantage of the potentialities of this progress. Each is seeking to formulate a scientific and technological policy, by which is meant the systematic consideration up to the highest levels of government of the relationship between scientific research and technological development, and the growth of the economy.

Throughout the post-war period Britain has been in the forefront—indeed it has been second only to the United States—in the proportion of its national wealth which it has devoted to research and development. In 1964–65 the total expenditure was about £750 million, some 2.6 per cent of the Gross National Product. The present figure is approaching £1,000 million per year. As yet no close correlation has emerged between this form of national expenditure and the Gross National Product. Otherwise our national economy would be more buoyant than it is. The fact is, of course, that the effect of research and development on the economy comes from the success with which its results are translated, through design and manufacture, into efficient, reliable and economically competitive new types of industrial products and improved forms of already well established ones. This is not to say that we should spend less on research and development. In fact, the cost of supporting the work of the individual research workers is rising steadily because of the increasing complexity and sophistication of the techniques and equipment nowadays required. But of what we spend, a larger proportion should be spent, in my opinion, within industry. I regard it as of great importance that there should be minimal physical separation between the processes of research and development and those of design and manufacture, although I shall introduce a reservation on this point later. I am therefore pleased that the self-financed expenditure within industry has increased considerably during recent years—from £77 million in 1955–56 to £320 million in 1964–65. To that latter figure was added about £180 million from government sources, mainly in aid of defence programmes, making a total expenditure of £500 million within the overall national figure of £750 million mentioned above.

We cannot reasonably expect, of course, to be in the forefront of all branches of technology. The electrical and chemical industries, for example, have grown considerably during the past few decades; others have remained stationary; and still others have declined in their contributions to industrial output and to the Gross National Product, and in the employment they provide. Industries on which the ascendancy and economic strength of this

country rested some decades ago are now finding it extremely difficult to compete with the corresponding productions of countries which were previously our customers. Clearly, there are many factors outside the scope of this address, including the structure of industry, the quality of its management, the availability of capital, the prevalence of restrictive practices, the increasing industrialization of other countries, which bear on this situation. But it is surely significant that whereas in 1962, the latest date for which I have found comparative figures, the electronics and chemical industries spent close on £200 per annum per employee on research and development, there were other large industries for which the figure was less than £20. I recognize the differences between industries, but if it is necessary in the national interest that our traditional industries should remain vigorously competitive, though perhaps on a reduced and more selective scale, it is surely beyond question that they must draw more fully on the potentialities of scientific knowledge and exploit it technologically with greater effectiveness.

The overall expenditure by government on research and development during 1964–65 was about £430 million of which in addition to the £180 million spent in private industry and public corporations, £250 million was devoted to work in the establishments of the various ministries, and in the universities. This large commitment raises questions for, and decisions by, government as to what the size of the overall budget for the succeeding year should be; the priorities between the different sectors and between competing projects within sectors; the location of work as between industry, government establishments and the universities; the overall co-ordination of the research and development programmes sponsored by different departments of government; the way in which government spending of other kinds should be used to stimulate the concentration of research and development work in industry; and the part which Parliament should play prior to the decisions of individual sponsoring ministries. These are illustrative of the questions which fall within the scope of scientific and technological policy.

I do not think I risk giving offence in saying that no country has yet progressed much beyond the experimental stage in devising means of formulating and implementing its scientific and technological policy. The recent setting up of a Central Advisory Council for Science and Technology, under the chairmanship of Sir Solly Zuckerman, to advise the Cabinet on national strategy for the co-ordination and utilization of the country's scientific and technological resources, is for this country an important further step forward in the process. The subject is not, however, the concern only of scientific and technological experts. It is crucial to the satisfactory progress of the process that Parliament—the representative body of the general public, and the one ultimately responsible to the public for the expenditure of public funds—should understand fully the basis and the criteria on which decisions

on the questions mentioned above have to be taken. It is essential that the members of Parliament should be informed, and become involved, early in the processes of analysis and appraisal leading to the taking of these important decisions. To a rapidly growing extent the background of these decisions is of a highly complex and sophisticated scientific, technical and economic nature. The usual and necessary procedure is for particular questions to be studied, and the case for ministerial and then governmental decision prepared, by the Civil Service staff of the ministry concerned, often with the help of advisory committees composed of experts drawn from outside government service. The danger is that unless Parliament finds a way of getting to grips more effectively with scientific and technological issues, its function will become little more than that of endorsing, on limited information, decisions already taken at ministerial level.

In these circumstances the role of the Parliamentary and Scientific Committee has become increasingly important. Established in 1939, it was until a few years ago a unique body of its kind. Its present membership includes some 130 members of the House of Commons, 60 members of the House of Lords, and nominated representatives of 130 scientific and technological organizations. The new House of Commons Select Committee on Science and Technology, which will be free to call for evidence from any quarter, is likely to exercise a much more direct influence on these matters. I hope that, in supplementation of the work of the Select Committee, the evidence it obtains will be used to stimulate discussion within the professional societies and institutions of scientists and technologists, and indeed within the wider sphere of the general public. A groundswell of informed public discussion in the formative stages would, it seems to me, be an invaluable guide to those responsible for formulating and deciding on major issues of scientific and technological policy.

### Policy for Basic Science

Within the overall national expenditure of £750 million on research and development in 1964-65, there were two components concerned mainly with basic research of long-term significance which call for special comment. One is the expenditure of the Science, Medical, Agricultural and Natural Environment Research Councils, amounting to about £44 million, of which some £28 million was spent in their own establishments, and the remaining £16 million in the universities in the form of research grants, training awards and the support of special units of the councils associated with the universities. The other is the money spent in the universities from their grants from the University Grants Committee, of the order of £40 million, giving a total university expenditure of about £56 million on scientific and technological research. The combined figure of £84 million has grown from about £25 million in 1955-56, at an annual rate substantially in excess of the rate for the overall national expenditure on research and development, and still more so of the rate for Gross National Product.

Inevitably, this has raised the question as to whether the recent rate of increase, around 13 per cent in real terms, can be maintained, and indeed on what criteria any particular rate can be justified. The responsibility for advising government on this aspect of scientific policy—that for science itself—rests with the Council for Scientific Policy, in collaboration with the research councils, and its report of May 1966 discussed the studies it was undertaking in the attempt to establish these criteria. Its task of quantifying and justifying desirable rising expenditure on basic research is indeed a difficult one, and one on which little guidance is available from the attempts of other countries faced with the same problem. Understandably, basic scientists are hesitant about making claims for the benefits resulting from their work, partly because they do not like it to be so assessed, and partly

because the benefits are often fortuitous and long delayed, and invariably depend on other factors falling outside their domain. Nevertheless, some striking examples of benefit to the community can be cited in the fields of all the research councils. I must restrict myself, however, to a brief reference to that of medical science.

Thus the death rate of children in the first year of life has been reduced over the past 50 years from 120 per thousand to 22 for boys, and from 98 to 17 for girls. On the assumption that on average people contribute usefully to the economy over 40 years of their lives, this improvement in infantile mortality now ensures some 3.5 million more years of work than would have been possible by the standards of 1912. Moreover, in 1964 there were alive some 70,000 men and women who would have died from tuberculosis but for the application of effective drugs from 1952. At a conservative estimate, the savings in the cost of hospital care and benefits alone—or their availability for other kinds of patients—are at least £30 million per year.

Many problems of equally great social and economic importance remain to be solved. One out of any five men over 40 years of age will develop coronary thrombosis before reaching the age of 65 years, and one-third of these are unlikely to survive the first attack. The cost to the Exchequer in terms of hospital care, sickness benefits, and widows' pensions was about £45 million in 1964, and the loss to national productivity about £90 million. One out of five women will develop cancer in the course of their lives, and in 1965 105,000 people died from this disease.

To attempt a quantitative justification for basic research in economic or sociological terms in whatever field is, however, precarious. The support for it and its pursuit must, to a large extent, be an act of faith. It may do no more than add to the body of scientific knowledge—to the seedbed from which, in due course, new ideas and new possibilities may hopefully be expected to emerge. There are no guarantees. This is especially the case with much of the research being undertaken within the universities, particularly within such fields as high energy nuclear physics, radio astronomy and microbiology. The leaders of research in these fields are literally probing the unknown, and in seeking support for their ideas and programmes they must rely on the acceptance by government, and by the community, of a number of arguments which may be found intangible. These are that the search for deeper scientific understanding for its own sake is a vital form of intellectual exercise; that the guidance and supervision of young research students are essential parts of the teaching function of the universities, and that, in the absence of research, teaching at the undergraduate level would rapidly be reduced to mere pedagogy; that basic research is a stimulant to, and affords the foundation for, the more objective pursuits of applied science and technology; and that it opens up, from time to time, a quite new field of technological development.

Yet those called on to afford this acceptance are faced with another set of intangibles. What total resources should be allocated to this basic research? How then should this be apportioned between the physical, biological and earth sciences? On what criteria should priorities be determined between the different facets of these main fields? To what extent should the claims of big science be allowed to jeopardize the support of small science? And on what grounds should a national programme be linked with, or subordinated to, participation in an international programme?

The scientific decisions, or recommendations, which lie within this formulation of a policy for science itself can, of course, be made only by scientists, and they are decisions which must now be made in the knowledge that the resources available cannot continue to grow at the rate of recent years, expressed in terms either of finance or, as I shall suggest, of qualified manpower. A continuing increase in expenditure on basic scientific research will be

possible only in so far as the successful exploitation of the results of research already completed makes increasing resources available. Regrettable though this may be to the scientist, the fact is that the strength of our national science has run ahead of the strength of our technology, and that, so long as this situation remains, the continuing strength of our science must be in jeopardy. Unfortunately, this danger is enhanced by the growing evidence that in some fields our technology has run ahead of our industrial and commercial expertise.

The argument has been used that the expanding needs of scientific research would be readily provided for by a tiny reduction in our defence budget, in the egg subsidy, or in our overseas aid. But it may also be argued that the facilities and freedoms which are rightly claimed by the outstanding scientist are regarded nowadays as matters of entitlement by many who are not outstanding and are unlikely to become so, and that we are spreading our available resources too thinly and over a wider range of science than is necessary or sustainable. In basic science, more than in most activities, quality is more important than quantity.

### Manpower Resources for Science and Technology

I believe that the prerequisite for the maintenance of our continued national progress in science and technology is an adequate supply from higher education of well qualified scientists and technologists. In other words, we cannot hope to implement a sound scientific and technological policy without a manpower policy involving the making of assessments, the forming of judgments, and the taking of decisions on the associated requirements for scientists and technologists, and on the means by which these requirements might be met.

Frankly, the present position and the future prospects are not encouraging. In its report of October last, the Committee on Manpower Resources for Science and Technology revealed that over the 1962-65 period the new supply of qualified scientists and technologists, especially of the latter, was inadequate to meet the overall national demand for them, particularly within industry and the schools. Unfortunately, the forecast data for the 1965-68 period afforded no promise of an improvement. Whereas the estimated overall demand of employers by 1968 was given as 24 per cent greater than the 1965 employment figure of about 330,000, it was stated that the new supply from higher education over 1965-68 would not raise this 1965 figure by more than 14.6 per cent. This means that against a requested rise of 7.4 per cent annually there is an assurance of growth from new supply of only 4.6 per cent. Moreover, the recent evidence of entry to the university departments of science and technology, and of the trend away from science in the sixth forms at school, suggests that this 4.6 per cent annual rate of growth is unlikely to be maintained beyond 1968. It is distressing for those who have striven to provide the greatly improved facilities that during the past academic year, according to the Universities Central Council on Admissions, there were 1,600 vacant university places for scientists and technologists. Clearly, employers will have to learn, and quickly, to use more effectively the scientists and technologists they already have.

In circumstances where it is all too easy to blame the schoolmaster, I want to pay tribute to what the quite inadequate number of science teachers have done in their response to the Nuffield Science Project, and in their search for means of teaching science in ways which reveal its technological significance. The development of experimental work aimed at stimulating an interest in technology, as described in the recent report of the Schools Council, *A School Approach to Technology*, is specially commendable.

I would also wish that the teaching of subjects such as history and geography might reflect more deliberately the ways in which scientific discovery and its technological

application have affected the progress of mankind and his use of the natural resources available to him.

The plain facts are that courses in science and technology, or the careers open to graduates in these subjects, are failing to appeal to a majority of the sixth form students who want to attend a university; that among the minority a majority are choosing science rather than technology; and that among the scientists a majority of the best academically are opting for research, preferably within a university. Unfortunately, many of the latter are prepared to emigrate if this wish cannot be met at home to their satisfaction. This is a serious state of affairs, since, uncorrected, it must lead to reduced national prosperity.

The factors affecting the choice of subjects in the schools and the universities are being studied by a committee under the chairmanship of Dr F. S. Dainton, vice-chancellor of the University of Nottingham; and those affecting the choice of first employment by science and technology graduates as between the schools, the universities, government service and industry, by another committee chaired by Dr Michael Swann, principal of the University of Edinburgh. I will content myself here with saying that I would like to see technology presented as a pursuit in which scientific, technical and economic considerations are intimately interwoven with concern for the human problems involved in its impact on the life of the community, both national and international.

There has been much talk in recent years of the two cultures. If these in fact exist, I see technology as a bridge between them, and I would wish it to be taught in a way which reveals and illustrates their interrelationships. I regard technology, so taught, not only as a necessary preparation for a wide range of vocational employments, but as a good preparation for life as it is now evolving. I therefore, as a technologist, welcome such steps as are being taken to reduce the degree of specialization in the final stages of school life and in undergraduate courses in science and technology. This means, of course, that the attainment of a degree in science or technology by no means represents the end of the educational process. I see the attention to changing specializations as a continuing process throughout a career and as involving a much closer partnership between employers and educationalists in the formulation and conduct of advanced courses than has yet been achieved.

And I attach no less importance to the continuation of scientific study at school and at the university by those who have no wish to prepare themselves for careers in science or technology as such. Many such people will inevitably be concerned with, and sometimes be required to take decisions in, situations created by the progress of science and technology, whether or not this country remains in the forefront of initiative in them. I appreciate the difficulties since, while the scientist and technologist can readily be helped to draw on the vast accumulation of literature in sociology and the arts, there is as yet little literature in science and technology which is well suited to the alternate purpose. Here is a field of research which should be tackled as a matter of urgency.

### Science, Technology and the General Public

Whatever may be done within the sixth forms of the schools, and in the undergraduate courses of the universities, cannot, however, increase the output of qualified scientists and technologists in the near future. It is essential, therefore, to focus attention on the effectiveness with which we are utilizing the ones we already possess. This involves consideration of whether these are distributed to the best national advantage between the schools, universities, government service and industry; whether there is adequate collaboration and mobility between the qualified manpower in these sectors; whether they are being given sufficient encouragement in status and salary and sufficiently attractive career prospects; whether



they are being assisted adequately by well educated and trained technicians and other supporting staff, etc. These are not, of course, new questions, and they are certainly not being neglected by those on whom the responsibility for decision and action rests. It may be doubted, however, whether we are moving rapidly enough towards their resolution.

And yet a basic trouble may be that notwithstanding our shortage of qualified scientists and technologists, the situation being created by those we already have is moving faster than lies within the competence of the community at large to comprehend and to adapt itself to. The community is expecting, if not demanding, a continuously rising standard of living without, apparently, understanding sufficiently the essential origins and the requirements of this improvement, and the consequences which are associated with it. The essence of the matter is that our expectations are rising faster than our effort and our co-operativeness.

Two of the post-war developments within my own subject which are changing the conditions of employment, and the kinds of skill relevant to it, for many people are those of automation and the electronic digital computer. Basically, there is nothing new in this situation; it has characterized the progress of technology, and the process of mechanization, throughout history. What is new is the rapidity with which the change is occurring, and the scale and urgency of the problems it is posing for attention. Popular opinion has it that the automatic control of industrial processes merely reduces the number of people employed, and therefore is a cause of unemployment. Indeed, as the Duke of Edinburgh said in a recent speech, "In this country the labour employed for the same output has been reduced to between one-tenth and one-fifteenth since the turn of the century". But he then said: "The point, of course, is in the words 'for the same output'. Without technological advance and automatic control we would need a labour force of hundreds of millions to produce the volume of consumer goods which we now take for granted." And I might myself add that many, or most, of them would not be available at all.

The Industrial Training Boards set up for different industries to implement the Industrial Training Act are as yet preoccupied with the formulation of schemes and the provision of facilities for the satisfactory initial training and associated further education of new recruits to industry at the craft, technician and professional levels. A primary aim of these schemes must be to ensure ready adaptability to changing situations and an appreciation of the likelihood that re-preparation for different work will arise periodically in later life. I understand that in Sweden 1 per cent of the working population are regularly in process of re-training. We are a long way from this, and we shall be wise to prepare to deal with a still larger proportion of our own. It is important that the provision should not be wholly vocational since a further consequence of technological progress is increasing leisure—and not least for many who have not yet learned to use to good communal advantage the leisure time they already possess.

### Conclusion

I have been able here to touch on only a few of the problems which the progress of science and technology creates for society. This progress impinges on, and raises issues of, moral as well as material concern over the whole range of human interest and activities. The destructive potentialities of nuclear power, the availability of harmful drugs, the increasing sophistication of crime, and the fantastically expensive aero-space race of the United States and Russia, while vast problems of human welfare are in urgent need of resolution even in their own countries, are a few examples.

A likely reason why science and technology are proving

less attractive to young people than I have been arguing is essential for this country on economic grounds may well be that they, reflecting a widespread feeling within the community, sense a lack of concern by scientists and technologists for these wider issues. In my experience this is by no means the case. Nevertheless, it must often appear, and indeed it is not infrequently a fact, that the anticipation of and the preparation for the consequences of technological change lag considerably on the urge to achieve it. The Select Committee of the House of Commons on Science and Technology, to which I referred earlier, carries a heavy responsibility in respect of Government sponsored projects in this regard. But in my belief the technologists themselves have an important part to play, by focusing early attention on the likely sociological effects of what they are seeking to achieve, and by ensuring that the knowledge and experience of sociologists and others are brought to bear in joint consideration of the problems involved. We must aim for a much closer identification of the social scientists with emergent technological possibilities, so that there may grow alongside innovation and change the ability to anticipate and to plan ahead the resolution of the human problems associated with it.

I wish I were competent to portray the kind of community we should be seeking to create with the aid of the immense potentialities of science and technology. I am sure that the strengthening of our technology is only one of the means of its attainment. But I am convinced that it is an indispensable means, and that only through the material resources that will flow from successful technology shall we be able to afford to do the things that most need doing. What I feel the public lacks is a clear enough conception of the things that most need doing and an understanding of the part which technology would play in the doing of them.

To make my final point, prominent among these I would place the need to continue to help the emergence of the developing countries, and to exercise the influence in international affairs for which I believe we have a special responsibility and talent. Our scientists and technologists have become not only the guardians and guarantors of our national economy, but also the agents of a new diplomacy in which much more than an expanding export trade is at stake. They will have an increasing part to play as advisers and teachers in the progress of the developing countries, and must not only carry conviction as experts in their respective scientific and technological specialities, but must also gain respect as ambassadors of broad and sympathetic vision, sound judgment and unassailable integrity. This country has a great international job still to do—unfortunately, it is at present uncertain whether we shall be able to find enough scientists, and more particularly technologists, to help ensure that we remain in a position to do it.

For over a century the British Association for the Advancement of Science has attempted to inform the general public of new developments in science and technology, and to stage discussions on the impact this is having, or is likely to have, on the life of the community. Its work in these respects is now being greatly supplemented by the increasing attention being paid to science and technology by the sound broadcasting and television services and by the daily Press. In close collaboration with these agencies the Association still has an extremely important part to play, and the six symposia, on matters of great public moment, which have been included in the programme of this Leeds meeting, and other developments being planned for next year in Dundee, are evidence of its continuing vitality. My concluding sentence must be to express my sense of the honour which attaches to the Presidency of this historic and distinguished Association; to thank those who were responsible for inviting me to occupy this office; and to wish the Association a long and successful future.

# Effects of the Numbers and Sizes of Platelet Aggregates on the Optical Density of Plasma

by

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The formation of platelet aggregates in plasma affects its optical density. The relationship between the optical density and the numbers and sizes of aggregates has been explored.

In 1962 a photometric method was introduced for the quantitative investigation of the aggregation of blood platelets<sup>1,2</sup>. A beam of light is directed through a suspension of platelets in plasma or in an artificial medium, the optical density of which is continuously recorded by measuring the transmitted light. The suspension is stirred at a rate rapid enough to make the collision rate of the platelets non-limiting but not enough to damage them. When the platelets aggregate the amount of transmitted light increases and when the aggregates disperse the amount decreases. With this method and its modifications (for example refs. 3-5) much new knowledge has been obtained about the mechanism and significance of platelet aggregation. The method itself, however, remained entirely empirical in so far as nothing was known about the way in which changes in light transmittance depended on the concentration and magnitude of the platelet aggregates formed. The experiments described here were carried out to provide information about this.

Blood was collected by puncture of the antecubital vein and mixed with one-tenth the volume of 3.8 per cent trisodium citrate (0.129 molar). The blood was centrifuged at room temperature for 10 min at 226*g*. The supernatant of platelet-rich plasma was transferred with a Pasteur pipette to siliconed glass bottles. The plasma was kept at room temperature until used. Platelet aggregation was measured in a modified EEL long-cell absorptiometer differing from the apparatus used by Haslam<sup>5</sup> in that it had in the sample tube a magnetic stirrer covered with polyethylene which was driven from below by a magnet rotating at about 1,000 r.p.m. A simple attenuator and back-off circuit was employed to connect the output from the photocell to a pen recorder of either 10 mV or 1 mV full-scale sensitivity. Samples of 1 ml. of platelet-rich plasma were pipetted into glass tubes treated with silicone, 10 × 50 mm, which were then warmed at 37° C for 5 min and inserted into the water-jacketed compartment, also at 37° C, of the absorptiometer. The recorder chart was calibrated between 10 and 70 per cent transmission with platelet-free plasma from the same blood sample set at 100 per cent.

Reagents were added to the plasma samples in the absorptiometer in volumes of 50  $\mu$ l. or less with microsyringes. Adenosine diphosphate (ADP) sodium salt was stored in the deep freeze as a 50 mmolar solution neutralized to pH 7.0 with sodium hydroxide. Dilutions of 1 mmolar were made in *tris*-saline (139 mmolar sodium chloride, 15.4 mmolar *tris*-(hydroxymethyl)aminomethane hydrochloride, pH 7.4) and stored in the deep freeze for up to 1 month. Further dilutions were made in *tris*-saline on the day of use. Desmethylinipramine (Pertofran, Geigy) was dissolved in *tris*-saline; stock solutions of 10 mmolar were stored in the deep freeze.

Before and at different times after adding ADP, aggregation was arrested by adding one-tenth of the

volume of 10 per cent formaldehyde in saline. At the moment of addition, the pen-recorder tracing showed an injection-dilution artefact; all change in light transmittance ceased within 2 sec, indicating that mixing was then complete. The formalinized samples were diluted with 1 per cent formaldehyde in saline up to five-fold, depending on the light transmittance at the time the reaction was stopped. This was done to facilitate counting and to aid in deciding whether adjacent platelets were actually adhering to each other in aggregates or were merely lying close together.

Platelets and aggregates were counted in the improved Neubauer phase contrast haemocytometer. The haemocytometer was cleaned successively with soap and water, methanol and acetone, and wiped dry with an electrostatic cloth. The cover slip and the chamber were examined with a hand magnifying glass to ensure that particulate matter was not present. To determine the initial platelet count, a sample of platelet-rich plasma was diluted 100-fold with 1 per cent ammonium oxalate. Fifty small boxes of squares were counted (both sides of the chamber), usually about 850 platelets in all.

The diluted formalinized plasma samples were thoroughly mixed and transferred to the exact centre of the haemocytometer. Using small forceps, the coverslip was carefully placed on the chamber so as to avoid trapping air bubbles or asymmetrically displacing the sample. The chamber was then examined under the phase contrast microscope to make sure that the distribution of aggregates in the central area was representative of the entire sample and that no bubbles or particles of lint were in this area. Two hours were permitted to elapse for the complete settling of platelets and aggregates. If by this time all single platelets did not lie within the same plane of focus, further time was allowed for settling. The entire central ruled area, that is twenty-five small boxes of squares, was photographed together with a microscopic calibration scale. In samples showing maximum second-phase aggregation the photographed area was doubled. Prints were made of the whole area at a final magnification of  $\times 250$  and joined in a montage. Parallax was minimal and did not affect the number of cells counted. Individual platelets together with those in small aggregates containing between two and eight platelets each were referred to as "countable platelets". These were counted in one section of each of the photographs of all samples, and the results were recorded before counting the next adjacent section of all samples in turn. This counting procedure was used to minimize bias, and the results of the counts were added at the end.

Aggregates in which individual platelets could not be counted reliably had to be measured differently. The photographic negatives were projected at the same magnification ( $\times 250$ ) onto sectional graph paper. The

periphery of each aggregate was traced and the enclosed area was measured in  $\mu^2$  by reference to the calibration scale. All measurements were then corrected for dilution.

The dense aggregates were irregular in shape. By dividing the total area of mapped aggregates by their number, an area in  $\mu^2$  was calculated for a hypothetical "mean circular aggregate". From the radius of the "mean circular aggregate" the volume in  $\mu^3$  was derived of a hypothetical "mean spherical aggregate". The number of platelets in all the dense aggregates was estimated by subtracting the number of countable platelets from the platelet count of the original suspension. This estimate was divided by the number of aggregates, and the resulting number was divided by the volume of the "mean spherical aggregate" to obtain an estimate of its density, expressed in platelets/ $\mu^3$ .

An important discovery made recently by means of the photometric method was the demonstration that the specific aggregation by ADP of human platelets at 37° C occurs in two phases<sup>8</sup>. The first phase is reversible and competitively inhibited by certain substances closely related to ADP<sup>2,7,8</sup>. The second phase is caused by the release of ADP from the platelets (unpublished work of Mills and Roberts) in a chain reaction<sup>9</sup>; it is irreversible but can be prevented by chlorpromazine, imipramine, and some related drugs<sup>10</sup>. It was clearly necessary to establish the relation between aggregates and light transmission in both phases separately; this was done as follows.

The concentrations of ADP added to different plasmas were chosen to permit the taking of samples at different points during the changes in light transmission which indicate the first and second phases of aggregation. In the first experiment (Fig. 1a), ADP was added at a concentration of 1  $\mu$ molar which, in this plasma, brought about a slow increase in light transmission amounting to only 7 per cent after 75 sec. By this time, 87 per cent of countable platelets had disappeared and many larger aggregates had formed. The density of these aggregates showed no significant change.

In the second experiment (Fig. 1b), desmethylinipramine was added to the plasma at a concentration of 50  $\mu$ molar to inhibit the second phase of aggregation. When ADP was then added at a concentration of 2  $\mu$ molar, light transmis-

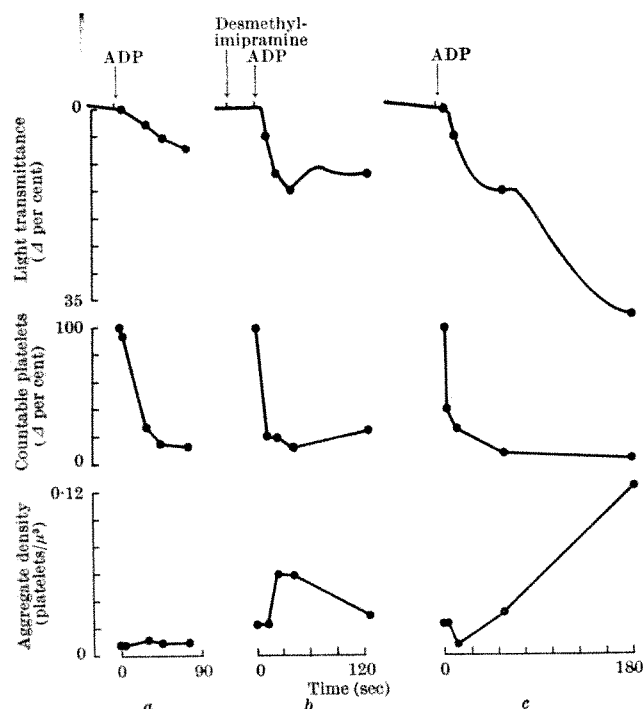


Fig. 1. Light transmittance, countable platelets and aggregate density related to time after adding ADP (see text).  $\Delta\%$  = change expressed as per cent of original value.

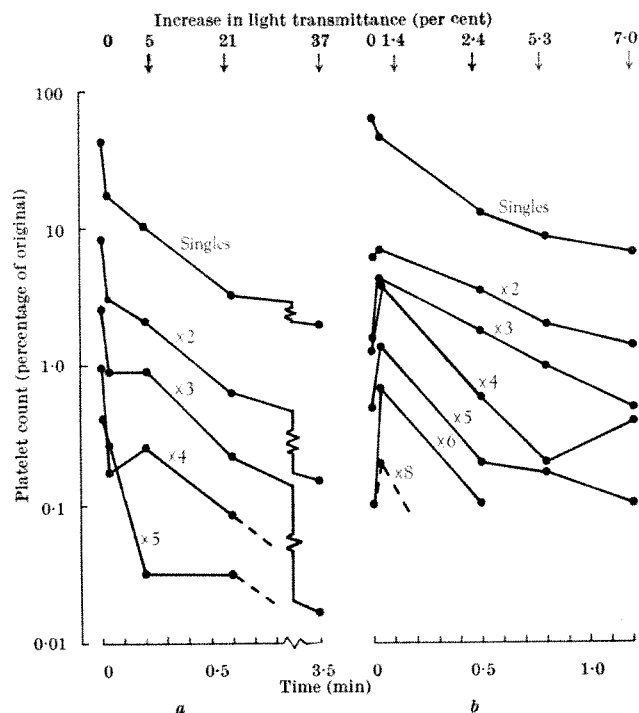


Fig. 2. Platelets countable as singles, doubles ( $\times 2$ ), triples ( $\times 3$ ), and so on related to change in light transmittance and time after adding ADP (see text).

sion increased by 15 per cent in 40 sec after which it decreased again indicating reversal of aggregation. During the increase in light transmission the number of countable platelets decreased by 90 per cent; during the reversal it increased by 15 per cent. This time the increase in light transmission was accompanied by a marked increase in the density of the aggregates which also reversed subsequently. During the reversal there was an increase in the number of aggregates, which indicated that they were fragmenting and releasing countable platelets.

Another sample of the same platelet-rich plasma was used to find out what occurred during the second phase induced by the addition of ADP in a concentration of 1  $\mu$ molar without the previous addition of desmethylinipramine (Fig. 1c). The optical record was similar to that shown in Fig. 1b for the first 45 sec; thereafter the light transmittance, instead of decreasing, continued to increase. During the first phase the number of countable platelets diminished, as before, by about 90 per cent. During the second phase, a further small diminution in the number of countable platelets was accompanied by a striking increase in the density of the larger aggregates.

The effect of ADP on the distribution of countable platelets is shown in Fig. 2. Immediately after the addition of ADP, the number of single platelets diminished sharply. At the same time, there was a transitory increase in the number of aggregates containing from two to six platelets. This was seen at a time when the light transmittance had hardly begun to increase (Fig. 2b). By the time the transmission increased by more than about 2 per cent, the number of these small aggregates was already diminishing (Fig. 2a and b). As light transmission increased further the number of single platelets as well as the number of small aggregates continued to decrease.

The experiments were done in an attempt to interpret the changes in light transmittance which form the basis of the photometric method for measuring platelet aggregation in terms of the numbers and sizes of the aggregates formed. It was possible to count platelets in small aggregates containing no more than six to eight platelets but not in larger aggregates which had therefore to be measured in a different way. On the assumption that the average diameter

of the large aggregates was the same in all dimensions, the total area of these aggregates was measured to provide a basis for calculating their total volume. From this it was possible to calculate the density with which the platelets were packed in the aggregates. We therefore counted the platelets in the small aggregates and measured the total area of the larger aggregates. Both measurements could be used throughout the period of aggregation; however, there was no simple way of combining and relating them together to the light transmittance at any given moment.

The results showed that the increase in light transmittance was associated with two effects, namely, the formation of aggregates and their increasing density. Formation of platelet aggregates began before there was any change in light transmittance. During this time the concentration of single platelets decreased steeply as they associated to form small aggregates. The number of these aggregates passed through a maximum about the time when the light transmittance began to increase. The subsequent increase in transmittance was associated with a progressive diminution in the numbers of both single platelets and small aggregates and the formation of fewer, larger aggregates in which the platelets were no longer countable. During early aggregation the density of the aggregates was low, indicating that the platelets were packed loosely with considerable space between individual platelets. This agrees with measurements of the plasma space in early aggregates which is 0.3–0.4  $\mu\text{l.}/10^8$  platelets or 30–40 per cent by volume (unpublished work of Born).

So long as light transmittance increased only slightly, the diminution in countable platelets was not accompanied by a change in the total area of the large aggregates. By contrast, when the light transmission increased rapidly, the total area of the large aggregates diminished markedly, indicating that the density of the aggregates was increasing. These observations establish that the initial velocity of increase in light transmittance is a measure not of the

rate of formation of small aggregates from single platelets but of large aggregates from small aggregates. Furthermore, a high initial velocity is caused much more by aggregate contraction than by aggregate formation.

The observed increase in aggregate density has two possible explanations. Either the volume of the individual platelets decreased, for which the experiments provide no evidence; or the distance between the platelets in the aggregates became smaller. The latter hypothesis is supported by the following consideration. Normal human platelets have a mean volume of  $5.8 \mu^3$ ; in the presence of ADP (0.5–2  $\mu\text{molar}$ ) the volume increases to 7.2 (ref. 11). From this it is possible to calculate the density of aggregates in which the platelets are so tightly packed that no space remains between them. The reciprocal of 7.2 is 0.139 which is the number of platelets/ $\mu^3$  under the influence of ADP, that is, when aggregated. In the experiment shown in Fig. 1c, the corresponding calculation at maximum second phase aggregation gave a value of 0.125 platelets/ $\mu^3$ ; this approaches the theoretical value for a tightly packed aggregate. Whether or not this explanation accounts for the contraction of the large aggregates, its cause remains to be established.

Our findings suggest that any mathematical expression for the relation between the light transmission and the aggregation process is bound to be complex.

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## Surface Topography of Ice Sheets

by

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The idea of predicting the thickness of an ice sheet directly from surface slope can be misleading. The slope of the surface now appears to be related not only to the thickness of the ice but also to changes of longitudinal stress resulting from movement of ice over varying bottom slopes.

THE hypothesis<sup>1,2</sup> that, provided conditions are uniform around the point of observation, the thickness of an ice sheet or glacier is inversely proportional to the surface slope has proved a useful approximation in many areas. Over certain regions of polar ice sheets, however, the hypothesis does not fit the observations<sup>3,4</sup>. The use of methods of continuous radio echo sounding<sup>5</sup> has now provided information which makes a more detailed study of this problem practicable along a line of ice flow to the south of Camp Century (77° 11' N., 61° 08' W.) in North Greenland. We have found that surface slopes can vary rapidly in response to changing longitudinal stresses caused by ice moving over undulations of the sub-glacial floor. A relationship between surface slopes and variations of stress along the line of flow is derived from elementary considerations. This relationship provides a satisfactory numerical explanation of observations when stress changes are averaged over a distance of the same order as the depth of ice. This article describes the basic concept which is used and shows how it works in practice. I. F. Collins (personal communication) has used a rigorous mathematical approach to the same problem and showed the approximations involved in using the simple concept.

It has been generally accepted, following papers by Nye<sup>1,2,6</sup>, that the shear stress  $\tau$  on the bed of a glacier is given by the relationship

$$\tau = \rho g h \alpha \quad (1)$$

where  $\alpha$  is the surface slope,  $\rho$  the density of ice,  $g$  the value of gravity and  $h$  the thickness of ice. The reason why the surface slope rather than the bottom slope controls the shear at the bed is because ice moves as an incompressible fluid. One readily appreciates that a surface slope is necessary to cause water to flow, whatever the slopes of a lake or river bed may be.

When considering glacier flow, however, we are dealing with a non-Newtonian viscous material which can support stress gradients along the line of flow. A recent theory of glacier flow<sup>6</sup> takes account of stresses associated with longitudinal strain rates throughout a glacier. In Nye's model, these strain rates do not vary with depth or along the line of flow and thus longitudinal stress gradients are not considered. The theory provides an explanation of the deformation of a borehole which is more consistent with laboratory studies of the deformation of ice<sup>7</sup>, however, than the first attempts to



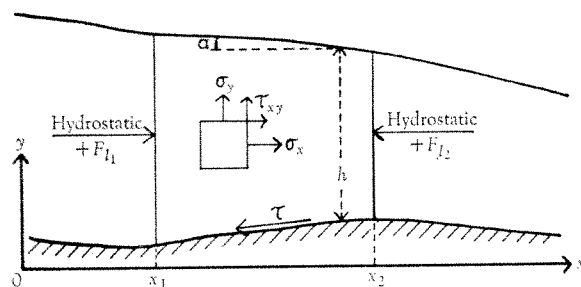


Fig. 1. Forces and stress components involved in the flow of an ice sheet over bedrock of varying slope.

explain borehole results on the basis of simple pseudo-hydrostatic theory<sup>8</sup>. Even with the inclusion of a constant longitudinal strain rate, equation (1) still holds, but as in earlier papers the stipulation for this to apply is that the physical conditions (for example, surface slope and ice depth) must not vary much in a distance of order  $h$  around the point of observation. In practice there are many places on glaciers and ice sheets where conditions do vary considerably over distances of this order. In order to tackle the problem we must take account of the variation of longitudinal strain rates and stresses along the line of flow of a glacier.

Longitudinal strain rates in a glacier (Fig. 1) are produced by the differences of stress in the  $x$  and  $y$  directions ( $\sigma_x - \sigma_y$ ). The basic assumption which is needed to justify the approach and procedure of this paper is that the stress difference ( $\sigma_x - \sigma_y$ ) is large compared with the shear stress  $\tau_{xy}$  throughout the ice, or in terms of mean values that  $\sigma_x - \sigma_y \gg \frac{1}{2} \tau$ . Our numerical analysis indicates that  $\sigma_x - \sigma_y$  lies between 1.8 and 3.0 bars for 93 per cent of our calculated values while  $\frac{1}{2} \tau$  varies from 0.3 to 0.4 bars; thus the basic assumption is reasonable. Collins shows that in this case we can consider the vertical stress as equal to the weight of overlying ice at all points, provided that bedrock slopes are not too great (say,  $< 10^\circ$ ). Consequently, the most important variations of ( $\sigma_x - \sigma_y$ ) along the line of flow are caused by changes of  $\sigma_x$ , and to emphasize this we will write  $(\sigma_x - \sigma_y) = \sigma_x^\circ$  where  $\sigma_x^\circ$  thus represents the departure of the longitudinal stress ( $\sigma_x$ ) from the weight of overlying ice at any point. Because longitudinal strain rates vary as ice moves over the varying slopes of bedrock (Fig. 1), so must our value of  $\sigma_x^\circ$ .

To relate  $\sigma_x^\circ$  to the surface slope, consider the forces on a slab of unit width along the line of ice flow extending from  $x_1$  to  $x_2$  (Fig. 1). We can consider the forces on either end as resulting from a hydrostatic term plus a longitudinal component force  $F_L$  due to the stresses defined by  $F_L = h \bar{\sigma}_x^\circ$  where the bar denotes the mean value throughout the ice thickness. If we add these forces to equation (1), which already incorporates the effect of hydrostatic pressure, we obtain

$$\alpha g h \alpha = \tau - \frac{dF_L}{dx} \quad (2)$$

We can then write

$$\frac{dF_L}{dx} = \frac{d(h \bar{\sigma}_x^\circ)}{dx} = \bar{\sigma}_x^\circ \frac{dh}{dx} + h \frac{d\bar{\sigma}_x^\circ}{dx}$$

It is clear from an evaluation of our results from Greenland that variations of longitudinal stress along the line of flow are the dominant cause of variations of our component longitudinal force  $F_L$ , that is  $\bar{\sigma}_x^\circ \frac{dh}{dx} \ll h \frac{d\bar{\sigma}_x^\circ}{dx}$ , so to a first approximation we can write equation (2) as

$$\alpha = \frac{\tau}{\rho g h} - \frac{1}{\rho g} \frac{d\bar{\sigma}_x^\circ}{dx} \quad (3)$$

The approximations involved in use of this equation will be set out in detail by I. F. Collins (in a future paper). Equation (3) has, in effect, been used by Lliboutry<sup>9</sup> as a more precise means of determining  $\tau$  than is given by equation (1). He did not, however, discuss the approximations involved or check his conclusions by direct measurement of  $\tau$ . The attraction of expressing equation (3) in terms of surface slope is that this can be measured and used to check the equation. The last term of equation (3) which we write as

$$\alpha_F = - \frac{1}{\rho g} \frac{d\bar{\sigma}_x^\circ}{dx} \quad (4)$$

shows the effect of longitudinal stresses on surface slopes. If the longitudinal stresses are of constant value along a flow line,  $d\bar{\sigma}_x^\circ/dx = 0$  and equation (1) applies as already mentioned.

Our derived values of  $\alpha_F$  will depend on the type of flow law used to relate  $\bar{\sigma}_x^\circ$  to the strain rate, in order to evaluate the term  $d\bar{\sigma}_x^\circ/dx$ . For example, with a Newtonian flow law,  $\bar{\sigma}_x^\circ$  would vary linearly with strain rate, and variations of surface slope would be directly proportional to variations of bottom slope, even in regions when the strain rate changed sign. Alternatively, if ice is treated as a simple plastic solid with a yield stress in shear of 1 bar, the only permitted values of  $\bar{\sigma}_x^\circ$  on our simplified model would be  $\pm 2$  bars. The term  $d\bar{\sigma}_x^\circ/dx$  would then only affect surface slopes when longitudinal stresses changed sign, the total stress change being  $\pm 4$  bars so that the effect on  $\alpha_F$  would be large. Use of a more realistic flow law, which involves a strain rate dependent on the fourth power of stress, will produce an effect intermediate between the plastic and Newtonian cases. Slope variations ( $\alpha_F$ ) of limited magnitude will be present in regions where the strain rate does not change sign, while large changes of slope are to be expected when strain rates change sign. This fits the character of our observations in Greenland.

The chief difficulty to be faced with our data is that of producing realistic values of longitudinal strain rates ( $\dot{\epsilon}_x$ ) as no direct measurements are available along our profile. We can, however, produce estimates if we make the steady state assumption that the thickness of ice does not change with time, so that the known rate of accumulation ( $\dot{a}$ ) over this part of the ice sheet<sup>10</sup> is compensated at all points by the outward velocity ( $v$ ) of ice movement. Keeping to a two dimensional approach, we have

$$v = \frac{1}{h} \int_0^x \dot{a} dx \quad (5)$$

where  $x$  is the distance from the start of ice movement—which is considered to begin at the crest of the ridge close to Camp Century, as shown on contour maps<sup>10</sup>. Because we know both the thickness of ice  $h$  and the rate of change of ice thickness,  $dh/dx$ , we can also use the steady state assumption to estimate the strain rate  $\dot{\epsilon}_x$ , assumed constant at all depths, from

$$\dot{\epsilon}_x = \frac{1}{h} \left( \dot{a} - v \frac{dh}{dx} \right) \quad (6)$$

To convert  $\dot{\epsilon}_x$  to a stress we use Glen's experimental data<sup>7</sup> relating strain rates to a uniaxial stress. We express this in the form

$$\bar{\sigma}_x^\circ = (\sigma_x - \sigma_y) = b(\dot{\epsilon}_x)^{\frac{1}{4}} \quad (7)$$

where  $b$  is treated as a constant, obtained by extrapolation of Glen's data to appropriate strain rates and ice temperatures. A correction of +20 per cent has to be added to the uniaxial stresses of Glen's data because in an ice sheet we assume the strain rate in the third dimension to be zero.

This procedure neglects the effects of shear stresses  $\tau_{xy}$  in deriving values of  $\bar{\sigma}_x^\circ$ . In principle, the method of success-

ive approximations described by Nye<sup>11</sup> could be used to take into account the effect of shear stresses but this was not practicable. Neglect of this factor could cause errors of up to +20 per cent in our estimates of  $\bar{\sigma}_x$ .

Although strain rates are assumed constant at all depths, the calculations of stress take account of variations in temperature with depth as estimated from the analysis of steady state temperature profiles given by Robin<sup>12</sup>, using data for Camp Century. Recent direct measurements of temperatures down to bedrock (Hansen, private communication) show that observed temperatures are similar to estimated, the maximum difference being 4° C at bedrock. These differences will not significantly affect the value of "b" derived for Camp Century which is 14.8 bar yr<sup>-1</sup>. This is very close to the value of 14.6 bar yr<sup>-1</sup> obtained from Nye's more rigorous calculations of  $(\sigma_x - \sigma_y)$  mentioned here, which were made for thicker ice but a similar temperature profile. Although temperatures deep in the ice must vary somewhat along the line of our profile (Fig. 2) the temperatures at 10 m depth only change from -24.5° C at 0 km to -20.1° C at 48 km; thus we have treated "b" as constant throughout these

calculations. Having obtained a series of values of  $\bar{\sigma}_x$  from (7) we then calculate slope variations from (4).

Our calculations showed that velocities of movement given by the steady state assumption of equation (5) vary from 1 m/yr at 0 km on the profile in Fig. 2 to 10 m/yr at 25 km and 24 m/yr at 46 km. Smoothed values of accumulation were used and ice thicknesses were read off the continuous film record every 220 m of traverse. The mean value of ice thickness from the outward and return journeys was used to determine values of  $\delta h/\delta x$  over distances  $\delta x = 880$  m, which are of similar magnitude to the ice thickness. Overlapping intervals were used to obtain a value of  $\delta h/\delta x$  every 220 m.

In calculating  $\alpha_F$  from (4) we again use overlapping intervals of  $\delta x = 880$  m and calculate  $\alpha_F$  every 220 m. Because small errors or irregularities in the bed produce considerable fluctuations in  $\alpha_F$ , we introduce further smoothing by taking a running mean of  $\alpha_F$  over five adjacent points, as shown in Fig. 2C.

Over the section of the profile from 27 to 44 km the calculated values of  $\alpha_F$  shown in Fig. 2C were found to vary much more than the observed surface slopes; therefore a

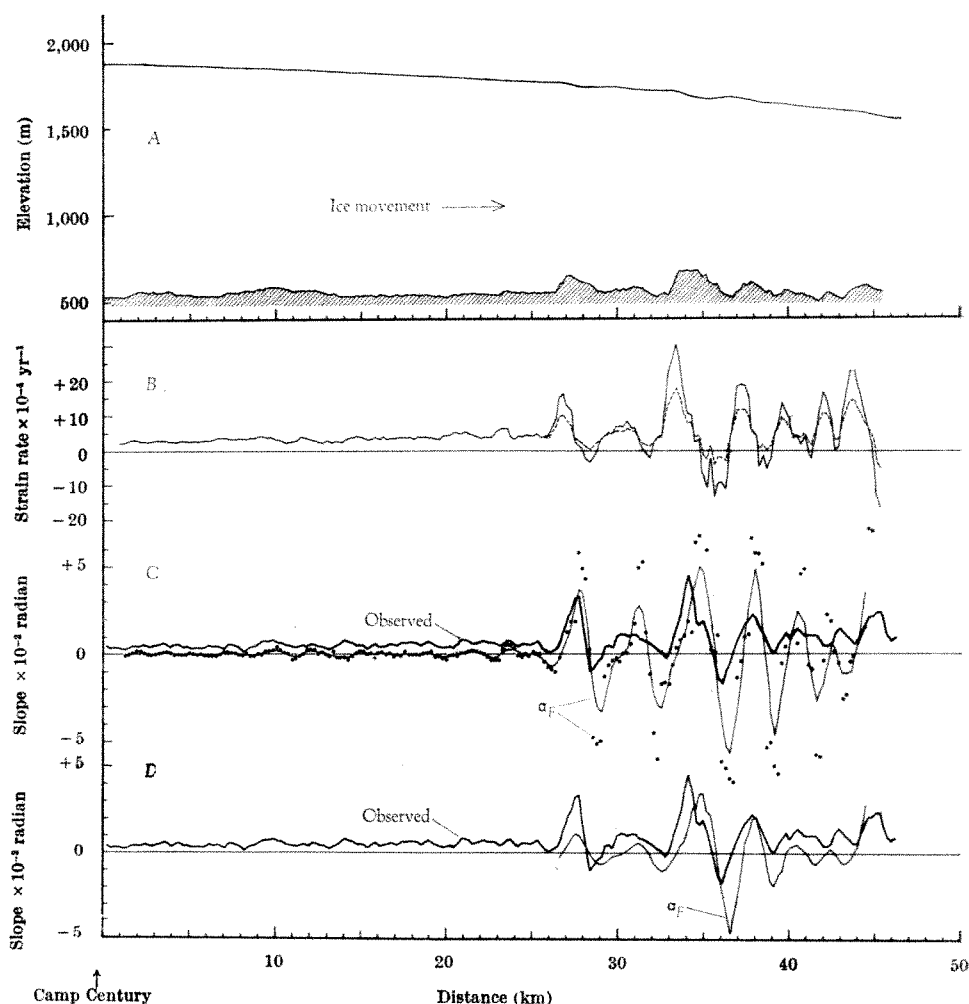


Fig. 2. A, Profile of the Greenland ice sheet along the estimated line of ice flow for a distance of 46 km south of Camp Century (77° 11' N., 61° 08' W.). B, Estimated strain rates ( $\dot{\epsilon}_x$ ) along the profile of A above. The full line shows strain rates calculated from equations (5) and (6) on the steady state assumption. The dashed line shows strain rates calculated on the assumption that velocities of ice movement are one half of those given by the steady state assumption. C, Comparison of observed surface slopes (heavy line) with the slope variations  $\alpha_F$  (thin line and points) calculated on the assumption of a steady state model. Calculated slope variations ( $\alpha_F$ ) derived from equation (4) are indicated by the points. They are based on the change of ice thickness between soundings 880 m apart. The continuous thin line shows smoothed values of  $\alpha_F$  obtained from a running mean of five adjacent points. D, Comparison of the same observed surface slopes (heavy line) with calculated slope variations (thin line) of 27-45 km using half the velocities of ice movement given by the steady state assumption (see dashed line in B).

second set of calculations was made for this section using the same accumulation rate but half the velocities of ice movement given by the steady state assumption. The calculated slope variations caused by these slower ice velocities are shown in Fig. 2D.

A profile of the top surface was obtained by using two Wallace and Tiernan altimeters, read by separate observers at intervals of 220 m along the route. Slopes have been calculated from the changes of altimeter readings over 440 m intervals centred on each 220 m. The difference between slopes measured on the outward and return journey over the section from 0 to 27 km averaged  $2 \times 10^{-3}$ . Figs. 2C and 2D show the mean of the slopes measured in the two directions. Isolated changes of slope  $2 \times 10^{-3}$  may result from random errors, but the errors of mean slope over 1 km should not exceed  $10^{-3}$ .

Fig. 2A shows the ice sheet profile along the estimated line of ice flow south of Camp Century and Fig. 2B the estimated strain rates, while Figs. 2C and 2D compare the observed surface slopes with calculated values of  $\alpha_F$ . The vertical displacement between the curves in both (C) and (D) is due to the term  $\tau/\rho gh$  in equation (3) which is the term usually considered to control surface slopes.

From 0 to 25 km along the profile the strain rates are positive at all points, and no major changes of surface slope are present. There is rough agreement between observed and calculated slope variations, possibly as much as can be expected from a two dimensional study of a problem which should really be studied in three dimensions.

The large changes of surface slope of 26–46 km are primarily associated with changes of sign of the estimated strain rates. A large rise in the positive strain rate between 26.0 and 26.8 km only decreases the smoothed value of  $\alpha_F$  by  $7 \times 10^{-3}$  whereas  $\alpha_F$  rises to  $27 \times 10^{-3}$  at 27.7 km due to the strain rate becoming negative at 28.0 km (Fig. 2C).

The lower velocities used for calculating  $\alpha_F$  in Fig. 2D result in fewer changes of sign in the calculated strain rates (dashed line, Fig. 2B). This produces a significantly better fit from 29 to 46 km. This does not apply between 26 and 29 km where a better fit is given in Fig. 2C. We therefore suggest that the ice movement ranges from around 8 m/yr at 26 km to 12 m/yr at 46 km. These velocities are lower than those given by the steady state assumption, possibly because of channelling of ice flow away from our profile towards outlet glaciers, or because outflow of ice is less than the surface accumulation over the region.

Although the process of picking a suitable velocity of ice movement to produce values of  $\alpha_F$  which fit the observed slopes may seem an arbitrary way of producing the right answer, the velocities suggested appear to be of the correct order. An existing theory<sup>13</sup> based on equation (1), when dealing with ice flowing over a ridge of a height of one-tenth of the ice thickness, predicts a change of slope of only 14 per cent compared with an observed change of slope by a factor of six for such a ridge at 27 km on our profile. The factor of six is explained adequately by taking longitudinal stress variations into account.

A second type of theory which involves plastic bending of a glacier<sup>14</sup> also fails to predict the very large changes of surface slope which are involved when the longitudinal strain rates change sign. It is this crucial point which gives confidence in the explanation outlined here. Further testing of our deductions will be provided when measurements of surface velocities and strain rates along the profile in Fig. 2A are completed this year by Mock<sup>15</sup>.

In general terms, equation (3) appears to provide a satisfactory explanation of both local and regional slopes of polar glaciers. The term  $\alpha_F$  can govern slopes over short distances, but because  $\sigma_x$  is unlikely to exceed  $\pm 3$  bar average stress changes over long distances must be

small, as must average values of  $\alpha_F$ . Thus over distances of 100 km average slopes will depend primarily on the term  $\tau/\rho gh$ . The effect of velocity on strain rates, shown in equation (6), also implies that bottom irregularities will have less effect on surface slopes near the centre of ice sheets where movement is slow than towards the edge of ice sheets where movement will be greater. This fits the general character of the levelling profile across central Greenland carried out by the Expedition Glaciologique Internationale au Groenland in 1959 (ref. 16).

Another point which appears justified by our results is the use of Glen's flow law<sup>7</sup> at strain rates around  $10^{-4}$  yr<sup>-1</sup> in cold ice, even though this involves extrapolation to one-thousandth of the lowest strain rates in laboratory experiments.

It must also be pointed out that more severe restrictions should be placed on use of equation (1) than have been applied in practice during recent years. Clearly if the upper or lower surfaces of the ice show appreciable curvature, the term  $d\sigma_x/dx$  becomes significant and equation (1) is inapplicable. A better stipulation would be that  $dh/dx$  and  $\alpha$  should not change in a distance of order  $h$  around the point of observation, or more simply that curvature of the surface should be negligible over any area to which equation (1) is to be applied.

The inverse relationship between surface slope and ice thickness predicted by Nye<sup>1,2</sup> has been very useful for estimating the thickness of Pleistocene ice sheets from a knowledge of the position of their margins and their bedrock topography. The findings of this paper do not affect such generalized predictions of ice thickness and volume as they are not sensitive to localized variations of surface slope.

Revision of current thinking is, however, necessary when observed surface slopes are used to predict ice thicknesses. It now appears necessary to use mean surface slopes over distances an order of magnitude greater than  $h$ , except for those regions where curvature is negligible over a distance of order  $h$  as mentioned here. Any attempt to make detailed estimates of bedrock profiles from surface profiles will be complex and can only be justified if one has detailed knowledge of strain rates or of the rates of accumulation and velocities of ice movement over any area studied. The improved theory, however, makes some estimates of ice movement possible on the basis of surface profiles, accumulation and thickness data for regions which are far from fixed reference points on exposed rock.

This paper is based on results of a co-operative programme of field work on the ice sheet of north-west Greenland described briefly by Rinker<sup>17</sup>, and Bailey, Evans and Robin<sup>5</sup>. The work was supported by the US Army Electronics Laboratory, Fort Monmouth, NJ, the US Army Cold Regions Research and Engineering Laboratories, Hanover, NH, and the US Army Research Support Group which provided all logistic support for the work. I thank Dr S. Evans, Mr I. Collins and Dr J. F. Nye for discussion.

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# Carbon Isotope Fractionation in the System $\text{CO}_2(\text{gas})-\text{CO}_2(\text{aqueous})-\text{HCO}_3^-(\text{aqueous})$

by

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Carbon isotope fractionation between gaseous carbon dioxide and aqueous bicarbonate decreases from 9.2 to 6.8 parts per thousand over the temperature range  $0^\circ-30^\circ\text{C}$ . This fractionation occurs in the hydration stage, not in the passage of atmospheric carbon dioxide through the air-water interface.

It has long been recognized that atmospheric carbon dioxide is depleted in carbon-13 relative to the inorganic carbon pool of the oceans. Reid and Urey<sup>1</sup> were the first to measure the equilibrium fractionation in the exchange  $^{13}\text{CO}_2 + \text{H}^{12}\text{CO}_3^- \rightleftharpoons ^{12}\text{CO}_2 + \text{H}^{13}\text{CO}_3^-$ ; they arrived at a value of  $1.0120 \pm 0.0025$  at  $25^\circ\text{C}$ . Craig<sup>2</sup> found a  $^{13}\text{C}$  depletion of some 7 parts per thousand in atmospheric carbon dioxide relative to oceanic bicarbonate, and Abelson and Hoering<sup>3</sup> redetermined a value of 1.0083 for the fractionation in the foregoing equilibrium at  $25^\circ\text{C}$ . This latter value is apparently the only one derived from laboratory experiments using improved analytical techniques that have become available since the early work of Reid and Urey<sup>1</sup>. No experimental data at all seem to exist on the change of this fractionation over the temperature range  $0^\circ-30^\circ\text{C}$ , yet this is the most important temperature range in the atmosphere and the oceans. Data are also lacking on carbon isotope fractionation between oceanic bicarbonate and molecular carbon dioxide dissolved in the water. Both fractionation effects are of geochemical as well as biological interest. Knowledge of their magnitude may contribute, for example, to an understanding of whether certain marine plants utilize dissolved carbon dioxide or bicarbonate or possibly both species during photosynthesis. We therefore decided to perform several laboratory experiments to determine the size of these fractionations under equilibrium conditions.

Round-bottom, three-neck distilling flasks of 300 ml. capacity were used as reaction vessels. A gas sample tube, a liquid-sampling pipette which consisted of two stopcocks in series with a 3 ml. volume between them, a modified stopcock the bulb of which contained phosphoric acid and a main stopcock for isolating the whole system were attached to the flasks. All stopcocks and joints were lubricated with a minimum of silicone grease in order to avoid contact between solution and grease. The gas sample tube was filled with the desired amount of carbon dioxide and the pipette and the stopcock which contained phosphoric acid were evacuated and closed after the acid was thoroughly degassed. A 50 ml. aliquot of the sample solution was then pipetted into the flask and frozen by placing the flask on a mixture of dry ice and acetone. When the solution was completely frozen the flask was evacuated to a pressure of about  $10^{-3}$  torr and closed. After thawing the solution, the same cycle was repeated so as to remove most of the gases dissolved in the solution. After the second thawing, the carbon dioxide in the gas sample tube was released into the flask and the entire reaction vessel with attachments was immersed in a water bath the temperature of which was controlled to  $\pm 0.5^\circ\text{C}$ . A minimum of 18 h was allowed for equi-

libration during which time the vessel was shaken periodically.

At the end of a run, a portion of the solution was drawn into the pipette for later  $p\text{H}$  measurement and the gaseous carbon dioxide was condensed back into the gas sample tube. Both these steps, and also the  $p\text{H}$  measurement, were carried out at the particular temperature of the experiment. Following the isolation of the gas phase, the solution was acidified by opening and draining the stopcock containing the phosphoric acid. The evolved carbon dioxide was extracted and purified by two passes through a trap cooled with dry ice and acetone. The carbon dioxide constituting the equilibrium gas phase was subjected to the same purification before transfer to the mass spectrometer. The spectrometer used for the isotope ratio measurements is a conventional 6-in.  $60^\circ$  instrument with dual inlet system and double collector, manufactured by Nuclide Corporation.

In the first set of experiments,  $10^{-4}$  mole of carbon dioxide with a  $\delta^{13}\text{C}^*$  of  $-27.0$  parts per thousand was equilibrated with a solution of  $10^{-4}$  mole of sodium bicarbonate with a  $\delta^{13}\text{C}$  of  $-6.0$  parts per thousand. The  $p\text{H}$  of the solution was 8.4, ensuring that roughly 98 per cent of the carbon was present in bicarbonate<sup>4</sup>. The  $\delta^{13}\text{C}$  measured on the carbon dioxide derived by acidifying the solution after the run therefore closely represented the value for the bicarbonate in equilibrium with the carbon dioxide atmosphere above the solution. Fig. 1 shows the data obtained at  $0^\circ$  and  $30^\circ\text{C}$  equilibration temperatures. The reproducibility in all experiments described here is of the order of  $\pm 0.2-0.4$  parts per thousand for  $\Delta$ , that is, the difference between the  $\delta$ -values of the equilibrated phases. As the error in  $\Delta$  depends on two separate sample preparations and analyses in addition to the errors inherent in the equilibration and separation stages, this level of precision seems quite acceptable. Fig. 1 also includes the data obtained in a separate experiment, similar to the one previously mentioned, where the initial carbon dioxide was isotopically heavier ( $\delta^{13}\text{C} = +3.6$  parts per thousand) than the bicarbonate ( $\delta^{13}\text{C} = -6.0$  parts per thousand). This experiment was performed in order to approach equilibrium from the opposite direction. As is evident from Fig. 1, a switchover was achieved and after the run the carbon dioxide above the solution was 9.6 parts per thousand lighter than the bicarbonate. Within

$$^* \delta^{13}\text{C} = \left( \frac{(^{13}\text{C}/^{12}\text{C})_{\text{sample}}}{(^{13}\text{C}/^{12}\text{C})_{\text{standard}}} - 1 \right) \times 1,000$$

All values reported are relative to the Chicago  $P\text{DB}$  standard.



the limitations of these experiments, this value can be considered identical to the others obtained at the same temperature, namely, 9.3, 8.8, 9.1 parts per thousand. The  $^{13}\text{C}/^{12}\text{C}$  difference between atmospheric carbon dioxide and aqueous bicarbonate at  $0^\circ\text{C}$  may thus be taken to be  $9.2 \pm 0.4$  parts per thousand. Table 1 lists the experimentally determined differences and their mean values for  $0^\circ$ ,  $10^\circ$ ,  $20^\circ$  and  $30^\circ\text{C}$ . It also gives the fractionation factors  $\alpha$ , defined as

$$\alpha = \frac{(^{13}\text{C}/^{12}\text{C})_{\text{HCO}_3^-}}{(^{13}\text{C}/^{12}\text{C})_{\text{CO}_2}}$$

for each temperature. These values were calculated from the best fit to all the data in a plot of  $1,000 \ln \alpha$  against  $10^3/T^{-2}$ , where  $T$  is the absolute temperature.

Table 1. EQUILIBRIUM FRACTIONATION OF CARBON ISOTOPES BETWEEN ATMOSPHERIC CARBON DIOXIDE AND AQUEOUS BICARBONATE

Temperature ( $^\circ\text{C}$ )	$\Delta_{\text{CO}_2-\text{HCO}_3^-}$ ( $\text{‰}$ )	$\bar{\Delta}$ ( $\text{‰}$ )	$\alpha$ ( $\pm 0.0003$ )
0	9.3		
0	8.8		
0	9.1	9.2	1.0093
0	9.6		
10	8.1		
10	8.1	8.1	1.0084
20	7.6		
20	7.0	7.3	1.0076
30	6.6		
30	7.1	6.8	1.0069

Another series of experiments was aimed at determining the isotopic composition of dissolved carbon dioxide in equilibrium with aqueous bicarbonate and atmospheric carbon dioxide. To this end buffered solutions of pH 6.20–6.53 were allowed to equilibrate with a carbon dioxide gas. The buffers used were mixtures of  $\text{KH}_2\text{PO}_4$  and  $\text{Na}_2\text{HPO}_4$  dissolved in distilled water. The equilibration temperature used was  $0^\circ\text{C}$  in order to maximize fractionation effects. Preparation procedure was identical to the one described previously except that in these runs all the carbon was initially in the form of carbon dioxide gas. On bringing the gas and solution into contact some of the carbon dioxide entered the solution and partly hydrated and dissociated to protons and bicarbonate ions. The molar ratio of free carbon dioxide (this includes roughly 0.1 per cent undissociated  $\text{H}_2\text{CO}_3$ ) to the bicarbonate in the solution is dependent on the pH and the temperature and can be obtained from tables of values calculated by Saruhashi<sup>4</sup>. To determine the isotopic composition of the dissolved carbon dioxide it was then only necessary to measure the pH of the solution at the end of the experiment and the  $\delta^{13}\text{C}$  values of the gaseous carbon dioxide and of the total carbon in the solution ( $\delta\Sigma\text{C(aq)}$ ). The  $\delta^{13}\text{C}$  of the bicarbonate and of the aqueous

carbon dioxide were then calculated from the following equations

$$\delta\text{HCO}_3^- = \delta\text{CO}_2(\text{gas}) + \Delta \quad (1)$$

$$\delta\text{CO}_2(\text{aq}) = \delta\Sigma\text{C(aq)} - Q(\delta\text{HCO}_3^- - \delta\Sigma\text{C(aq)}) \quad (2)$$

where  $\Delta$  is the previously determined difference between the  $\delta$ 's of the carbon dioxide and bicarbonate in equilibrium (9.2 parts per thousand at  $0^\circ\text{C}$ ) and  $Q$  is the molar ratio of bicarbonate ions to carbon dioxide in the solution, a value obtained from Saruhashi's tables for the pH and temperature of the experiment.

Table 2 shows the data obtained in three runs in which  $Q$  varied from 0.41 to 0.89. In all three cases the calculated  $\delta\text{CO}_2(\text{aq})$  is identical to the measured  $\delta\text{CO}_2(\text{gas})$  to within  $\pm 0.5$  parts per thousand or better. Thus the agreement is excellent considering that the errors associated with  $\Delta$ ,  $Q$  and two independent sample preparations and analyses affected the calculation.

Table 2. COMPARISON OF  $\delta^{13}\text{C}$  VALUES ( $\text{‰}$ ) OF  $\text{CO}_2(\text{GAS})$  AND  $\text{CO}_2(\text{AQ})$  IN EQUILIBRIUM AT  $0^\circ\text{C}$

pH	Q	$\delta\Sigma\text{C(aq)}$ (measured)	$\delta\text{CO}_2(\text{gas})$ (measured)	$\delta\text{CO}_2(\text{aq})$ (calculated)	Difference
6.53	0.89	5.7	1.6	1.2	+0.4
6.27	0.48	5.5	2.8	2.4	+0.4
6.20	0.41	5.7	2.7	3.2	-0.5

To obtain a further check on the data presented in Table 2, one additional experiment was performed in which the pH of the solution was lowered to 5.2. At this low pH, 96 per cent of the carbon in solution is in the form of carbon dioxide and the bicarbonate fraction can be neglected for the purposes of the present experiment. In the terms of equation (2),  $Q$  was 0.04, which made the second term negligible and  $\delta\Sigma\text{C(aq)} \approx \delta\text{CO}_2(\text{aq})$ . As the solubility of carbon dioxide at pH 5.2 is appreciably lower than at the pH values of the other experiments the carbon dioxide pressure above the solution was raised by allowing 20 c.c. STP of carbon dioxide to equilibrate with the solution instead of the usual 2.25 c.c. On completion of the run great care was taken not to allow any of the dissolved carbon dioxide to escape the solution. The stopcock of the gas sample tube into which the gaseous carbon dioxide was being frozen was closed as soon as the first bubbles appeared in the solution, produced by the sudden drop in pressure above the solution. It is conservatively estimated that at least 99 per cent of the gaseous carbon dioxide was recovered and that no more than 1 per cent of the dissolved carbon dioxide escaped the solution before the stopcock was closed. The solution was then acidified to liberate the dissolved carbon dioxide. Comparison of the volumes after purification of the gas

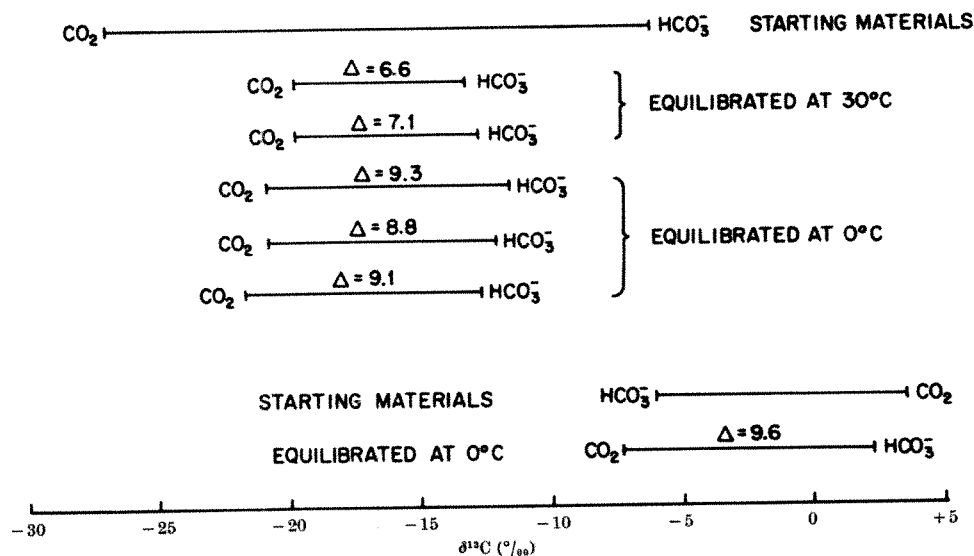


Fig. 1. Comparison of  $\delta^{13}\text{C}$  values of carbon dioxide and bicarbonate before and after equilibration at  $0^\circ$  and  $30^\circ\text{C}$ .

samples showed that about 16 per cent of the gas had gone into solution. The initial carbon dioxide of the experiment, comprising the total carbon involved, had a  $\delta^{13}\text{C}$  of  $-27$  parts per thousand. After equilibration the measured values for the gaseous and dissolved carbon dioxide were  $-26.5$  and  $-27.1$  parts per thousand, respectively, which are identical within the established limits of precision.

These experiments have shown that the fractionation of carbon isotopes between atmospheric carbon dioxide and aqueous bicarbonate decreases from  $9.2 \pm 0.4$  parts per thousand at  $0^\circ\text{C}$  to  $6.8 \pm 0.4$  parts per thousand at  $30^\circ\text{C}$  and that there is no measurable fractionation between atmospheric carbon dioxide and carbon dioxide dissolved in water. In the absence of complete stagnancy equilibrium appears to be established rather quickly so that the difference in the kinetics of passage through the air-water interface between the two isotopic species  $^{12}\text{CO}_2$  and  $^{13}\text{CO}_2$  cannot significantly contribute to the carbon dioxide-bicarbonate fractionation. It may be assumed that the fractionation occurs in the hydration step where carbon bonds are rearranged and not in the dissociation although this cannot be ascertained experimentally because of the low relative abundance of the undissociated phase. One biological implication of this is that it is possible to distinguish the inorganic carbon source of aquatic plants. If the plants utilize carbon dioxide only, their carbon should be isotopically identical to that of air breathing plants as long as the utilization proceeds along the same paths and the carbon dioxide can

equilibrate with the atmosphere. If a plant can utilize either carbon dioxide or bicarbonate, depending on their relative availability, the proportion of the two will be reflected in the plant's carbon isotope content. In the extreme case of exclusive bicarbonate uptake, the plant will, depending on mean growth temperature, be 7–9 parts per thousand heavier than in the case of pure carbon dioxide uptake. The apparent ease of equilibration and consequent insignificance of the kinetic effect in the passage of carbon dioxide molecules through the air-water interface also casts doubt on the reality of the large kinetic effect postulated for the passage of carbon dioxide from the atmosphere into the plant cytoplasm during photosynthesis.

In the light of these experiments, carbon isotope differences between most fresh waters (light) and marine waters (heavy) can no longer be interpreted to be exclusively a result of the large biogenic carbon dioxide contributions to rivers and lakes. The pH and consequently the ratio of dissolved carbonic acid derivatives appear to be principal factors in determining the ultimate  $\delta^{13}\text{C}$  in a given water body.

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## Isotropic Gamma-ray Intensity in an Expanding Universe

by

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The intensities of isotropic gamma-rays produced by the inverse Compton process in an expanding universe are calculated, including the effect of red-shift on the radiation field. The results may give information concerning the universal black-body radiation field and the intergalactic electron flux intensity.

THIS article presents a calculation of the mean free path and the intensities of isotropic gamma-rays in an expanding universe. Similar calculations<sup>1</sup> have been made, but none has included the effect of the red-shift of the radiation field. We assume, along with Fazio, Stecker and Wright<sup>2</sup>, that the mechanism producing the isotropic gamma-rays is the inverse Compton process that takes place between the high energy cosmic electrons and the assumed black-body radiation field, which is at a temperature of  $3.5^\circ\text{K}$  in the neighbourhood of Earth at the present epoch<sup>3</sup>.

In an isotropic, homogeneous universe, the line element has the familiar Robertson-Walker form

$$ds^2 = c^2 dt^2 - R^2(t) \left[ \frac{dr^2}{1 - kr^2} + r^2 (d\theta^2 + \sin^2 \theta d\varphi^2) \right]$$

We have the relation between  $E_{0\gamma}$ , the energy of the gamma-rays observed at the present epoch  $t_0$  and  $E_\gamma$ , and the energy at the epoch  $t$ , given by the expression

$$E_\gamma = (1 + Z) E_{0\gamma} = E_{0\gamma} \frac{R(t_0)}{R(t)}$$

where  $Z$  is the red-shift. Suppose the cross section of the inverse Compton effect between the black-body radiation field, with a photon density  $n_{\text{ph}}(\epsilon)$ , and cosmic electrons,

with an intensity spectrum  $I_\epsilon = K_\epsilon E_\epsilon^{-\alpha}$ , is  $\sigma(E_\gamma, \epsilon, E_\epsilon)$ , then the gamma-ray source function for the energy  $E$ , produced in a spherical shell at the "time"  $t$ , is given by the equation

$$S(E_\gamma, t) dV(t) = dV(t) \int_{E_\epsilon} I_\epsilon dE_\epsilon \cdot \sigma(E_\gamma, \epsilon, E_\epsilon) \cdot n_{\text{ph}}(\epsilon) d\epsilon$$

where  $dV = -4\pi r^2 R^2(t) dt$  is the volume of the shell. Summing over the contributions from all the shells, we find the intensity of the observed gamma-ray energy,  $E_{0\gamma}$ , at "time"  $t_0$  is

$$I(E_{0\gamma}) = \int_t S \left( E_{0\gamma} \frac{R(t_0)}{R(t)}, t \right) dV(t) / 4\pi D^2(t)$$

where  $D(t) = rR^2(t_0)/R(t)$  is the luminosity distance of the source<sup>4</sup>, and  $E_\gamma$  is replaced by  $E_\gamma = E_{0\gamma} R(t_0)/R(t)$  in the source function  $S$ .

The integral involving the inverse Compton cross section in  $S(E_\gamma, t)$  has been given by Ginzburg and Syrovatskii<sup>5</sup>. We find then that intensity is

$$I(E_{0\gamma}) = -f^1(\alpha) \cdot K_\epsilon \cdot T(t_0)^{\frac{\alpha+5}{2}} E_{0\gamma}^{-\frac{\alpha+1}{2}} c \int_t \left( \frac{R(t)}{R(t_0)} \right)^3 dt$$

where we have used the relation  $T(t)R(t) = T(t_0)R(t_0)$  for the temperature of the radiation field, and  $\alpha$  is the electron flux index. This relation is true so long as the radiation

Table 1.  $Z_c$  AND  $D_I$  AS A FUNCTION OF  $E_{\gamma}$  FOR OPEN AND FLAT UNIVERSES

Observed energy of gamma-rays, $E_{\gamma}$ in eV	$10^4$	$10^5$	$10^6$	$10^{10}$	$10^{14}$	$10^{16}$
$Z_c$ (open)	$7 \times 10^4$	$7 \times 10^3$	$8 \times 10^2$	79	7	0.08
$D_I$ (open, in cm)	$3.2 \times 10^{27}$	$3.2 \times 10^{27}$	$2.15 \times 10^{27}$	$3.15 \times 10^{27}$	$3.1 \times 10^{27}$	Local
$Z_c$ (flat)	$7.4 \times 10^4$	$7.8 \times 10^3$	$8 \times 10^2$	85	8	0.1
$D_I$ (flat, in cm)	$2.7 \times 10^{27}$	$2.7 \times 10^{27}$	$2.7 \times 10^{27}$	$2.65 \times 10^{27}$	$2.65 \times 10^{27}$	Local

field and the matter field have de-coupled, and each undergoes adiabatic cooling independently, as we have assumed in the present calculations. The function  $f^1(\alpha)$  is

$$f^1(\alpha) = f(\alpha) \frac{2}{3} \sigma_T a (mc^2)^{1-\alpha} \left( \frac{4}{3} \times 2.7 k \right)^{\frac{\alpha-3}{2}}$$

where  $f(\alpha) \simeq 1$  for  $\alpha$  in the range 1-2. The integral in the expression for  $I(E_{\gamma})$  is defined as

$$D_I = -c \int_{t_0}^{t=t_c} \left( \frac{R(t)}{R(t_0)} \right)^2 dt$$

and is integrated from the present epoch  $t_0$  to a cut-off "time"  $t_c$ , which is a function of the gamma-ray energy, because different gamma-ray energies have different opacities. This integral can be interpreted as the farthest "distance" we can "see" into the past through each channel of different gamma-ray energies. We now proceed to calculate the mean free paths of the gamma-rays, and hence  $t_c$  and  $D_I$ .

$$\lambda_{\text{open}} = 1.64 \times 10^{19} E_{\gamma} \frac{Z}{1+Z} \left( \exp \left[ -\frac{3.12 \times 10^3}{E_{\gamma}(1+Z)^2} \right] \left( \frac{3.12 \times 10^3}{E_{\gamma}(1+Z)^2} + 1 \right) - \exp \left[ -\frac{3.12 \times 10^3}{E_{\gamma}} \right] \left( \frac{3.12 \times 10^3}{E_{\gamma}} + 1 \right) \right)^{-1} \text{ cm}$$

The most effective absorption of the gamma-rays takes place by pair production, which in our case results from the interaction between the gamma-rays and the cosmic black-body radiation field, which had a higher energy density in the past than in the present. This higher energy density lowers the threshold energy so that the gamma-rays more easily produce the electron-positron pairs, and thus the removal of the gamma-rays is facilitated. The interaction between gamma-rays and cosmic electrons, which is the Compton effect, is about a hundred times less efficient than pair production, and we shall therefore neglect it in calculating the mean free paths.

For gamma-rays traversing a black-body radiation field, which has a photon number density  $n_{\text{ph}}(\epsilon)$  for a distance  $dl(t)$ , we have the differential optical depth  $d\tau(t)$

$$d\tau(t) = dl(t) \int_{\epsilon} n_{\text{ph}}(\epsilon) \sigma(E_{\gamma}, \epsilon) d\epsilon$$

where  $\sigma(E_{\gamma}, \epsilon)$  is the cross section of pair production. Notice that we have neglected the angular dependence of the interaction and assumed that all the interactions are head-on collisions. This will introduce little error, for the gamma-rays and the black-body radiations are isotropically distributed. The cross section of the interaction, which has a maximum value of  $0.7 \pi r_0^2$  ( $r_0 = 2.8 \times 10^{-13}$  cm is the classical radius of the electron) at the black-body photon energy  $\epsilon_{\text{max}} \simeq 2.25 (mc^2)^2/E_{\gamma}$ , is significant in the interval  $\Delta\epsilon \simeq 2\epsilon = 2 (mc^2)^2/E_{\gamma}$  for a given energy  $E_{\gamma}$  of the gamma-ray<sup>6</sup>. The threshold energy relation is  $E_{\gamma}\epsilon = (mc^2)^2$ . We can therefore approximate the cross section as a square pulse of height  $\frac{1}{2} \pi r_0^2$  in the interval  $\Delta\epsilon$ , and zero elsewhere. Using the formula for the black-body radiation photon density,

$$n_{\text{ph}}(\epsilon) = 1/[\pi^2 (c\hbar)^3 (e^{\epsilon/kT} - 1)]$$

we obtain, for an observed gamma-ray energy  $E_{\gamma}$ , the total optical depth from the distance of red-shift  $Z$

$$\begin{aligned} \tau(E_{\gamma}, Z) &= \int_Z \int_{\Delta\epsilon} \frac{c}{H_0} \frac{dZ}{(1+Z)^2 (1+2q_0 Z)^{1/2}} \cdot \frac{1}{\pi^2 (c\hbar)^3} \cdot \left( \frac{1}{e^{\epsilon/kT} - 1} \right) \cdot \frac{1}{2} \pi r_0^2 d\epsilon \\ &\simeq 4 \times 10^{23} \frac{c}{H_0} \cdot \frac{10.12 (mc^2)^6}{E_{\gamma}^3} \int_{Z=0}^Z \frac{1}{(1+Z)^5 (1+2q_0 Z)^{1/2}} \exp \left( -\frac{2.25 (mc^2)^2}{E_{\gamma} kT(t_0) (1+Z)^2} \right) dZ \end{aligned}$$

where we have neglected 1 in the denominator of the Planck function and have used the formula

$$dl = \frac{c}{H_0} \cdot \frac{dZ}{(1+Z)^2 (1+2q_0 Z)^{1/2}} \quad (\text{ref. 7})$$

where  $q_0$  is the cosmological deceleration parameter. This approximation is good as long as we have  $Z \leq 10^7$  for  $E \simeq 10^8$  eV, extending to  $Z \leq 0.01$  for  $E \simeq 10^{15}$  eV, which we shall see is in our range of  $Z$  in the subsequent calculations.

Now defining the mean free path of a gamma-ray photon as

$$\lambda = \frac{Z}{0} \int dl / \tau(E_{\gamma}, Z)$$

we can easily calculate  $\lambda$  by using the expression for  $\tau$  and the formula for  $dl$  as given above. We calculate  $\lambda$  as a function of  $Z$  for an open universe where  $q_0 = 0$ ,  $k = -1$ ; and for a flat universe, where  $q_0 = \frac{1}{2}$ ,  $k = 0$ . Using a value  $H_0 = 100$  km/sec-megaparsec, we have for an open universe

and for a flat universe

$$\begin{aligned} \lambda_{\text{flat}} &= 3.5 \times 10^{15} E_{\gamma}^2 \left[ 1 - \frac{1}{(1+Z)^{3/2}} \right] (1+Z)^{5/2} \\ &\quad \exp \left[ \frac{3.12 \times 10^3}{E_{\gamma}(1+Z)^2} \right] \text{ cm} \end{aligned}$$

From the foregoing formulae we can determine the cut-off red-shift  $Z_c$  by using the condition  $\lambda(Z_c) = l(Z_c)$  for a given observed gamma-ray energy (Fig. 1). In other words, for a given energy the gamma-rays are "visible" to the red-shift  $Z_c$ , beyond which the mean free path of the gamma-ray becomes less than the proper distance from the observer to the source, and hence "invisible". Once we know  $Z_c$ , it is a straightforward matter to calculate the "distance"  $D_I$ . The results for  $Z_c$  and  $D_I$  are shown in Table 1. In the integration of  $D_I$  we have used the relation  $t_c = t_0/(1+Z_c)$  for an open universe, and  $t_c = t_0/(1+Z_c)^{3/2}$  for a flat universe. In Fig. 2 we plotted  $E_0$  against  $Z_c$  for an open universe. It can be seen that the lower the gamma-ray energy, the larger is the cut-off red-shift  $Z_c$ . For gamma-rays of energies greater than  $10^{14}$  eV, the contributions from the distant past of the universe are insignificant, and we can observe only those gamma-rays that originated in our immediate vicinity, that is, our galaxy. This is because of the very high opacity for high energy gamma-rays. Table 1 shows that the distance  $D_I$  changes very little between the lower energies and the energies up to  $10^{14}$  eV, because of the very rapid rise of the cosmic proper distance  $l(Z)$  as a function of  $Z$ , so that  $D_I$  is not sensitive to the change of  $Z$  once  $Z$  becomes greater than unity. The intensities of the gamma-rays can be calculated from the values  $D_I$  and the formula for  $I(E_{\gamma}, Z_c)$ . The value of  $K_e$ , the electron density in intergalactic space, is assumed to be  $10^{-2}$  times

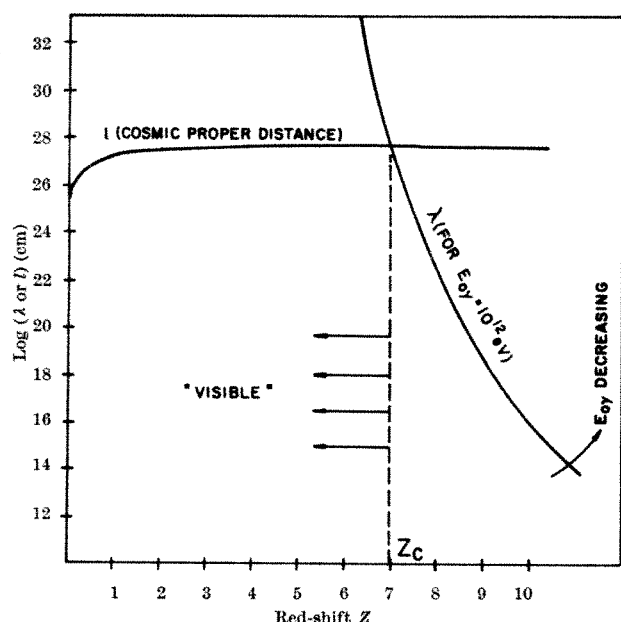


Fig. 1. Optical depth of gamma-rays of observed energy  $E_{0\gamma} = 10^{12}$  eV and the cosmic distance  $l$  are plotted as functions of red-shift  $Z$  for the case of an open universe ( $k = -1$ ,  $q_0 = 0$ ).

that observed in the neighbourhood of the Earth, where the electron flux density is  $K_e = 11 \times 10^{3a-7}$  (MeV) $^a$  (cm $^2$  sec sterad MeV) $^{-1}$ , as given by L'Heureux and Meyer<sup>8</sup>. The results are given in Tables 2 and 3.

In Fig. 3 we plotted the gamma-ray intensity  $I_0$  against the gamma-ray energy  $E_{0\gamma}$ ; also included are the observational spectrum and a theoretical spectrum<sup>2</sup>. The calculated intensity spectrum maintains its slope up to the energy of  $10^{12}$  eV, but is shifted parallel upward from that obtained by Fazio, Stecker and Wright. The parallel shifting of the spectrum without change of slope in an expanding universe has also been pointed out by Felton and Morrison<sup>9</sup>. In our case, this perseverance of the spectrum is due to the large values of  $Z_c$  for  $E_{0\gamma} \leq 10^{12}$  eV and the insensitivity of the cosmic proper distance to large red-shift. The spectrum will not continue to maintain its local shape to very high energy beyond  $E_{0\gamma} \geq 10^{14}$  eV, where, because of the high opacities at these energies, the spectrum will become steeper. The calculated intensities are seen to be in fair agreement with observations in the lower energy range ( $E_{0\gamma}$  approximately  $10^4$  eV), but greater than the observational values by a factor of 100 in the higher energy ranges. Some general conclusions can be drawn from a comparison of theoretical with experimental intensity values.

If we assume that the isotropic gamma-rays are produced throughout the whole universe via the inverse Compton effect between assumed cosmic electrons and the cosmic black-body radiation field, then we get too

Table 2. GAMMA-RAY INTENSITY AT EARTH ( $E_{0\gamma}$ ) IN AN OPEN UNIVERSE (IN PHOTONS/CM $^2$  SEC STERAD MEV)

	$E_{0\gamma}$ (eV)				
Electron flux index	$3 \times 10^3$	$10^4$	$10^5$	$10^{10}$	$10^{12}$
1.1	$3.5 \times 10^3$	7.8	$6.1 \times 10^{-2}$	$4.8 \times 10^{-4}$	$3.8 \times 10^{-6}$
1.6	$3.7 \times 10^3$	7.9	$4.9 \times 10^{-3}$	$1.2 \times 10^{-5}$	$3.1 \times 10^{-8}$
2.1	$4.1 \times 10^3$	0.5	$3.9 \times 10^{-4}$	$8.2 \times 10^{-7}$	$2.6 \times 10^{-10}$
Experimental	$2.0 \times 10^3$	$\approx 10^{-2}$	$\leq 3.0 \times 10^{-4}$	None	None

Table 3. GAMMA-RAY INTENSITY AT EARTH ( $E_{0\gamma}$ ) IN A FLAT UNIVERSE (IN PHOTONS/CM $^2$  SEC STERAD MEV)

	$E_{0\gamma}$ (eV)				
Electron flux index	$3 \times 10^3$	$10^4$	$10^5$	$10^{10}$	$10^{12}$
1.1	$3.0 \times 10^3$	6.6	$5.2 \times 10^{-2}$	$4.1 \times 10^{-4}$	$3.2 \times 10^{-6}$
1.6	$3.2 \times 10^3$	1.6	$4.1 \times 10^{-3}$	$1.0 \times 10^{-5}$	$2.6 \times 10^{-8}$
2.1	$3.4 \times 10^3$	0.4	$3.3 \times 10^{-4}$	$2.7 \times 10^{-7}$	$2.2 \times 10^{-10}$
Experimental	$2.0 \times 10^3$	$\approx 10^{-2}$	$\leq 3 \times 10^{-6}$	None	None

high a gamma-ray intensity in the neighbourhood of the Earth when compared with the experimental values. This discrepancy can be resolved if we assume an electron flux  $10^{-4}$  smaller than that observed at the Earth, instead of  $10^{-2}$ , as used in the foregoing calculations. Note that we have assumed that the electron flux is constant in time; this may not be true, and it may have been higher in the past than it is now. A greater electron flux would result in a further increase of the gamma-ray intensity. The uncertainty in the electron flux seems to present some difficulties in the extragalactic model.

Apart from the uncertainty of the cosmic electron flux, the high gamma-ray intensities calculated previously suggest some doubt about the existence of a universal black-

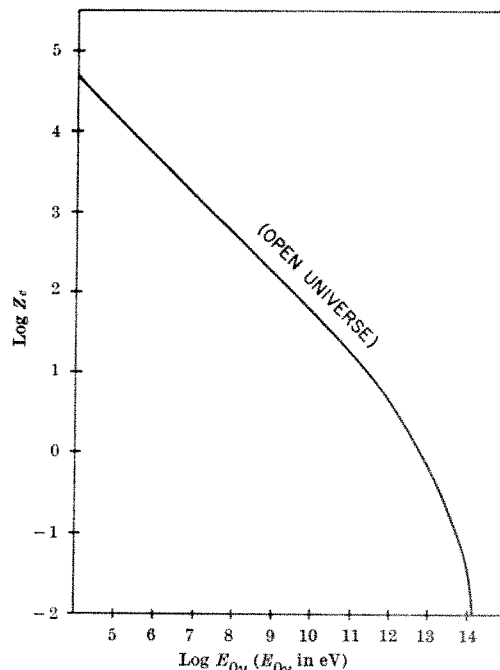


Fig. 2. Cut-off red-shift  $Z_c$  plotted as a function of observed gamma-ray energy  $E_{0\gamma}$  for an open universe.

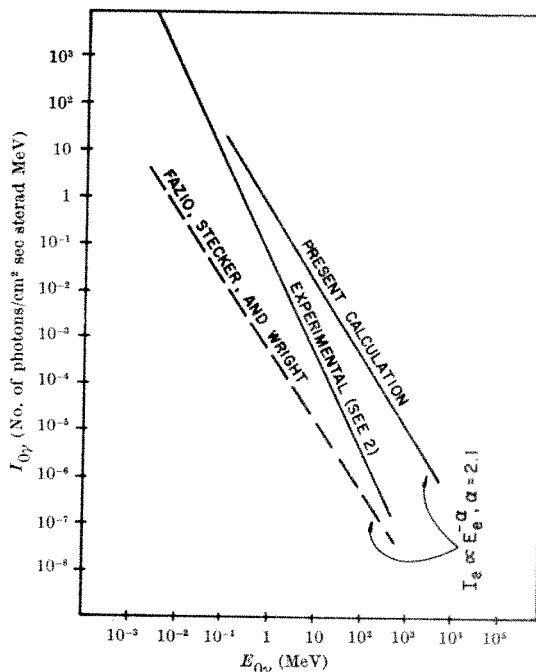


Fig. 3. Intensity spectra of gamma-rays. The calculated spectrum is for an open universe.



body radiation field, if there is a cosmic electron flux greater than  $10^{-4}$  times the flux observed at the Earth. Recently, Daniel and Stephens<sup>10</sup> also raised this doubt, from a comparison of the predicted and the observed cosmic electron spectrum at high energies. Further observations are obviously needed. Finally, the isotropic gamma-rays may not be produced uniformly throughout the whole universe as we suppose here, but may be partially due to unresolved individual gamma-ray sources.

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## Enhancement of Respiration and Fermentation in Algae by Blue Light

by

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In the dark, algae live on their reserve substances and respire very slowly. In this state a little blue light can stimulate their dark metabolism considerably. This regulatory effect may be the basis for several responses of plants to blue light.

WHEN micro-organisms run out of food, their respiration continues at the expense of reserve substances, but the rate of this endogenous respiration declines after several hours and finally continues at a fraction of the rate in optimal conditions. This is true for heterotrophs in a mineral environment as well as for photo-autotrophs kept in the dark<sup>1,2</sup>. We have reported that blue light prevents the decline of endogenous respiration in "resting" cells, or, after it has gone down, restores it to a remarkably high value<sup>3</sup>. This effect of blue light was independent of photosynthesis. It still existed when the latter had been abolished either by poisoning with DCMU or by mutation. The effect was found while we were searching for a more immediate metabolic response of algae to blue light than their known peculiar deviation in protein synthesis<sup>4</sup>. We report here on the progress of these investigations.

Most of the experiments were carried out with a mutant of *Chlorella vulgaris* (211-11h/20 of the culture collection of algae at the University of Göttingen) which was free from chlorophyll but contained carotenoids. This organism is most convenient to use because no special precautions are needed to avoid complications with light absorption and light reactions of the chloroplast system.

As Fig. 1 shows, the effect of blue light on the uptake of oxygen by this yellow mutant could be easily seen either with the Warburg manometric technique or with an "oxygen sensor". In the latter case the current between a gold cathode and a silver anode changes in proportion to the partial pressure of oxygen in the suspension of algae. Depending on the degree of starvation, blue light enhanced the rate of the endogenous respiration up to five times, and maintained it at such a rate for a day or longer. In one experiment, which lasted 41 h, the enhanced respiration began to decline markedly after 20 h of uninterrupted illumination. The relative rates after 2 h were dark 15, blue 29; after 21 h they were dark 4, blue 21; and after 41 h they were dark 2, blue 13. We have seen no case where the induced rate surpassed the maximal rate of respiration in the presence of sufficient exogenous glucose or acetate, or a rate stimulated by optimal concentrations

of dinitrophenol. To obtain good effects, the glucose grown algae were therefore starved for a day or two in plain buffer before the experiments.

The extra oxygen consumption in blue light was a true respiration. Its respiratory quotient was approximately unity and did not change with the increase in rate; that is the release of carbon dioxide followed exactly the increase in oxygen uptake. The temperature dependence of the slow respiration in the dark and that of the fast, light induced respiration was the same. The blue light effect was perfectly reversible. Between 15 and 30 min after "light off" the respiration was almost back to its normal dark level. Such "on" and "off" tests could be repeated many times.

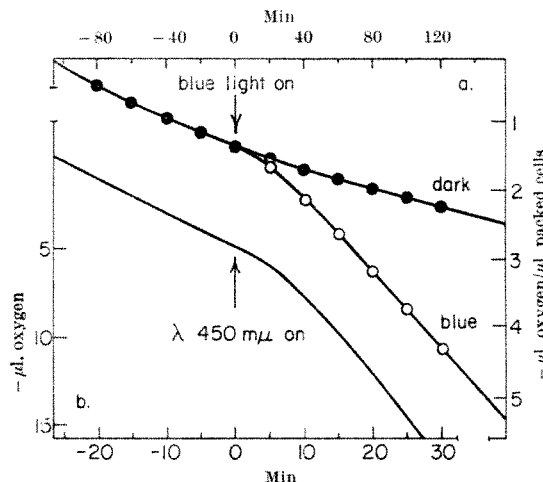


Fig. 1. Acceleration of endogenous respiration by blue light in the yellow *Chlorella* mutant 211-11h/20 suspended in 0.1 molar phosphate buffer, pH 6.5, at 30° C. (a) Oxygen uptake measured manometrically. Light: broad band  $\lambda > 366 - < 550$  m $\mu$ ,  $\sim 3 \times 10^3$  ergs sec<sup>-1</sup> cm<sup>-2</sup>; 70  $\mu$ l. of packed cells  $\sim 15$ -5 mg dry weight/vessel. (b) Oxygen uptake as recorded with an "oxygen sensor". Light:  $\lambda$  450 m $\mu$  interference filter 9 m $\mu$  halfwidth.  $\sim 500$  ergs sec<sup>-1</sup> cm<sup>-2</sup>.

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So far we have found conspicuous differences between the normal and the light induced respiration in the respective responses to the nature and the pH of the suspension medium. The endogenous respiration was markedly slower in 0.1 molar phosphate buffer than in 0.1 molar bicarbonate both at pH 8.5, and also slower in phosphate at pH 6.5 than at pH 8.5. The enhanced respiration in blue light, however, showed virtually no influence of either pH or bicarbonate versus phosphate—the rates remained equally fast<sup>5</sup>. Consequently, the difference between the uptake of oxygen in the dark and that in the light was the more impressive the slower the rate of the former.

In contrast to photosynthesis and more like photoperiodism and phototropism, the amount of light needed to give a measurable enhancement of oxygen uptake in the conditions of our experiments was quite small. At  $\lambda$  450 m $\mu$  50 ergs cm<sup>-2</sup> sec<sup>-1</sup> sufficed for half-saturation, and 500 ergs cm<sup>-2</sup> sec<sup>-1</sup> for complete saturation of the blue light effect, while photosynthesis with blue light in green plants needs from 10,000–50,000 ergs cm<sup>-2</sup> sec<sup>-1</sup>.

An action spectrum for the enhancement of respiration has been determined by Kowallik<sup>6</sup> using the oxygen sensor technique. Far-red, red and yellow light were completely ineffective even at ten times the intensity which was sufficient to saturate the effect in blue light. The maximum activity for equal flux of incident quanta was found in a broad peak around  $\lambda$  460 m $\mu$ . The efficiency dropped steeply towards the green with no activity beyond  $\lambda$  520 m $\mu$  and towards the ultra-violet, but there a second, smaller peak appeared around  $\lambda$  375 m $\mu$ . This action spectrum is almost identical with that for the phototropic responses of *Phycomyces* sporangiothecae<sup>7</sup> and of *Avena coleoptiles*<sup>8</sup>, for carotenoid formation in *Fusarium*<sup>9</sup> and

for the retardation of flower opening in *Oenothera*<sup>10</sup>. Rau believes that his action spectrum indicates the function of a flavin. The other authors independently agree that these action spectra do not show whether the active pigment is a flavin or a *cis*-carotenoid.

Flavin is a part of the respiratory chain and so the question arose as to whether the effect of blue light on respiration was directly on the oxidative electron transport chain. If this was so the effect might disappear in anaerobic conditions. Furthermore, a direct photochemical influence on the respiratory mechanism should become apparent immediately after exposure to blue light. This, however, was not the case. In dozens of experiments of the type shown in Fig. 1, it took between 10 and 20 min from "light on" until the new steady rate of gas exchange was reached. And this was similarly true, as already mentioned, for the return to the dark rate after "light off". These transient periods were therefore much longer than those in photosynthesis. Obviously some slow and complex metabolic reaction preceded the visible increase in respiration. Further experiments in the absence of oxygen confirmed this.

If algae in phosphate buffer of pH 5.0 were kept anaerobically in darkness or blue light only a small positive gas pressure change could be seen. There was, however, a measurable pH change in the medium. This fits the well known observation that fermenting green algae and their mutants chiefly produce acids, little free carbon dioxide. The amount and the kind of such fermentation end-products may vary considerably among different species and depend largely on culture conditions<sup>11</sup>. Which acids are formed in our case we may leave aside for the moment.

Under nitrogen the pH of a suspension of the yellow *Chlorella* mutant in 0.002 molar phosphate buffer changed in the dark from 6.45 to 6.38; in blue light, however, to 6.29 in 50 min. This light-enhanced production of acids in anaerobiosis could also be demonstrated with 0.01 molar sodium bicarbonate as suspension medium: in blue light about nine times more carbon dioxide was released from the bicarbonate by the acids produced than in darkness. Blue light thus increased fermentation by this *Chlorella* mutant in these conditions.

Further proof that blue light is responsible for the release of more fermentation products when algae are incubated anaerobically is given in Fig. 2. Four equal samples of algae were kept under nitrogen for 140 min. One was left in the dark during the entire period, and the others were illuminated for 10, 30 or 60 min immediately before being brought back into aerobic conditions. In all four samples the ensuing respiration in the dark was greater than before. But the rate of oxygen uptake in the samples which were exposed to light under nitrogen was faster than that of the dark control. The difference between the amounts of oxygen consumed by the dark control and the samples exposed to blue light was proportional to the time of illumination (see Table 1). In the absence of oxygen blue light had produced something which served afterwards as substrate for respiration in the dark. In these circumstances the enhanced respiration started without an induction period at its highest rate, and, as usual, declined in the course of time. Further experiments showed that the substances made available to the respiratory system by anaerobic pre-treatment with blue light were very stable. It made no difference whether air was re-admitted afterwards in the dark immediately or 1 or 2 h later.

The anaerobic metabolism enhanced by blue light had a pronounced dependence on temper-

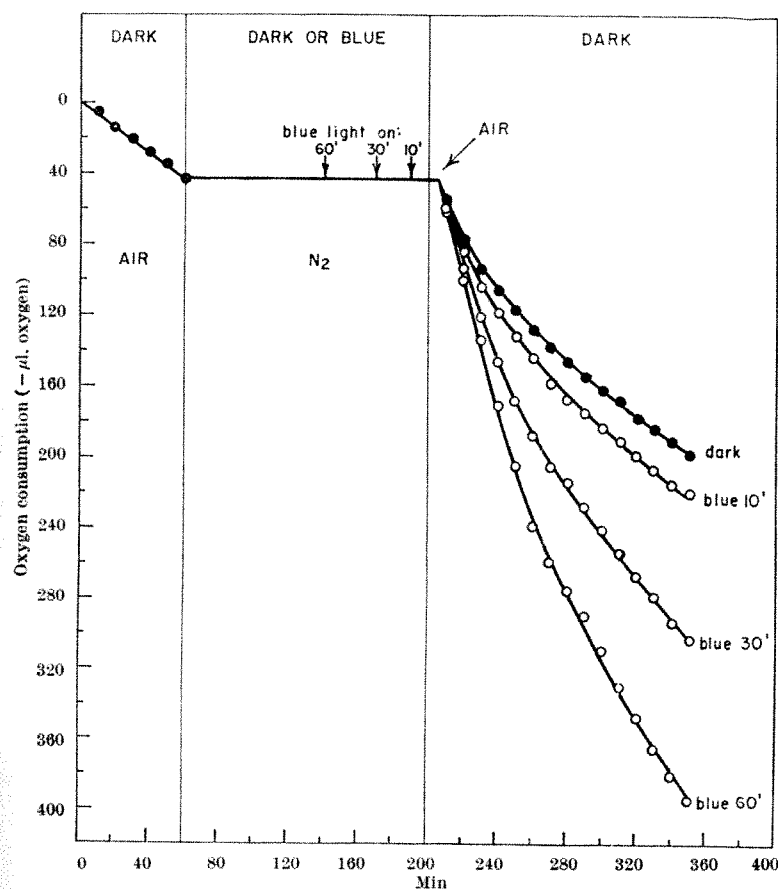


Fig. 2. Enhancement of dark respiration in a yellow *Chlorella* mutant after an anaerobic incubation period during which one sample remained dark, the others were exposed for 10, 30 or 60 min respectively to blue light. 300  $\mu$ l. of packed cells in 3 ml. of 0.1 molar phosphate buffer, pH 6.5, at 30–4° C.

ature. In the experiment shown in Fig. 3 the organisms were first exposed to light in anaerobic conditions at a temperature of 0° C instead of 30° C. When they were afterwards tested aerobically at 30° C in the dark the usual difference in respiratory rates between unilluminated and samples exposed to blue light did not show up. This experiment shows that no primary products are formed which live long enough to influence the metabolism some time later. We conclude that the blue light establishes or regulates conditions which permit a normal endogenous metabolism to proceed faster while the light is on.

Reports of the influence of light on respiration date back to the second half of the nineteenth century. The vast literature which has accumulated since that time is full of contradictory statements, even concerning the same type of experiment with the same living object (for literature review see ref. 12). If we restrict our discussion to earlier results which showed a positive light effect on the respiration of plants, we may eliminate from this group all enhancements obviously caused by recent photosynthetic production of new carbon compounds. Among the remaining ones we may ignore a stimulation of oxygen uptake as the consequence of injury by ultra-violet radiation or effects attributed specifically to far-red light.

Thus we are left with very few experiments which indicate an effect of blue light exclusively on respiration<sup>13-15</sup>.

One of the clearest earlier observations is that of Emerson and Lewis<sup>14</sup>. While trying to determine the right correction for respiration in their measurements of the quantum efficiency of photosynthesis at different wavelengths, they observed that the oxygen consumption in the dark had increased after illumination with blue light at  $\lambda$  480 m $\mu$ , but not after illumination with light at  $\lambda$  435 or 560 m $\mu$ . They also saw that during the light periods photosynthesis increased slowly in blue-violet or yellow light, but decreased in the blue. The authors concluded "that the alteration in rate of pressure change during the early part of the (blue) light period was due to an increase in respiration rather than a decrease in photosynthesis", and wrote further "a strictly photochemical effect of this kind would not be expected to carry over into the ensuing dark period, nor should it be so slow in starting". Very likely 24 yr ago Emerson and Lewis came across the same phenomenon we have been watching directly in the absence of a photosynthetic activity. The reason why their findings were never followed up was probably the difficulty of reproducing them.

This brings us to the fact that our effect of blue light was most pronounced in very peculiar conditions. The farther removed they were from those considered optimal for the life of the cell, without actually being harmful, the easier it was to find the difference between metabolic rates in darkness and in weak blue light. One cannot readily see what advantage should accrue to a resting cell by being stimulated into using up its reserves when it has no further means of subsistence. The true usefulness of the blue light must be to serve as a regulating factor in circumstances where this regulation cannot readily be observed. This regulation of respiration by blue light does not seem to occur universally. So far we did not see an effect in *Rhodotorula*, *Prototheca*, and in some blue green algae, while we found it in several strains of green and red algae.

Besides those cases mentioned here, where virtually identical action spectra bespeak metabolic relationships<sup>6-10</sup>,

Table 1. PROPORTIONALITY BETWEEN TIME OF BLUE LIGHT TREATMENT IN NITROGEN AND SUBSEQUENT EXTRA OXYGEN UPTAKE IN AIR IN THE DARK				
Time (min) of light exposure in nitrogen	10	30	60	
Excess $\mu$ l. of oxygen absorbed	30 min	6	14	33
in darkness after:	60 min	10	35	62

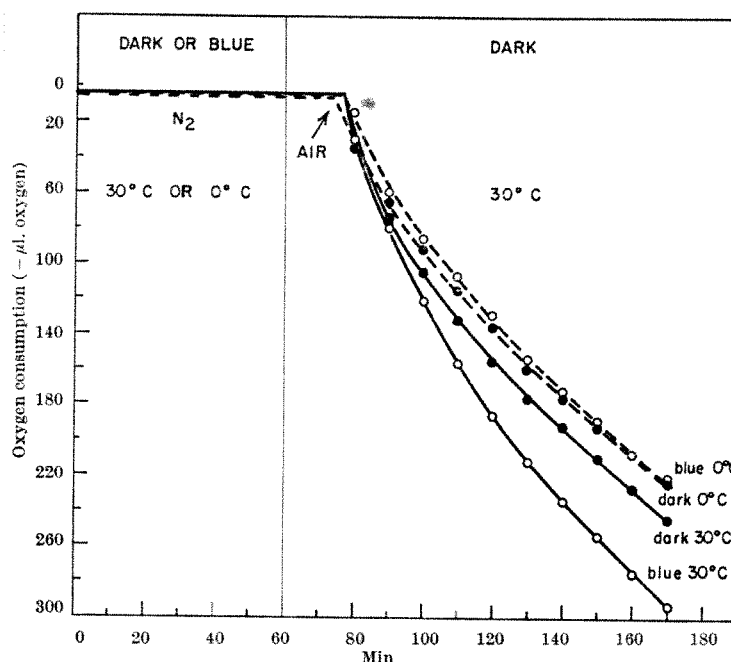


Fig. 3. Comparison between the oxygen uptake at 30° C in darkness by algae which had been in the dark or exposed to blue light under nitrogen at 0° and at 30° C. ~300  $\mu$ l. of packed cells of yellow *Chlorella* mutant 211-114/20 suspended in 0.1 molar phosphate buffer at pH 6.5.

we should mention the following observations where the blue light effect we have described might be involved. Clauss<sup>16</sup> found that *Acetabularia* would eventually stop growing in pure red light, and Terborgh<sup>17</sup> adduces evidence that this had its counterpart in a decline of photosynthetic capacity. The addition of a little blue light overcame the inhibition of photosynthesis in Terborgh's experiments, and that of growth in those of Clauss. A similar claim for the need of traces of blue light in the photosynthesis of *Chlorella* was put forward some years ago by Warburg *et al.*<sup>18</sup> (but see the analysis of this paper by Kok<sup>19</sup>).

Considering the mechanism of the blue light effect, it seems fairly certain that it does not act directly on respiration or on fermentation, but rather on the release of some carbohydrate by surprisingly small amounts of light. Thus we might consider changes in permeability or in phosphorylation or both. The next experimental attack ought to be on the nature and the site of the pigment, the nature of the substance released and the primary photochemistry.

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# Nature of the Scrapie Agent

There is evidence to suggest that the infectious disease scrapie is caused by an agent which does not depend on a nucleic acid for its ability to replicate. In the first of the two following articles it is suggested that scrapie can best be considered to arise from a replicable change in the structural pattern of a commonly occurring unit membrane. In the second article it is suggested that the agent is a protein and three possible mechanisms for its self-replication are proposed.

DESPITE the fact that the agent of scrapie has some unusual properties, it has been usual to consider it a member of the class of "filter-passing viruses"<sup>1</sup>. This class of agent, however, has been defined structurally by Wildy and Watson<sup>2</sup> and according to current conception it should always contain nucleic acid. The recent experiments of Alper *et al.*<sup>3</sup> have clearly shown that the agent of scrapie is much more resistant to the action of ionizing and ultra-violet radiation than any known viral agent, and these authors have suggested that the agent of scrapie probably contains no nucleic acid at all.

Even before these remarkable observations were published, it had become evident that the agent of scrapie was somewhat exceptional, and indeed the viral nature of the agent had been questioned at an earlier stage<sup>4</sup>. A summary is given in Table 1 of the behaviour of the agent towards chemical reagents and under a variety of physical conditions. Quantitative information has been obtained from studies with a form of the agent that has been adapted to mice<sup>5</sup>, because only in the mouse (and possibly in the rat or hamster) is it routinely practicable to carry out titration experiments. A cursory examination of Table 1 shows that, for a self-replicating agent, scrapie has rather bizarre properties. There are substantial difficulties in identifying the agent as a virus, a nucleic acid, a protein or a polysaccharide, and we suggest here that the presence of the agent of scrapie in a cell may represent an alteration in the basic three-dimensional configuration of a commonly occurring unit membrane structure. Such an alteration would not necessarily require the introduction of any new molecular components into the cell affected by scrapie.

In a field such as this, speculation is easier to come by than good experimental data. Nevertheless, it is perhaps useful at this stage to assemble the existing information, including some recent unpublished results, relevant to the chemical nature of the scrapie agent.

(1) *Virus hypothesis.* It is still possible to make out a case that the agent of scrapie is a virus: scrapie disease is caused by a filtrable and transmissible agent<sup>1</sup> and in some respects exhibits the typical development of a neurotropic virus multiplying at an unusually slow rate<sup>17</sup>. The agent of scrapie is unusually resistant to formalin. It should be borne in mind, however, that twin-stranded nucleic acid molecules react very slowly with formaldehyde and that polyoma virus is relatively resistant to the action of this chemical<sup>18</sup>. To explain the behaviour of the agent of scrapie, it is only necessary either that it should readily release its nucleic acid, or that its outer surface should be unusually resistant to the action of formalin, as are some protein and many carbohydrate molecules.

Attempts to isolate or to visualize the scrapie agent have failed to provide support for the virus hypothesis. Similarly, a detailed investigation<sup>19</sup> of nucleic acid metabolism in scrapie brain has not revealed the presence of a nucleic acid specific to scrapie. The strongest evidence against the virus hypothesis comes, however, from the experiments on ultra-violet irradiation. It is difficult to dismiss these, and the simple explanation that the scrapie

agent contains no nucleic acid is probably correct, especially as the results obtained from the use of ionizing radiation suggest that if it is a virus then it is unusually small. Two principal possibilities remain, however, both of which would be consistent with a viral-type agent. (i) There may be an unusually active nucleic acid repair system operating in brain. (ii) The agent may contain a nucleic acid of unusually simple sequence, such that small sections of the polynucleotide chain retain the full informational sequence of the agent.

While neither of these suggestions seems very plausible, they are certainly not impossible and, in view of the difficulties inherent in the assignment of an informational role to any other type of molecule, the virus hypothesis cannot be entirely dismissed.

(2) *Protein hypothesis.* Crick<sup>20</sup> has considered the problem of whether a protein can carry genetic information and considers it intrinsically improbable. Nevertheless, as he points out, it is not impossible to construct hypotheses whereby a protein could provide genetic information within the framework of modern molecular biology. No such situation is known to exist. Proteins vary considerably in the degree of their resistance to physical and chemical agents and many of the results listed in Table 1 would be consistent with a protein character for the agent of scrapie. One would certainly not expect, however, a

Table 1. RESPONSE OF THE AGENT OF SCRAPIE TO VARIOUS PHYSICAL AND CHEMICAL TREATMENTS

Treatment	Effect	References
Ionizing radiation	$D_0 = 4.3$ Mrads	3
Ultra-violet radiation at 240, 254, 265, 280, 290, 315 and 330 m $\mu$	Negligible loss of titre with incident doses up to $5 \times 10^4$ ergs/mm <sup>2</sup>	3
Heat at 100° C for 10-60 min	Substantial, but incomplete loss of titre	6, 7
Heat at 80° C for 60 min	Negligible loss of titre	6, 7
Treatment with fluorocarbon at 0°-4° C	Negligible loss of titre	8
Heat at 80° C for 20 min after fluorocarbon treatment	Substantial loss of titre	9
Treatment with ether at room temperature	Partial loss of titre	10, 11, 12
Acid and alkali	Essentially stable in pH range 2.5-10.5. Destroyed by strong acid and alkali	11, 13, 14
Formalin, 0.5-18 per cent	Highly resistant. Some loss of titre, but no accurate quantitative data available	4, 15
$\beta$ -Propionolactone, 1 per cent	Slight loss of titre	14
0.01 molar periodate at pH 4 at room temperature	Substantial loss of titre	9
6 molar or 8 molar urea	Substantial loss of titre	7, 9
90 per cent phenol	Substantial loss of titre	7, 9
Strong salt solutions (for example, 6 molar lithium chloride, caesium chloride and ammonium sulphate)	Titre readily lost under various conditions	8, 13
Detergents	Relatively stable in the presence of neutral detergents and 0.01 molar sodium deoxycholate	6, 8
Proteolytic enzymes	Partial loss of titre after fluorocarbon treatment, but very little before it	8, 9, 11
DNase, RNase, lipase, phospholipase $\alpha$ , phospholipase $\epsilon$ , neuraminidase, $\beta$ -glucuronidase	No effect on titre of crude preparations	7, 8, 16



protein sensitive to the action of strong solutions of urea and phenol to show such a marked resistance to the action of heat and formalin; nor would most proteins be so sensitive to the action of periodate under the conditions applied to the agent of scrapie<sup>21</sup>. Treatment with proteolytic enzymes would be expected to produce more marked effects than have been observed if the agent were a simple protein.

Only proteins with rather special amino-acid composition would exhibit the observed degree of resistance to ultra-violet light at certain wavelengths and, furthermore, unlike the agent of scrapie<sup>22</sup>, most proteins of a size indicated by the degree of sensitivity to ionizing radiation would be highly antigenic. Basic nucleoproteins, however, are usually deficient in aromatic amino-acids and are also poor antigens and they are in intimate contact with gene DNA. There is evidence that they are concerned in gene expression<sup>23</sup>. The activation by nucleohistone of a gene responsible for its own synthesis has not been reported but, nevertheless, if the nucleic acid hypothesis must be abandoned, nucleohistone is perhaps one of the most likely substances to fulfil a self-replicating role. The agent of allergic encephalomyelitis is certainly a basic polypeptide, and some workers have pointed out resemblances between this agent and that of scrapie<sup>24,25</sup>.

(3) *Carbohydrate hypothesis*. The biosynthesis of polysaccharide, glycoprotein and protein-polysaccharide complexes follows a course quite distinct from that of protein synthesis. These substances are apparently constructed by the transfer of sugars or small oligosaccharide units (frequently by way of a phospholipid intermediate<sup>26</sup>) from an appropriate sugar nucleotide to an acceptor molecule. The acceptor site will usually be a free hydroxyl group of a sugar in a pre-existing poly- or oligo-saccharide chain. With polysaccharides there is provision for the initiation of synthesis and, with protein-polysaccharide complexes and glycoprotein, transferases which will use an amino-acid residue (serine, threonine or asparagine) as acceptor. It is the synthesis of the sugar transferring enzymes that is under direct genetic control, rather than the synthesis of oligosaccharide or polysaccharide<sup>26-28</sup>.

The hypothesis was therefore formulated that a foreign oligosaccharide or polysaccharide or glycoprotein may induce the synthesis of polysaccharase which would also be able to function as a transferase; in this way a polysaccharide or oligosaccharide would appear to be self-replicating. While it is true that the synthetic activity in, for example, induced disaccharases has not yet been demonstrated, it is clear that, if the agent of scrapie is devoid of nucleic acid, some such novel process which results in apparent self-replication must exist. Increases in the activity of three carbohydrases in scrapie brain have in fact been reported<sup>29</sup>. Suggestions that the agent of scrapie may contain carbohydrate<sup>29,30</sup> can in this way be provided with a theoretical basis and other related hypothetical schemes can be constructed.

The experiment in which a scrapie preparation was oxidized by periodate<sup>9</sup> was designed as a partial test of this hypothesis and indeed the agent of scrapie is labile to this mild and rather specific glycol-cleaving agent. Polysaccharides and oligosaccharides are rather stable substances and resist treatment with formaldehyde and ultra-violet light. We must conclude, however, that scrapie cannot be caused by a simple oligosaccharide or polysaccharide, because if it were it would surely survive treatment with phenol and strong urea—reagents widely used in the isolation of polysaccharides.

(4) *Membrane hypothesis*. Another approach to the problem is to fractionate tissue infected with scrapie and to determine whether activity is associated with any particular organelle or class of biochemical substances. This approach has been singularly unfruitful, and all fractions obtained by differential centrifugation techniques are infective<sup>31</sup>, even the final cell sap supernatant,

although this can be largely cleared of activity by prolonged ultra-centrifugation. It is possible to remove the bulk of the brain lipids without much loss of activity<sup>9,13</sup> and it is easy to produce partially cleared suspensions containing small amounts of activity after shaking the ground tissue with a variety of aqueous solutions<sup>8</sup>. This can be done continuously, and even after fifty such "extractions" further small quantities of activity can be removed from the coarser debris by shaking with water or saline<sup>28</sup>. Attempts to dissociate completely the activity from tissue debris using detergents, enzymes, solvents and chromatographic techniques have been unsuccessful<sup>6,8</sup>, however, and one is forced to conclude that there is an extremely tenacious association of the agent with almost every type of cell component. But there is one part of the cell which is often ignored but which does behave in the manner observed, that is cell membrane.

Known membrane constituents, for example the mouse histocompatibility antigens<sup>32</sup>, do behave on fractionation or extraction of tissue components in much the same way as does the agent of scrapie, although some of these antigens differ markedly from scrapie in their overall stability. The membrane appears to break up into fragments of greatly varying sizes which contaminate all the subcellular fractions. The idea that the infective agent may be part of the structure of the cell membrane is at least worth further examination and could well account for conflicting observations of the size of the agent<sup>11,33</sup>. In their simplest form, cell membranes appear in electron micrographs as double-layered structures the components of which are protein and phospholipid<sup>34</sup>. The membrane is not a uniform structure; there are regions responsible for the active transport of ions and other substances into and out of the cell. Specific antigenic substances are also located on the membrane and in the most thoroughly investigated cells (the erythrocyte and the Ehrlich ascites tumour cell) sugars are prominent among these antigenic determinants<sup>35</sup>. Neuraminic acid has also been associated with an active transport site<sup>36</sup>, and an isolated histocompatibility antigen<sup>37</sup> has been found to possess enzyme activity suggestive of the behaviour of ion transport systems. Although the activities can be separated, antigenic and transport sites may be functionally related.

If the determining factor in scrapie pathogenesis is a steric arrangement of the membrane—and we specifically suggest that it is likely to be a re-arrangement in the sugar or oligosaccharide residues attached to it—then the agent of scrapie would behave, when subjected to the chemical and physical treatments listed in Table 1, in the very curious ways observed. Membranes are usually rather resistant to the action of proteolytic enzymes and heat, presumably as a result of the protection afforded by the lipid layers. These phospholipid layers would be disturbed and perhaps partially removed together with the bulk of the neutral lipid by emulsification with fluorocarbon. While the protein would probably react with formaldehyde, provided the membrane fragment was not lysed, the steric arrangement in anything attached to it would be preserved. Because solutions of urea and phenol are potent breakers of hydrogen bonds, however, they will disperse and solubilize the membrane and destroy its specific molecular arrangement.

There are some additional more speculative points concerning the replication and pathogenicity of the agent which are perhaps worth noting. One may take advantage of our ignorance of the way in which membrane is synthesized to suggest that it may in fact be produced by a self-copying process not directly involving nuclear DNA, and that a foreign membrane fragment may become incorporated and later copied. Studies on one highly developed living cell, *Paramecium*, suggest that precisely such a process can occur<sup>38</sup>. Alternatively, the suggestion put forward for the self-replication of polysaccharide is equally applicable to an oligosaccharide on the surface of a membrane. Turning to the pathogenicity of the agent,

it should be pointed out that no antibody against scrapie has yet been detected, and the agent seems to multiply in tissues outside the nervous system<sup>17</sup> without causing obvious damage. There are, however, indications of association between sugars on the cell surface and ion transporting systems and, while cells of all tissues possess such systems, the ion transport system in nerve cells is extremely specialized, sensitive and rapid. Tentatively, then, it is suggested that the alteration in the arrangement of sugars attached to the membrane causes some impairment in the efficiency of ion transport, but is insufficient to be immunologically recognizable. Some alteration in the degree of antigenicity might nevertheless be expected and has in fact been observed<sup>22</sup>. Such a slight change in membrane structure as that postulated may be disastrous only in central nervous tissue.

These conclusions, based on a study of scrapie disease, may have implications for workers in the field of human nervous disease, other slow virus infections and cancer research. The interested reader is referred to the excellent short review article recently published by Gajdusek<sup>23</sup>.

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## Self-replication and Scrapie

It has been suggested that the agent responsible for scrapie, which affects sheep, has a very low molecular weight ( $\sim 2 \times 10^5$ ) and is probably a protein without nucleic acid<sup>1,2</sup>. It can infect goats, rats, mice or hamsters as well as sheep<sup>3,4</sup>. This behaviour would not have been surprising had scrapie contained DNA or RNA because any cell contains the machinery to copy arbitrary sequences of nucleotides. It is not generally thought, however, that they can copy polypeptides and the idea that scrapie is a protein therefore presents difficulties. In this article I discuss the self-replication of proteins and argue that there are at least three distinct kinds of way in which it could occur. This shows that there is no reason to fear that the existence of a protein agent would cause the whole theoretical structure of molecular biology to come tumbling down.

**The First Way.** It is generally supposed that a large number of the genes of a mammalian cell—differentiated or otherwise—are switched off by the action of repressors. It is possible that, through a chance mutation, a gene *G* may arise which is switched off in all cells of a particular animal. If this mutation is not selectively disadvantageous, it will simply persist. Suppose, furthermore, that *G* codes for a protein *S* which acts as an inducer for *G*. Then *S* could act as an infective agent because it is normally never present but, if it is introduced, it will reproduce itself.

We now consider various properties of scrapie on the hypothesis that it is such an *S*. The infectivity of scrapie depends on the rate at which it is induced in a given cell and the rates at which it leaks out through the cell membrane and in through other membranes. In the light of this, we would not be able to predict the long period of induction (months-years)<sup>5</sup> but we would not be surprised by it.

A considerable range of sensitivity and clinical symptoms is observed in sheep and two distinct limiting forms of the disease have been recognized<sup>6</sup>. These breed practically true in the goat<sup>6</sup> but probably do not in the sheep<sup>5</sup> and these facts introduce a curious complexity into the picture. We shall consider first the difference in sensitivity between individual sheep.

Because the protein *S* is never normally formed, further mutations of *G* itself can occur harmlessly. Some sheep might therefore code for an *S* which acts as an inducer and some for an *S* which does not. So far as cells within a given sheep are concerned, a difference in sensitivity might reflect either a difference in the damage done by the presence of *S* (which may be enzymatically active) or in the degree of repression of *G*.

The fact that other animals are also susceptible to scrapie may seem to imply that they contain identically the same gene *G*. This is not the case and all that is necessary is that the protein *S* (from, for example, a sheep) should induce a gene *G*<sup>1</sup> (in, for example, a goat) with similar properties to *G*. A common hereditary origin for *G* and *G*<sup>1</sup> is, however, suggested because the protein *S*<sup>1</sup> from *G*<sup>1</sup> can infect the sheep, that is, act as an inducer for *G*.

The occurrence of two distinct forms of the disease would seem to imply, on the present hypothesis, two genes *G*<sub>1</sub> and *G*<sub>2</sub> with corresponding *S*<sub>1</sub> and *S*<sub>2</sub>. In some cases *S*<sub>1</sub> could induce *G*<sub>2</sub>, and vice versa, and in some cases not.

There is some evidence that scrapie can arise spontaneously<sup>7</sup>. If so, we would interpret it by saying that *G*

is not repressed with absolute efficiency and so, even in a healthy animal, a molecule of S may occasionally be found.

The hypothesis is very similar to one of the suggestions made by Monod and Jacob about the mechanism of differentiation<sup>8</sup>. As in that case, it is certainly not the only possible one. For example, S could be an enzyme which catalyses the formation of a metabolite M, which is the inducer and not present in normal cells. If so, M as well as S would act as an infective agent, which does not seem consistent with the closely exponential radiation inactivation curve found by Alper, Haig and Clarke<sup>2</sup>. Then, again, any set of proteins would do provided the individual proteins are never produced simultaneously in any normal cell and that collectively they induce their own production. For example, it is easy to produce a model based on two proteins P<sub>1</sub> and P<sub>2</sub> such that each of P<sub>1</sub> and P<sub>2</sub> occurs separately in some normal cell and scrapie is P<sub>1</sub> and P<sub>2</sub> occurring together.

**The Second Way.** Self-replication need not involve any very intricate mechanism, provided that suitable components are available<sup>9</sup>. Penrose and Penrose illustrated this with a mechanical example<sup>10</sup> but we shall now see that a logically similar one could be made out of protein.

Given protein sub-units  $\alpha$  which can undergo the following reactions



it is clear that the net result is that the monomer  $\alpha$  gets converted into the dimer  $\alpha_2$ . If we suppose also that the reaction

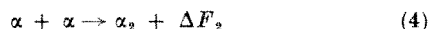


cannot take place directly, it follows that  $\alpha$  can only dimerize to  $\alpha_2$  under the catalytic influence of molecules of  $\alpha_2$  which are already present.

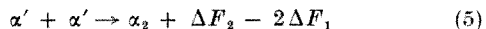
Next we show that this reaction scheme could be realized through the assignment of physically reasonable properties to the protein sub-units. Let the reactive sub-unit  $\alpha$  be a different conformation of the stable structure,  $\alpha'$ , say, of the protein. Then we have the equilibrium



The free energy change  $\Delta F_1$  could easily be so large (for example,  $>100$  kT) that the mean total number of sub-units in all cells in the animal, which are in the form  $\alpha$ , is much smaller than unity. We suppose, then, that all sub-units are in the conformation  $\alpha'$ . The reaction

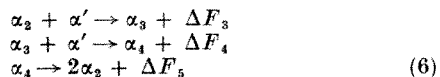


may then be very favourable to the formation of dimers but can never proceed because no molecules in the conformation  $\alpha$  are available. In fact if  $\Delta F_2 > 2\Delta F_1$ , the reaction



is thermodynamically favourable to  $\alpha_2$  but cannot actually proceed. (I mean by this, strictly, that it will be an enormously long time before even a single molecule of  $\alpha_2$  appears.)

The remaining reactions are



with  $\Delta F_3 > 0$ ,  $\Delta F_4 > 0$ ,  $\Delta F_5 > 0$ . The first two reactions of equations (6) involve the combined combination of  $\alpha'$  and change of its conformation. That this, unlike reaction (5), should proceed at measurable velocity is quite plausible because  $\alpha'$  can fold into its new conformation as it joins onto the "template"  $\alpha_2$  or  $\alpha_3$ . Finally, we can combine equations (5) and (6) to show that

$$\Delta F_5 = -2\Delta F_1 + \Delta F_2 - \Delta F_3 - \Delta F_4 > 0 \quad (7)$$

which is true if both  $\Delta F_3$  and  $\Delta F_4$  are small. The first of these conditions requires no justification. The second could be interpreted to mean that the tetramer  $\alpha_4$  is very

strained, the strain energy thus offsetting the energy of linkage between  $\alpha$  and  $\alpha_3$ .

Assuming that  $\alpha'$ , but not  $\alpha$  or  $\alpha_2$ , is a normal cellular constituent and that  $\alpha_2$  can penetrate the cell wall, then  $\alpha_2$  will act as an infective agent made of protein. With such an explanation of scrapie, it would be easy to understand the spontaneous appearance of the disease in previously unaffected animals: it could mean that the equilibrium (3) is not quite so unfavourable to the formation of  $\alpha$  as we have supposed. The existence of the two clinically distinct forms of scrapie could be explained if there were two similar (perhaps isozymic) sub-units  $\alpha'$  and  $\beta'$ , each satisfying similar reaction schemes and in some cases (for example, in sheep) being able to form mixed polymers such as  $\alpha_2\beta$  and in some cases not (for example, in goat).

Just as with the first way, more complicated reaction schemes along the same lines are possible. There is an obvious analogy between the idea presented here and the idea that a gas can only condense on nuclei which are already present: many of the more general schemes could be summed up by saying that the sub-units can only polymerize by utilizing "condensation nuclei" of polymer which are already there.

**The Third Way.** In the first two ways the infective agent used the products of a single cell to replicate itself. In the third way it uses apparatus belonging to the whole animal. To the animal, a foreign protein often acts as an antigen (A) which stimulates the production of an antibody (A'). A' is a protein which is normally different from A. It could happen that there exists an A for which A' = A. This gives our third way. The mechanism of production of antibodies is not understood in detail but there seems no reason to expect it to be inexplicable in terms of the ideas of present day molecular biology<sup>11</sup>. Thus the same would be true of an infective agent using the third way. It should be pointed out that this is not an "auto-immune" mechanism. Immunologists mean by that term that the animal makes antibodies against its own tissue constituents, not that the antibodies are chemically identical with the antigens. It seems probable, however, that scrapie does not use the third way because there is good experimental evidence that the disease is not antigenic<sup>12</sup>.

I have shown the existence of at least three classes of replication mechanisms and that, therefore, the occurrence of a protein agent would not necessarily be embarrassing although it would be most interesting. There is evidence that scrapie does not belong to the third of these classes. If it belongs to one of the first two classes, then it is a protein or a set of proteins which the animal is genetically equipped to make, but which it either does not normally make or does not make in that form. It may be passed between animals but be actually a different protein in different species. Finally, in either case, there is the possibility of spontaneous appearance of the disease in previously healthy animals.

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## LETTERS TO THE EDITOR

## ASTRONOMY

## Absorption Line Spectrum of the Quasi-stellar Object 3C 191

BAHCALL<sup>1</sup> has pointed out that in the absorption line spectrum for ions in the gaseous envelope of 3C 191, there is a correlation between the ionization potential and  $\Delta z$ , the difference between  $z$  (the red-shift of the absorption line spectrum for any ion) and  $\bar{z}_{em}$  (the mean red-shift of the emission line spectrum). Fig. 1 indicates, as a set of vertical lines, the spread in  $\Delta z$  values derived from curves fitted by Burbidge and Lynds<sup>2</sup> to four sets of measurements<sup>3,4</sup>. In the figure, the ionization potential plotted is that of the atom or ion having one more electron than the absorbing ion; while  $\Delta z$  is defined as  $\bar{z}_{em} - z$ .

In an attempt to explain this correlation we take the following simplified model of a quasi-stellar object: the line spectra are assumed to be produced by electron excitation of gaseous material which has a finite velocity of radial flow in the gravitational field of a large central mass. For flow velocities  $\sim 10^3$  km per sec and electron energies  $\leq 100$  eV, the motion of an atom suffers negligible perturbation in the excitation process, so that its initial velocity defines a Doppler component of red-shift for the line spectra produced. Because the velocity is a function of radius but independent of atomic (or ionic) mass, this component is the same throughout the spectra if the excitation occurs in a region of well defined radius, but different for different ions if their excitation conditions have a radial dependence.

We propose that the electrons are emitted from a small source<sup>5-7</sup> and have a narrow energy spectrum. A correlation between  $\Delta z$  and excitation energy then results if both the electrons and the atoms of the envelope are moving in the gravitational field of the massive object with which the central source of electrons and optical continuum radiation may be associated. Fig. 2 shows the essential features of the model. A central object  $M$  is surrounded by an envelope, a thin hollow shell of radius  $R$  across which the velocity of gaseous flow ( $\beta c$ ) and the kinetic energy of the electrons ( $E$ ,  $=\epsilon mc^2$ ) vary continuously. For observable line spectra to be produced, the shell must be optically

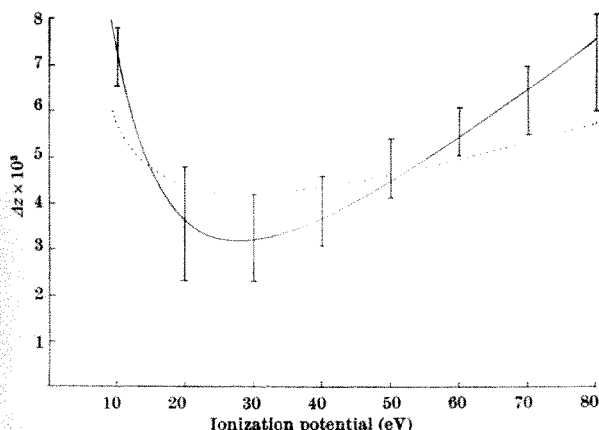


Fig. 1.

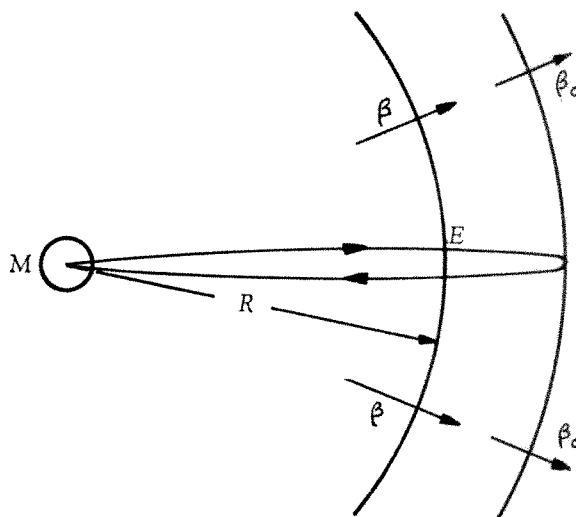


Fig. 2.

thin both to photons, in the visible region, and to electrons. Ions resulting from electron excitation may then produce absorption lines from the optical continuum, while emission lines arise from the recombination of these ions in the same envelope.

If it is assumed that the relevant physical parameters vary continuously throughout the envelope, without regard to the exact location of emission and absorption line regions, then a positive sign for  $\Delta z$  may be obtained for either of two possible systems: in one, the envelope is expanding, with decelerating motion, and has the absorption line region inside the emission line region; in the other, the envelope is contracting, with accelerating motion, and has the absorption line region outside the emission line region. Three aspects of the system have to be examined in order that a definite relationship between  $\Delta z$  and excitation energy may be established.

(a) In the particular case illustrated in Fig. 2, the electrons are emitted with less than their escape velocity and attain a maximum excursion radius, at which the gaseous flow velocity is defined as  $\beta_0 c$ , while the material in the shell, which in this case is taken to be expanding, has a velocity exceeding its escape value.

The total energy for an electron in the gravitational field may be written as

$$mc^2(1 + \epsilon) - \frac{mMG}{R} \quad (1)$$

Then the non-relativistic, ( $\beta \ll 1$ ), equation of motion for an atom of the envelope moving with velocity  $\beta c$  at radius  $R$ , when combined with an equation representing the conservation of the quantity (1) with respect to change in  $R$ , gives

$$\epsilon = \frac{1}{2} (\beta^2 - \beta_0^2) \quad (2)$$

(b) We take the excitation energy to be equal to the sum of the kinetic energies of electron and atom in their centre of mass system. Assuming that  $\epsilon \ll 1$ , we obtain for the excitation energy the approximation,

$$mc^2 \left[ \frac{1}{2} \beta^2 + \left(1 + \frac{1}{2} \beta^2\right)(\epsilon \pm \beta \sqrt{2\epsilon}) \right] \quad (3)$$

The double sign in the last term expresses the fact that two-way electron flow in the expanding envelope gives two different excitation energies for each value of  $\beta$ . From (3), and using (2), we may plot curves showing excitation energy as a function of  $\beta$  for any value of  $\beta_0$ . Each curve has a minimum at  $\beta = \beta_0$ .

(c) As we are concerned with observed differences in red-shift, each value of  $\Delta z$  has to be related to a velocity difference. We assume that the emission lines originate in



a region with mean velocity  $\beta_e c$ , and we define  $\Delta\beta$  as  $\beta - \beta_e$ . The corresponding red-shift difference depends not only on  $\Delta\beta$  but also on any recession velocity  $\beta_e c$  which the whole quasi-stellar object may have relative to the observer.

The usual formula for  $z$  as a function of  $\beta$  gives, on relativistic addition of velocities,

$$\Delta z \approx \left( \frac{1 + \beta_r}{1 - \beta_r} \right)^{\frac{1}{2}} \Delta\beta \quad (4)$$

From these considerations we may therefore derive  $\Delta z$  as a function of excitation energy, which is equivalent to the ionization potential, in terms of the parameters  $\beta_r$ ,  $\beta_0$  and  $\beta_e$ .

We make a tentative fitting to the experimental data of Fig. 1 for different values of  $\beta_0$  and  $\beta_e$ , and for two particular values of  $\beta_r$ .

For  $\beta_r = 0$ , representing a system with its centre stationary with respect to the observer, the best fitting curve shown as a dotted line in the figure is calculated for  $\beta_0 = 10.5 \times 10^{-3}$  and  $\beta_e = 6.3 \times 10^{-3}$ . It does not, however, give a  $\Delta z$  variation of the required magnitude for the range of ionization potential plotted.

For  $\beta_r = 0.79$ , corresponding to a recession velocity given by a Doppler interpretation of the mean emission line red-shift<sup>8</sup>  $z_{em} = 1.946$ , the best fitting curve—shown as a solid line in the figure—is calculated for  $\beta_0 = 10.5 \times 10^{-3}$  and  $\beta_e = 9.4 \times 10^{-3}$ ; it clearly shows satisfactory agreement with the experimental data. The parameters  $\beta_0$  and  $\beta_e$  have consistent signs only for the case of an expanding envelope, while the small difference between their values may be attributed to a finite diffusion distance between the points of ion production and recombination.

In our model there are two difficulties which could affect its validity. The first is that the mean free path for interaction of the incident electrons must be at least comparable with the thickness of the shell in which the line spectra originate. Currently accepted figures for interaction cross-sections and envelope densities give a shell thickness  $\leq 10^{10}$  cm. A supply of gaseous material with the required velocity of expansion at a given radius could not be maintained for any length of time by a spherical shell of this thickness, although it could perhaps be maintained by a filament of spiral form, such as a stream of material emitted from a central source which has a small velocity of rotation.

The second difficulty is that there must be some process for the production of electrons with a sufficiently narrow

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### Probable Values of the Time of Rise for the Forthcoming Sunspot Cycles

THE values of the time of rise corresponding to the successive sunspot cycles do not seem to show periodicity<sup>1</sup>. If we consider, however, the values of the time of rise for the cycles of the same polarity, that is, the cycles corresponding to the odd and even values of the current number  $N$  separately, we find a more or less clear tendency for the minima of the time of rise to recur every eight cycles for cycles with  $N = 2K + 1$  and every ten cycles for cycles with  $N = 2K$ ,  $K = 0, \pm 1, \pm 2, \dots$

I shall show here that each of these two groups of cycles,  $N = 2K + 1$  and  $N = 2K$ , can be subdivided into two subgroups each of which presents an individual periodic variation. The periods of these variations are very probably multiples of eight sunspot cycles for cycles with  $N = 2K + 1$  and ten sunspot cycles for cycles with  $N = 2K$ . In fact, if we consider the values of the time of rise  $T_R^*$  corresponding to the sunspot cycles  $N = -12$  (1610.8–1619.0) to  $N = 3$  (1775.5–1784.7), determined on the basis of the smoothed mean monthly relative sunspot numbers<sup>2</sup> as well as the values of the time of rise  $T_R$  corresponding to the latter sunspot cycles,  $N = 4$  to  $N = 19$ , determined on the basis of the observed mean monthly relative sunspot numbers<sup>1</sup>, then the existing observational data show that these values can be satisfactorily represented with the help of the following relations

#### (a) ODD CYCLES

$$T = \left[ a_1 - b(3 - \psi_1) \sin(N-1) \frac{2\pi}{64} \right] \cos^2(N-1) \frac{2\pi}{8} + \left[ a_2 - 2b \sin(N-1) \frac{2\pi}{24} + 4b \cos^2(N-1) \frac{2\pi}{24} \sin^2 \frac{N+1}{4} \frac{\pi}{2} + \psi_2 \right] \sin^2(N-1) \frac{2\pi}{8} \quad (1)$$

#### (b) EVEN CYCLES

$$T = \left[ a_3 - b \sin(N-5) \frac{2\pi}{40} + \frac{3}{2} b \sin(N-5) \frac{2\pi}{5} - \psi_3 \right] J + a_4 \left[ 1 - \frac{1}{4} \cos(N-1) \frac{2\pi}{20} \right] (1-J) \quad (2)$$

energy spectrum. The decay of  $\pi$  mesons<sup>9</sup>, if these are of low energy  $\ll 5$  MeV, may provide a suitable source.

We conclude that a correlation of the observed form existing between  $\Delta z$  and ionization potential can provide, in terms of the model described here, an estimate of recession velocity. In the particular case of 3C 191, the present experimental data are consistent with a Doppler interpretation of the mean emission line red-shift.

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where  $J = 1$  for the sunspot cycles with  $N = 10K$ ,  $10K-2$  and  $10K-4$ , and  $J = 0$  for the sunspot cycles with  $N = 10K-6$ ,  $10K-8$ ,  $K = 0, \pm 1, \pm 2, \dots$ .  $a_1 = 6.4$ ,  $a_2 = 5.0$ ,  $a_3 = 4.65$ ,  $a_4 = 4.4$  and  $b = 0.85$ .

In relations (1) and (2) the quantities  $\psi_1$ ,  $\psi_2$ , and  $\psi_3$  represent additional periodic terms acting only during certain sunspot cycles, that is

$$\psi_1 = 2 \left[ 1 - \frac{3}{4} \sin(N-7) \frac{2\pi}{40} \right] \sin^2 \frac{N-1}{4} \frac{\pi}{2}$$

$$\psi_2 = \frac{2}{3} b \cos N \frac{2\pi}{24} \cos K\pi, N = 4K-1, K = 0, 1(6, 7) 12, 13, \dots$$

$$\psi_3 = \frac{3}{2}b \cos N \frac{2\pi}{40} \cos K\pi, N = 2K-2, K = 1, 0 \quad (19, 20)$$

29, 30, ...

The continuous lines (a) and (b) in the upper part of Figs. 1 and 2 represent, respectively, the first and the second term of relations (1) and (2). In the lower part of these figures the small circles represent the observed values of the time of rise while the dots connected with the dashed lines illustrate the values of  $T$  computed with the help of relations (1) and (2).

Table 1 gives the values of  $T$  as well as the observed values of the time of rise  $T_R^*$  (for the sunspot cycles  $N = -12$  to  $N = 3$ ) and the time of rise  $T_R$  (for the sunspot cycles  $N = 4$  to  $N = 19$ ). It can be seen that relations (1) and (2) represent the values of  $T_R^*$  and  $T_R$  with an accuracy

equal to  $(1 - \frac{\sigma}{T_R})$  100 per cent = 97 per cent. Despite this

high degree of accuracy, however, the values of the time of rise for the forthcoming sunspot cycles found by extrapolating equations (1) and (2) should be considered with due caution, because in these relations some periodic terms with periods as long as sixty-four cycles are present, while the observational data on which relations (1) and (2) are based do not refer to more than thirty-two cycles.

I showed earlier<sup>3</sup> that the mean annual values  $[R_m]$  of the relative sunspot numbers during the year of maximum solar activity, as well as the maxima  $R_{max}$  and the minima  $R_{min}$  of the mean monthly relative sunspot numbers during the same year, can be satisfactorily represented as functions of the time of rise  $T_R$  with the help of the relations

$$[R_m] = C + 2 T_0 (T_0 - T_R)^2 + T_0 Y \quad (3)$$

$$R_{max} = a^2 + 2 T_0' (T_0' - T_R')^2 + a Y + 4 T_0' \sin \left( N - \frac{a}{2} \right) \frac{2\pi}{8} \quad (4)$$

$$R_{min} = T_0'^2 + (T_0' + 1) (T_0' - T_R')^2 + \frac{1}{2} T_0' Y \quad (5)$$

where  $T_0 = 5.76$ ,  $T_0' = 6.3$ ,  $a = 9.0$ ,  $C = 2T_0^2$  for the cycles  $N = 7-19$  and  $C = 2T_0(T_0 - 1)$  for the cycles  $N = -3$  to 6, but with cycles  $N = 0$  and  $N = 1$ ,  $C = 2T_0(T_0 + 1)$ . The term  $Y$  is an exponential term of the form

$$Y = \frac{n}{10-n} \exp(n-9)$$

where  $n = 0, 1, 2, \dots, 9$  for each decade of cycles, the value  $n = 0$  corresponding to the sunspot cycles  $N = -10, 0, 10, 20$ , etc.

It is therefore of interest to investigate to what extent the accuracy given by relations (3), (4) and (5) will be influenced if instead of  $T_R$  we use in these relations the new parameter  $T$  defined with the help of equations (1) and (2). The results are shown in Fig. 3, where the small circles connected with continuous lines show the values of  $R_{max}$ ,  $[R_m]$  and  $R_{min}$  respectively given by the observations, while the dots connected with dashed lines give the values of the same quantities computed with the help of relations (3), (4) and (5). The probable values of  $[R_m]$  for the forthcoming cycles  $N = 20, 21, \dots$  are also given in Fig. 3 and it can be seen that the representation of the already available values of these three quantities as functions of  $T$  is very satisfactory.

For the current cycle  $N = 20$  the value of  $T$  computed with the help of relation (2) is equal to  $4.1 \pm 0.1$ . If therefore we take into account that the minimum of cycle  $N = 20$  occurred in 1964.7, we reach the conclusion that the maximum of cycle

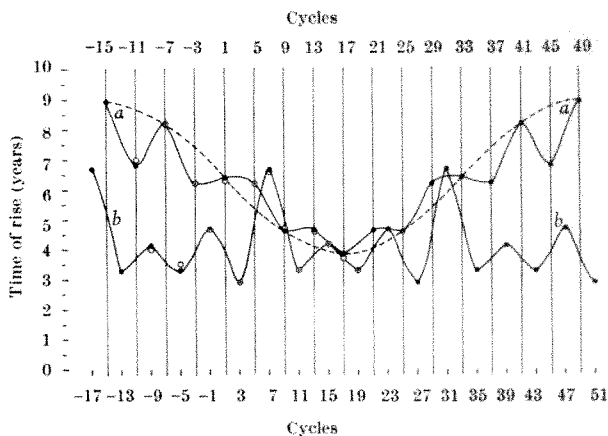


Fig. 1.

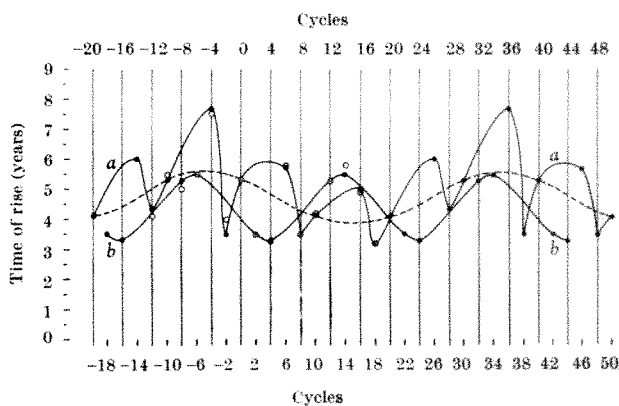


Fig. 2.

1830	Apr	July	1836	Apr	July	1842	Apr	July	1848	Apr	July	1854	Apr	July	1860	Apr	July	1866	Apr	July	1872	Apr	July	1878	Apr	July	1884	Apr	July	1890	Apr	July	1896	Apr	July	1902	Apr	July	1908	Apr	July	1914	Apr	July	1920	Apr	July	1926	Apr	July	1932	Apr	July	1938	Apr	July	1944	Apr	July	1950	Apr	July	1956	Apr	July	1962	Apr	July	1968	Apr	July	1974	Apr	July	1980	Apr	July	1986	Apr	July	1992	Apr	July	1998	Apr	July	2004	Apr	July	2010	Apr	July	2016	Apr	July	2022	Apr	July	2028	Apr	July	2034	Apr	July	2040	Apr	July	2046	Apr	July	2052	Apr	July	2058	Apr	July	2064	Apr	July	2070	Apr	July	2076	Apr	July	2082	Apr	July	2088	Apr	July	2094	Apr	July	2100	Apr	July	2106	Apr	July	2112	Apr	July	2118	Apr	July	2124	Apr	July	2130	Apr	July	2136	Apr	July	2142	Apr	July	2148	Apr	July	2154	Apr	July	2160	Apr	July	2166	Apr	July	2172	Apr	July	2178	Apr	July	2184	Apr	July	2190	Apr	July	2196	Apr	July	2202	Apr	July	2208	Apr	July	2214	Apr	July	2220	Apr	July	2226	Apr	July	2232	Apr	July	2238	Apr	July	2244	Apr	July	2250	Apr	July	2256	Apr	July	2262	Apr	July	2268	Apr	July	2274	Apr	July	2280	Apr	July	2286	Apr	July	2292	Apr	July	2298	Apr	July	2304	Apr	July	2310	Apr	July	2316	Apr	July	2322	Apr	July	2328	Apr	July	2334	Apr	July	2340	Apr	July	2346	Apr	July	2352	Apr	July	2358	Apr	July	2364	Apr	July	2370	Apr	July	2376	Apr	July	2382	Apr	July	2388	Apr	July	2394	Apr	July	2400	Apr	July	2406	Apr	July	2412	Apr	July	2418	Apr	July	2424	Apr	July	2430	Apr	July	2436	Apr	July	2442	Apr	July	2448	Apr	July	2454	Apr	July	2460	Apr	July	2466	Apr	July	2472	Apr	July	2478	Apr	July	2484	Apr	July	2490	Apr	July	2496	Apr	July	2502	Apr	July	2508	Apr	July	2514	Apr	July	2520	Apr	July	2526	Apr	July	2532	Apr	July	2538	Apr	July	2544	Apr	July	2550	Apr	July	2556	Apr	July	2562	Apr	July	2568	Apr	July	2574	Apr	July	2580	Apr	July	2586	Apr	July	2592	Apr	July	2598	Apr	July	2604	Apr	July	2610	Apr	July	2616	Apr	July	2622	Apr	July	2628	Apr	July	2634	Apr	July	2640	Apr	July	2646	Apr	July	2652	Apr	July	2658	Apr	July	2664	Apr	July	2670	Apr	July	2676	Apr	July	2682	Apr	July	2688	Apr	July	2694	Apr	July	2700	Apr	July	2706	Apr	July	2712	Apr	July	2718	Apr	July	2724	Apr	July	2730	Apr	July	2736	Apr	July	2742	Apr	July	2748	Apr	July	2754	Apr	July	2760	Apr	July	2766	Apr	July	2772	Apr	July	2778	Apr	July	2784	Apr	July	2790	Apr	July	2796	Apr	July	2802	Apr	July	2808	Apr	July	2814	Apr	July	2820	Apr	July	2826	Apr	July	2832	Apr	July	2838	Apr	July	2844	Apr	July	2850	Apr	July	2856	Apr	July	2862	Apr	July	2868	Apr	July	2874	Apr	July	2880	Apr	July	2886	Apr	July	2892	Apr	July	2898	Apr	July	2904	Apr	July	2910	Apr	July	2916	Apr	July	2922	Apr	July	2928	Apr	July	2934	Apr	July	2940	Apr	July	2946	Apr	July	2952	Apr	July	2958	Apr	July	2964	Apr	July	2970	Apr	July	2976	Apr	July	2982	Apr	July	2988	Apr	July	2994	Apr	July	3000	Apr	July	3006	Apr	July	3012	Apr	July	3018	Apr	July	3024	Apr	July	3030	Apr	July	3036	Apr	July	3042	Apr	July	3048	Apr	July	3054	Apr	July	3060	Apr	July	3066	Apr	July	3072	Apr	July	3078	Apr	July	3084	Apr	July	3090	Apr	July	3096	Apr	July	3102	Apr	July	3108	Apr	July	3114	Apr	July	3120	Apr	July	3126	Apr	July	3132	Apr	July	3138	Apr	July	3144	Apr	July	3150	Apr	July	3156	Apr	July	3162	Apr	July	3168	Apr	July	3174	Apr	July	3180	Apr	July	3186	Apr	July	3192	Apr	July	3198	Apr	July	3204	Apr	July	3210	Apr	July	3216	Apr	July	3222	Apr	July	3228	Apr	July	3234	Apr	July	3240	Apr	July	3246	Apr	July	3252	Apr	July	3258	Apr	July	3264	Apr	July	3270	Apr	July	3276	Apr	July	3282	Apr	July	3288	Apr	July	3294	Apr	July	3300	Apr	July	3306	Apr	July	3312	Apr	July	3318	Apr	July	3324	Apr	July	3330	Apr	July	3336	Apr	July	3342	Apr	July	3348	Apr	July	3354	Apr	July	3360	Apr	July	3366	Apr	July	3372	Apr	July	3378	Apr	July	3384	Apr	July	3390	Apr	July	3396	Apr	July	3402	Apr	July	3408	Apr	July	3414	Apr	July	3420	Apr	July	3426	Apr	July	3432	Apr	July	3438	Apr	July	3444	Apr	July	3450	Apr	July	3456	Apr	July	3462	Apr	July	3468	Apr	July	3474	Apr	July	3480	Apr	July	3486	Apr	July	3492	Apr	July	3498	Apr	July	3504	Apr	July	3510	Apr	July	3516	Apr	July	3522	Apr	July	3528	Apr	July	3534	Apr	July	3540	Apr	July	3546	Apr	July	3552	Apr	July	3558	Apr	July	3564	Apr	July	3570	Apr	July	3576	Apr	July	3582	Apr	July	3588	Apr	July	3594	Apr	July	3600	Apr	July	3606	Apr	July	3612	Apr	July	3618	Apr	July	3624	Apr	July	3630	Apr	July	3636	Apr	July	3642	Apr	July	3648	Apr	July	3654	Apr	July	3660	Apr	July	3666	Apr	July	3672	Apr	July	3678	Apr	July	3684	Apr	July	3690	Apr	July	3696	Apr	July	3702	Apr	July	3708	Apr	July	3714	Apr	July	3720	Apr	July	3726	Apr	July	3732	Apr	July	3738	Apr	July	3744	Apr	July	3750	Apr	July	3756	Apr	July	3762	Apr	July	3768	Apr	July	3774	Apr	July	3780	Apr	July	3786	Apr	July	3792	Apr	July	3798	Apr	July	3804	Apr	July	3810	Apr	July	3816	Apr	July	3822	Apr	July	3828	Apr	July	3834	Apr	July	3840	Apr	July	3846	Apr	July	3852	Apr	July	3858	Apr	July	3864	Apr	July	3870	Apr	July	3876	Apr	July	3882	Apr	July	3888	Apr	July	3894	Apr	July	3900	Apr	July	3906	Apr	July	3912	Apr	July	3918	Apr	July	3924	Apr	July	3930	Apr	July	3936	Apr	July	3942	Apr	July	3948	Apr	July	3954	Apr	July	3960	Apr	July	3966	Apr	July	39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Table 1

Cycles <i>N</i>	<i>T</i> (in years)	<i>T<sub>R</sub></i> <sup>*</sup>	Cycles <i>N</i>	<i>T</i> (in years)	<i>T<sub>R</sub></i>	Cycles <i>N</i>	<i>T</i> (in years)
-12	4.3	4.7	4	3.3	3.3	20	4.1
-11	6.8	7.0	5	6.2	6.2	21	4.6
-10	5.3	5.5	6	5.7	5.8	22	3.5
-9	4.2	4.0	7	6.7	6.6	23	4.7
-8	5.3	5.0	8	3.5	3.5	24	3.3
-7	8.2	8.2	9	4.6	4.7	25	4.6
-6	5.5	5.5	10	4.1	4.2	26	6.0
-5	3.3	3.5	11	3.3	3.3	27	2.9
-4	7.7	7.5	12	5.3	5.3	28	4.3
-3	6.2	6.2	13	4.6	4.6	29	6.2
-2	3.5	4.0	14	5.5	5.8	30	5.3
-1	4.7	4.7	15	4.2	4.2	31	6.7
0	5.3	5.3	16	5.0	4.9	32	5.3
1	6.4	6.3	17	3.8	3.7	33	6.4
2	3.5	3.5	18	3.2	3.2	34	5.5
3	2.9	2.9	19	3.3	3.3	35	3.3

$N=20$  will occur probably in  $1968.8 \pm 0.1$ . According to relations (3), (4) and (5) the values of  $[R_m]$ ,  $R_{\max}$  and  $R_{\min}$  for this cycle will be respectively

$$[R_m] = 98.1 \pm 4.0 \quad R_{\max} = 132.3 \pm 8.0 \quad R_{\min} = 75.0 \pm 5.0.$$

It should be noted that King-Hele<sup>4</sup> has recently found that the time of rise for the sunspot cycles  $N=20$  and 21 will be equal to  $T_R=3.4$  and 3.8 years, respectively. On the other hand, for  $T_R=4.1$  years, King-Hele's relation  $(R_m - 16)T_R^2 - 10^{-5} (R_m - 100)^4 \approx 1401$  gives two values for  $R_m$  of which the smallest (99.3) differs little from the value of  $[R_m]$  reported here (98.1) for the sunspot cycle No. 20.

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<sup>2</sup> Waldmeier, M., *The Sunspot Activity in the Years 1610-1960* (Zurich, 1961).

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## PLANETARY SCIENCE

### Rotational Speed of the Upper Atmosphere: an Increase with Height above 200 km

THE average speed of rotation of the upper atmosphere can be determined by analysing small changes in the inclinations of satellite orbits to the equator. Previous studies<sup>1,2</sup> have indicated that, on average, the upper atmosphere at heights of 200-300 km is rotating faster than the Earth: the mean value obtained for the ratio  $\Lambda$  of the

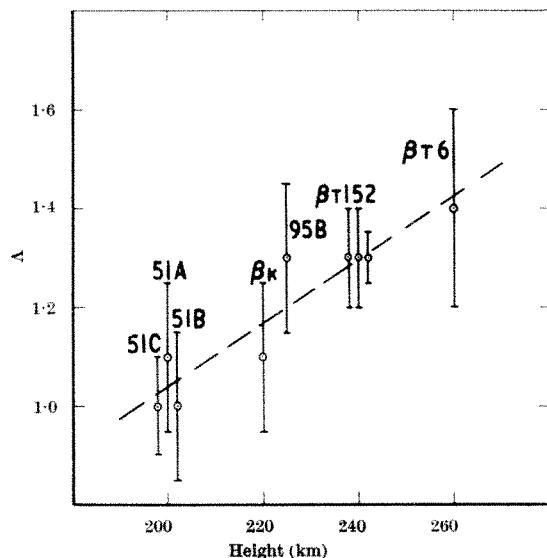


Fig. 1. Values of  $\Lambda$  obtained.

rotational speed of the atmosphere to that of the Earth was  $\Lambda = 1.27$ , which corresponds to a mean west-to-east wind speed of 100 m/s in middle latitudes. These previous studies, however, were not accurate enough to allow any definite conclusions to be drawn about the variations of  $\Lambda$  with height, time or latitude.

Recently we completed an analysis of the changes in the orbital inclinations of the following nine satellites: 1962 $\beta$ x, 1962 $\beta$ 1, 2, 5 and 6, 1965-95B, and 1966-51A, B and C. Their inclinations range between 49° and 90°, and the heights to which the values of  $\Lambda$  apply, taken as half a scale height above the mean perigee height, range between 200 and 260 km.

The values of  $\Lambda$  obtained, which are more accurate than before, are plotted against height in Fig. 1, with their estimated standard deviations and an unweighted least-squares line through the points. Fig. 1 justifies the conclusion that the average angular velocity of the upper atmosphere increases from about 1.1 at a height of 210 km to about 1.4 at 260 km. The corresponding mean west-to-east wind speeds in middle latitudes would be 30 m/s at 210 km, increasing to 130 m/s at 260 km. The values obtained previously<sup>1,2</sup>, although less accurate, are also consistent with this conclusion.

For two of the satellites in Fig. 1, 1966-51B and C, for which the average value of  $\Lambda$  over the whole lifetime is 1.0, it has been possible to obtain values of  $\Lambda$  over a much shorter time interval centred in August 1966 when perigee was near the sunset line, with the local time between 18 and 21 h (ref. 3): over this time interval both satellites yield values of  $\Lambda$  near 1.5, which suggests that the west-to-east motion is greatest in the early evening.

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<sup>1</sup> King-Hele, D. G., *Plan. Space Sci.*, **12**, 835 (1964).

<sup>2</sup> King-Hele, D. G., and Scott, D. W., *Plan. Space Sci.*, **14**, 1339 (1966).

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### Plasma Resonances of the Magnetosphere

THERE have been many investigations of resonance phenomena in geophysics, for example, the Schumann resonances of the Earth-ionosphere cavity which is excited by lightning flashes. Theoretical attempts have been made to determine possible hydromagnetic resonances of the magnetosphere which should be excited by the solar wind. Under conditions of axial symmetry it is possible to obtain uncoupled solutions for poloidal and toroidal modes. Carovillano and Radoski<sup>1</sup> have recently considered a perfectly conducting plasma, magnetized by a dipole field, in which the plasma density function was expressed in a generalized form, thus allowing their model to incorporate some of the observed features of the magnetosphere. Their calculations for the toroidal modes, and also for the poloidal modes, indicate that the eigenperiods for such a model magnetosphere should lie in the geomagnetic micropulsation range, in agreement with the conclusions reached by a number of other workers.

In practice there are considerable departures from axial symmetry in the magnetosphere and so there arises the question of whether it is possible to observe anything which approximates to axially symmetric resonant modes. The vast amount of rather confusing and often contradictory information in the literature on the characteristics of micropulsations recorded at different localities has so far left this question unanswered.

Using techniques similar to that described by Mainstone and McNicol<sup>2,3</sup>, daily micropulsation spectra in the form of sonagrams covering the Pc3-Pc4 range have been produced



at a number of stations, particularly at Esk near Brisbane, Toolangi near Melbourne, in the eastern part of Australia, and in the United States at Cougar Mountain near Seattle. A careful examination of these sonagrams provides strong evidence in favour of the existence of hydromagnetic resonances associated with (to all intents and purposes) axially symmetric modes; as well as these there are other resonances which are most probably not axially symmetric.

Figs. 1 and 2 are examples of sonagrams of  $Pc3$ – $Pc4$  activity at Esk and Seattle on days of weak geomagnetic agitation. Despite the differences of latitude, longitude, hemisphere and time of year, a recognizable modal pattern can be traced in the frequency range 0–50 mc/s, consisting of two predominant spectral lines and evidence of a third. The two lower frequency modes are usually detectable on sonagrams throughout the entire day while the upper one is more readily seen by day than by night—this behaviour is illustrated in Fig. 3.

The excitation of the various modes is rather variable from day to day, but the frequencies remain relatively constant. When gross changes in frequency do occur they

are usually smooth and may take place over 3 or 4 h as in Fig. 2. As far as can be ascertained, on any one day the frequencies of corresponding modes observed at Esk and Toolangi are identical; however, their relative amplitudes at the two places are not constant. The same resonances have been seen on sonagrams from Townsville in North Queensland, at a relatively low geomagnetic latitude of  $28^\circ$  S. For the purposes of discussion these apparently invariant features of magnetospheric activity will be designated  $X$  modes.

The sonograph spectrum analyser allows spectra to be produced either with coarse frequency resolution ("wide-band" as in the illustrations in this paper), or alternatively with better frequency resolution, and consequent degradation of timing, using the "narrow-band" filter. Sonagrams made by the latter method indicate the existence sometimes of sharp single lines corresponding to the  $X$  resonances, but usually some splitting occurs and this gives rise to a fine structure of closely spaced lines.

The advantages of the sonagram method of dynamic spectrum presentation lie in the optical integration which

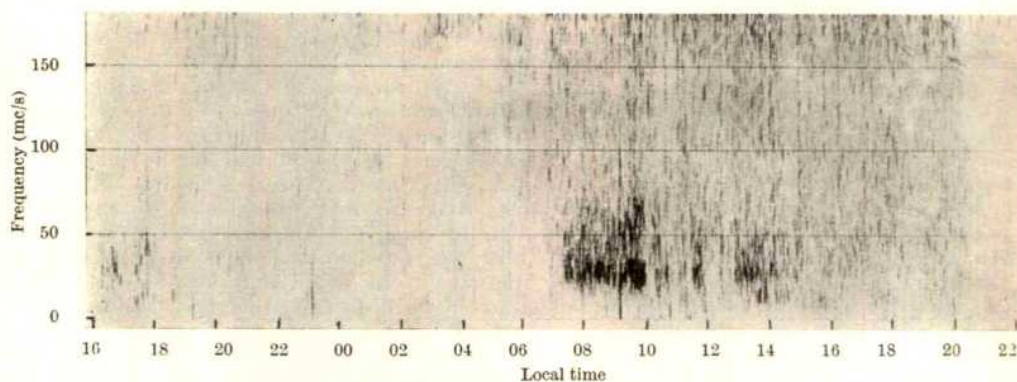


Fig. 1. Esk. June 11/12, 1963.  $E_y$  component. Very weak excitation of  $X$  modes during the day.

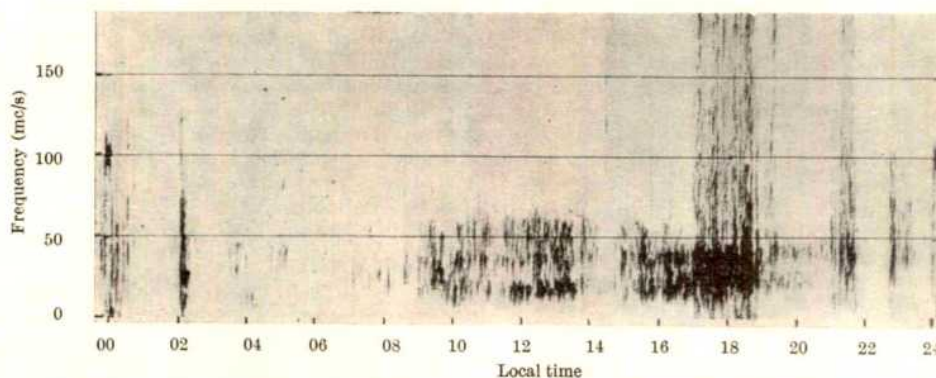


Fig. 2. Cougar Mountain. January 20, 1964.  $H_x$  component. Excitation of  $X$  modes with slow change in frequency.

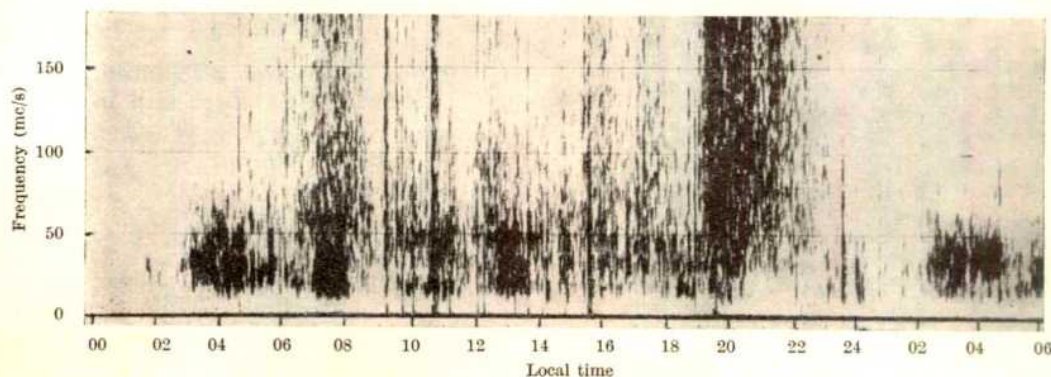


Fig. 3. Esk. November 4/5, 1963.  $H_x$  component.



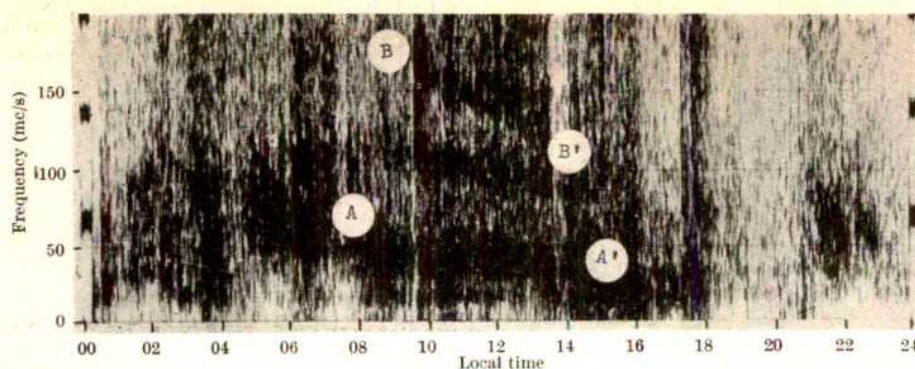


Fig. 4. Toolangi, September 22, 1963.  $H_x$  component. A complex pattern of excitation of  $X$  and  $Y$  modes during a severe magnetic storm.

is inherent with this type of display, making the recognition of invariant features relatively simple. Recent work, however, of a different kind by Fooks and Morgan<sup>4</sup>, who have published several examples of micropulsation power spectra, and by Nwaigwe, Hall and Usher<sup>5</sup>, may reasonably be interpreted as evidence of the detection of  $X$  modes in England. The latter authors give an example of different N.-S./E.-W. behaviour of simultaneous oscillations having frequencies which correspond to the resonances identified here; what might be described as the "E.-W. morning enhancement" which they observe is also a prominent feature on the chart records at Esk.

It should be noted that the  $X$  resonances lie in both the  $Pc3$  and  $Pc4$  domains. On occasions there is some evidence on the sonagrams of a still lower frequency mode, particularly during the day—this is in the  $Pc5$  range but as the equipment concerned has a deliberate cut-off below about 5 mc/s, information on the behaviour of this mode is rather scant. On the other hand the weak excitation of higher order modes is not uncommon.

At times of greater geomagnetic agitation such as in the stormy period after sudden commencement the micropulsation spectrum can become very complex. The activity at Toolangi on such a day, September 22, 1963, is shown in Fig. 4. Type  $X$  modes are present but there are also modes characterized by a slowly falling frequency during the daylight hours. The mode  $AA'$  is centred at about 50 mc/s; the second harmonic about 100 mc/s is visible, and possibly also the third at about 150 mc/s. In addition there is another mode,  $BB'$ , the frequency of which falls more rapidly during the day. These will be designated  $Y$  modes.

The complex pattern of excitation in Fig. 4 does not appear to be arbitrary. Duncan<sup>6</sup> has published two sonagrams for Adelaide covering consecutive highly disturbed days in September 1960. The first day, September 6, 1960, shows an exceedingly detailed agreement with Fig. 4. On the second day, September 7, the geomagnetic agitation was somewhat less and accordingly the activity was chiefly confined to the lowest of the type  $Y$  modes, a behaviour entirely consistent with that deduced from Toolangi sonagrams during September 1963. Duncan's data refer to the vertical component measured by a large ground loop; at Toolangi recordings have been made of only the horizontal N.-S. component.

The lowest of the  $Y$  modes exhibits internal consistency within a given season. There is, however, a noticeable change in characteristics from one season to another. When this mode appears at a slightly lower frequency than in the example shown it becomes difficult to separate from the  $X$  modes. The excitation of both types of resonance in an overlapping pattern probably explains the bulk of the daily spectra.

From records obtained at Esk and Toolangi there is little to suggest that there is any change in the frequencies of corresponding  $Y$  modes with latitude, although the

well-known general diminution of amplitude towards the equator is observed. Sonagrams from Townsville indicate that the dominant patterns of activity in mid-latitudes are not so easily recognizable at low latitudes, at least in the horizontal N.-S. component, and that there may be another, more variable, source of  $Pc3$ - $Pc4$  pulsations near the equator.

The  $Y$  modes cannot be traced reliably through a 24 h period, because at all times of the year there appears to be a cessation of this type of activity by 18-19 h local time. The spectra of the normal night time  $Pi2$  pulsations indicate a connexion with  $X$  modes. Strong night time  $Pc$  activity, however, can sometimes occur in mid-latitudes and, for example, in the interval 21-23 h on September 22, 1963 (Fig. 4), where it is obviously different in appearance from the  $X$  modes, it may be related to the  $Y$  modes. Some of the activity in the very disturbed early morning hours in Fig. 4 may represent  $Y$  mode excitation. Although spectra of  $Y$  modes from stations at other longitudes would be needed to establish the point conclusively, at this stage it seems most unlikely that such modes represent axially symmetric resonances.

There is insufficient evidence to identify any of the modes discussed here as poloidal or toroidal, but further detailed studies of the mode structure of micropulsations may well help to resolve some of the long-standing problems in this field.

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<sup>4</sup> Fooks, G. F., and Morgan, D. P., *J. Atmos. Terr. Phys.*, **29**, 201 (1967).

<sup>5</sup> Nwaigwe, C. N. C., Hall, S. H., and Usher, M. J., *Nature*, **214**, 1319 (1967).

<sup>6</sup> Duncan, R. A., *J. Geophys. Res.*, **66**, 2087 (1961).

### Radio Anomalies associated with an Ephemeral Satellite still in Orbit

ATTENTION was recently directed in *Nature* to some radio reception anomalies observed in 1962, 1963 and 1965 at several different locations receiving the VLF stations *GBR* (Rugby) on 16.0 Kc/s and *NBA* (Panama Canal Zone) on 18.0 Kc/s. These anomalies consisted of an interfering signal just off the nominal transmitted frequency by some  $10^{-7}$  parts, sometimes above the nominal value and sometimes below it. The interfering signal was often as strong as or stronger than the *GBR* or *NBA* transmissions, depending on the location of the receiving station. Two of the possible explanations for these



Table 1

Duration of observation period	Observed between (U.T.)	Average period between HF enhancements (h)	Average daily advance (h)	Average duration of HF enhancements (h)	Implied sidereal period (h)
Feb. 9, 1962	2200	4.25	-0.40	1.0	4.72
Mar. 12, 1962	0700				
Jan. 20, 1963	0100	4.38	-0.63	0.9	4.67
Mar. 3, 1963	0700				
Nov. 13, 1965	2200	3.78	0.00	1.3	4.00
Dec. 12, 1965	0700				
Dec. 5, 1966	0100	2.53	-1.20	1.0	3.26
Feb. 8, 1967	0700				
Apr. 24, 1967	0100	2.76	-0.50	0.8	2.94
May 5, 1967	1330				

All observations were made at 118.5 W. longitude, 34.0 N. latitude. A positive daily advance signifies a later time on each succeeding day.

anomalies have been discussed—nearby unknown transmitters operating on the same frequencies<sup>1,2</sup>, and an alternate antipodal transmission path<sup>3</sup>. I have studied the *Nature* report and others<sup>2-5</sup> and offer some independent evidence of high frequency radio reception anomalies and a third possible explanation.

Early in January 1961 I listened to the high frequency time signals of *CHU* (Ottawa, Canada) on 7.33 Mc/s at Pacific Palisades, California. Occasionally, for periods of 30 min and more the signal, usually weak at my location, was strong enough not to require an external antenna. To examine this phenomenon further, I monitored *CHU* on 7.33 Mc/s almost continuously each evening during February and March 1962. There were many brief periods when the signal-to-noise ratio became very large, sometimes reaching 18 dB (Table 1, line 1, except last column).

At first, I assumed these enhancements would correspond with the onset of evening twilight at a skip zone, but my early 1962 records clearly demonstrated that this was not the case for more than 80 per cent of the enhancements. *CHU* was monitored again in early 1963 between January 20 and March 3. Once more I found a definite pattern of reception enhancements, but it differed from that of early 1962 (Table 1, line 2). I tried unsuccessfully to correlate the times of enhancements with overhead passages of *ECHO* I and other artificial satellites.

During November and December 1965 I made reception measurements of *CHU* on 7.33 Mc/s and again found periodic enhancements. More than 90 per cent of them had a very definite pattern which differed from the previous patterns (Table 1, line 3). Additional data for *CHU* were taken during December 1966 and January 1967 by B. A. Bagby and myself at Pacific Palisades. Again there was a very definite pattern to most of the enhancements, which differed from all preceding ones (Table 1, line 4). It was obvious that the time between enhancement periods was different at each succeeding epoch, and that the daily advance was also changing. If these enhancements were due to reflexions from the passage of a particular Earth satellite through the upper atmosphere, then that satellite had a changing orbital period. I tried to correlate the various reception enhancements with the osculating orbit of the natural Earth satellite previously discussed in *Nature*<sup>6</sup>, but because I was unable to account for all the various radio enhancement periods as being due to the natural satellite, I put the task aside temporarily.

I have re-evaluated the *CHU* radio data, and have found that the source of the earlier difficulty was the very elliptical orbit of the natural object. I had assumed that the apparent period deduced from the radio anomalies would always be the simple reciprocal addition of the object's sidereal period and that of the Earth's rotation, which is true only for a circular orbit. Allowing for the orbital situation at each radio epoch, I related the *CHU* radio enhancement records on 7.33 Mc/s in Table 1 to the osculating orbit of the natural satellite on each occasion (Table 1, last column).

I recently attempted to find a correlation between the *GBR* and *NBA* radio reception anomalies and those found

for *CHU*, and tentatively concluded that the VLF radio anomalies could also be caused by the natural satellite. This would be confined to radio reflexions taking place near the object's perigee. Here the charged cloud associated with the passage of the body through the upper atmosphere would be large enough<sup>7</sup> to reflect 16 and 18 Kc/s signals. This would temporarily provide an alternate transmission path similar to, but shorter than, that suggested by Isted<sup>3</sup> as the cause of the VLF interferences. I computed the geocentric latitude and longitude of the perigee of this satellite on the various occasions of VLF anomalies and found that exceptionally favourable conditions were present during both the May-June 1962 and November-December 1965 periods. Fair conditions were present during the April 1963 period. For June 11, 1962, the perigee dip occurred near 14° S., 167° W. at 0300 U.T. For November 13, 1965, the perigee dip was near 30° N., 149° E. at 0800 U.T. These positions are approximate to the estimated locations of the postulated second transmitter<sup>1,2</sup> in each case. The frequency shifts observed ( $\approx 10^{-7}$  parts) on 16 and 18 Kc/s for the secondary VLF signals<sup>1,2</sup> could have been due to the satellite's approaching or recessional velocity relative to both the receiving and transmitting stations. The rarely occurring maximum possible displacement, due to this Doppler effect, would be  $\Delta f/f = 5.4 \times 10^{-5}$ . More often this value would be much lower, and would have varied slightly during the brief enhancement period, as was observed<sup>1</sup>.

Allowing for differences in the orbital situation, the *GBR* records of November 1965 (ref. 1) are almost replicas of the *CHU* records for that same period, with reference to time of day and periodicity. The 1962 and 1963 VLF data and the 1961 HF data are too meagre for such a positive comparison.

In order to compare the radio anomalies with the orbit of the natural satellite beyond the period of time covered in my earlier paper<sup>6</sup>, I made more optical observations. With the aid of the indirect *CHU* radio evidence, this was accomplished photographically near the object's apogee on both January 18 and 20, 1967. Further optical observations are given in Table 2. Because the *CHU* radio experiment was also being run during January 1967 the two sets of data could be directly compared. The successful pairs of plates (out of sixty pairs taken) were each exposed shortly after a radio enhancement period had begun at Pacific Palisades.

With the recent optical observations, orbital elements have been derived to cover the time period since October 25, 1965 (Table 3). The solution was quite unique, converging very rapidly during the trial and error computational process. The slowly changing inclination is not without precedent. This effect, due to the upper atmosphere near perigee, has been discussed at length by Nigam<sup>8</sup> and others<sup>9</sup>. After the orbit solution was com-

Table 2

Date	Time (U.T.)	Right ascension	Declination	Magnitude
Jan. 18, 1967	05.18.35	5h 00m	+46.0°	8
	05.23.00	4h 30m	+42.5°	8
	05.24.45	4h 25m	+41.0°	8
Jan. 20, 1967	05.24.45	3h 57m	+38.2°	8
	05.28.45			
Mar. 22, 1967	13.17.20	205°	6.5°	1
	13.18.25	231°	6.2°	3
		Azimuth	Elevation	

All observations were made from 118.5 W. longitude, 34.0 N. latitude. The epoch of the celestial co-ordinates is 1920.

Table 3

$$\begin{aligned}
 a &= 12,860 - 0.784 (t)^{1.27} \\
 e &= 1.000 - (6.530/a) \\
 i &= 137.0 - 6.64 \times 10^{-4} t \\
 \Omega &= 340 + 1.096t + 1.49 \times 10^{-5} (t)^{2.28} \\
 \pi &= 316 - 0.159t - 3.64 \times 10^{-7} (t)^{2.75} \\
 \text{Period} &= 10.72 (7.993 - 4.87 \times 10^{-4} t^{1.27})^{1/2} \\
 \text{Epoch} &= \text{October 25.0, 1965}
 \end{aligned}$$

In the equations: the semi-major axis,  $a$ , is in kilometres; the eccentricity,  $e$ , would be 0.00 for a circular orbit and 1.00 for a parabolic orbit; the inclination,  $i$ , exceeds 90° due to the retrograde sense of the orbit; the longitude of perigee,  $\pi$ , is the algebraic sum of the right ascension of the node,  $\Omega$ , and the argument of perigee; the anomalistic period is in mean solar minutes; and the symbol  $t$  is the number of mean solar days elapsed since the epoch.

pleted a radio enhancement check of the orbit was made between April 24, 1967, and May 5, 1967 (Table 1, line 5). This confirmed the orbital parameters as extrapolated from Table 3 for that epoch.

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<sup>8</sup> Nigam, R. C., *Research in Space Science*, Special Report 112, Smithsonian Astrophys. Obs., Cambridge (1963).

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### Mid-Ocean Ridge in the Mouth of the Gulf of Aden

For the past thirty years it has been assumed that the seismically active structure of the Carlsberg Ridge is connected with the seismically active East African Rift System through the Gulf of Aden. Details of this connexion have emerged during the International Indian

Ocean Expedition<sup>1-3</sup>, but sounding and magnetic profiles in the entrance to the Gulf of Aden have been too sparse to determine the line of the median structure. The track that R.R.S. Discovery followed during Cruise 16 in March 1967 (Fig. 1) was designed to criss-cross the epicentre belt and fill in this gap. Bathymetric and magnetic data obtained confirmed the existence of a mid-ocean ridge structure in the Gulf of Aden west of the Owen Fracture Zone; we suggest the name "Sheba ridge" be given to this feature.

Profiles obtained along the sections B-X of the track are presented in Fig. 2. Depths are plotted in corrected fathoms and the magnetic anomalies are shown after deduction of the regional gradient taken from ref. 4 but without correction for daily variation. The soundings have been combined with other soundings in the area and used to revise the relevant portions of contour charts based on British Admiralty 1 : 1,000,000 plotting sheet areas 158 and 159 (A. S. Laughton, personal communication). The significant features of these charts are summarized in Fig. 1.

The median valley has been traced as a continuous structure (with one displacement) from the Alula-Fartak trench to the Wheatley Deep, a trough on the Owen Fracture Zone 600 fathoms below the Indus abyssal plain<sup>3</sup>. Its trend changes continuously from E.-W. at 55° E. to N.W.-S.E. at the Owen Fracture Zone. The

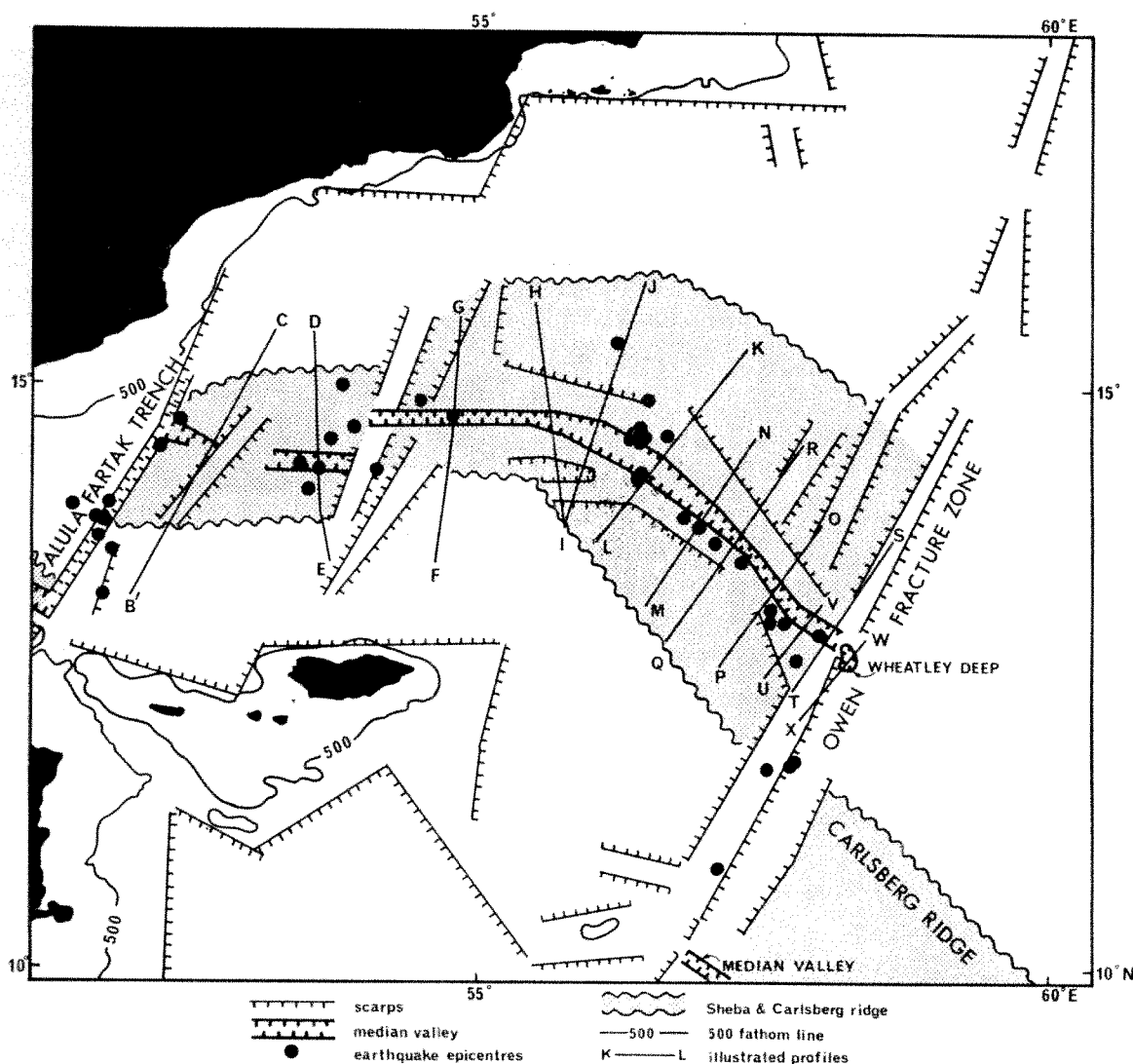


Fig. 1. Structural features of the Gulf of Aden and epicenters from Alula-Fartak trench to Owen Fracture Zone, and positions of profiles illustrated in Fig. 2.

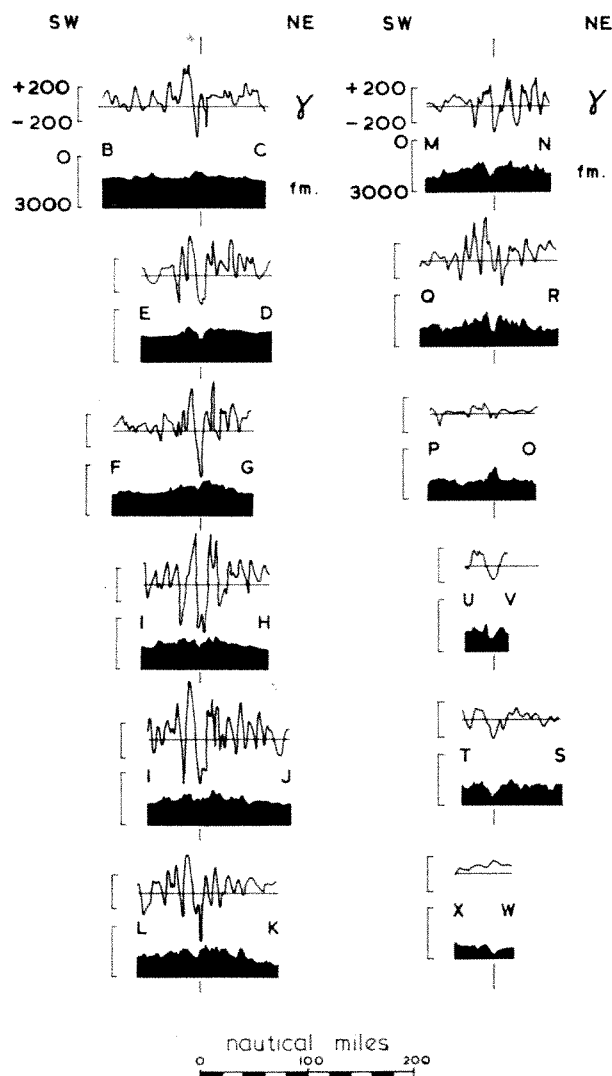


Fig. 2. Bathymetric and magnetic profiles B-X inclusive.

median valley is clearly recognizable on all the profiles except profiles BC and PO; Fig. 1 shows how it corresponds with the epicentre belt. In the six western profiles, as far as LK, the valley is clearly identified by a large negative median magnetic anomaly. On profiles MN and QR there is no unique large anomaly while on profile PO there is no large anomaly at all. The median anomaly reappears with smaller amplitudes on the short sections UV and TS. These profiles are in the Owen Fracture Zone: Matthews, Vine and Cann<sup>6</sup> have suggested that the pattern of magnetic anomalies is expunged in a fault zone by extensive brecciation and hydrothermal alteration of the permanently magnetized rocks.

The track WX lay just to seaward of the Owen Fracture Zone and crossed the south-eastern edge of the Wheatley Deep which has a maximum depth of 3,100 fm.; no significant magnetic anomalies appear along the part of this track opposite the faulted end of the median structure. Steaming south from X, the ship passed over the foothills of the Carlsberg Ridge just east of the Owen Fracture Zone and over the median anomaly at 10° N., 57° E. at a point which lies precisely on the line of the median structure at the northern end of the Carlsberg Ridge<sup>3</sup>. This result confirms the measured displacement of the axis of the ridge as 170 nautical miles right lateral.

The median line of the ridge is displaced 100 miles to the left at the Alula-Fartak trench (Fig. 1), and Sykes<sup>6</sup> has identified this feature as a transform fault<sup>7</sup>. Examina-

tion of the contoured charts of magnetic anomalies and of topography (unpublished) makes it clear that another transform fault lies on a line between the kink in the edge of the continental shelf just west of Socotra and a corresponding kink in the Arabian coastline near 17° N., 55° E. The displacement of the axis of the ridge is 30 miles left lateral at this fracture, which is marked by earthquake epicentres.

The redrawn topographic contour chart shows a strong lineation of ridges and troughs running parallel to the median valley in the western part of the area. Near 13.5° N., 57.5° E. (on profile QR) this trend is crossed by a system of larger and smoother features running perpendicular to the first, parallel with the Owen Fracture Zone. The evidence indicates that the median valley transects these Owen Fracture Zone ridges and terminates in the Wheatley Deep.

The contoured chart also shows that the median valley breaks through the block faulted ridge immediately east of the Alula-Fartak trench at 14.5° N., 52.5° E. and enters the trench itself near its northerly end. If one makes the very reasonable assumption that the system of ridges parallel to the Owen Fracture Zone and to the Alula-Fartak trench are horst and graben features associated with the faulting in these shear zones, then it is clear that the latest phase of opening along the median line of the mid-ocean ridge postdates the formation of the fracture zones, and that the horst and graben features postdate the formation of the principal part of the central rough zone in the Gulf of Aden. These horst and graben blocks which run N.E.-S.W. parallel to the fracture zones form the dominant topographic lineation in the part of the Gulf of Aden west of the Alula-Fartak trench. It can also be argued that the formation of the Wheatley Deep must be post-Pleistocene because it has not been filled by the sediments of the Indus Cone. These sediments must have been deposited during the Pleistocene because the absence of turbidity currents (deduced from the absence of broken cables, Heezen—personal communication) across the cone suggests that they are not being deposited at present. If that is so the following sequence of events has occurred in the Gulf of Aden. Formation of the Sheba Ridge structure parallel to the median line; formation of the horst and graben N.E.-S.W. structures (Pleistocene or earlier) and formation of the median valley (post-Pleistocene).

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## PHYSICS

### Effect of Pressure on the Viscosity of Water

ACCURATE values for the viscosity of water at various temperatures and pressures are necessary both to the chemist, who may require to relate viscosity with other phenomena, such as electrical conduction in aqueous solutions, and to the engineer, who may require the information in the solution of heat transfer and flow problems. It has been observed that above about 33° C



the viscosity of water increases with pressure, and that below this temperature, initially the pressure effect is negative but at about 1,000 kg/cm<sup>2</sup> the viscosity relative to 1 atm. exhibits a minimum. Presumably at this pressure the structured regions occurring in water have been partially destroyed and the behaviour is more like that of a "normal" liquid.

Horne and Johnson<sup>1</sup> have recently published results obtained with a rolling-ball viscometer in the range 1–2,000 kg/cm<sup>2</sup> and in the temperature range 2° to 20° C. The results disagree with the low temperature measurements of Bett and Cappi<sup>2</sup>. While Cappi's results suggest that the rate at which the negative coefficient changes between 20° C and 2° C is fairly uniform, Horne and Johnson's results indicate a gradual change until 4° C is reached, and below this temperature the pressure coefficient is shown to increase abruptly. This is apparent in Fig. 1 where it can be seen that Cappi's 10° C isotherm has a steeper slope than the 4° C and 6.2° C isotherms of Horne and Johnson, while Cappi's 20° C isotherm corresponds to the 10° results given by Horne and Johnson.

The temperature of 4° C is significant for water because at this point the effect of thermal expansion becomes greater than the contraction due to the thermal destruction of ordered regions of molecules. This gives rise to the well known minimum in the specific volumes at this temperature. Horne and Johnson's results indicate that at 4° C there is a sudden increase in the effect of pressure on viscosity while Cappi's observations show no sign of this.

I have made preliminary measurements using a rotating cylinder viscometer, which has been previously described<sup>3</sup>, at pressures up to 230 kg/cm<sup>2</sup> and at temperatures between 12.5° C and 108° C. At low temperatures it can be seen that these results are in agreement with those of Bett and Cappi rather than with those of Horne and Johnson. At higher temperatures measurements are in agreement with other workers, including those of Moszynski<sup>4</sup>.

Discrepancies such as these present problems in the setting up of formulae representing the properties of water. The anomalous behaviour of water at low temperatures is not correctly indicated by published tables of values, examples of these being the Skeleton Tables of the International Steam Conference<sup>5</sup> and the more recent correlation of Bruges, Latto and Ray<sup>6</sup>. There appears to be a need for more measurements at low temperatures and up to 1,000 kg/cm<sup>2</sup> to remove any doubts which may still exist.

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Received July 31, 1967.

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<sup>4</sup> Moszynski, J. R., *J. Heat Transfer*, **83**, 111 (1961).

<sup>5</sup> Sixth Intern. Conf. on the Properties of Steam, Suppl. on Transport Properties, publ. in *Engineer*, **219** (1965).

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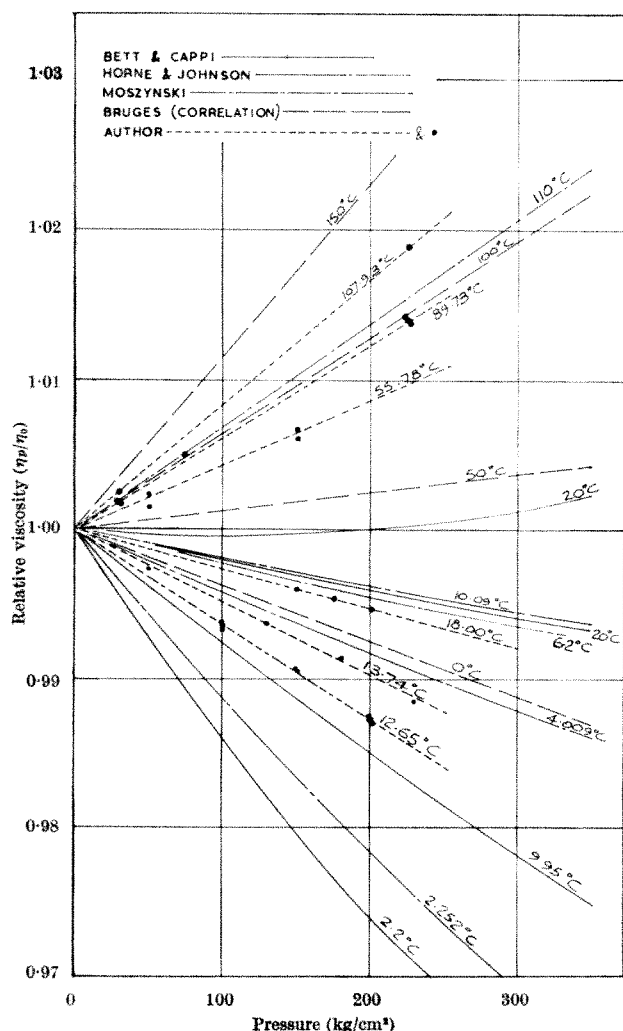


Fig. 1. Relative viscosity of water.

### Wettability and Adhesion of Polyethylene

THE linear relationship found experimentally by Barbarisi<sup>1</sup> between bond strength and  $(1 + \cos \theta)$ , where  $\theta$  is the contact angle for epoxy-polyamide adhesive on polyethylene, is of technological importance and merits more careful analysis.

The relationship was arrived at on the basis of the well-known equation between the reversible work,  $W$ , required to separate liquid from 1 cm<sup>2</sup> of a solid surface, leaving adsorbed film on the surface in equilibrium with the liquid, and the contact angle

$$W = \gamma_{LV}(1 + \cos \theta) \quad (1)$$

where  $\gamma_{LV}$  is the surface tension of the liquid. This equation indicates that for a given liquid, the greater the degree of wetting of a surface, the stronger is the bond; a chosen solid which is completely wetted by the liquid will give  $W = 2\gamma_{LV}$  (experimentally found bond strengths are invariably much less than theoretical values)<sup>2</sup>.

Low energy solids, for example, polymers, can be characterized by their critical surface tension  $\gamma_c$ , a quantity which was found empirically by Zisman and co-workers<sup>3</sup>. The precise significance of  $\gamma_c$  is still uncertain, but Fowkes<sup>4</sup> has shown that it is almost equal to  $\gamma_s^d$ —the dispersion force contribution to the surface tension of the solid. For polyethylene  $\gamma_c = 31$  dyne/cm and  $\gamma_s^d = 35$  dyne/cm. Because the surface tension of polyethylene will come from dispersion forces, it is justifiable in this case to write

$$\gamma_s = \gamma_s^d \approx \gamma_c \quad (2)$$

Only in the absence of better information is it reasonable to approximate  $\gamma_c$  for  $\gamma_s$  when dispersion forces are known to dominate.

Barbarisi<sup>1</sup> makes the statement that contamination on the surface (of polyethylene) will in general lower the value of  $\gamma_c$ , that is, reduce the ability of a liquid to wet the solid, and therefore recommends pretreatment of the surface. The following arguments demonstrate that

(Continued on page 1071)

## BRITISH ASSOCIATION SUPPLEMENT—LEEDS, 1967

## "THE ADVANCEMENT OF SCIENCE"

CONVENTION has often required, in recent years, that people should shake their heads with regret and a little scorn at the supposed incomprehensibility of modern science. "Science is fragmented", the saying goes. One way and another there is a widespread belief that the condition of science was much healthier a century or so ago, when it was often possible for intelligent country gentlemen, occupied for most of their time with doctoring or industry, to keep up with what the natural philosophers were writing and often to take issue with them as well. And it is true, of course, that circumstances were very different then. There was less science. The pace of advance was comparatively leisurely, so that there was time for new concepts to be assimilated by people not actively working in a field before they were in turn discarded by the professionals. But even in those supposedly halcyon days, understanding was not always easy to come by. Darwin was in everybody's mind, but appreciation of the more abstract work on which Faraday was engaged was inevitably much more restricted, for example. Since then, there has been no real qualitative change in the character of scientific advance, but merely such a dramatic acceleration of the pace of discovery that things appear to be quite different from what they were.

In circumstances like these it makes no sense to complain about specialization as such. For centuries, and for long before the beginning of modern science, scholars have found that the interesting problems are only solved when effort is concentrated on them. Mediaeval scholars in particular were often firmly wedded to specific tasks—the translation of the Bible from one language to another. For all the ease with which Victorian gentlemen could participate in science, specialists were plentiful in the nineteenth century. A good many of them were as single-minded as any now alive. Just as at present, they would often fail to communicate exciting news to specialists in other fields. In other words, it seems that when it comes to making progress, specialization is not merely a necessity but often a virtue without which scholarship would be diminished. It follows that the only complaint against the specialists which can, in some circumstances, be sustained is that they take too little trouble to acknowledge that the essence of what they do can be enormously interesting and important to other kinds of people. It is not too fanciful to think that the advance of science as a whole consists of the integration into a whole of the separate discoveries of scientists working in fields which seem, at first sight, quite unrelated.

The annual meeting of the British Association, at Leeds this year, is a good time for remembering this, for the association has its roots in the days when it was possible—but, even then, not common—for amateurs to make contributions to the advance of science ranking in importance with the contributions of those who laboured full-time at some laboratory bench. The

records of the old meetings show how easily scientific issues could become matters for public debate between men (but few women) with widely different issues. Over the years, the association has quite properly changed its character with the acceleration of discovery, but it remains, as it should, a means by which a public wider than that of the full-time professionals can be kept abreast of new developments. In doing so it has come up directly against the difficulty of distilling from the continuing torrent of professional activity those developments which may in some sense be called essential. This problem is, of course, one that the association shares with a great many other organizations—responsible newspapers, for example, and the growing number of magazines designed to bridge the gap between the full-time profession as such and the great company of those outside.

The problem is that of reading the fine print and picking out the points at which matters of general interest escape almost without assistance from the technicalities with which they are inevitably accompanied. There is probably a case for saying that the professionals do too little to help outsiders and each other by drawing attention to matters of great importance. Yet there is much that can easily be done, as it is hoped the following pages will show. They are intended to pick out for the interest of a wider readership than that usually concerned with individual articles in this journal problems which seem to have become of special interest and excitement in the past year or so. The selection, it will be seen, is biased. It is mostly concerned with issues which have been raised in this journal, but it does not do justice to all the matters which are at present of great importance—the physiology and chemistry of vision, the mechanism by which platelets clump together in blood and a great many other topics.

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## COSMOLOGY

# Radiation from the Origin of Time

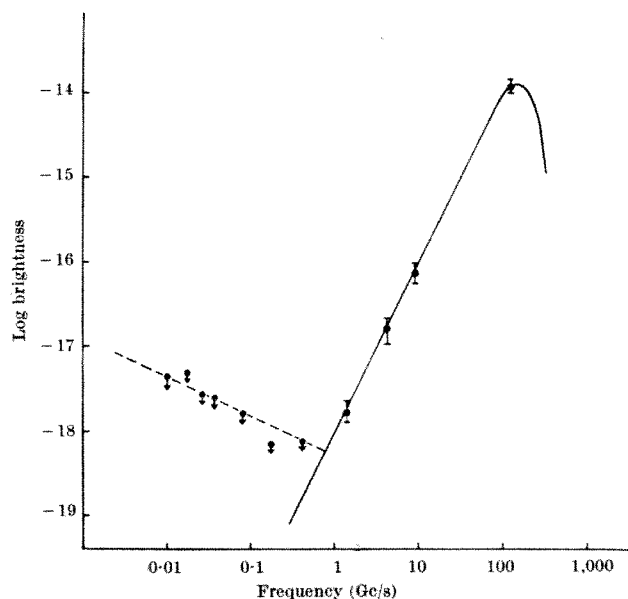
THE discovery which has done the most to electrify research in the past year is probably the recognition that the Universe is filled with microwave radiation which is probably in one sense or another a relic of the beginning of time. At present, this bath of radiation seems to be that appropriate to a black-body with a temperature of  $3^\circ\text{K}$ . The supposition is that it is what is left of the exceedingly energetic radiation—gamma rays and the like—that would have been generated if the universe were once compacted into a relatively tiny volume. After roughly  $10^{10}$  years in an expanding universe, the gamma rays have been degraded into microwaves. If this interpretation is correct, the microwave radiation constitutes evidence that the Universe has aged with time, and thus cuts the ground from beneath the theory of continuous creation, one of the objectives of which is to permit a universe whose intensive properties do not change with time. It does not follow, however, that the microwave radiation is final support for the big bang picture of a universe with a beginning in some cataclysmic phenomenon. A pulsating universe in which light elements were refashioned out of heavy elements at each compressed extreme of the cycle would be equally consistent with the existence of the microwave radiation.

The search for the microwave background, and its detection in the past two or three years, owes much to R. H. Dicke and his colleagues<sup>1</sup> at Princeton University. The essence of their position is that the origin of the Universe must have been a state of affairs very much like thermal equilibrium but corresponding to such a high temperature that all elements except hydrogen (and possibly deuterium and even helium) would have evaporated. This implies a temperature of some  $10^{10}$  degrees K, and therefore a black-body radiation with a peak consisting of X-rays somewhat more energetic than those from the annihilation of positrons and electrons. The rate at which this radiation will degrade in wavelength must depend on the rate at which the material universe is expanding. As luck will have it, the atmosphere of the Earth is comparatively transparent to microwaves between one and 20 centimetres, and Dicke's first calculations suggested that the degraded radiation from the primeval universe would fall in this range, which meant that there could easily be profit in a search for this radiation.

In the event, the first direct observations of the contemporary background radiation appeared in the background noise detected by the horn antenna built by the Bell Telephone Laboratories to collect signals from Echo satellites<sup>2</sup>. But the group at Princeton used a more specific piece of apparatus designed earlier by Dicke to pick out spectrally broad sources of radiation in the presence of noise from radio receivers, and which has the further advantage of making a

direct comparison between the microwave background and an artificial source of thermal radiation kept at only a few degrees above  $0^\circ\text{K}$ . The result<sup>3</sup> was a direct confirmation of the horn antenna at the Bell Telephone Laboratories though at a different wavelength, and also some evidence to suggest that the microwave background is isotropic, which is of course essential if the radiation is somehow characteristic of a symmetrically expanding universe. Since then, other points have been obtained on what seems to be a straightforward Planck curve describing the intensity distribution of radiation of different wavelength from a black-body with a temperature of about  $3.3^\circ\text{K}$ , or to a wavelength of roughly two millimetres. The most intriguing method<sup>4</sup> depends on the fact that molecular cyanogen has an electronic excitation with an energy corresponding to a wavelength of 2.6 millimetres, which means that some proportion of the interstellar cyanogen will exist in the excited state, and that the proportion of such molecules—which can be estimated from the strength of the absorption lines due to the two forms of cyanogen in stellar spectra—is a measure of the temperature of the microwave background. Unless there is some quite unexpected error of interpretation, it looks as if the existence of an all-pervading bath of microwave radiation has been proved up to the hilt.

The solid curve in the accompanying diagram (from Howell, T. F., and Shakeshaft, J. R., *Nature*, **210**, 1318; 1966) shows the spectrum of the microwave background in the light of four separate measurements available a year ago. The solid line itself is the theoretical brightness expected from a black-body at a tempera-



ture of  $3^\circ$  K. Brightness is measured in units of ergs  $\text{sec}^{-1} \text{cm}^{-2} (\text{c/s})^{-1} \text{ster}^{-1}$ . The experimental measurement at the peak of the curve is that representing measurements of absorption of light from the stars by cyanogen molecules. The lowest experimental measurement on the solid curve was made at the Mullard Radio Astronomy Observatory, and the other points in the United States at Princeton and the Bell Telephone Laboratories. To the left of the solid line are a number of points representing background microwave radiation from the galaxy which must be expected to swamp the cosmological microwave background at frequencies lower than about 1,000 mc/s. One study of this kind has already been described<sup>5</sup>. There seems at least to be a possibility, much in need of confirmation before it can be considered as anything like a fact, that the effective temperature of the microwave radio background is greater in two directions in the Universe, one above and one below the galactic equator, and there is just a possibility that these apparently preferred directions may somehow be linked with places in the sky at which certain exceptional quasars are located (see page 1059).

What does all this mean? The most immediate value of these new developments is that they provide a further means of access to cosmological questions. One possibility, for example, is that the mere existence of the microwave background will set a limit to the energy with which protons in the cosmic radiation can reach the Earth. Too much energy will imply too great a chance of interacting with some photon from the microwave background. Fortunately the discovery that energetic cosmic ray showers in the atmosphere produced by the arrival of single energetic particles from outside are accompanied by the emission of pulses of radio waves may provide a means of testing whether the limit of energy lies at  $10^{21}$  electron volts,  $10^{22}$  electron volts or at some other level. Another possibility, being followed energetically at Princeton and elsewhere, is that it may be possible to measure directly the motion of the Earth through the microwave background by a sufficiently sensitive measurement of the apparent intensity of the radiation from various directions fixed in space. Although to a first approximation the radiation is isotropic, the velocity of the Earth within the Solar System should be enough to produce a detectable imbalance, leading to a greater flux of radiation from the direction towards which the Solar System is moving, even if this galaxy as a whole is at rest with respect to the microwave background. But what if this galaxy is itself moving relative to the background? Studies like these, in other words, could provide direct evidence about the way in which the galaxy was formed.

Whether the existence of the microwave radiation will quickly help to resolve the differences between competing cosmologies is another matter. But on the face of things, the microwave background does seem to be a piece of evidence that the Universe has changed in the course of time, which seems to argue against steady state theories in which the local character of the Universe remains unchanged from one millennium to the next. It is, however, worth remembering that there may conceivably be other explanations of the microwave background than the degradation of the radiation in an initial big bang—and that the supporters of steady state theories are resourceful people. But

even if the Universe is at present ageing, it does not follow that the origin was a big bang. It could also have been the extreme phase of a huge oscillation. As yet it is not clear how far the microwave radiation will help to distinguish possibilities such as these.

<sup>1</sup> Dicke, R. H., Peebles, P. J. E., Roll, P. G., and Wilkinson, D. T., *Astrophys. J.*, **142**, 414 (1965).

<sup>2</sup> Penzias, A. A., and Wilson, R. W., *Astrophys. J.*, **142**, 419 (1965).

<sup>3</sup> Roll, P. G., and Wilkinson, D. T., *Phys. Rev. Lett.*, **16**, 405 (1966).

<sup>4</sup> Field, G. B., Herbig, G. H., and Hitchcock, J., reported at Amer. Astro. Soc. Meeting, Berkeley, December, 1965.

<sup>5</sup> Wilkinson, D. T., and Partridge, R. B., *Nature*, **215**, 719 (1967).

## RADIO-ASTRONOMY

### Smaller Quasars

THE radio sources which are quasars are distinguished from other radio sources in the sky by their small angular diameter. The precise measurement of an extremely small radio source requires a radiotelescope of great size operating as an interferometer, and techniques for doing this have been worked out over the past several years at the Nuffield Radio-astronomy Laboratories at Jodrell Bank by a group of people under Dr H. P. Palmer<sup>1</sup>. Over a period of several years the components of a pair of radiotelescopes have been moved further and further apart until the distance separating them was as great as half a million wavelengths of radiation at 21 cm. The technique consists of comparing the signals from two separate radiotelescopes so as to extract information not merely about intensity but phase as well. Where the distances are so great, it is necessary to use radio links for comparing the signals. By this means radio sources smaller than a quarter of a second of arc have been measured. But is there any possibility of making interferometers with a still greater base length? One difficulty is that even the best microwavelengths and landlines tend to lose quality after some hundreds of kilometres.

It now appears that a way round the problem does exist. A group of radio-astronomers from Canada<sup>2</sup> have been able successfully to use magnetic tape recorders capable of recording accurately a wide band of radio frequencies in such a way as to produce records from two independent radiotelescopes which can later be accurately compared with each other. The standard of time is provided by independent local oscillators controlled by rubidium frequency standards. In one series of observations, two radiotelescopes were separated by 3,000 kilometres or the equivalent of more than 4.5 million wavelengths at a frequency of 448 megacycles per second. With this arrangement, it has been demonstrated that the diameter of at least one quasar (3C 273B) is less than 0.02 seconds of arc.

Plainly this is an important development of technique which is certain to lead to still more refined measurements of the diameters of quasars.

<sup>1</sup> Palmer, H. P., Rowson, B., Anderson, B., Donaldson, W., Miley, G. K., Gent, H., Adgie, R. L., Slee, O. B., and Crowther, J. H., *Nature*, **213**, 789 (1967).

<sup>2</sup> Broten, N. W., Legg, T. H., Locke, J. L., McLeish, C. W., Richards, R. S., Chisholm, R. M., Gush, H. P., Yen, J. L., and Galt, J. A., *Nature*, **215**, 38 (1967).



## ASTRONOMY

# Why is the Sun Flat?

OBSERVATIONAL tests of Einstein's general theory of relativity are exceedingly few and far between, and since the twenties observations of the rotation of the orbit of the planet Mercury have provided the most direct support for the theory as a whole. This is why great interest attached to an announcement earlier this year by R. H. Dicke and his colleagues<sup>1</sup> at Princeton of some measurements of the flatness of the Sun, and to their suggestion that this by itself could account for a part of the rotation of the orbit of the planet which has so far been attributed to Einstein's theory. If this assertion is true, it is plain that support for the general theory of relativity will be undermined. In the event, however, admiration for the ingenuity and the accuracy of the measurements of the flatness of the Sun has not been accompanied by a general acceptance of Dicke's reasons for believing the Sun should be flattened. It remains to be decided whether the new evidence goes against Einstein's theory.

The facts are these. The orbit of Mercury rotates slowly with respect to the fixed stars chiefly because of the influence of the other planets, but when these effects have been allowed for there remains a rotation of 43.11 seconds of arc a century to be accounted for. When this was first appreciated at the beginning of the century, a search was made for other explanations than the disturbing influence of the other planets. One possibility then considered was that the Sun might be flattened in such a way as to affect the position of the orbit, although people found it hard to believe that this could happen without more obvious disturbances of the orbit of Mercury than the rotation of the perihelion. In any case, precise measurements of the shape of the Sun were not then practicable. In the event, it may have seemed almost too good to be true that the strictly relativistic calculations of Einstein's theory should imply that there should be a rotation of the orbit of Mercury amounting to 43 seconds of arc a century.

Dicke<sup>2</sup> was the first to suggest, as early as 1964, that this agreement between experiment and observation might not be as comforting as people had supposed. He pointed out that a flattening of the Sun might well account for a good part of the rotation of the perihelion of Mercury. Dicke and his colleagues have now built and operated an exceedingly clever instrument by means of which light from the outer rim of the Sun is scanned electronically around the circumference in such a way that disturbances due to the Earth's atmosphere can be eliminated. The result, which appears to be beyond dispute, is that the radius of the Sun is greater towards the equator than towards the poles by 34 kilometres, or by roughly five parts in a hundred thousand.

What does this imply for the rotation of the orbit of Mercury? Everything depends on the success with which the gravitational forces of the Sun can be inferred from the shape which is observed. If, of course, the Sun were a mass of fluid held together by its own

gravitation, but otherwise at rest, it would be a perfect sphere and it would be possible to infer that the gravitational potential is the same at every point on the surface. (What seems to be the surface would not be a sharp discontinuity between different phases as at the interface between the Earth and its atmosphere, but it would instead be a surface on which the density of solar matter is constant.) The rotation of the Sun as a whole complicates the position, and Dicke says that this accounts for 7 km of the observed flattening, leaving 27 km to be accounted for in other ways. For him, this implies that the external gravitation forces of the Sun are not those corresponding to a perfectly spherical distribution of matter but, rather, to one which is significantly asymmetrical. And if this is indeed the case, some of the observed rotation of the orbit of Mercury—3.4 seconds of arc a century—is to be attributed to flattening of the Sun and not to Einstein's theory of relativity.

This is not a big discrepancy. Even if it is safe to assume that the shape of the Sun is a guide to its gravitational influence on the planets, observation and the predictions of theory would differ by only 8 per cent. This, however, is more than the cosmologists could comfortably ignore, and in any case there remains the puzzle of how to account for flattening of the surface. Dicke suggests that it is enough to suppose that the interior of the Sun is rotating a little more quickly than the surface (which makes one revolution in 25.4 days). One immediate difficulty is that any hypothesis like that must somehow be linked with theories of the rotation of the Sun—and in particular with the awkward question of how since the beginning of the Solar System the Sun can have lost enough angular momentum to explain the comparatively slow rotation now observed.

In the circumstances it is not surprising that Dicke's argument is being studied with the greatest care. Professor I. W. Roxburgh<sup>3</sup> has raised a number of objections, and has, for example, pointed out that if the inside of the Sun were rotating more quickly than the surface, the temperature on the Sun would probably differ by 10 degrees between the equator and the poles. For Roxburgh, temperature gradients within the Sun could account more satisfactorily for the flattening which Dicke has measured and, on that interpretation, observed flattening should have no effect on the gravitational influence of the Sun. Dicke<sup>4</sup> has refuted some of these arguments, but it would be a great surprise if this important question were now forgotten. In the long run, the controversy could do more to improve understanding of the rotation of the Sun and the structure of its interior than to upset the foundations, such as they are, for the general theory of relativity.

<sup>1</sup> Dicke, R. H., and Goldenberg, H. M., *Phys. Rev. Letters*, **18**, 313 (1967).

<sup>2</sup> Dicke, R. H., *Nature*, **202**, 433 (1964).

<sup>3</sup> Roxburgh, I. W., *Nature*, **213**, 1077 (1967).

<sup>4</sup> Dicke, R. H., and Goldenberg, H. M., *Nature*, **214**, 1294 (1967).

## ASTRONOMY

## Continuing Problems about Quasars

THIS has been a frustrating year for those who look for explanations of what quasars are or, even more modestly, for some simple information about the location of these puzzling objects. The outstanding problems remain much as they were described six months ago by Professor F. G. Smith<sup>1</sup>. It is, however, extremely odd that each fresh attempt to test some hypothesis about quasars tends to be inconclusive or even to produce some kind of contradiction.

The first quasars were recognized by the intensity of their radio emission and by their unusual compactness—a few seconds of arc or less. Radio sources like these are often associated with optical sources, and spectral lines tend to be shifted enormously towards the red. Known quasars are now numbered in hundreds. Some of them have optical but not radio emission. Others may be invisible radio sources. The oddness of the quasars is well shown up by the way in which, in the same optical spectrum, absorption and emission lines may be shifted towards the red to different degrees.

The interpretation of the red-shift seems still to be uncertain. If quasars were like ordinary nebulae outside this galaxy, the existence of a red-shift would imply recession and its degree would be a measure of the velocity of recession. In that case, quasars would be extremely energetic sources of radiation at exceedingly great distances. But may the red-shifts be accounted for in some other way, possibly by recognizing that in theories of gravitation, extremely massive bodies would redden light escaping from them? On that interpretation of the red-shift, quasars would be less energetic and less massive but some means would have to be devised for explaining why light from them is shifted to the red—and why so many of them put such a large proportion of their energy output into radio waves. The fact that the output of radiation, light as well as radio, can vary significantly within a few days is something else to be accounted for.

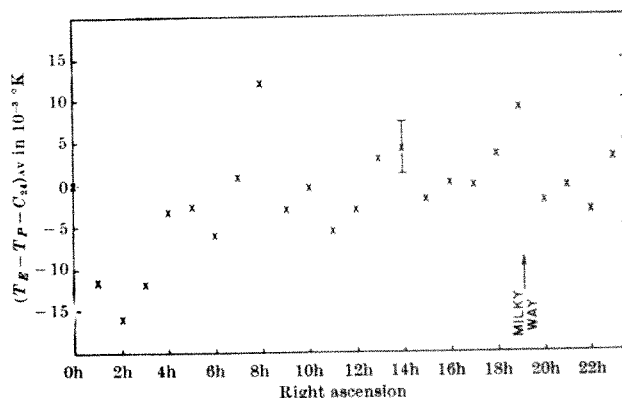
From the beginning there have been hopes that some understanding of the quasar problem would be provided if there were some relationship between the brightness of the quasars and their red-shifts. If, for example, red-shift is a measure of distance, then the faintest quasars should be those with the greatest red-shifts. When there was only a handful of quasars to include in a statistical analysis, the absence of a convincing correlation was not surprising. Now, however, that there are many more objects with which to work, such conclusions as have been drawn are not cheerfully in agreement with each other. Thus one group has claimed that radio observations, at an unusually high frequency, show signs that brightness decreases with increasing red-shift<sup>2</sup>. (The diagram on the next page shows the relationship between red-shift and brightness at 5,000 Mc/s.) Another group, working principally with optical observations, has pointed out that a relationship between red-shift and brightness need not necessarily imply that the quasars

with the greatest red-shifts are also the most distant<sup>3</sup>. How this will be resolved is not at this stage clear, but obviously it would be a great advantage if there were some way of deciding unambiguously how the red-shift of quasars is to be interpreted.

There has also been a search for evidence to suggest that quasars may be distributed in patches in the sky, but this, too, has been inconclusive. On the whole, there is as yet no compelling reason for thinking that the quasars now known are distributed irregularly. At least two attempts have been made to account for what appear to be patches of the sky in which quasars are unexpectedly abundant<sup>4,5</sup>, but it has also been argued that the statistics are not yet good enough to endow the appearance of patchiness with undeniable authenticity.

It remains, however, puzzling that quasars with very large red-shifts (in which the wavelength in Angstroms of the emitted light is multiplied by a factor of three or thereabouts) seem to stand out from the others partly by what seems to be a clustering at one end of the range of red-shifts and partly because they seem to be associated with two patches in the sky above and below the plane of this galaxy. If it should turn out, when there are more quasars with which to make a statistically convincing analysis, that there is indeed some variation of the distribution of quasars with direction in the sky, people will be forced to the conclusion either that quasars are not placed towards the outer regions of the universe or that the universe has some entirely unexpected property—either an anisotropy<sup>4</sup> or an inhomogeneity<sup>5</sup>.

But how to test for some departure of the universe from complete uniformity? The cosmological microwave background has been suggested as one means of doing this, and Wilkinson and Partridge<sup>6</sup> have now made a search for differences in the apparent temperature of this radiation in different directions in the sky. As it happens, they have found that the apparent temperature of the radiation appears to be  $0.016^\circ\text{K}$  higher than the average in a direction roughly corresponding to that in which the quasars with the biggest red-shifts seem to be concentrated. The accompanying diagram shows the results obtained by combining the

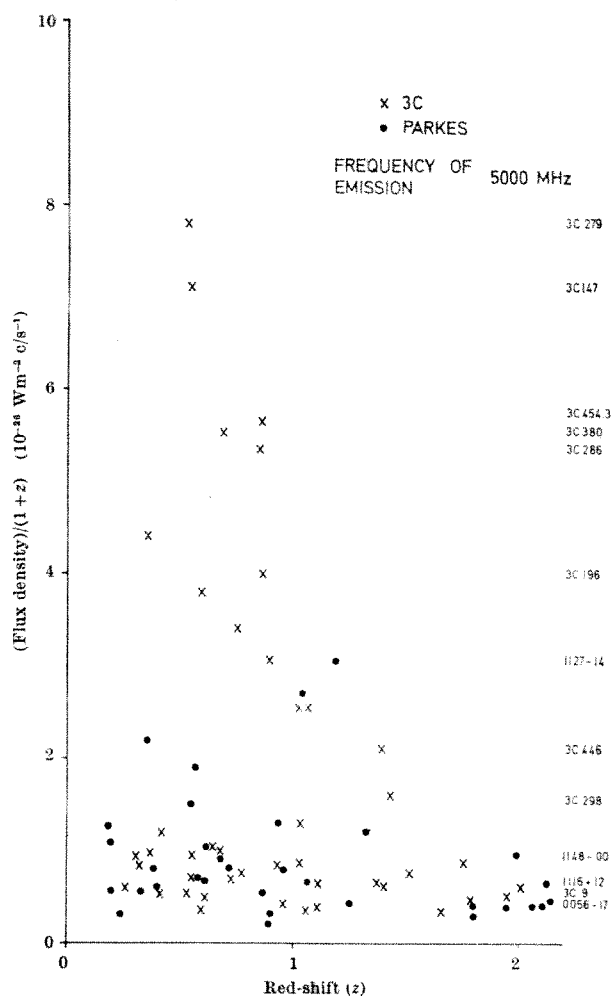


results of 80 sweeps, each lasting 24 hours, along a circle in the sky at a declination of  $-8^\circ$ . Wilkinson and Partridge warn their readers of the need not to take this coincidence for proven. It could easily turn out to be a statistical fluke. But evidently there is plenty of scope, and incentive, for detailed statistical analyses of where quasars are to be found.

The question of what quasars are remains remarkably open. It seems, however, to be agreed that individual quasars cannot be single massive stars producing their red-shifts gravitationally. For that to be possible, the stars would have to be so massive and so dense that it is hard to see how large amounts of radiation could ever escape from them. Hoyle and Fowler<sup>7</sup> have suggested one way out of this difficulty by means of a model in which the quasar radiation is assumed to come from a cloud of gas trapped in the gravitational field of a large number of objects, stars or even neutron stars, bound together by their mutual attraction. Objects like these need not be placed at the distant parts of the Universe. Their production of energy could, for example, be accounted for by interactions between the stars which they include, which in turn implies a structure dense enough for interactions between them to be frequent.

This, however, is only one of many possibilities. Other suggestions have been based on the observation that the radiation from quasars may fluctuate rapidly in a comparatively short time. There is no doubt that, in these circumstances, the region in which the fluctuation occurs must be small by astronomical standards. The size of the region from which the radiation comes must, indeed, be less than the distance travelled by light in the time in which the fluctuations occur. This implies that the sizes of those regions of space responsible for the emission of radiation must be measured in light years or even light days, or that their size lies between 1,000 million km and perhaps 100 times as much. These distances are comparable with the dimensions of the Solar System and are, of course, much smaller than those of the nebulae. The rapidity of the optical variation of the quasar 3C 446 led Cannon and Penston<sup>8</sup> to suggest that the energy comes from a small central source radiating energy at a more or less constant rate, and that clouds of gas would from time to time prevent the escape of the radiation. This view is consistent enough with the argument that quasars should be small objects. The trouble, however, is that if the clouds of gas which obscure the radiation from the central source are moving with the comparatively small velocities which observation suggests are also necessary, the clouds will have to be placed about as far from the central object as the Earth is from the Sun, which will in turn enormously exaggerate the problem of explaining how it is possible for such an object to radiate energy at a rate calculated by Burbidge<sup>9</sup> to be such that each  $\text{cm}^3$  of space around the quasar would contain roughly two Joules of energy. In conditions like that the radiation would be self-destructive, and much of its energy would be carried away by electrons accelerated by the inverse of the Compton effect.

One way out of the difficulty of explaining how quasars can seem at once small and exceedingly energetic is the possibility that they may be comparatively transient phenomena. There is, of course, no means of testing such a possibility directly without



waiting a considerable time to see what happens to individual quasars in the course of several years. Recently, however, a group at the Mullard Radio Astronomy Observatory in Cambridge has been able to show that a certain radio source with all the properties of a quasar has no small blue star associated with it<sup>10</sup>. The question naturally arises of whether the optical component of what may at one stage have been a simple quasar is by now burned out. The difficulty with this interpretation, of course, is that it implies that quasars should be comparatively common phenomena.

This exemplifies the perplexing character of the problem of quasars. Time after time it seems as if some bright idea will provide a rational explanation of the radiation from the quasars, and with comparable regularity it turns out that the explanation entails some unacceptable or at least distasteful consequence. Although opinion seems to favour most of all the possibility that quasars are likely to be exceedingly compact nebulae beyond the limits of this Galaxy, that hypothesis has the status of an intelligent guess.

<sup>1</sup> Smith, F. G., *Nature*, **213**, 967 (1967).

<sup>2</sup> Horton, P. W., and Daintree, E. J., *Nature*, **215**, 917 (1967).

<sup>3</sup> Longair, M. S., and Scheuer, P. A. G., *Nature*, **215**, 919 (1967).

<sup>4</sup> Strittmatter, P., Faulkner, J., and Walmesley, M., *Nature*, **212**, 1441 (1966).

<sup>5</sup> Rees, M. J., and Sciama, D. W., *Nature*, **213**, 374 (1967).

<sup>6</sup> Wilkinson, D. T., and Partridge, R. B., *Nature*, **215**, 719 (1967).

<sup>7</sup> Hoyle, F., and Fowler, W. A., *Nature*, **213**, 373 (1967).

<sup>8</sup> Cannon, R. D., and Penston, M. V., *Nature*, **214**, 256 (1967).

<sup>9</sup> Burbidge, G., *Nature*, **214**, 1213 (1967).

<sup>10</sup> Macdonald, G. H., and Kenderdine, S., *Nature*, **215**, 603 (1967).

## GEOPHYSICS

## Continental Drift Comes True

By the end of the century it will seem quite remarkable that the doctrine of continental drift, hotly disputed for half a century since Wegener launched it on a sceptical profession, should have been generally accepted as correct in just about half a decade. Yet this is what has happened. Several independent lines of evidence have conspired to make continental drift respectable, and in the process have borne out Wegener's own statement, quoted regretfully in the preface to his book *Die Entstehung der Kontinente und Ozeane* (Vieweg, Braunschweig, 1928), that the question of continental drift would not be decided by the necessarily qualitative arguments of the geologists and the palaeobotanists but only when it was possible to apply some quantitative geophysical argument to the problem. But the past few years have done much more than make continental drift acceptable. They have also provided evidence to show how the drifting apart of the continents is linked with the existence and the activity of the great ocean ridges—the mid-Atlantic ridge, for example. Indeed, the fashionable phrase is no longer continental drift but ocean floor spreading. Evidently the years ahead will be great fun for those who will be concerned to relate phenomena such as earthquakes and even mountain building with the natural forces, not yet clearly understood, which drive the continents apart.

The origin of the most recent wave of controversy about continental drift lies in the work in the early fifties on the permanent magnetization of all kinds of rocks. Before that, even those temperamentally in favour of radical causes had been forced to admit that the topographical, geological and botanical arguments in favour of continental drift could not in themselves be conclusive. Given the lack of a plausible mechanism whereby the continents might be driven along, it was clear that the circumstantial evidence would have to be quite convincing. Palaeomagnetism has made good the earlier deficiencies of the case for continental drift, though it is only within the present decade that those who work on rock magnetism have been agreed that movement of the continents is the best way of accounting for their measurements.

To be fair, the interpretation of the earliest palaeomagnetic measurements was complicated by the co-existence of two anomalies. First, the direction of the permanent magnetization of a rock might differ markedly from the direction of the contemporary magnetic poles, which suggests that the poles have not always been where they seem to be now or, what comes to the same thing, that the land masses have moved, at least by rotation, since they were formed. But the direction of permanent magnetization can also be entirely reversed, which implies a quite unexpected geophysical phenomenon. It is understandable that people should have sought to avoid both sets of unpleasant consequences by asking whether the permanent magnetization of the rocks was really as permanent

as it was assumed to be—a vein of scepticism which has now been worked out. Both field reversals and the movement of land masses are real phenomena. Studies of successions of lava flows, as in Iceland, have made it possible to trace in detail several successive field reversals, and there is now an even more dramatic demonstration of several cycles of field reversal in long cores recovered from oceanic sedimentary deposits by the Lamont Geophysical Observatory, New York.

Using the magnetic data to work out the positions of the land masses in the geological past has necessarily been a more difficult task, however, for this has entailed the matching together of the apparent movement of the poles as seen from several places on the present surface of the Earth, which raises problems of correlating in age rocks in widely separate places. So, in a sense, it is not surprising that even though maps showing how the poles seem to have wandered as seen from one place on the Earth have been drawn for several years, it is only within the past three or four years that these have presented a convincing picture of the past 100 million years or so.

But how, in any case, could the continents move? It seems that the late Sir Arthur Holmes was the first to suggest that there may be convection currents in the mantle of the Earth involving the bodily movement of material on a time scale long enough for plastic flow to be sustained. More recently, Runcorn invoked convection currents as an explanation of continental drift<sup>1</sup>, and put forward a detailed scheme by means of which a small number of convection cells reach down to the base of the mantle. On this view, one possibility would be that material from the mantle would be carried to the surface of the Earth at the mid-oceanic ridges, and that the continents bordering the Atlantic on west and east would then be found at the places where convecting material would be carried downwards again. Although it now seems that mantle deep convection is unlikely because the viscosity of the deep rocks is too great, the complex of theories now coming into its own is intimately bound up with convection currents of some kind.

The great novelty in the past year or two has been the direct study of the consequences of these lateral movements of material in the surface of the Earth. The starting point seems to have been an argument by Dietz<sup>2</sup> that if, indeed, material from the mantle is welling up towards the surface at the mid-ocean ridges, material should be flowing laterally away from the ridges, from which a number of observable consequences follow. Dietz in 1961 drew attention, in particular, to the way in which ocean floor spreading should mean that the ocean floor is geologically young, and, as it happens, much of the confidence now placed in the reality of continental drift stems from accurate dating of sediments and other features associated with the mid-oceanic ridges. Then Dietz also pointed out that if the mid-oceanic ridges are indeed the sites at which



two long ribbons of material from the mantle are being continually extruded on to the ocean floor, and because these rocks have characteristic magnetic properties, it would not be surprising to find that the pattern of magnetic anomalies associated with traverses of the mid-oceanic ridges would have much in common the whole way along. It was not very long before Vacquier and his colleagues found a striking north-south regularity in surveys of magnetic anomalies off the continental shelf of North America. In other words, accurate magnetic surveys capable of showing up variations of field strength of a few gamma (one gamma =  $10^{-5}$  gauss) seemed to yield contour lines running predominantly north-south, or roughly parallel to the mid-Atlantic ridge lying much farther out to sea.

But why, in any case, should there be such pronounced magnetic anomalies over the deep ocean bottoms? By 1963 it was evident that the magnetic field strength could fluctuate by five or six hundred gamma with a few tens of kilometres immediately above the mid-Atlantic ridge. It is hard to account for variations on such a small scale by differences in the composition of material. So may it be that blocks of material on the ocean floor are magnetized in different directions? This was the question which Vine and Matthews<sup>3</sup> asked themselves, and they pointed out that if, indeed, the direction of the magnetic field of the Earth reverses at intervals, then successive stripes of rock on the ocean floor should be magnetized in opposite directions.

The accuracy of this prediction has now been amply confirmed by several groups of workers at the Lamont Geological Observatory, New York. A year ago, for example, Pitman and Heirtzler<sup>4</sup> described the results of several traverses of the Pacific Antarctic Ridge in the South Pacific on which depth and magnetic surveys were carried out. It turns out that both surveys yield patterns which are remarkably symmetrical on both sides of the ocean ridge, and it is possible to determine with surprising clarity the peaks in the magnetic pattern which lie on each side of the oceanic ridge and which correspond to the same condition of the Earth's magnetic field at some epoch in the past.

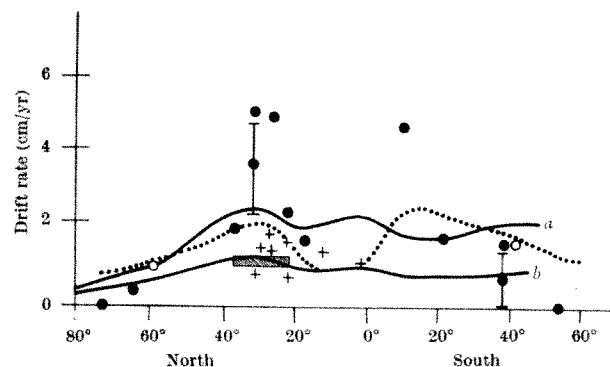
This is, of course, a remarkable conclusion. What it implies is that by simply driving a ship on a course at right angles to a mid-oceanic ridge, it is possible to recover a magnetic record which represents on one chart both the reversals of the Earth's magnetic field in the geological past and the spreading of the ocean floor. And because the times at which reversals of the Earth's field occurred are now known with considerable

accuracy—there have been 35 reversals of the field in the past ten million years or so—it is possible without recovering samples from the ocean floor to fit a time scale to the process of ocean spreading. Moreover, by following the pattern of magnetic anomalies sufficiently far back in time, it should be possible to go at least some way towards a reconstruction of the lines on which the continents have drifted away from each other in the past hundred million years or so. The conclusions drawn from these arguments have been clinched by palaeological sediments from the Atlantic bottom which have been recovered by Ewing and his collaborators<sup>5,6</sup>; for one thing, these provide a more continuous record of the variation of the Earth's magnetic field than can be obtained from the necessarily intermittent flow of lava on the surface of the Earth. Furthermore, it has been possible to show directly how the date of the sediments on the ocean floor increases steadily away from the centre of an oceanic ridge.

All this amounts to a consistent picture of how the ocean floor is spreading. One way and another, it appears that the rate of spreading can vary between 1 and 5 cm a year from one place to another along a mid-oceanic ridge. These figures agree well with the estimates which have been made by the examination of samples from the ocean floor of the dates on which the submarine rifts began to function. On the whole, it seems as if there have been 250 million years since the beginning of the mid-Atlantic ridge, which would in turn agree well with the geological evidence which suggests that if Europe and Africa have drifted apart from the Americas, the process probably began in the Upper Cretaceous 250 million years ago or thereabouts.

The accompanying diagram shows various estimates by Vogt and Ostengo<sup>7</sup> of the rate of ocean floor spreading along the length of the mid-Atlantic ridge. Rates are calculated, in cm/yr, from the assumption that the whole Atlantic has opened in  $120 \times 10^6$  yr (solid line *a*) and  $240 \times 10^6$  yr (solid line *b*). The open circles represent estimates by the Lamont group based on magnetic reversals. The solid circles are rates of spreading calculated from the ages of the oldest rocks on certain oceanic islands in the Atlantic.

Inevitably, the recognition that ocean floor spreading is a real and measurable phenomenon will prompt many further studies. One obvious need is to learn more about the pattern of convection, and it is possible that seismology or even deep drilling will identify which layers of the outer mantle are involved. Questions will also arise about the formation of crustal material and what Heezen has called "micro-continents" such as the Seychelles. One interesting suggestion already to emerge is that the strange buried islands called guyots which seem like atolls buried some hundreds of metres in the oceans near mid-oceanic ridges may have arisen from volcanic atolls originally near the top of an oceanic ridge which have moved down its slopes under the influence of crustal spreading<sup>7</sup>.



<sup>1</sup> Runcorn, S. K., *Nature*, **193**, 311 (1962).

<sup>2</sup> Dietz, R. S., *Nature*, **190**, 854 (1961).

<sup>3</sup> Vine, F. J., and Matthews, D. H., *Nature*, **199**, 947 (1963).

<sup>4</sup> Pitman, W. C., and Heirtzler, T. R., *Science*, **154**, 1164 (1966).

<sup>5</sup> Ewing, M., LePichon, X., and Ewing, J., *J. Geophys. Res.*, **71**, 1611 (1966).

<sup>6</sup> Ewing, J., Worzel, J. L., Ewing, M., and Windisch, C., *Science*, **154**, 1125 (1966).

<sup>7</sup> Vogt, P. R., and Ostengo, N. A., *Nature*, **215**, 810 (1967).

## SPACE RESEARCH

# Rocket Satellites and Research

In the United States, roughly \$5,000 million a year is being spent on space research and development of all kinds and much of it—\$694 million in the current financial year—is devoted to what may be described as research unrelated to practical objectives. It is inevitable that expenditure on this scale should have influenced considerably the pattern of academic research in the United States and elsewhere, and that it should have reaped a rich harvest of data. Although there may be something in the argument that similar benefits could have been won more cheaply in other ways, nobody denies the value of what has been done in the past decade by rockets and satellites. Dr Homer Newell of the National Aeronautics and Space Administration had no need to overstate his case when he applied on behalf of the administration to Congress for funds with which to keep NASA going for another year.

Something of the scale of the operation in support of research being carried out in the United States can be gathered from the numbers of rockets launched under the Space Science and Applications Program in recent years. Since the beginning of the sixties, the numbers of rockets have fluctuated around an average of 14 or 15, and are expected to reach a peak of 20 during the current year. These figures do not include the great numbers of sounding rockets, costing \$22 million a year, launched from the United States for a variety of reasons, often in meteorology.

What have been the results? Dr Newell claimed that the rockets and satellites had provided new tools for the study of old problems in geophysics, had opened up entirely new areas of research such as in the belts of radiation surrounding the Earth, and had for the first time made it possible to study directly the physics of the plants in the Solar System. He went on to suggest that space research would resolve a number of important issues in physics such as the correctness or otherwise of current notions of relativity, and predicted that space techniques would have an equally profound influence on astronomy. The discovery of X-ray stars is the proudest boast in this connexion.

In reality, of course, the discovery of X-ray sources in the sky was made with comparatively simple rockets (in 1962 by Herbert Friedman) at the Naval Research Laboratory in Washington. The first sources to be recognized were in Scorpio, Cygnus and Taurus. Altogether more than 20 X-ray sources are now known. They lie in the plane of the Milky Way, which implies that they belong to the galaxy. (The external galaxy M87 also emits X-rays.) Occultation of the X-ray sources by the Moon has shown that they are not starlike, but, rather, distributed. Some are now known to be old novae. Dr Newell in his evidence pointed out that a great deal of work had been carried out with

sounding rockets provided by NASA. By now, of course, other agencies elsewhere in the United States are using rockets like these for the study of X-ray sources.

X-rays from the Sun are another feather in NASA's cap, although these were first discovered a decade ago before the space agency had been established and when the US Navy was supporting the research directly from its own funds. In recent years photographing of the Sun with X-ray plates carried in rockets has become sufficiently a matter of routine for useful correlations to be made between X-ray emission and other features of the solar surface—sunspots, for example. One part of the programme being planned for and carried out consists of the orbiting solar observatories intended to carry large packages of instruments into long-lasting orbits about the Earth. But NASA is also planning what are called orbiting telescopes—devices which will be in part optical telescopes and in part radiotelescopes of various kinds.

The exploration of the Solar System has been undertaken largely with the help of long-ranging rockets launched from the Earth. As yet, however, it does appear that the data gathered by the Mariner II flight past Venus did not produce conclusive results about the constitution of the atmosphere of the planet. Rocket engineers are not the ones to blame for this. There is such a wide range of models for the atmosphere of Venus, and such a variety of estimates of the thickness of the atmosphere, that it seems that rocketry will not be able to contribute much more than conventional forms of astronomy at least until it is possible to launch rockets into orbits about Venus.

NASA's contribution to the understanding of Mars depends on the flights of rockets past the planet, the first of these in 1965 at a distance of 4,000 miles. This has produced photographs with a resolution of roughly two miles. It will be interesting to see how quickly the budgetary pressures to which it is now being subjected will be eased to the point at which NASA will be able to use larger Saturn rockets for launching their ambitious devices towards Mars. One way and another, it seems that NASA hopes its contribution to the accurate description of the planets lies in the future—possibly the comparatively near future.

In its evidence to Congress, however, NASA can make a strong case for the value of its work in throwing light on the region called the magnetosphere around the Earth in which the Earth's magnetic field is a dominating influence, and on the interaction between this region and the flux of atomic particles from the Sun which is called the Solar Wind. The magnetosphere as such is by now, of course, an ancient concept. It has been a feature of a great many theories to explain such

phenomena as the variation of the strength of the Earth's magnetic field as observed on the ground with activity on the surface of the Sun. But van Allen in 1957 discovered that atomic particles, protons and electrons, are trapped by the magnetic field of the Earth. In the years since then, a great many space rockets have been able to map out not merely the average shape of the trapped radiation but also have been able to describe its variation from one time to another. But the Solar Wind is something else again—nothing was known of it but some speculation before 1958. It consists of electrified particles travelling outwards from the Sun with speeds of 300 and 700 kilometres a second when they cross the orbit of the Earth. The Solar Wind has something between 3 and 60 protons in each cubic centimetre. Because of the rotation of the Sun, it sweeps out in a spiral fashion which is evident enough now that rockets are able to chart the directions in which the Solar Wind appears to be moving with considerable precision.

The interaction between the magnetosphere and the Solar Wind is by now familiar; the speed of the outward moving stream of particles is supersonic in the medium, which in turn implies the formation of a shock wave at the outer boundary of the magnetosphere. In practice, the existence of the Earth's magnetic field provides a means of warding off the Solar Wind, which instead sweeps back behind the Earth to leave a wake or shadow stretching backwards for perhaps several hundred Earth radii. So much at least is apparent from the record of the flight of the rocket Pioneer VII launched in the autumn of 1966. Plainly, until more is known about the balance of forces within the Solar Wind and the magnetosphere, rockets will play an indispensable part in the delineation of its properties.

There remains the contribution which NASA and its works have made to the understanding of the atmosphere of the Earth. As soon as the first satellites appeared a decade ago it was clear that their motion would be markedly influenced by such things as the density in the high layers of the atmosphere. The first suggestion that the density above 100 kilometres was greater than had first been expected has now been followed by the recognition that temperature increases more rapidly than had been thought, at least in the first 200 kilometres above the Earth. Thereafter, and out to whatever may be the limits of the atmosphere, the temperature remains substantially constant, but at a value which may vary between 1,000° C and 1,500° C according to the activity of the Sun.

Dr Newell is entirely right to claim, as he does, that NASA has contributed directly to the spate of discovery by rockets and satellites which have been launched from the United States in the past decade. Satellites such as Explorer IX (1961), which consisted of a sphere 12 feet across, were designed deliberately as a means of measuring atmospheric density. Explorer XVII (1962) carried mass spectrometers for the first time, and the use of those instruments confirmed an earlier suggestion by Nicolet that there is a layer of the atmosphere in which helium predominates above 1,000 kilometres. More recently, NASA has been launching much heavier satellites such as the orbiting geophysical observatories, the first of which appeared in 1964, and the similar devices intended to carry out astronomical work. Devices like these will consume

an increasing share of the budget which NASA has to spend on space research. In the present financial year, for example, \$20 million will be spent on orbiting geophysical satellites to be launched at various times in the next five years, and \$40 million on astronomical observatories. NASA itself would not wish to deny how much uncovenanted use has been made of its rockets and satellites in the past decade—it remains a striking fact that some of the most interesting studies of such things as the variation of the density of the upper atmosphere from one time to another have been made by people not directly concerned with the design of experiments for inclusion in satellites. There are some circumstances when access even to such rudimentary information as the orbital elements of satellites can be of value in throwing light on the character of the upper atmosphere.

In the years ahead, it is plain that NASA's scientific programme—the enterprise to carry men to the Moon comes under another heading in the budget—will be increasingly concerned with matters such as the despatch of rockets towards the planets and the Moon. There is, for example, the Voyager programme as a part of which rockets will be sent into orbits around Mars and Venus or landed without damage on the planets. The first flight of such a rocket will be towards Mars in 1973, and it is a striking commentary on the length of time over which plans for space research must be laid that the design of the Voyager satellites will consume several million dollars in the current financial year. By means of these programmes it is hoped that it will be possible to land packages of instruments weighing up to half a ton on the surfaces of the planets Mars and Venus so as to continue there the kind of work at present being done or to be attempted in the next few years on the surface of the Moon. Between now and 1973, however, NASA will use less sophisticated means of sending rockets to Mars and Venus under the heading of what is called the Mariner programme, by means of which it has already been possible to gather important information about the character of the atmosphere of Venus. Photographs of the surface of Mars have come out of the same Mariner programme.

Not all the new plans will cost enormous sums of money, however. There is, for example, an imaginative scheme designed by a group at the Massachusetts Institute of Technology intended to use the comparatively cheap Scout rocket for sending instruments into an orbit about the Sun. The first launchings of these devices may be made in 1968. Before then, at least two more Earth satellites intended to gather information about the Sun will have been put into orbits, and it is intended to use these devices to keep a continuous and detailed watch on the Sun during the period of maximum solar activity in 1969.

One feature of the NASA plan which will not be much welcomed in the universities is the way in which the growing pressure on the budget as a whole—and particularly the pressure to reduce the cost of sending men to the Moon—has forced a reduction of the amounts of money being allocated to the support of university programmes in the United States. Training grants in the present financial year are down to less than a third of what they were two years ago. NASA will spend \$20 million on the support of work in universities in the year ahead, which is less than a half of its expenditure two years ago.

## MOLECULAR STRUCTURE

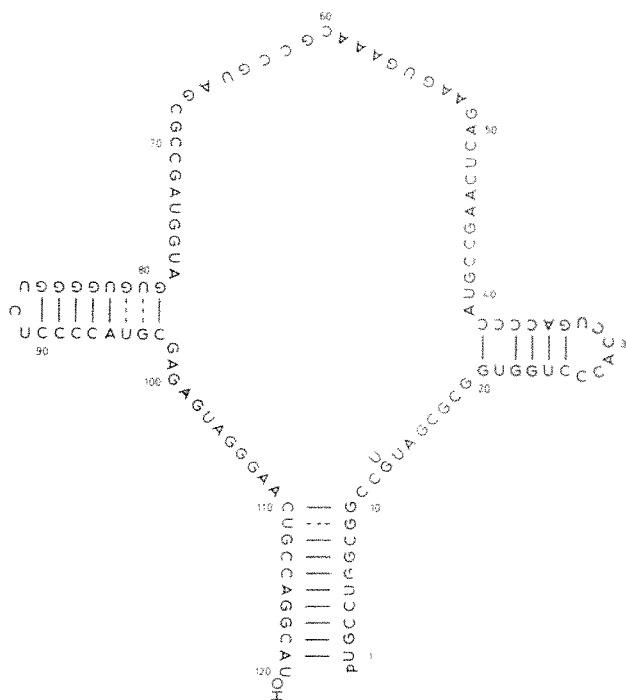
# How One RNA Molecule is Strung Together

It is now almost a matter of routine to work out how the amino-acids in a protein molecule are arranged along its length—and there are even machines which are said to do the job automatically<sup>1</sup>. Working out the arrangement of units along the length of a nucleic acid molecule is much more difficult for several reasons. For one thing, in a protein molecule there may be up to twenty different amino-acids, many of which are easily distinguishable from the others; in nucleic acids such as DNA or RNA, by contrast, there are usually only four possible different units from which chain-like molecules may be assembled, which means that if a molecule is broken chemically into smaller pieces, these may be difficult to tell apart. One consequence is that the structures of all but the smallest and simplest molecules of nucleic acids have so far been inaccessible except by inference.

This is why it is an important landmark in molecular biology that Dr F. Sanger and his colleagues<sup>2</sup> at the MRC Laboratory of Molecular Biology at Cambridge have been able to work out the sequence in which no fewer than 120 sub-units are arranged in a particular molecule of RNA. Their work carries significantly further the work of Holley and his collaborators<sup>3</sup>, who showed two years ago how to obtain the sequence of sub-units of a molecule of RNA half the size of that which has now been analysed but in which the occurrence of certain easily distinguished and unusual units served to simplify the problem.

The material which Sanger and his colleagues have used is interesting in its own right, for they have chosen for their chemical analysis a molecule of RNA which is a functional component of the molecular apparatus for manufacturing protein in the bacterium *Escherichia coli*—the workhorse of molecular biology. Specifically, the molecule is the smallest of the three nucleic acid components of the ribosomes at which protein molecules are assembled from amino-acids. Altogether it consists of no fewer than 120 sub-units strung together.

The technique used for this complicated analysis will undoubtedly be widely used in the years ahead. So as to be able to work with extremely small quantities of the RNA, Sanger and his colleagues have used material in which the nucleotide units in the chain—there are four kinds of them altogether—are heavily laden with radioactive phosphorus-32. The next step is that already used in the analysis of simpler RNA molecules—they are broken into smaller pieces by treatment with forms of the enzyme ribonuclease which have the effect of consistently breaking the RNA molecule at the links between specific pairs of nucleotides in the chain. The various fragments have been separated from each other by various forms of chromatography which yield a two-dimensional pattern of spots on a piece of filter paper, each spot being recognized by its radioactivity and characteristic of some fragment of the whole molecule. An essential part of the job has been to find ways of incompletely breaking



down the RNA molecule by partial digestion with ribonuclease so as to be able to decide how the smallest fragments are linked together.

In many ways the whole procedure is closely analogous to the now standard methods used for working out the arrangement of amino-acid units in the long chains of protein molecules, and it is, of course, more than just a coincidence that Sanger played a central part in the development of those more than a decade ago.

The outcome of this analysis is embodied in the accompanying diagram. The symbols A, C, G and U stand for the four nucleotides adenine, cytidine, guanine and uridine which can be linked together to form molecules of RNA. The direction in which the units are strung together is identified by the chemical symbols for phosphorus and hydroxyl which appear at one or other of the two ends. The chemical analysis by itself provides only the order in which the nucleotide units are arranged along the chain, but it can be inferred from this sequence that the molecule has three separate regions which are cross linked by hydrogen bonding. In these regions the character of the nucleotides is such that they will of their own accord coil into the spirals analogous to those in a DNA molecule.

Sanger himself has pointed out that substantial sections of the molecule repeat each other—the sections beginning with the units numbered 10 and 61, for example—and he suggests that this may have an evolutionary significance. At one time there may have been organisms in which the corresponding RNA molecules were only half the size. But it may also be

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## PROTEIN STRUCTURE

# Structure and Function of Proteins

THE past few months have brought a rich harvest for those concerned with the three-dimensional structure of protein molecules. Within a few weeks, earlier this year, structures were obtained by X-ray diffraction for several important molecules—the enzyme ribonuclease (in two versions) and the enzyme chymotrypsin. And there is the promise of similar information about other enzyme molecules soon to come.

The interest of this work is two-fold. It is important to know why large protein molecules, with 100, 200 or more amino-acid units strung together in a chain, should consistently form themselves always into the same specific three-dimensional pattern. What is it about the chemical constitution of a protein molecule that determines what its overall shape should be? But there are more practical reasons than these for hoping for a great deal from the detailed information now accumulating. In particular there is at least a sporting chance that detailed structures of some enzyme molecules will help to explain how these molecules function in real life.

At the beginning of this year, only the structures of myoglobin and lysozyme were known with such detail that the positions of individual atoms in a three-dimensional structure could be placed with certainty. (A good deal is also known about the three-dimensional structure of the haemoglobin molecule in various stages of its combination with oxygen.) Molecular structures can be obtained for protein molecules by X-ray diffraction only when crystalline forms of them can be prepared. To give the molecules characteristics which can easily be identified in the X-ray diffraction patterns, it is necessary somehow to prepare crystalline derivatives in which heavy atoms such as mercury are associated with the molecules almost as if in a chemical combination. The technique for carrying out this kind of analysis was first developed at the Cavendish Laboratory, Cambridge, by Dr M. F. Perutz and, later, by Dr J. C. Kendrew.

The X-ray analysis of the ribonuclease structure may be of especial value in working out a relationship between structure because of the detailed chemical studies which have already been carried out. Altogether there are 124 amino-acid units in the protein chain, and the chemical properties of the molecule indicate that the chain is folded in such a way that it is linked internally at four places.

But ribonuclease is also important because of its biochemical function—it is a means by which nucleic acids are hydrolysed *in vivo* and *in vitro*. Obviously there is great interest in the details of precisely how it functions.

In March, Carlisle and his colleagues<sup>1</sup> described a structure for ribonuclease *A* at the comparatively low resolution of 5.5 Å. This was closely followed by the publication of a structure at 2 Å resolution by Harker and his colleagues<sup>2</sup>, and by Wyckoff and Richards' announcement<sup>3</sup> of a molecular structure, at 3.5 Å resolu-

tion, of ribonuclease *S*—a derivative of ribonuclease *A* produced by subtilisin. The degree of agreement between the structures proposed by Harker's group and by Wyckoff and Richards is gratifying, especially because the two groups used different heavy atom substitution derivatives and different solvents. Further, the differences between the Harker and Carlisle structures, which arose from differences of interpretation rather than of data, have now been reconciled.

The polypeptide chain of ribonuclease *A* is folded to form a more or less kidney-shaped molecule, 38 × 28 × 22 Å, with a deep cleft in the centre. On either side of the cleft the loops of the polypeptide chain are linked together by chemical bridges, and there is a short segment of the protein molecule standing apart from the rest of the molecules, and overhanging the cleft.

What is the chance of relating this three-dimensional structure to the biochemistry of ribonuclease? By chemical studies, the two American groups have been able to show that the cleft of the molecule is indeed the site of the activity of the enzyme, where there is now seen to be a cluster of groups derived from the amino-acids lysine and histidine. These units in the long protein chain seem to be intimately involved in the functioning of the enzyme—for example, they are equipped with side chains which can combine with materials which inhibit the enzyme, presumably by filling up the cleft of the molecule with inactive chemical groups.

All this agrees well with chemical evidence collected much earlier, and which had always been hard to understand in the absence of a three-dimensional structure of the molecule. For one thing, it was clear that two histidine residues near the opposite ends of the protein chain are both a part of the active centre<sup>4</sup>, which implies that the molecule is somehow wrapped up on itself. Hirs<sup>5</sup> showed that a lysine group a third of the way along the chain is essential to the enzyme activity. How could such widely separate parts of the molecule be simultaneously involved? That is a question that the biochemists were forced to ask themselves. It now emerges that the two histidine groups are close together on one side of the cleft in the molecule, and that the lysine group is placed directly opposite.

These correlations are comforting, but they are only the beginning of a full explanation of how the enzyme molecule functions. One of the difficulties with ribonuclease is that it has not yet been possible to make a crystalline form free from extraneous chemical groups attached to the active site. There has been more success with lysozyme, which also has a cleft in the otherwise blob-like shape into which the protein chain is wrapped, for there it has been possible to follow the changes of shape which occur when the enzyme molecule associates with molecules of substrate or inhibitor. In the long run, the objective will be to understand

how the parts of enzyme molecules remote from the active sites somehow play a part in enzyme function.

The shapes of protein molecules will not easily be understood in detail, but a few general principles seem to have emerged from a comparison of all the X-ray structures now available. One of the structures most recently to have been determined—that of chymotrypsin—resembles the structure of molecules such as lysozyme in that the long protein chain is curled up in such a way that the amino-acid groups which are chemically hydrophobic tend to be buried within the molecule. By contrast, hydrophilic groups lie on the outer surface. That is reasonable enough, although it does raise the question of how the shape of a protein molecule is influenced by such things as the acidity of the medium. It will also at some stage be important to know why the structure of chymotrypsin differs from that of most of the other proteins so far mapped in detail in that only a very small part of the chain is coiled into the helical form which protein molecules tend naturally to form.

What lies ahead? To begin with, the structures of more protein molecules will be determined, and it is only seemly to remember that a full-scale X-ray analysis is still a tedious and even an uncertain venture. That said, detailed structures of the protein antibody molecules would clearly be of the greatest interest now that the chemistry of these materials is being understood. And for the rest it is abundantly clear that the X-ray structures of protein molecules which have appeared in the past few months are not an end point in themselves but only incentives for other investigations.

<sup>1</sup> Avey, H. P., Boles, M. O., Carlisle, C. H., Evans, S. A., Morris, S. J., Palmer, R. A., Woolhouse, B. A., and Shall, S., *Nature*, **213**, 557 (1967).

<sup>2</sup> Kartha, G., Bellow, J., and Harker, D., *Nature*, **213**, 862 (1967).

<sup>3</sup> Quoted by Barnard, E. A., *Nature*, **215**, 6 (1967).

<sup>4</sup> Moore, S., and Stein, W. H., *Methods in Enzymology*, **6**, 819 (1963).

<sup>5</sup> Hirs, C. H. W., *Brookhaven Symp. Quant. Biol.*, **15**, 154 (1962).

<sup>6</sup> Matthews, B. W., Sigler, P. B., Henderson, R., and Blow, D. M., *Nature*, **214**, 652 (1967).

## CELL BIOLOGY

# Synthesis of an Enzyme

Nirenberg and Matthaei provided an invaluable method for the investigation of protein synthesis when they discovered that in cell free systems—essentially the pulp of ground-up cells containing ribosomes, transfer RNA and other components necessary for protein synthesis together with an added source of energy—peptide bond formation occurs and amino-acids are incorporated into polypeptides under the direction of endogenous or added messenger RNA. As a result, cell free systems have been increasingly used for studies of protein synthesis *in vitro* and they have yielded otherwise unobtainable data. But there has always been a nagging doubt that since the conditions in a cell synthesizing protein are so different from those in a test-tube, what occurs *in vitro* in a cell free system may not occur *in vivo* within the cell. Such doubts were not diminished when it was found that in a cell free system programmed with a particular messenger RNA, the amino-acids incorporated into polypeptides vary with the ionic environment and especially with the concentration of  $Mg^{++}$  ions.

It was something of a relief, therefore, when it was shown that haemoglobin and some RNA phage proteins synthesized *in vitro* have a similar peptide composition to the natural proteins. Unfortunately, however, these proteins are not enzymes; they cannot be assayed for biological activity and so the fidelity of *in vitro* protein synthesis cannot be properly assayed. It is quite possible that even if a protein synthesized *in vitro* had the same amino-acid sequence as the natural product, it could not, in the artificial environment of a test-tube, fold to assume the secondary and tertiary structures which determine its biological activity. Hence the recent demonstration by Salser, Gesteland and Bolle (*Nature*, **215**, 588; 1967) of *in vitro* synthesis of biologically active  $T_4$  bacteriophage lysozyme assumes a double importance. Apart from providing an experimental system for precisely defining the components and conditions necessary for synthesis of an active enzyme their result raises the general level of confidence that can be placed in *in vitro* observations.

$T_4$  lysozyme, an enzyme made by the phage fairly late in the infection cycle, causes the dissolution of the bacterial cell wall and the release of the progeny particles. It has several obvious advantages for study *in vitro*. There is a very sensitive assay for it; it consists of a single polypeptide chain and is a fairly small protein (molecular weight 18,000) and so must be coded for by a small messenger RNA molecule. This is important because it increases the chances of isolating the messenger RNA undegraded.

Salser *et al.* isolated the RNA made in phage infected *E. coli* and used this to programme a cell free system. The protein made was assayed for lysozyme and showed activity. Moreover the amount of lysozyme made *in vitro*, about 4 per cent of the total protein synthesized, is similar to the amount made *in vivo*. Several controls showed the enzyme had been made *de novo*. For example, RNA from cells infected with a mutant phage that lacks the gene for lysozyme failed to stimulate lysozyme synthesis *in vitro*, and puromycin and chloramphenicol, drugs that inhibit protein synthesis *in vivo*, abolished the *in vitro* synthesis of lysozyme.

It is clear from these experiments that in the environment of a cell free system, the translation of the information encoded in RNA into the sequence of amino-acids in a protein can be sufficiently accurate to yield an active enzyme.

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that this symmetry has something to do with the geometry of the ribosomes from which the RNA molecule has been derived<sup>1</sup>. Another feature of the shape of the molecule to which Sanger has drawn attention is the occurrence of internal regions in which the molecule is coiled into a spiral form. In this respect the long RNA molecule has something in common with the structure of the smaller molecules of what is called transfer RNA first worked out by Holley and his collaborators, the function of which seems to be to involve individual amino-acid molecules in the construction of a protein.

<sup>1</sup> See *Nature*, **214**, 865 (1967).

<sup>2</sup> Brownlee, G. G., Sanger, F., and Barrell, B. G., *Nature*, **215**, 735 (1967).

<sup>3</sup> Holley, R. W., Appgar, J., Everett, G. A., Marquisee, M., Merrill, S. H., Penswick, J. R., and Zamir, A., *Science*, **147**, 1462 (1965).

<sup>4</sup> *Nature*, **215**, 690 (1967).

## CYTOLOGY

# Organization of Chromosomes

As the genetic code is solved in more and more detail the organization of the genes on the chromosome is also being closely attended to. A model proposed by Callan<sup>1</sup> involves serially repeated nucleotides, or gene copies, which form a terminal master gene with a number of slave genes. This model helps to explain the behaviour of some chromosomes during the reduction division—meiosis—which involves recombination between chromosomes and gives rise to the haploid gametes of diploid organisms.

Cytological evidence shows a very wide variation in the content of DNA—together with protein, the raw material of chromosomes—in the nuclei of gametes of related diploid organisms. This would be explained if chromosomes were multistranded—that is, if they contained several parallel DNA fibres—for some could then contain more fibres than others. This, however, seems unlikely on genetical evidence; multistrandedness does not fit in with semi-conservative DNA replication or with the phenomena of mutation and recombination, all reliably demonstrated long ago. This dissimilarity of DNA content is much more acceptably explained in the light of Keyl's observations<sup>2,3</sup> of the salivary gland chromosomes of *Chironomus*, a midge. He found that the ratios of DNA contents in homologous bands on the chromosomes conformed to a geometric doubling series 1 : 2, 4, 8 and 16. Keyl<sup>4</sup> has shown that this is not the result of unequal DNA replication in different bands, and the explanation seems to be that there is a serial replication of genetic units along a chromosome, different amounts of duplication leading to more or less DNA in a chromosome.

Callan<sup>1</sup> developed this idea to propose that each chromosome may have several copies of the same gene of which the terminal one, by virtue of its position, acts as the master gene while the others are slaves. This would explain Callan's own observations of the loops on lampbrush chromosomes of amphibian oocytes. The formation and regression of these loops in different places along the chromosome can be explained if after recombination between chromatids of homologous chromosomes, restricted to the master genes, the nucleotide chains of the slave genes are matched with those of the master gene, so that the slave nucleotides come to lie adjacent to those of the master. If necessary slave nucleotides are corrected so that their nucleotide sequences conform with those of the master. Each slave sequence is matched in turn, and the uncoiling involved would produce the loops which are observed. Only slave genes would act as templates for DNA synthesis, which would occur while they are exposed in the loop. This agrees with the known function of the loops, to produce ribonucleoprotein.

Whitehouse<sup>5</sup> has pointed out that the necessary separation of neighbouring master genes does not conform with data on recombination in *Aspergillus*

*nidulans* which imply the presence of only one copy of each master gene, each of which must be in contact with its neighbours. To reconcile this disparity Whitehouse has modified Callan's model and suggests that during meiosis, slave genes are removed from the chromatid by a crossover between the first and last members of the linear series of identical genes. While detached from their chromatid the gene copies are in the form of a closed circle. After crossing over has occurred between homologous chromatids the gene copies are replaced in their chromatid by the same mechanism by which they were removed. In a further modification of Callan's model, he suggests that only one nucleotide chain of a slave gene—the one which is a template for RNA synthesis—is matched to the master gene. Matching would always occur before the slaves function as templates.

This model now has to be tested experimentally. Hotta and Bassel<sup>6</sup>, for example, have electron microscope evidence that part of the DNA of the sperm of the boar, *Sus domesticus*, is circular, and some amphibian nucleoli have been shown to contain circular DNA<sup>7</sup>. Other such evidence should not be difficult to obtain. Chromosomes could be examined, by electron microscopy and special staining techniques, during the reduction division of meiosis, and this might reveal circular DNA molecules in all kinds of organisms. Similar investigations might also give more information about the proposed matching of master to slave in the amphibian lampbrush chromosomes. Further information about the part of these chromosomes which is responsible for the production of the nucleolus, and which is known to replicate many times and produce rings of DNA, could also be obtained. Reliable results from work such as this could be very instructive in substantiating this model for the organization of chromosomes, which is as yet very speculative.

This is an attractive model; it accounts for a variety of apparently contradictory genetical and cytological data. Fincham<sup>8</sup> has used it to account for the almost continuously graded variation of derivatives of mutable genes—genes which show a much higher frequency of mutation than is usual. As a result of a mutation in the master gene the linear gene series becomes internally heterozygous, for the slaves remain normal. The instability characteristic of mutable genes would be provided by the replacement of defective nucleotides in a master by normal nucleotides from a slave, and this could be brought about by intrachromosomal crossing over and/or correction of master by slave.

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## ECOLOGY

## Intense Selections and the Speed of Evolution

THE Darwinian theory of organic evolution is so widely accepted that it is easy to forget that the direct evidence for it is limited to a few cases in which evolution has been studied in the wild. Perhaps the best known of these is that of the peppered moth, *Biston betularia*, whose black form has become abundant in Britain since the Industrial Revolution.

It had been thought that advantageous characters only slightly increased the viability of an animal; selective advantage was low, and evolution invariably slow. Recent work has shown that this assumption was unjustified. A common British butterfly of grassland, the meadow brown, *Maniola jurtina*, bears a series of spots on the underside of its wing. The number of spots, which is controlled by many genes, varies from none to five. A team of workers led by Ford<sup>1</sup> discovered that the distribution of spot frequencies for female butterflies remained strikingly constant over most of England, despite great differences in climate. In south-west England, however, the spot frequency distribution was different, and a larger number of insects had two spots. At the boundary of these two areas one would have expected populations with intermediate spot-frequency distributions, and a gradual transition between the two main types of distribution over, perhaps, tens of miles. Instead, the boundary line was found to be a hedge, over which insects were flying freely.

This combination of stability over a huge area, and sudden transition to an alternative distribution, is a result of very high selection pressures. This has been confirmed by work in the Isles of Scilly, where each island is characterized by a particular spot-frequency distribution of *Maniola*. Great changes in spot-frequency distribution have been seen to take place within a year in association with ecological changes. On the other hand, under constant ecological conditions, when the population of *Maniola* was reduced to a few hundred individuals, in the next generation the population had regained not only a considerable size but also its characteristic spot distribution. The selection had been strong enough to overcome chance effects acting on the reduced population, such as the "accidental" death of insects possessing advantageous genes. It has been calculated that as many as 80 or 90 per cent of animals of certain spot-numbers may be selectively eliminated within a few months.

It is most unlikely that this very high death rate is caused by the spots themselves. The genes which produce the spots must be responsible for more important characters. Work is going on to discover what these are<sup>2</sup>. It has been shown that animals with more spots are susceptible, in some unknown way, to the attacks of a parasitic insect, *Apanteles*. There is also a possibility that the bacteria in the intestines of *Maniola* may be concerned with the selection against certain forms.

A similar situation has recently been shown to exist in populations of a grass, *Agrostis tenuis*<sup>3,4</sup>. This plant has various forms characterized by their hardiness in particular climatic or chemical conditions. Some are able, for example, to survive in windy conditions, or in the face of sea spray, or where the soil concentration of metals is high. These conditions may change over a few yards, as where slag from a mine has been heaped up in a field where the soil has not been contaminated by metal. Because of the very high selective pressures acting to eliminate forms which are inviable in the particular microhabitat, adjacent small populations of *Agrostis* consist of very different forms, even though there is cross pollination between the two populations. In this case, then, strong disruptive selection opposes and overcomes the effects of gene flow.

Another example of rapid evolution was investigated by Dobzhansky and Pavlovsky in California<sup>1</sup>. One of the chromosomes of the fruit fly, *Drosophila pseudoobscura*, is marked by the reversal of the genetic material at one point. Many such inversions, as they are called, appear in the chromosomes of this species, this one being known as Pike's Peak (P.P.). In the 1940s the frequency of this inversion was so low that it was effectively absent in California. Since then, however, there has been a rapid increase in the percentage of animals bearing the inversion, and a corresponding decrease in the percentage of animals bearing another important inversion. This major change in the genetic configuration of the species does not seem to have been a product of any gross meteorological change. Yet an increase in the frequency of P.P. has taken place in areas of the state with very different climates. Nor is the phenomenon associated with towns or industries; there seem to be no obvious differences between populations in country and town. Migration cannot explain the phenomenon, and it is unlikely that increased radiation levels are its cause. And it is a mystery not only why the increase came about, but how the P.P. inversion has become prevalent throughout so vast a space in so short a time.

It was long ago pointed out by Ford that evolution is most rapid, and can be most easily studied, when populations are small, when there exist two or more forms of the same species within the same habitat, or when the selective advantage of a character is great. It now appears that these conditions are fulfilled more often than had been supposed. It is therefore necessary to revise views of the speed of evolution and of the part which selection plays. Selection has always been thought to have been important, but in many cases it may be overwhelmingly so.

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<sup>3</sup> Gregory, R. P. J., and Bradshaw, A. D., *New Phytol.*, **64**, 131 (1965).

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## MOLECULAR BIOLOGY

## Isolation of Genetic Repressors

IN such a rapidly expanding and fiercely competitive field as molecular biology, comparisons are bound to be invidious, but few would deny that the outstanding achievements in the past 12 months have been the isolation of two repressors and the consequent confirmation of Jacob and Monod's model of genetic regulation.

A brilliant series of deductions led Jacob and Monod from their kinetic data on enzyme induction in *E. coli* to the operon concept and to a model of genetic regulation in which operon-specific repressor molecules prevent the expression of sets of genetic information—the operons—until these are required by the cell. The classic example is the negative control of the lactose operon which envisages a repressor molecule preventing the expression of three genes involved in lactose metabolism, until the inducer, normally lactose, is presented to the cell, whereupon the genes are derepressed, enzymes are made and the lactose metabolized. The simplest hypothesis to account for the action of inducer and repressor proposes that the repressor actually binds to the DNA, preventing transcription, and that the inducer by binding to the repressor somehow modifies it, perhaps by allosteric changes, so that it can no longer bind to the DNA.

Confirmation of this hypothesis, which has become central to much of molecular biology, awaited the isolation of a repressor molecule and there was understandably great excitement when Gilbert and Müller-Hill announced (*Proc. US Nat. Acad. Sci.*, **56**, 1891; 1966) that this had been done for the lactose operon repressor. The approach was elegant and direct, based on the minimal assumption that repressor and inducer interact. Gilbert and Müller-Hill reasoned that because an uninduced *E. coli* must contain some lactose repressor molecules, it should be possible to isolate them simply by fractionating the total cell protein. That done, the repressor fraction could be identified by its ability to bind inducer specifically and reversibly. The isolation of suppressible amber mutants of repressor genes had already shown that repressors are proteins, not nucleic acids. Gilbert and Müller-Hill made cell extracts of a mutant strain of *E. coli* with a lactose repressor which appeared to bind inducer more tightly than wild-type repressor, and after a crude fractionation they dialysed the fractions against a radioactive inducer, not lactose but an analogue. With minds sharpened by many months of negative results, they immediately grasped the significance of one fraction which bound inducer very slightly more than all the others above the background level. Further fractionation of this material yielded the repressor.

Two other mutant strains provided negative controls. First, Gilbert and Müller-Hill could not detect binding between their inducer and fractions from a strain of *E. coli* which always makes the lactose enzymes even in the absence of lactose. These cells evidently lack

the repressor. Second, all the fractions from a mutant strain which has a modified repressor molecule and is not induced *in vivo* by the inducer they were using failed to bind the same inducer.

The lactose repressor has a sedimentation coefficient of 7 to 8S, and an estimated molecular weight of 150,000 to 200,000. It is a rather large protein. Gilbert and Müller-Hill also estimated that the repressor corresponds to only about one part in  $10^4$  of the total cellular protein. This gives an indication of the magnitude of their achievement in isolating it.

Closely following this success, Ptashne (*Proc. US Nat. Acad. Sci.*, **57**, 306; 1967), from the same Harvard laboratory, isolated the  $\lambda$  phage repressor which is responsible for completely repressing the entire phage genome, allowing the establishment of lysogeny, and for the immunity of lysogenic bacteria to super-infection. By taking advantage of a strain of *E. coli* which can, after massive ultra-violet irradiation, maintain phage protein synthesis even though its own protein synthesis is greatly reduced, and by making use of super-infection immunity, Ptashne isolated the  $\lambda$  repressor using column chromatography and a double-labelling technique. He identified the repressor fraction as such by showing that it is not made by  $\lambda$  phage with an amber mutation in the repressor gene and that phage with a temperature sensitive mutation in this gene make a modified form. The  $\lambda$  repressor is an acidic protein and, with a sedimentation coefficient of 2.8S and an estimated molecular weight of 30,000, it is much smaller than the lactose repressor.

Ptashne has gone on to show that the  $\lambda$  repressor does indeed bind to DNA (*Nature*, **214**, 232; 1967). A phage known as  $\lambda$  imm<sup>434</sup> lacks a small region of the wild-type genome including both the repressor gene itself and the site which determines the sensitivity of the phage to  $\lambda$  repressor. In other words,  $\lambda$  imm<sup>434</sup> neither makes  $\lambda$  repressor nor is repressed by it. The protein Ptashne had isolated and identified as the  $\lambda$  repressor binds to  $\lambda$  DNA but not to  $\lambda$  imm<sup>434</sup> DNA. This simple experiment not only proves that the isolated protein is in fact the specific repressor but also strongly suggests that the repressor acts by binding directly to DNA and thus preventing transcription. Further support for this conclusion comes from Gilbert and Müller-Hill's demonstration that the lactose repressor binds to lactose operon DNA and moreover can be removed from the DNA by an inducer. Neither of these observations, of course, proves that the binding of repressor to DNA prevents transcription, but Gilbert and Müller-Hill are now in a position to show this unambiguously with *in vitro* experiments.

There is no doubt that this important work has opened the way to a complete elucidation of the molecular events that occur during genetic repression and induction.

(Continued from page 1054)

the untreated surface used by Barbarisi is typical of polyethylene and that the treatments given increase  $\gamma_c$  above the literature value of  $\gamma_c = 31$  dyne/cm.

Fowkes<sup>4</sup> has shown that the angle of contact is related to the dispersion force contributions,  $\gamma_s^d$  and  $\gamma_{LV}^d$ , to the surface tensions of solid and liquid

$$\cos \theta = \frac{2(\gamma_s^d \gamma_{LV}^d)^{1/2}}{\gamma_{LV}} - \frac{\pi}{\gamma_{LV}} - 1 \quad (3)$$

where  $\pi$  is the spreading pressure (Zisman has shown that for low energy surfaces  $\pi \approx 0$  if  $\theta > 0$ ). For the epoxy-polyamide adhesive used by Barbarisi  $\gamma_{LV} = 41.7$  dyne/cm. Equation (3) can therefore be used to calculate the expected contact angle for the adhesive on polyethylene, provided  $\gamma_{LV}^d$  can be obtained. The maximum value of  $\gamma_{LV}^d$  is  $\gamma_{LV}$ , and on the basis of comparison with the contribution of  $\gamma_{LV}^d$  to  $\gamma_{LV}$  of other organic liquids<sup>4</sup>, a minimum value of about  $0.75 \gamma_{LV}$  or 30 dyne/cm is reasonable. When  $\gamma_{LV} = \gamma_{LV}^d = 41.7$  dyne/cm, equation (3) gives  $\theta = 33.3^\circ$  and, when  $\gamma_{LV}^d = 30$  dyne/cm,  $\theta = 56.6^\circ$ . The values of  $\theta$  given by Barbarisi are  $35.4^\circ$  on untreated polyethylene and  $18.5^\circ$  on polyethylene wiped with acetone and treated with acid (unknown) for 5 min at  $70^\circ$ . The untreated surface therefore most resembles polyethylene—a conclusion reached without invoking the concept of  $\gamma_c$ .

The empirical linear relation found by Zisman and co-workers<sup>3</sup> can be written as

$$\cos \theta = 1 + b\gamma_c - b\gamma_{LV} \quad (4)$$

where  $-b$  is the slope of  $\cos \theta$  against  $\gamma_{LV}$  plots;  $\gamma_c$  will equal  $\gamma_{LV}$  when  $\theta = 0$ . This shows that the contact angle exhibited by a given liquid on different solids will depend on  $b$  as well as on  $\gamma_c$ . Equation (4) can be used to estimate the surface tension of a liquid which gives  $\theta = 35.4^\circ$  on polyethylene, for Zisman<sup>3</sup> established that  $b = 0.026$ . The result,  $\gamma_{LV} = 38.1$  dyne/cm, compares favourably with  $\gamma_{LV} = 41.7$  for epoxy-polyamide adhesive. It also indicates that the untreated surface is that of polyethylene. (It is also clear that the surface tension of the adhesive arises essentially from dispersion forces:  $\gamma_{LV}^d \approx \gamma_{LV}$ .)

The increased adhesion due to successive treatments of polyethylene must therefore be due to changes in its surface. (On the rough assumption that  $b$  does not alter, a surface giving  $\theta = 19^\circ$  has  $\gamma_c \approx 40$  dyne/cm.) The most comparable case found involves the results of de Bruyne<sup>6</sup>. The failing stress of epoxy resin/polyethylene bonds increased with decrease in the contact angle of water on surfaces oxidized with chromic acid, a trend which Zisman<sup>5</sup> attributed to an increase in  $\gamma_c$ .

The considerations discussed help to give insight into factors involved in adhesion to plastics. It must, however, be mentioned that thermodynamic arguments give little direct information about the magnitude of shearing forces parallel to the surface required to break adhesive bonds, and that with polymers, part of the energy required to break an adhesive bond may be expended in causing deformation of the solid<sup>2</sup>.

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## CHEMISTRY

## Band Width of the OH-stretching Vibration in Solid Alcohols

THERE have been many theories<sup>1</sup> advanced to explain the extremely broad OH-stretching vibration ( $\nu_{OH}$ ) in hydrogen bonded molecules. None of these theories fully explains the  $\nu_{OH}$  band width for all types of hydrogen bonded systems and many of the theories cannot be tested experimentally. We wish to report experimental evidence that the OH-stretching vibration in solid alcohols is not inherently broad, and that the breadth normally observed results from crystal interactions through nearest neighbour or first order coupling between OH groups along the hydrogen bond chain. Spectra of single crystals were obtained in a diamond window high pressure cell with techniques previously described<sup>2</sup>. Low temperature spectra were obtained in a conventional Dewar cell.

It has been well established<sup>3</sup> that intermolecular interactions associated with crystallinity can best be detected by isotopic dilution and mixed crystal studies. Fig. 1 shows such pertinent spectral data for various degrees of deuteration of solid *n*-decanol. With increasing dilution by *n*-decanol-OD, the  $\nu_{OH}$  sharp-broad doublet coalesces, and at high dilution becomes a singlet. At the same time,  $\nu_{OD}$  changes from a narrow singlet at low concentration to a sharp-broad doublet at high concentration analogous to the shape of  $\nu_{OH}$  in the pure OH compound. These deuteration studies leave no doubt that the appearance of two bands was caused by crystal splitting. Another equally important point is that the decoupled singlet OH band is quite narrow. In the lower spectrum of Fig. 1 we measured a half-band width of  $30\text{--}40\text{ cm}^{-1}$  even though the deuteration was not sufficient to decouple completely the OH vibration.

The sharp-broad character of the coupled doublet is seen more clearly in Fig. 2. This shows polarized spectra of single crystals of *n*-decanol at both low and high pressure. At low pressure, the bands are completely separated by polarization into a sharp band at  $3,410\text{ cm}^{-1}$  and a broad band near  $3,320\text{ cm}^{-1}$ . At high pressures, both  $\nu_{OH}$  components shift to lower frequencies, but the broad, lower frequency component shows a greater shift.

The explanation of these experimental observations can be seen with the aid of Fig. 3. Here we have assumed a

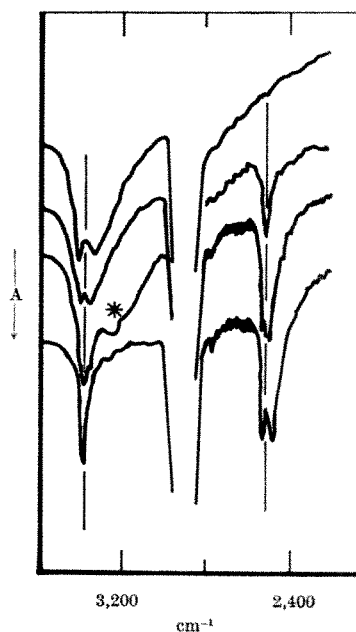


Fig. 1. Infra-red spectra of low temperature solids of deuterated *n*-decanol. OH/OD: 100/0, 85/15, 45/55, 25/75. \*Water.

planar hydrogen bonded chain of alcohol molecules (ROH) where the hydrocarbon portion (R) is considered as a point mass. In Fig. 3, the large circles represent R, dark circles represent oxygen atoms and the small, light circles represent hydrogen atoms. There are two vibrations due to the phase relationship of adjacent OH movements along the hydrogen bonded chain.

It is apparent from Fig. 3 that the in phase vibration would favour a tautomerization to the lower equivalent structure, whereas the opposite movement of the protons in the out of phase vibration would not favour such a tautomerization. It would be expected that such a tendency towards tautomerization would lead to a broadened absorption band for the in phase vibration, while the lack of tautomeric contribution in the out of phase vibration would lead to a more normal, narrow band. While compression because of increasing pressure would shorten the hydrogen bonds and lead to low frequency shifts for both components of  $\nu_{OH}$ , the pressure shift would be greater for the in phase vibration.

In addition, the resultant dipoles of these two vibrations would be very nearly at right angles to each other, thus

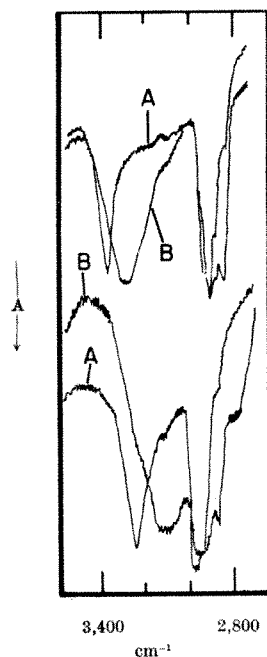


Fig. 2. Polarized infra-red spectra of a single crystal of *n*-decanol in diamond-window high-pressure cell. The upper curves are spectra (electric vector  $90^\circ$  apart) at low pressure. The lower curves are spectra (electric vector  $90^\circ$  apart) of the sample at high pressure.

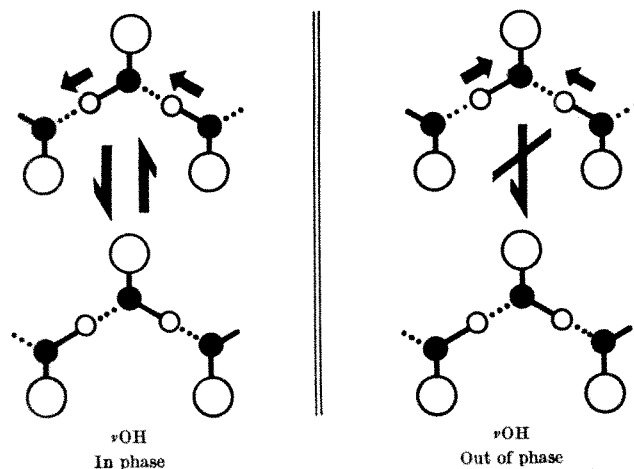


Fig. 3. Coupled OH vibrations of hypothetical alcohol solid.

accounting for the experimentally observed polarization data.

We therefore conclude that experimentally observed splitting of  $\nu_{OH}$  in a solid alcohol arises from nearest neighbour or first order coupling of adjacent OH groups along the hydrogen bonded chain. This splitting alone leads to an increased band width for  $\nu_{OH}$ . In addition, coupling favours tautomeric forms which contribute to an increased band width for some components of the split OH vibration. Interaction with low frequency hydrogen bond modes ( $\nu_{OH} \dots O$ ) may also account for some of the breadth of  $\nu_{OH}$ . The coupling and tautomerism must be present, however, for this interaction to take place. Thus nearest neighbour coupling is the underlying reason for the breadth of  $\nu_{OH}$  in solid alcohols.

The decoupled OH-stretching vibration of a strongly hydrogen bonded solid alcohol gives a narrow singlet absorption with a half band width as low as  $30 \text{ cm}^{-1}$  and possibly even lower. Thus the OH vibration is not inherently broad. This narrow band shape may be general for many hydrogen bonded systems. This is now being investigated.

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### Electronic States of Gaseous Fluorides of Scandium, Yttrium and Lanthanum

SOME of the more obvious features in the spectra of scandium fluoride ( $\text{ScF}$ )<sup>1,2</sup> and yttrium fluoride ( $\text{YF}$ )<sup>2,3</sup> have been described. The absorption and fluorescence spectra of  $\text{ScF}$  in low temperature matrices have now been observed<sup>4</sup>, and calculations<sup>5</sup> support the experimental finding that the ground state of  $\text{ScF}$  is  $^1\Sigma^+$ . Some analogies<sup>6</sup> may be drawn between the states of  $\text{ScF}$  and those of the isoelectronic molecule  $\text{TiO}$ . We have now carried out rotational analyses of a number of bands which have not been described before, and the purpose of this paper is to summarize the present position. For the most part the spectra have been observed in absorption in a carbon tube furnace at about  $2,000^\circ \text{C}$ , but some bands have also been photographed in thermal emission.

$\text{ScF}$  shows absorption systems based both on  $X^1\Sigma^+$  and on an excited  $^3\Delta$  state. No intercombination bands have yet been seen. Data on the singlet and triplet states are given in Tables 1 and 2.

A surprising feature of the state  $B^1\Pi$  in  $\text{ScF}$  is that the  $\Lambda$  doubling changes rapidly with  $v$ : in  $v=2$ ,  $q = -0.00046$ , and becomes  $-0.00105 \text{ cm}^{-1}$  in  $v=0$ . The doubling is in the sense requiring a  $^1\Sigma^-$  state lying below  $B^1\Pi$ . Such a state is hardly to be expected on any view of the likely low lying electron configurations in this molecule, and it

Table 1. SINGLET STATES OF  $\text{ScF}$

State	$T_{00}$	$\Delta G_0^\dagger$	$\lambda_{\text{wave}}$	$B_0$	$10^3 a$
$^1\Pi^*$	38,806.1	—	—	0.3671	—
$^1\Pi^*$	34,920.7	570	—	0.378	—
$^1\Pi^\dagger$	26,809.6	565.3	—	0.3449	2.5
$E^1\Pi^*$	20,326.8	614.7	3.7	0.3615	2.96
$C^1\Sigma^\dagger$	16,092.0	534.3	2.64	0.3461	2.4
$B^1\Pi^\dagger$	$\leq 10,661.3$	$\geq 581.9$	2.28	$\geq 0.3416$	2.48
$X^1\Sigma^\dagger$	0	728.0	3.8	0.3937	2.66

$\mu = 13.359 \text{ A.M.U. } r_0 (X^1\Sigma^+) = 1.791 \text{ \AA}$ .

\* These states all show perturbations.

$^\dagger q_0 = B_{11}^\dagger - B_{11} = -0.0020 \text{ cm}^{-1}$ .

$^\ddagger$  Values of  $q$  in this state may need revision upwards.  $q_0 = -0.00105$ ,  $q_1 = -0.00071$ ,  $q_2 = -0.00046 \text{ cm}^{-1}$ .

Table 2. TRIPLET STATES OF SCF

State	$T_{00}$	$\Delta G_{\frac{1}{2}}$	$x_c\omega_e$	$B_0$	$10^3 a$
$^3\Phi_1$	27,202.2 + $x_2$	—	—	0.3463	—
$^3\Phi_2$	27,171.1 + $x_2$	—	—	0.3441	—
$^3\Phi_3$	27,138.2 + $x_1$	—	—	0.3413	—
$(^3\Pi_2)$	18,361.4 + $x_2$	—	—	0.3677	—
$(^3\Pi_1)$	18,336.0 + $x_2$	—	—	—	—
$^3\Phi_4$	15,317.6 + $x_2$	—	—	0.3530	3.10
$^3\Phi_5$	15,277.5 + $x_2$	564.5	2.96	0.3511	—
$^3\Phi_6$	15,234.4 + $x_1$	—	—	0.3490	—
$^3A_1$	$x_3$	643.05	—	0.3693	2.58
$^3A_2$	$x_2$	642.92	3.03	0.3652	2.54
$^3A_1$	$x_1$	642.85	—	0.3610	2.50

The rotational constants given are effective, case  $a$  values: however, the energy levels do not entirely follow simple case  $a$  formulae. The constants given for  $^3\Pi_2$ — $^3A_1$  follow from an incomplete analysis of a system with closely spaced and perturbed rotational structure. At least three other systems with structure which seems to be too complex for singlet transitions have been observed.

may be rather that the perturbing state is the  $\Omega=0$ -component of a  $^3\Sigma^+$  state.

A strong O—O sequence exists at 21,927  $\text{cm}^{-1}$ , the structure of which suggests  $^3\Delta$ — $^3\Delta$  in which the coupling constants in the two states are almost equal. The bands are degraded  $V$ , and if the lower is still  $^3\Delta$ , then the upper state has  $\Delta G_{1/2} = 717.4$

$$x_c\omega_e = 3.64 \text{ cm}^{-1}$$

Secondly, there is a complex group at 26,300  $\text{cm}^{-1}$ , degraded  $R$ , and finally there are violet degraded bands with O—O head at 35,942.0  $\text{cm}^{-1}$ .

In YF, singlet systems dominate the absorption spectrum, and the one triplet system so far known, and assigned as  $^3\Phi$ — $^3\Delta$ , partly by analogy with ScF, appears rather weak in absorption. Thus the  $^3\Delta$ — $^1\Sigma^+$  separation may be a little bigger in YF than in ScF. Constants for YF are given in Tables 3 and 4.

Table 3. SINGLET STATES OF YF

State	$T_{00}$	$\Delta G_{\frac{1}{2}}$	$x_c\omega_e$	$B_0$	$10^3 a$
$^1\Pi$	31,205.8	536.3	2.13	0.27545	2.28
$^1\Sigma^+$	27,986.9	547.5	2.69	0.2741	—
$^1\Pi^+$	26,046.4	—	—	0.2685	—
$^1\Pi^+$	25,464.4	—	—	0.2706	—
$^1\Pi^+$	25,324.9	—	—	0.2680	—
$^3\Sigma^+$	19,190.3	527.2	2.45	0.2657	1.74
$^3\Pi^+$	15,885.8	534.7	2.35	0.26631	1.56
$X^1\Sigma^+$	0	631.3	2.50	0.28960	1.63

$$\mu = 15.658 \text{ A.M.U.}, r_0(X^1\Sigma^+) = 1.928 \text{ \AA}.$$

\* The constants for this state are from ref. 3.

† This region contains several overlapping bands and it is not yet certain how many states are involved.

$$\dagger q = B_{\Pi^+} - B_{\Pi} = -0.00013 \text{ cm}^{-1}.$$

Table 4. TRIPLET STATES OF YF

State	$T_{00}$	$\Delta G_{\frac{1}{2}}$	$x_c\omega_e$	$B_0$
$^3\Phi_1$	15,028.0 + $x_3$	—	—	—
$^3\Phi_2$	14,842.8 + $x_4$	531.3	2.41	0.277
$^3\Phi_3$	14,635.5 + $x_1$	—	—	—
$^3A_1$	$x_3$	578.5	2.49	—
$^3A_2$	$x_2$	577.5	2.42	0.285
$^3A_1$	$x_1$	576.4	2.39	—

$$r_0(^3A_2) = 1.94 \text{ \AA}.$$

The vibrational constants are from ref. 3.

A preliminary study of the absorption spectrum of LaF has revealed bands in the region 7650–8650  $\text{\AA}$ . The rotational analysis of two bands, red degraded, with single  $R$  and  $P$  branches, has been completed. Constants are given in Table 5. They are most probably the 1—O and O—O bands of a  $^1\Sigma^+$ — $^1\Sigma^+$  system. The lower state is probably the ground state of LaF, but a  $^3\Delta$  state may also be expected to give rise to absorption bands. Rotational structure may also be seen in red degraded bands at 13,059 and 13,029  $\text{cm}^{-1}$ : these bands seem to belong to a second infra-red system of LaF.

Table 5. STATES OF LaF

State	$T_{00}$	$\Delta G_{\frac{1}{2}}$	$B_0$	$10^3 a$
$^1\Sigma^+$	11,661.9	489.4	0.2273	1.1
$X^1\Sigma^+$	0	550*	0.2458	—

\* From Kratzer's relation.

$$\mu = 16.718 \text{ A.M.U.}, r_0 = 2.026 \text{ \AA}.$$

Violet degraded emission bands between 3200 and 3625  $\text{\AA}$  have also been ascribed<sup>7</sup> to LaF. They do not seem to

bear any relation to the bands described here, and some of the vibration frequencies derived, 682 and 657  $\text{cm}^{-1}$ , for example, which are larger than in the ground state of YF, seem to be too big for LaF.

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## CYTOLOGY

### Antigen and Antibody Localization in Hassall's Corpuscles

HASSALL's corpuscles in the guinea-pig thymus take up carbon<sup>1,2</sup> and Evans blue<sup>3,4</sup> from the circulation. In man, these corpuscles contain gamma globulin<sup>5-7</sup>. We therefore studied the localization in the thymus of albumin and globulin injected into the circulation by autoradiography and compared it with that in other lymphoid tissues.

Twelve immature female guinea-pigs (Hartley strain), weighing 260–320 g, were grouped into pairs of similar weight. Potassium iodide (50 mg/l.) and sodium chloride (4.5 g/l.) were added to the drinking water from the day before the experiment began, in order to block iodide uptake by the thyroid. The thymus was locally X-irradiated and received a tissue dose of 300 r., as described before<sup>1</sup>. Six animals were irradiated—three were allowed to survive for 2 days and three for 8 days. The same number of paired control animals was used.

Within 1 h of irradiation, animals were injected by the intracardiac route with one of the following: human serum albumin (HSA) (Hoechst) (eight animals) or guinea-pig gamma<sub>2</sub> globulin (GGG) (four animals). The latter was highly purified and showed a single precipitin band on immunoelectrophoresis. Each protein was trace-labelled with iodine-125 (R.C.C., Amersham, IMS-3) by the method of McFarlane<sup>8</sup>. With HSA, specific activities of 6.6 and 4.4 mc./mg were obtained, and giving 4–6  $\mu\text{c./g}$  body-weight produced good autoradiographs. Four animals received the smaller dose and four others the larger. The latter group was given, in addition, one intraperitoneal injection of 3 mg rabbit anti-HSA antibody plus 5 mg of mepyramine maleate 1 or 2 days before being killed. The labelled GGG had a specific activity of 0.3 mc./mg. Four animals each received 4  $\mu\text{c./g}$  body-weight.

Animals that had not received antibody were anaesthetized, perfused with saline through an intracardiac cannula to remove labelled protein from the circulation, and immediately killed with ether. Tissues were fixed in 10 per cent formol saline and processed. Sections were cut (5–7  $\mu$ ) from two blocks: one contained the two lobes of the thymus and a cervical node, the other the spleen and a mesenteric node. Autoradiography on histological sections was carried out with Kodak 'AR 10' stripping film, exposed for 2–6 weeks, developed and stained with methylene blue.

Two days after injection, the label from both proteins was diffusely distributed over thymic lobules with accentuation over interlobular septa and blood vessels. The



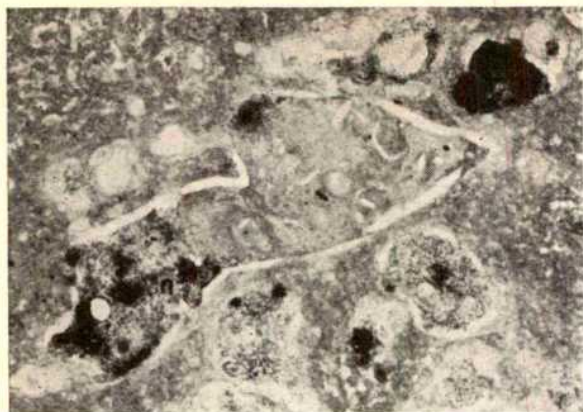


Fig. 1. Autoradiograph of X-irradiated thymus from an animal that was injected with HSA- $^{125}\text{I}$  2 days before death and with rabbit anti-HSA antibody 1 day before death. There is selective accumulation of HSA in parts of Hassall's corpuscles. A "compound" corpuscle shows uptake of the protein at one end. Other corpuscles show variable amounts of labelling (exposure time for ARG 4 weeks, methylene blue,  $\times c. 30$ ).

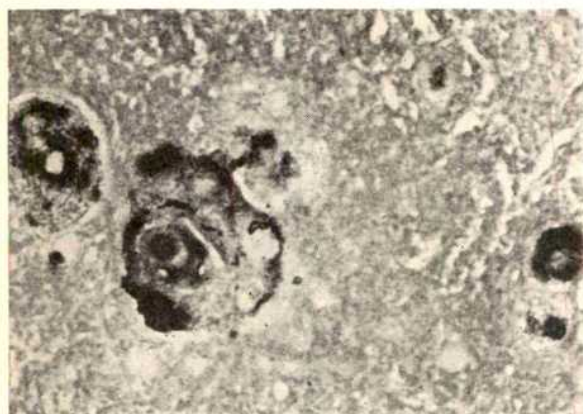


Fig. 2. Another area from the same animal as Fig. 1, showing concentric lamellar pattern over one corpuscle, while others show heavy confluent aggregations of silver grains (exposure time for ARG 4 weeks, methylene blue,  $\times c. 30$ ).

greatest accumulation of protein, however, was seen in Hassall's corpuscles. Irradiation further enhanced the uptake by the corpuscles (Figs. 1 and 2) and many became hyperplastic. The appearance suggested active concentration. At this time, the proteins were not localized in germinal centres in spleen or lymph nodes, but were diffusely spread over the parenchyma of these organs with marked accentuation of vascular channels and lymphatic sinuses (Fig. 3).

After 8 days the concentration of proteins was of lesser intensity, but still uniformly distributed over thymic lobules, with the exception of the corpuscles which were still prominent (Fig. 4). The concentration in the spleen and lymph nodes was similar to that of the thymus. In two animals that had had antibody 2 days earlier, there was virtually no label over the capsule, interlobular septa or cortex of the thymus. Only a small amount was present in the medulla but there was intense circumscribed labelling that clearly delineated the corpuscles (Fig. 5). Proof that this was caused by antigen and not by released iodine was provided by the follicular pattern in spleen and lymph nodes (Fig. 6) as described by McDevitt *et al.*<sup>9</sup>.

The present investigation demonstrates the rapid entry of homologous and heterologous serum proteins into the thymus of the guinea-pig. The intensity of label over the gland is of the same order as that found over lymph nodes and spleen. This agrees with previous quantitative studies<sup>4</sup>. Furthermore, there is focal concentration in

Hassall's corpuscles independent of previous sensitization. Irradiation increases the size and number of corpuscles that are "active" in the uptake of proteins, only a few remaining "quiescent", similar to the behaviour found in the phagocytosis of carbon<sup>2</sup>. These observations conflict with studies of antigenic localization in the thymus after intravenous injection in the mouse and the rat<sup>10-12</sup>. In these animals, however, the thymus is devoid of Hassall's corpuscles.

The speed of localization of antigen in Hassall's corpuscles of the immature guinea-pig compares with that found in germinal centres of the spleen of unprimed chicks aged 4-7 weeks<sup>13</sup>. In the latter, antigen was found as early as 2 days after intravenous injection, long before antibody had formed locally. Comparing the Hassall's corpuscles of immature guinea-pigs and germinal centres of the chick spleen, it is seen that both readily take up

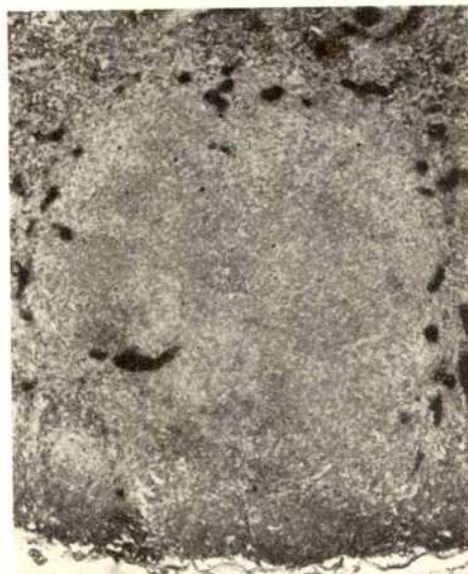


Fig. 3. Autoradiograph of a cervical lymph node from the same animal as in Fig. 1. The foreign protein is present in lymphatic channels around, but not inside, the germinal follicle (exposure time for ARG 4 weeks, methylene blue,  $\times 12$ ).

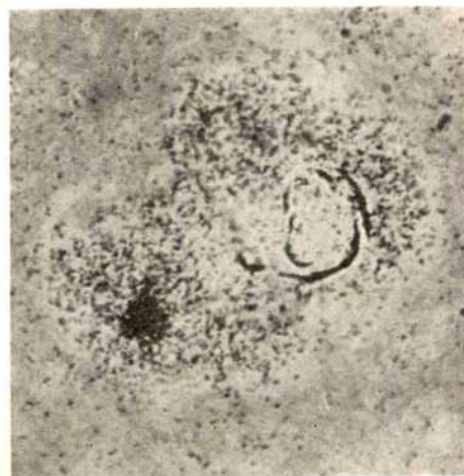


Fig. 4. Autoradiograph of thymus from a control animal that was injected with GGG- $^{125}\text{I}$  8 days before death. Heavy labelling is seen over a corpuscle which also shows an autoradiographic artefact. Such artefacts are not uncommon and have also been observed over other concentric structures such as aorta (personal communication from C. W. M. Adams). Labelling of lesser intensity is also seen in the adjacent medulla (exposure time for ARG 3 weeks, methylene blue,  $\times 120$ ).





Fig. 5

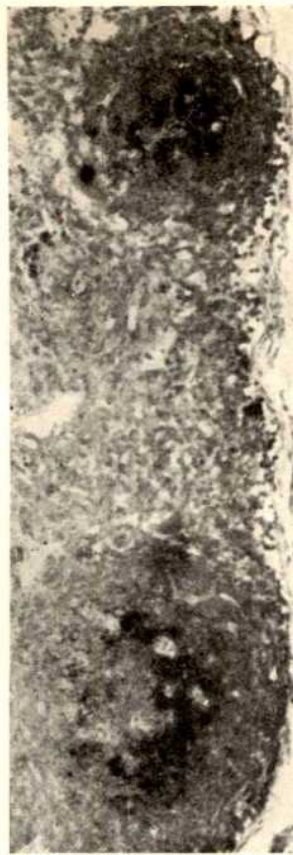


Fig. 6

Fig. 5. Autoradiograph of X-irradiated thymus from an animal injected with HSA-<sup>125</sup>I 8 days before death and rabbit anti-HSA antibody 2 days before death. Foreign protein is present in Hassall's corpuscles but of lesser intensity than seen 2 days after injection (exposure time for ARG 4 weeks, methylene blue,  $\times c. 30$ ).

Fig. 6. Autoradiograph of cervical lymph node from same animal as illustrated in Fig. 5. Concentration of antigen inside germinal follicles is clearly seen (exposure time for ARG 4 weeks, methylene blue,  $\times c. 30$ ).

circulating antigen in the unprimed state and both, at a later stage, contain gamma globulin. This may be relevant to the human foetal thymus where gamma globulin containing corpuscles are found in the eighth month of intrauterine life<sup>6</sup>.

The present investigation fails to support the concept of a barrier between blood and thymus<sup>11</sup>, at least with regard to serum proteins in the guinea-pig. If these findings are applicable to man, it suggests that a proportion of the gamma globulin found in human Hassall's corpuscles might come from the circulation and may not be elaborated locally.

These results have affirmed that Hassall's corpuscles in the guinea-pig thymus are by no means effete vestigial structures—on the contrary, they are sites of intense activity, showing phagocytosis, antigenic localization, uptake of gamma globulin and rapid changes in size caused by accretion of cells and cellular debris<sup>2</sup>. The rapid entry into the thymus of serum proteins of differing molecular sizes argues against a blood-thymus barrier in the guinea-pig.

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### Deletion of the Long Arm of Chromosome 16 and an Unexpected Duffy Blood Group Phenotype reveal a Possible Autosomal Linkage

CHROMOSOME deletions are ideal material for identifying gene loci, by the correlation of the absence of part of a chromosome with a particular phenotype. We have recently studied a child who demonstrates this cytogenetic principle. A 26 month old mildly retarded Caucasian female was found to be heterozygous for a deletion of the long arm of chromosome 16. Analysis of autosomal marker systems in the propositus and her family suggests that the location of the genes determining the Duffy blood system might be on the chromosome 16.

The propositus was a well developed, well nourished female who was below the third percentile in size. She had low set ears with flattening and over-rolling of the superior helix, epicanthic folds, widely spaced nipples, tapered fingers and a valgus deformity of the second toe crossing the third. There was notable dimpling over the shoulders,

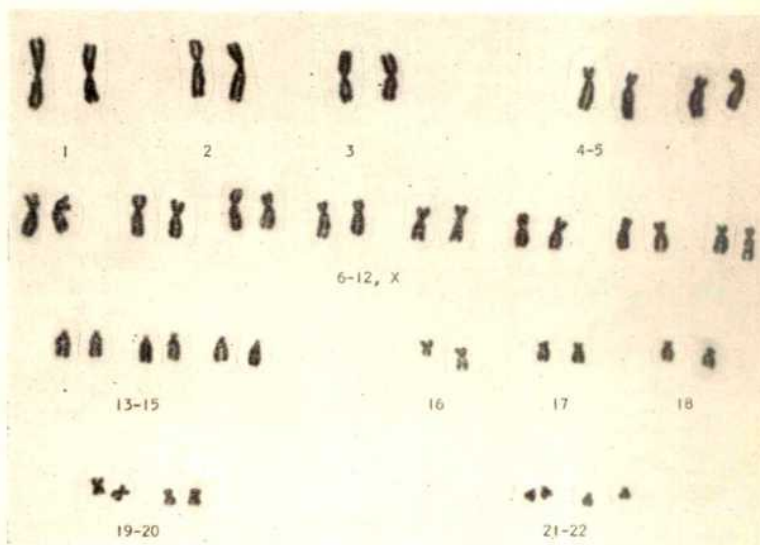


Fig. 1. Karyotype from the propositus. Note the deleted long arm on the left hand chromosome of pair 16. (Lymphocyte culture.)

elbows, knees, knuckles, sacrum and the spinous processes of L-2 and L-3. Routine laboratory and X-ray studies were within normal limits, and her dermatoglyphics were not unusual. The child had secondary bilateral optic pallor. She also had hearing loss, with serviceable hearing in the right ear and profound loss in the left.

The propositus had forty-six chromosomes, with a single heteromorphic pair. One chromosome 16 was shorter than its normal homologue (Fig. 1). This abnormality was visible in every cell. A secondary constriction was frequently observed in the shorter arm of the anomalous chromosome 16, which we interpret as having a deletion (either interstitial or terminal) distal to the secondary constriction of the long arm of the normal chromosome 16.

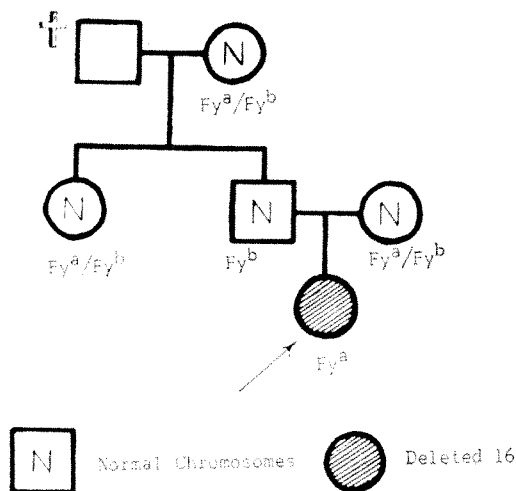


Fig. 2. Pedigree of the W. family. The Duffy blood group phenotypes are indicated.

Fifteen autosomal marker systems were investigated to see whether the loss of one or more was associated with loss of chromosomal material in the child. Heterozygosity allows us to state that the loci of the MNS and Rh red cell antigens are not on the deleted chromosome. Only the Duffy blood group gave unexpected results (the pedigree is given in Fig. 2). The propositus should be either  $Fy^bFy^b$  or  $Fy^aFy^b$ . Her  $Fy^a$  phenotype suggested that she was hemizygous for the locus. The father, however, could be heterozygous for  $Fy^b$  and the  $Fy$  allele for which the corresponding antibody has not been found. (The  $Fy$  gene, which has a frequency of 83–90 per cent in Negroes, is estimated to have a frequency as high as three per cent in Europeans<sup>1</sup>.) If that were the case the father of our propositus would be  $Fy^bFy$  and the propositus  $Fy^aFy$ . Dr Bruce Chown of the Rh laboratory, Winnipeg, Canada, kindly supplied an anti- $Fy^b$  serum (Sturg.) which when used neat also reacts with  $Fy$ . The cells of the mother and father reacted strongly with this serum, but the cells of the child were non-reactive. The Duffy phenotypes were checked with five anti- $Fy^a$  sera and five anti- $Fy^b$  sera (and all gave identical results). Dosage effects could not be obtained with these sera. The other blood group systems provide no evidence that this child is extramarital.

We therefore conclude that either the propositus has a previously undescribed  $Fy$  allele or that the absence of the expected  $Fy^b$  allele in the child is the result of the loss of chromosomal material which includes the locus of the Duffy gene.

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### Monolayer Cultures of Insect Cell Lines and their Inoculation with a Plant Virus

TWENTY-SEVEN years after Trager<sup>1</sup> reported short term survival and growth of silkworm cells from dissected female gonads, Grace successfully established continuous insect cell lines *in vitro* from ovarian tissues excised from diapausing pupae of *Antheraea eucalypti*<sup>2</sup>. The various attempts, during the interim, to improve *in vitro* growth of insect cells have been critically reviewed<sup>3-5</sup>.

Cultivation of leafhopper cells is of special interest, because it may provide, for those plant viruses that multiply in their vectors, a method of study which has long been available for researchers on phage and vertebrate viruses. Recently there have been successful attempts to obtain growth of cells of several leafhopper species by explanting tissues from certain nymphal or imaginal organs<sup>6,7</sup> or young embryos<sup>7-9</sup>. Plasma cultures derived from the ovary, hypodermis and digestive tract of a few cicadellids survived limited transplantations with evidence of mitosis<sup>6</sup>. Primary cultures in liquid media from embryonic tissues of *Macrosteles fascifrons*, *Dalbulus maidis*, *Agallia constricta*<sup>7</sup> and of *Nephotettix cincticeps*<sup>9</sup> could be maintained for several months. Mitsuhashi and Maramorosch<sup>7</sup> have reported that cells which formed extensive sheets surrounding tissue explants in the primary cultures grew very poorly when subcultured, despite their immediate attachment to glass surfaces. Such cells, separated from tissue explants, soon stopped growing and degenerated. Recently, primary tissue cultures of *Agallia constricta*, which transmit wound tumour virus (WTV), were inoculated with WTV and infection demonstrated by fluorescent antibody staining and infectivity tests<sup>10</sup>. There had been previous reports of the inoculation of leafhopper tissue cultures with aster yellows virus<sup>11</sup> and rice dwarf virus<sup>12</sup>; however, the evidences of infection seem inconclusive and the recovery of virus from inoculated cultures was either not attempted or was unsuccessful. Here we describe the establishment and maintenance of cell lines from *A. constricta*, the formation of monolayers by the cells and their inoculation with WTV.

To obtain primary coverslip cultures, young embryos of *A. constricta*, dissected from eggs laid 7 days previously in the petioles of crimson clover plants (*Trifolium incarnatum* L.), were explanted as described by Chiu *et al.*<sup>10</sup>. In order to attain a sufficient cell population per culture, 30 or 60 mm sealable Petri dishes were used. Usually, twenty or more pieces of embryonic tissue were placed in a small quantity of medium in the centre of each Petri dish and incubated at room temperature (about 24°C). Treatment of the pieces of embryo for 15 min in a 0.25 per cent trypsin solution before explanting improved initial growth of cells but was not essential. Initially, the medium developed by Mitsuhashi and Maramorosch<sup>7</sup> was found satisfactory for starting primary cultures, but later the growth medium described here was found to give equally good results and, indeed, for continuous culture was better.



Within a few hours after the cultures were started, attachment of dissociated cells and small groups of cells could be seen. These isolated cells and small groups, however, seldom survived long. Successful cultures were dependent on the attachment of the larger explanted tissue pieces to the glass. New cells began to appear from attached embryo fragments in 24–48 h. As these cells increased in number, cell sheets began to form, to expand gradually and eventually to engulf the original tissue explants. Such cultures could be maintained for several months by changes of medium at weekly intervals. The cell types that have been described in primary cultures of *Macrosteles fascifrons*<sup>7,13</sup> were also observed in those of *A. constricta*.

Early attempts to subculture the cells with the medium of Mitsuhashi and Maramorosch failed. Successful subculturing was obtained later with a medium based on Schneider's salt solution<sup>14</sup> at half strength. Each 100 ml. of our medium contained 400 mg *D*-glucose, 105 mg sodium chloride, 80 mg potassium chloride, 185 mg  $MgSO_4 \cdot 7H_2O$ , 30 mg calcium chloride, 30 mg potassium dihydrogen phosphate, 35 mg sodium bicarbonate, 650 mg lactalbumin hydrolysate, 500 mg 'Yeastolate', 17.5–20 ml. foetal bovine serum, 10,000 U penicillin, 10,000 µg streptomycin, 5,000 µg 'Neomycin', 250 µg 'Fungizone'. The inorganic salts can be prepared in two solutions: (a) a solution of all salts, except sodium bicarbonate, at five times the final concentration (sterilized by filtration) and (b) a solution of sodium bicarbonate at ten times the final concentration (sterilized by autoclaving). Stock solutions of glucose (4 g/100 ml.), lactalbumin hydrolysate (3.25 g/100 ml.) and 'Yeastolate' (5 g/100 ml.) can be sterilized by filtration. The foetal bovine serum is heated at 56° C for 30 min shortly before its incorporation in the medium. In preparing the medium, one must be careful to avoid concentrations of any of the ingredients which will cause irreversible precipitations. To make 100 ml. of the medium the various stock solutions of components may be added to 20 ml. of water in the order given. Antibiotics in stock solutions can be added just before supplying water to make a final volume of 100 ml. The medium has a pH of about 7.0 without adjustment.

Cell growth was less satisfactory in early subcultures without the substitution of 2.5 ml. of insect or lobster haemolymph for a like quantity of foetal bovine serum. (Haemolymphs were also heated at 56° C for 30 min before incorporation.) This medium supported vigorous

cell growth after cell dispersion after trypsinization. In one case medium that contained 20 per cent foetal bovine serum but no insect or lobster haemolymph yielded a cell line from *A. constricta*. Once adapted, the cultures grew continuously and well without insect or lobster haemolymph.

Nine cell lines of *A. constricta* have been passed through 7–55 subcultures made at intervals of 4–8 days. Details will be given here for the establishment of only one of these cell lines, namely, AC2 (an abbreviation for *Agallia constricta* cell line 2) which has been subcultured more than fifty-five times. On December 16, 1965, the primary AC2 culture was started by explanting embryonic tissues into a 60 mm sealable Petri dish. This culture was maintained in Mitsuhashi and Maramorosch's medium until early March when a decreasing rate of cell growth and darkening of the explanted tissues became apparent. On March 7, 1966, after the medium had been removed, the culture was exposed for 10 min to 0.05 per cent trypsin in Rinaldini salt solution free of calcium and magnesium ions. Trypsinization was stopped by the addition of an equal volume of growth medium containing foetal bovine serum (17.5 per cent) and haemolymph of *Antheraea eucalypti* (2.5 per cent). Gentle pipetting at this stage aided cell dispersion. After at least 6 h, when most of the living cells had become attached to the glass surface, the medium was changed and the floating dead cells together with some tissue pieces were removed. Subculturing by the same procedure was later repeated four times at 7–8 day intervals with the Petri dish unchanged but with the medium wetting greater areas each time. The brief trypsinization adopted did not suffice to disperse the cells completely and this proved to be advantageous because better growth was noticed at this stage from cells in clumps or from pieces of tissue.

Beginning on April 4, 1966, subculturing was carried out regularly by a procedure which involved trypsinization for 6–8 min in 0.05 per cent trypsin to dissociate the cells, centrifugation for 2 min at 200*g* and dispersion of the sedimented cells in medium for distribution to suitable containers. With a seeding density of  $4.8 \times 10^6$  cells in 4 ml., confluent monolayers were obtained in 3 days at 27° C in disposable plastic bottles with a useful flat surface of 25 cm<sup>2</sup>. In a 50 mm disposable plastic Petri dish 3 ml. of such inoculum was adequate and growth was obtained without adjustment of the supply of carbon dioxide. It should be noted that the period between starting the primary culture and the first subculturing was unnecessarily long in this case. Later experience indicated that successful subculturing could be achieved when extensive cell growth was attained about 3–4 weeks after starting primary cultures and established cell lines could be attained in another month.

All nine cell lines of *A. constricta* were comprised predominantly of epithelial types of cells (Fig. 1), although some variations in cell morphology and growth habit were noted among the lines. One cell line of *A. constricta* had distinctly more slender cells than did other lines. Although a steady growth rate was reached, none of the nine cell lines attained uniformity in cell morphology. Giant cells were intermingled with the predominant epithelial cells. Groups of cells with distinctive features emerged here and there when subculturing was infrequent. We made no attempt to determine whether these various cell types contained the symbionts which occur in leafhoppers and which may be retained in cultured cells derived from them<sup>7</sup>.

All the established *A. constricta* cell lines were susceptible to WTV prepared either

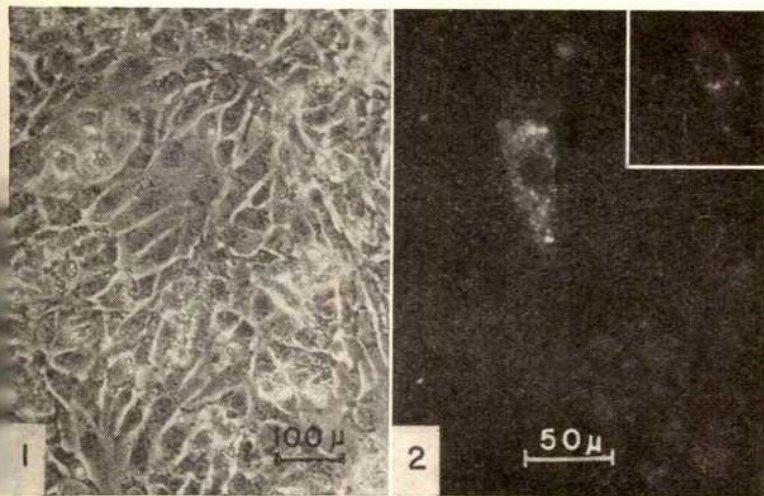


Fig. 1. A portion of a monolayer culture of *Agallia constricta* cells grown in a disposable Petri dish for 72 h.

Fig. 2. Cells of *A. constricta* stained with fluorescent antibody 48 h after inoculation with WTV. Non-infected cells which did not fluoresce are not clearly seen in the picture. Inset: Cell of *A. constricta* stained with fluorescent antibody 18 h after inoculation. Note the perinuclear location of WTV antigens.



from infected insects or from root tumour tissue of clover plants as previously described<sup>10</sup>. By the direct method of fluorescent antibody staining, virus antigen could be detected 12 h after inoculation and incubation at 30° C. Later, infected cells fluoresced more intensely and discrete stained spots appeared in the cytoplasm (Fig. 2), frequently in perinuclear locations (Fig. 2, inset). Infected lines, with 75 per cent or more of the cells positively stained, showed a growth rate comparable with the healthy lines. This became obvious when cultures inoculated with WTV were compared with healthy cultures of the same cell line, AC2, for ten consecutive subcultures. Seeding density ( $N_0$ ) was adjusted to  $1 \times 10^6$  cells per ml. or to a close approximation of this concentration. After the cells had attached at room temperature, cultures were placed in an incubator at 27° C. Cell counts were made for two 30 ml. bottles the next day ( $N_1$ ) and again for two bottles 4 days after subculturing ( $N_4$ ). There was no significant difference between the healthy and infected cultures in the ratios of  $N_4/N_1$  which were  $1.817 \pm 0.233$  for the healthy and  $1.765 \pm 0.101$  for the infected, respectively. The  $t$  test revealed a significantly lower ratio of  $N_1/N_0$  for the inoculated culture and this seems to indicate less satisfactory attachment of the infected cells during subculturing.

The method of establishing *A. constricta* cell lines and the use of our growth medium are applicable to other leafhopper species. We have succeeded in establishing two cell lines of *Agallia quadripunctata*, now at the ninth and twentieth passages. Cultures of *Aceratagallia sanguinolenta* and *Agalliopsis novella* are in early stages of subculturing.

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### Ion Transport and Phosphorylation in Dried and Rehydrated Yeast Cells

BAKER's yeast, consisting of almost 70 per cent water, can be dried to a moisture content of 7–8 per cent of dry weight without serious impairment of viability or fermenting capacity<sup>1,2</sup>. The cell membrane in such cells seems to be altered in such a way that considerable leakage of cytoplasmic material occurs when the cells are suspended in water at or below room temperature. Suspension of the cells in water at 35°–45° C prevents excessive loss of cytoplasmic constituents<sup>3</sup>. The "active dry yeast" to be considered here is dried yeast rehydrated at 35° C in which the cellular permeability barrier has been restored. The lipid composition of yeast cells can be altered by

this process<sup>4</sup>. I have investigated the question of whether such cells are still capable of active ion transport, which is one of the normal membrane functions<sup>5–7</sup>.

Baker's yeast was washed by repeated centrifugation and resuspension, and brought to a "starved" condition by bubbling air through a 15 per cent suspension overnight. Packed cells were suspended in distilled water to give a 60 per cent suspension (0.6 g of fresh yeast/ml.).

To prepare dried yeast the packed cells were spread as a 1–2 mm layer on filter paper and dried in a thermostated box by circulating air at 35° C for about 3 h, until the moisture content was 7 per cent. On a dry weight basis 3 g of fresh yeast corresponds to 9 g of dry yeast. Resuspension of the dry yeast was effected at 35° C; subsequent fermentation experiments were carried out at 30° C. The course of transport of hydrogen ions to the medium, on the addition of glucose, was measured at constant pH by means of a Radiometer titrator-titrigraph, using *tris* (hydroxymethyl) aminomethane as the titrant. At intervals during fermentation 0.5 ml. of yeast suspension was pipetted into 4 ml. of ice-cooled 10 per cent trichloroacetic acid. The supernatant obtained after centrifuging the trichloroacetic acid suspensions was buffered with 15 ml. of 0.2 molar sodium acetate. Orthophosphate, in the presence of labile phosphates, was determined according to Lowry and Lopez<sup>8</sup> and Peel *et al.*<sup>9</sup>. Adenosine triphosphate was determined in the same solution by an enzyme method, using phosphoglycerate kinase and glyceraldehyde-3-phosphate dehydrogenase (Boehringer). In separate experiments, the time course of formation of inorganic polyphosphates was determined by a method developed by Lohmann and Langen<sup>10</sup> and modified by van Steveninck<sup>11</sup>.

Figs. 1 and 2 illustrate the differences in hydrogen ion production between normal, and dried and rehydrated, cells in comparable conditions. The stimulation of transport of hydrogen ions by potassium ions for normal cells is manifest in a considerable upward shift of the line giving total hydrogen ion production ( $\Delta H^+$ ) as a function of pH. No potassium-stimulation of hydrogen ion transport occurs in dried and rehydrated cells, and there is no uptake of potassium ions. There is still transport of hydrogen ions, but the slope of the line giving  $\Delta H^+$  as a function of pH is different. For the whole range of pH investigated production of carbon dioxide was virtually constant for both kinds of yeast.

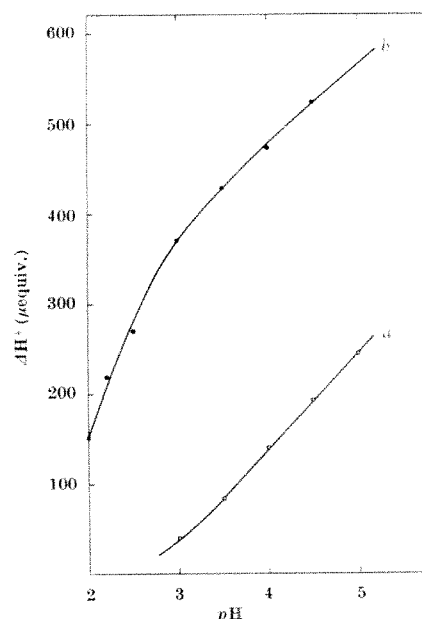


Fig. 1. Normal yeast. Acid production as a function of pH; 0.5 ml. of 30 per cent glucose was added to 15 ml. of 60 per cent yeast suspension plus 3 ml. of water (a). (b) Water (1 ml.) plus 2 ml. of 1 molar potassium chloride was added before glucose addition ( $N_2$ , 30° C).

On the supposition that ion transport depends on a metabolic energy supply, factors influencing ion transport should have an effect on energy production and/or utilization. In this connexion the course of phosphate incorporation during fermentation is of some interest.

Time curves were obtained for intracellular orthophosphate ( $P_i$ ) during fermentation. The decrease in  $P_i$  immediately after glucose has been added to the yeast suspension is normally divided into two distinct phases. The initial fall in  $P_i$  was found to be virtually independent of the glucose quantity; at its end steady state concentrations of various phosphorylated intermediates of fermentation and of adenosine triphosphate are reached. The secondary decrease in  $P_i$  increases when larger quantities of glucose are added. As Fig. 3 shows, a constant concen-

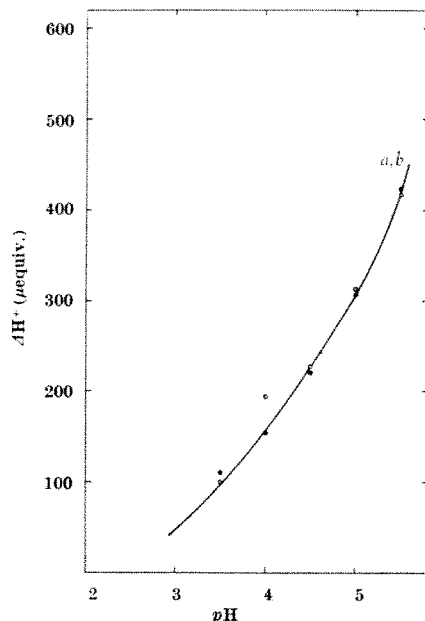


Fig. 2. Dried and rehydrated yeast. Acid production as a function of pH: 0.5 ml. of 30 per cent glucose was added to a suspension of 3 g of dried yeast in 15 ml. of water (a). (b) The suspension was made in 13 ml. of water and 2 ml. of molar potassium chloride was added before glucose addition. ( $N_2$ , 30° C.)

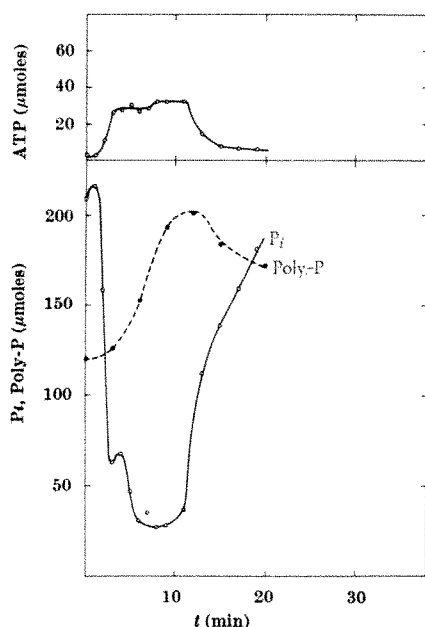


Fig. 3. Normal yeast. Changes in orthophosphate ( $P_i$ ), inorganic polyphosphate (poly-P), and ATP during fermentation; 30 ml. of 60 per cent yeast; at  $t = 0.2$  ml. 30 per cent glucose was added.

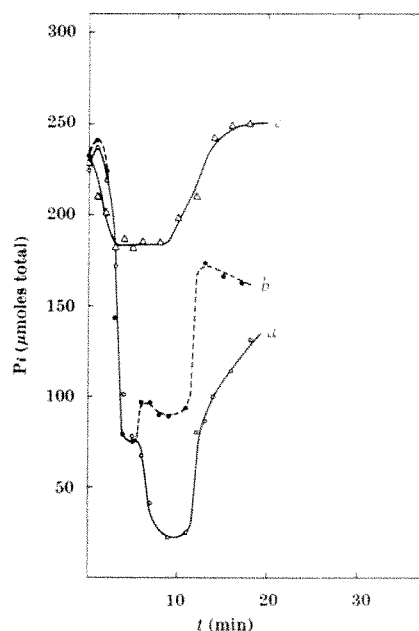
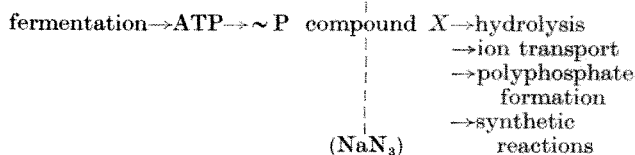


Fig. 4. Comparison of normal yeast, azide-poisoned yeast and dried and rehydrated yeast. Orthophosphate ( $P_i$ ) time curves during anaerobic fermentation in conditions corresponding to those in Fig. 3. (a) Normal yeast; (b) normal yeast, with 1 mmolar  $NaN_3$ ; (c) dried and rehydrated yeast.

tration of ATP in this phase is maintained until all the glucose added is used up; then there is a return to a relatively low concentration of ATP. While ATP remains constant there is a steady rise in inorganic polyphosphate. Hydrogen ion transport occurs chiefly in the period between 5 and 8 min after glucose addition, that is the period of the secondary fall in  $P_i$  (ref. 7). Inhibitors of potassium-stimulated hydrogen ion transport, such as azide and 2,4-dinitrophenol, eliminate the secondary fall in  $P_i$  (Fig. 4).

Experiments with dried and rehydrated yeast cells gave  $P_i$  curves which were shifted upward when compared with the  $P_i$  curves for normal yeast, and resembling the  $P_i$  curves for yeast poisoned with azide.

Theoretically for each mole of glucose fermented 2 moles ATP can be produced. The actual rise in the concentration of ATP corresponds, however, to only a small fraction of the theoretical quantity. Apart from dissipation of energy as heat,  $\sim P$  appears to be utilized in a number of processes as outlined below:



In the presence of azide and similar inhibitors the time curve for ATP remains virtually unchanged. The high-energy intermediate indicated as  $\sim P$  compound  $X$  may become unstable; uncoupling of ion transport and polyphosphate formation from fermentation would be the result. In dried and rehydrated cells as well as in cells poisoned by azide, polyphosphate formation is in fact eliminated (my unpublished results).

Dehydration influences a number of processes requiring an intact membrane structure, "Active dry yeast" has an impermeable cell membrane, and hydrogen ion transport during fermentation is still possible. On the other hand, potassium ions do not enhance hydrogen ion transport, and are no longer taken up in an exchange between potassium and hydrogen. Inorganic polyphosphates, an important fraction of which has been localized at the cell membrane<sup>10,12,13</sup>, are no longer synthesized during

fermentation. Both with regard to an altered phosphorus metabolism and with regard to an impaired exchange between potassium and hydrogen "active dry yeast" resembles azide- or dinitrophenol-poisoned yeast.

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## GENETICS

### Fundamental Theorem of Natural Selection

EDWARDS<sup>1</sup> has recently criticized Li's description<sup>2</sup> of his formula for the rate of change of mean fitness in a population as the "fundamental theorem of natural selection" on the grounds that Fisher's derivation<sup>3</sup> of the fundamental theorem used overlapping generations, whereas Li uses separate generations. Because Fisher<sup>3</sup> said that his theorem held "the supreme position among the biological sciences", and because at one time he worked on one of the many organisms which in the wild have non-overlapping generations<sup>4</sup>, it seems very unlikely that he thought the generation-system had any essential effect on his theorem, and it is not possible to exclude from consideration formulations with non-overlapping generations when discussing the generality of the theorem. True or untrue statements with one system of generations are likely to be just so with the other.

Although Fisher spoke of the "rigour of the demonstration"<sup>5</sup> of his theorem, its presentation is so opaque, even to mathematicians<sup>6</sup>, that it is hard to be sure it is rigorous; in view of the cases in which the theorem is not strictly true, it is better regarded as a brilliant creative approximation than a rigorous piece of mathematics. Thus Fisher thought it was true with or without dominance, with any number of loci and with any system of mating; in fact it is absolutely true only for a single locus without dominance under random mating<sup>2</sup>. When there is dominance the change in fitness lies between  $\frac{1}{2}$  and  $n$  times Fisher's value, where  $n < \infty$ . I shall discuss elsewhere some aspects of dominance and multiple loci<sup>6,7</sup>; there is no recent treatment of non-random mating, and it is instructive to consider the simplest case—inbreeding. Following Kimura<sup>8</sup>, the rate of change of mean fitness,  $\bar{M}$ , with time,  $t$ , is given, for two alleles, by

$$d\bar{M}/dt = 2q_0q_1(M_0 - M_1) \left( \frac{1}{2} d\bar{M}/dq_0 \right) \quad (1)$$

where  $q_0, q_1$  are the gene frequencies and  $M_0, M_1$  are the average fitnesses of the genes, and we ignore the effects of dominance.  $(M_0 - M_1)$  is Fisher's "average excess" ( $\alpha$ ) and if  $\frac{1}{2}(d\bar{M}/dq_0)$  were Fisher's "average effect" ( $\alpha$ ), then (1) would be identical with Fisher's  $2q_0q_1\alpha\alpha$ , which he called the genetic variance in fitness and which is always positive. But the quantity in (1) is not necessarily positive. Thus at a non-trivial equilibrium the rate of change of gene frequency

$$dq_0/dt = q_0q_1(M_0 - M_1) \quad (2)$$

is zero, and so also is the quantity<sup>9,10</sup>

$$d(\bar{M} + F\bar{M}_I)/dq_0 \quad (3)$$

where  $F$  is the inbreeding coefficient and  $\bar{M}_I$  the mean fitness of the inbred portion of the population. With inbreeding therefore  $(d\bar{M}/dq_0)$  is not zero at a non-trivial equilibrium. Now if the equilibrium is stable, (2) will be positive for gene frequencies lower than equilibrium and negative for higher gene frequencies. Because  $(d\bar{M}/dq_0)$  is likewise positive below and negative above its zero point, and because this zero point does not coincide with the equilibrium, it follows that in the region between these points one of the quantities  $(M_0 - M_1)$  and  $\frac{1}{2}(d\bar{M}/dq_0)$  is positive, the other negative. Thus equation (1) is negative and the change in fitness is not equal to Fisher's positive quantity  $(2q_0q_1\alpha\alpha)$ . The same applies to an unstable equilibrium. With random mating  $(M_0 - M_1) = \frac{1}{2}(d\bar{M}/dq_0)$  and (1) is the same as Fisher's expression. Inclusion of dominance adds a small correction analogous to that under random mating<sup>2</sup>, but does not alter the conclusions.

These manipulations can be made also for non-overlapping generations; as Edwards<sup>1</sup> and Li<sup>2</sup> say, Fisher's theorem seems to be at best approximate under general conditions.

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### Caffeine-induced Mutagenesis in *Drosophila*

ANDREW<sup>1</sup> reported that caffeine is mutagenic for the *Drosophila* male when administered either by a larval feeding or by an adult injection method. Yanders and Seaton<sup>2</sup> later repeated these experiments, and found no indication of a mutagenic effect for caffeine by either method of administration. As far as the present communication is concerned, the interest of these reports lies in the conflicting results of the larval feeding treatments.

Andrew's larval feeding treatment<sup>1</sup> was carried out with a medium composed of treacle, semolina and agar (not seeded with yeast) to which 0.25 per cent caffeine was added, whereas Yanders and Seaton's medium<sup>2</sup> containing 0.25 per cent caffeine was composed of sucrose, dextrose and brewers yeast. In view of the observation by Novick and Szilard<sup>3</sup> of an antimutagenic effect of certain natural purine ribonucleosides (adenosine, guanosine and inosine) on the mutagenic effect of certain *N*-methylxanthines (including caffeine) on *Escherichia coli* cells grown in continuous (chemostat) culture, it seems feasible that the higher concentration of RNA and nucleotides (from the yeast) in Yanders and Seaton's medium might be masking the mutagenic effect of caffeine observed by Andrew on his medium. On the basis of this consideration, we have repeated the feeding treatment of *Drosophila* (Oregon-K) larvae with caffeine on a chemically defined and axenic medium from which all purine and pyrimidine sources may be omitted<sup>4</sup>. The experiments have been designed to test for a mutagenic effect of caffeine in the absence and in the presence of exogenous purine and pyrimidine sources (as supplied by yeast RNA): the toxic concentration of caffeine on the chemically defined medium is lower than on the usual *Drosophila* media, particularly in the absence of RNA.

The treatment medium was prepared by adding a Seitz-filtered solution of caffeine to the autoclaved chemically defined medium at 60° C. The medium was then dispensed as 25 ml. portions into small Petri dishes, and one hundred newly hatched axenic<sup>4</sup> larvae were added to each culture. The larvae were treated for 48 and 72 h with medium containing 0.05 per cent caffeine, and for 48, 96 and 240 h with medium with 0.075 per cent caffeine, after which they were transferred to a corresponding medium without caffeine to complete their development.

On emergence, males were individually mated to two Muller-5 females for 3 days for the detection of  $F_1$  complete sex-linked recessive lethal mutations, and one (or more) non-lethal  $F_2$  culture arising from each treated male was sampled for evidence of  $F_1$  sex-linked recessive lethal-mosaicism. This latter procedure detects those lethals which are not completely established throughout the gonad of the  $F_1$  female (and not detected as  $F_1$  complete lethals), but are lethal mutations which occur after a delay and are established in only a fraction of the gonad of the  $F_1$  female ( $F_1$  lethal-mosaic)<sup>5</sup>.

Table 1. COMPLETE AND  $F_1$  MOSAIC SEX-LINKED RECESSIVE LETHAL FREQUENCIES IN *Drosophila* MALES AFTER LARVAL FEEDING TREATMENT WITH CAFFEINE

	Control		Treatment			
	Concentration of caffeine (per cent)		0.05			
	—	—	0.03	0.6	—	—
RNA concentration (per cent)	0.3	—	0.3	0.6	—	—
Duration of treatment (h)	48	48	48	48	72	—
Survivors (per cent)	89	72	76	81	64	—
No. males examined	43	123	88	48	36	—
No. chromosomes examined	511	1,111	747	478	477	—
Complete lethals (per cent)	0.0	—	0.45	0.27	0.21	0.0
No. non-lethal $F_2$ cultures examined	48	50	—	53	—	—
	(arising from 43 males)	(arising from 50 males)	—	(arising from 48 males)	—	—
Average No. females examined/non-lethal $F_2$ cultures	9	10	—	9	—	—
No. non-lethal $F_2$ cultures yielding at least one lethal in $F_2$ set	1	1	—	0	—	—
Cultures showing $F_1$ lethal-mosaicism (per cent)	2.0	2.0	—	0.0	—	—
Total No. $F_2$ females examined	451	493	—	472	—	—
No. lethal-bearing $F_2$ females	1	1	—	0	—	—
Lethals in $F_2$ (per cent)	0.22	0.20	—	0.0	—	—

Table 2. COMPLETE AND  $F_1$  MOSAIC SEX-LINKED RECESSIVE LETHAL FREQUENCIES IN *Drosophila* MALES AFTER LARVAL FEEDING TREATMENT WITH CAFFEINE

	Control		Treatment			
	Concentration of caffeine (per cent)		0.075			
	—	—	0.6	0.6	—	—
RNA concentration (per cent)	0.6	—	0.6	0.6	—	—
Duration of treatment	48 h	48 h	48 h	96 h	10 days	—
Survivors (per cent)	93	68	67	51	42	—
No. males examined	53	45	56	29	46	—
No. chromosomes examined	510	401	386	647	551	—
Complete lethals (per cent)	0.19	0.25	0.0	0.15	0.18	—
No. non-lethal $F_2$ cultures examined	53	40	51	—	—	—
	(arising from 53 males)	(arising from 40 males)	(arising from 51 males)	—	—	—
Average No. females examined/non-lethal $F_2$ culture	10	11	10	—	—	—
No. non-lethal $F_2$ cultures yielding at least one lethal in the $F_2$ set	1	1	1	—	—	—
Cultures showing $F_1$ lethal-mosaicism (per cent)	1.9	2.5	2.0	—	—	—
Total No. $F_2$ females examined	556	449	509	—	—	—
No. lethal-bearing $F_2$ females	1	1	1	—	—	—
Lethals in $F_2$ (per cent)	0.18	0.22	0.19	—	—	—

Tables 1 and 2 illustrate the results obtained for sex-linked recessive lethal mutations after larval feeding treatments at two concentrations of caffeine (0.05 and 0.075 per cent) in the chemically defined and axenic medium. There is no indication that caffeine increases the frequencies of  $F_1$  complete or  $F_1$  mosaic sex-linked recessive lethal mutations either in the absence or in the presence of RNA (0.3 or 0.6 per cent). These experiments thus lend no support to the observation by Andrew<sup>1</sup> that caffeine is mutagenic for the *Drosophila* male larva.

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## Chromosome Marker Studies in the Irradiated Chick Embryo

THE repopulation of the irradiated adult animal by injected myeloid cells has provided a considerable amount of qualitative and quantitative data on haemopoietic stem cells<sup>1</sup>. The work reported here was designed to investigate the capacity of cells of various embryonic organs to populate the haemopoietic tissues of the irradiated embryo. The chick embryo is especially suited to such a study, first because haemopoietic cells can be injected intravenously into it, and second because the sex chromosome difference between male (ZZ) and female (ZW) cells provides a marker method for distinguishing the contributions of host and donor to haemopoietic recovery.

We have confirmed the acute mortality which has been reported<sup>2</sup> in chick embryos after irradiation, and this limits the total amount of radiation to which embryos can be subjected. After preliminary experiments, using various dosages of radiation, a total of 800 rads (dose rate 66 rads/min from a cobalt-60 source) was administered as a standard procedure to embryos which had been incubated for 13 days. In the strain of bird used (Rhode Island and White Leghorn) there was an acute mortality of about 20 per cent and about 75 per cent of remaining embryos hatched out. Some of the irradiated embryos were injected after incubation for 14 days with cell suspensions prepared from either 7 day yolk sac, 14 day spleen, 16 or 18 day bone marrow, or 14 or 18 day thymus. A total of  $10 \times 10^6$  cells of male sex (ZZ) were injected intravenously in each case. In an additional experiment, quantities of 0.1 ml. of whole blood removed from 13 day male embryos were injected into irradiated hosts.

After various intervals, but usually at 2 days after hatching, chicks which had been irradiated and injected with cells were treated with 'Colcemid' (Ciba) and killed 2 h later. If they were female (ZW), chromosome spreads were prepared from thymus, bursa of Fabricius, spleen, and bone marrow by a technique described previously<sup>3</sup>. These organs, removed from both injected and non-injected birds, were also examined histologically.

The results of the chromosome analysis are presented in Table 1. In the very short time period studied, there was a partial recolonization of haemopoietic tissues by yolk sac, spleen, bone marrow or blood cells. With the exception of one embryo which showed a low level of bursal chimerism, however, no comparable repopulation was found after injections of thymocytes.

Although haemopoietic recovery was incomplete in the thymus, bursa of Fabricius and spleen at the times of chromosome analysis, the results presented here do allow some tentative conclusions to be made about the potentialities of embryonic stem cells for haemopoietic development. After 7 days of incubation, the yolk sac is the only important site of haemopoiesis in the embryo and its activity is mainly directed towards the production of erythrocytes. Yolk sac cells from this stage, however, are



Table 1. CHROMOSOME MARKER ANALYSIS OF IRRADIATED CHICK EMBRYOS (FEMALE) INJECTED WITH EMBRYONIC HAEMOPOIETIC CELLS (MALE)

Age at sampling	Age of donor (days)	Thymus		Marrow		Bursa		Spleen	
		No. cells scored	% Donor	No. cells scored	% Donor	No. cells scored	% Donor	No. cells scored	% Donor
Yolk sac injection ( $10 \times 10^6$ cells into each embryo)									
2 days (after hatching)	7	50	24	100	12	50	16	—	—
2 days (after hatching)	7	100	8	100	46	100	20	—	—
2 days (after hatching)	7	50	36	100	32	50	24	—	—
Spleen cell injection ( $10 \times 10^6$ cells into each embryo)									
2 days (after hatching)	14	100	88	100	25	50	98	25	48
2 days (after hatching)	14	25	60	50	58	25	44	25	48
2 days (after hatching)	14	25	48	25	56	25	40	—	—
Bone marrow injection ( $10 \times 10^6$ cells into each embryo)									
20 days (embryo)	16	100	12	100	32	50	26	—	—
20 days (embryo)	16	50	14	100	20	50	16	—	—
2 days (after hatching)	18	100	16	100	28	50	8	—	—
2 days (after hatching)	18	100	24	100	44	50	12	—	—
7 days (after hatching)	18	100	30	100	29	50	24	20	40
Thymus cell injection ( $10 \times 10^6$ cells into each embryo)									
2 days (after hatching)	14	50	0	100	0	100	0	—	—
2 days (after hatching)	14	50	0	100	0	100	0	—	—
2 days (after hatching)	18	50	0	100	0	100	6	—	—
2 days (after hatching)	18	25	0	50	0	25	0	25	0
2 days (after hatching)	18	25	0	50	0	25	0	—	—
Embryonic blood injection (0.1 ml. blood into each embryo)									
2 days (after hatching)	13	50	28	100	20	100	32	—	—
2 days (after hatching)	13	50	14	50	10	50	20	—	—

capable of populating both myeloid and lymphoid organs (thymus and bursa) in the irradiated embryo, which suggests that there are cells in the yolk sac which are pluripotential. Alternatively it might be suggested that there are within the organ distinct categories of stem cell already committed to particular types of haemopoietic differentiation. This seems less likely because the 7 day stage precedes both marrow and spleen haemopoiesis as well as bursal and thymic lymphopoiesis.

Although the yolk sac is the most likely primary source of stem cells with a range of developmental capacities, the results suggest that at later stages spleen, bone marrow and blood also contain these stem cells. Thus spleen cells which were mainly granuloid at the time of injection can populate both myeloid and lymphoid tissues. Cells which enter the thymus, however, lose their potentiality for alternative types of differentiation.

These results agree with other experimental data on embryonic haemopoiesis collected in this laboratory, which have shown that the development of the thymus<sup>1</sup>, bursa of Fabricius<sup>2</sup>, and spleen and bone marrow<sup>3</sup> are all dependent on the inflow of blood-borne stem cells. The cell type common to all these organs during the initial stages of haemopoiesis and present in the circulation throughout embryogenesis is a large basophilic cell with prominent nucleolus and irregular outline. Autoradiography suggests this cell is most likely to be the blood-borne stem cell (our unpublished results). If, as the present experiments suggest, the blood-borne stem cell is initially uncommitted, then its later differentiation into distinct cell types may depend on an inductive influence exerted by the organ anlage (epithelial in the thymus and bursa, mesenchymal in the spleen and marrow) into which it migrates.

In conclusion, the population of haemopoietic organs of the irradiated embryo by injected myeloid cells supports the role of blood-borne cells in embryo haemopoiesis and suggests that the embryo stem cell has a wide range of developmental capacities.

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## MICROBIOLOGY

### Thermophilic Bacterium isolated on *n*-Tetradecane

GROWTH at the expense of hydrocarbons occurs in a wide variety of micro-organisms; Fuhs<sup>1</sup> listed more than 100 yeasts, bacteria and fungi with this characteristic. Scant information is available concerning the influence of temperature on microbial hydrocarbon oxidation. Four strains of micrococci have been checked for their ability to grow at the expense of octane, nonane and decane at 20° and 25° C (ref. 2). At the lower temperature there was good utilization of decane but none of the strains grew on this hydrocarbon at 25° C. Possibly such differences in growth response are related to an increase in vapour pressure of the *n*-alkane with an increase in temperature. According to Allen<sup>3</sup>, most if not all of the most important types of metabolism carried on by mesophilic bacteria can also be found among thermophilic bacteria. A variety of carbon sources can be utilized by thermophilic bacteria<sup>4</sup>, and some years ago a general survey of thermophilic bacteria was made using enrichment culturing<sup>5</sup>. No bacterium was selected from soil, fresh water or marine mud, or dry plant material when paraffin served as the carbon source. Presumably the paraffin was a mixture of long-chain saturated hydrocarbons.

A sample of mud from an oil-covered inlet of Lake McBride near Iowa City was inoculated into a basal salts medium<sup>6</sup> amended with 2 per cent *n*-tetradecane. After transfers in liquid media a pure culture was obtained by streaking an inoculum from the liquid enrichment medium on basal salts-agar plates to which hydrocarbon was added to filter paper placed in the cover of an inverted Petri dish. All incubations were at 60° C.

The organism obtained was a Gram-positive bacillus, 2.8 to 4.2  $\mu$  long by 0.5 to 0.7  $\mu$  wide, bearing terminal spores. Growth occurred in a temperature range of 45°–70° C, with optimum growth between 55° and 60° C. At the optimal temperature range good growth was observed in 24 h on basal salts-agar slants to which a layer of hydrocarbon was added to the base of the slant. On transfer to liquid medium containing hydrocarbon, visible growth was not seen until after 96 h, which seemed to indicate that a stimulatory growth factor was present in the agar.

Commercially available agars and agar subjected to various extraction procedures were used in growth experiments to ascertain if a stimulatory factor was present. The effect of these agars on growth was measured at 60° C in a basal salts medium to which 1.5 per cent of the various agar preparations was added. *n*-Tetradecane was

Table 1. GROWTH RESPONSE OF A THERMOPHILIC BACTERIUM TO *n*-TETRADECANE ON BASAL SALTS MEDIUM PLUS VARIOUS PREPARATIONS OF AGAR

Solidifying agent	Growth		
	24 h	48 h	72 h
(1) Agar (Difco)	2	3	3
(2) Ion agar-2 (Oxoid)	2	2	2
(3) Noble agar (Difco)	0	1	2
(4) Special-purified agar (Difco)	0	0	1
(5) Agar (Difco) dialysed	2	3	3
(6) Agar (Difco) dialysed, CHCl <sub>3</sub> -CH <sub>3</sub> OH extracted	1	1	2
(7) Agar (Difco) cold water-extracted	2	3	3
(8) Agar (Difco)-CHCl <sub>3</sub> -CH <sub>3</sub> OH extracted	2	3	3
(9) Agar (Difco)-water ethanol, acetone, pyridine-extracted	1	3	3
(10) Silica gel ('Ludox')	0	0	2

0, No growth; 1, slight growth; 3, abundant growth.

added to the base of each agar slant. Results are shown in Table 1. Preparation No. 5 was dialysed against deionized water for 7 days and a portion of this was dried and extracted with methanol-chloroform for 7 days (No. 6). The washing procedure outlined by Dworkin and Foster<sup>6</sup> was followed for preparation 7. Thiamine and biotin are apparently removed by the extraction procedure used for No. 9 (ref. 7). Agar was replaced by silica gel as a solidifying agent in No. 10 (ref. 8). Only the purification procedure used by the manufacturer of "special-purified agar" appeared effective for the removal of growth stimulatory factor or factors from agar. It appears that growth of this thermophilic bacterium on *n*-tetradecane is unique in that it occurs without the addition of organic growth factors. Growth is obtained on a solid medium of silica gel and basal salts and in a liquid medium of basal salts with *n*-tetradecane as the only source of carbon in both cases.

The factor of aeration was considered in attempts to shorten the lag period because at 60° C the solubility of oxygen in water is decreased. When humidified air was sparged through the medium containing hydrocarbon there was no growth. Incubation in a gyrotory water-bath shaker (200 r.p.m.) at 60° C also inhibited growth. In similar conditions in a more complex medium—meat infusion broth—growth was observed within 24 h.

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## Biochemical Balance and Synchronized Cell Cultures

In cell biochemistry, it is desirable to have criteria for identifying abnormal changes resulting from experimental manipulation, especially during the induction of synchrony. For this purpose, use has been made of the concept of unbalanced growth, first introduced by Cohen and Barner<sup>1</sup>, to explain the death of a thymine-requiring mutant of *Escherichia coli* as a result of continued cytoplasmic synthesis in the absence of proportional DNA synthesis. Most people who use this concept are doubtless aware that its foundation must be in the biochemical processes within individual cells and that the fundamental definition of "balance" should be at the cellular level. There are, however, examples in the literature in which it is concluded, on the basis of changes in the gross composition of mammalian cell cultures in which the age distribution of cells is changing, that growth is "unbalanced" without proper consideration of the possibility that the changes might

result merely from redistribution of individual cells in the several phases of the life cycle, rather than from significant changes in their composition.

These conclusions are based on the Campbell definition<sup>2</sup> that "growth is balanced over a time interval if, during the interval, every 'extensive' property of the system increases by the same factor". For large populations with an invariant distribution of ages, this formulates the results expected for a particular type of macro-system. As Zeuthen<sup>3</sup> points out, however, such a definition automatically categorizes all synchronous cell cultures as unbalanced because of periodic variations in their properties, although individual cells of the culture may be normal at all times. Similarly, it is impossible for a single mammalian cell to display a "balanced" growth pattern by Campbell's definition, although the individual cells determine the properties of the culture. This paradox results from the fact that the gross constancy of large populations results from the combination of two distinct requirements: (a) the periodic biochemical pattern of individual cells as they traverse the life cycle must be time-invariant (at least as averaged over an adequate number of cells and/or cycles); and (b) the fraction of cells in each of the several phases of the life cycle must be time-invariant. Clearly, the first requirement is fundamental to the concept and mechanism of balanced growth, but the second is, in this connexion, superficial and relates only to demographic properties of the culture.

We propose, therefore, that balanced states be defined in terms of the properties of individual cells and that the culture as a whole be classified on the basis of summation over the biochemical states of its members. For a single cell, an unbalanced state is one in which it has a composition not shown by any cell in the course of the "normal" life cycle. To the extent that all cells follow an identical sequence of development, each state corresponds to and succinctly defines a single "age" in the life cycle. Because of random fluctuations (for example, in generation time, in mass at birth and division), it is clear that a simple one to one correspondence between age and state does not exist. Rather, there are many possible alternative states between birth and division, and a given cell will traverse only a limited number of these states in the course of its life cycle. The totality of accessible states, however, can be identified in principle and the probability of alternates determined. Unbalanced growth then is growth which leads the cell into a state of abnormal composition.

For a culture of many cells, the state of the culture is determined by the distribution of the states of its component cells and is specified by enumerating the number of cells which are in each cellular state. The "balance" of a synchronous culture will depend not on the numerical distribution of cells among the available cell states but, rather, on whether or not some cells are in states not attained by any cell in the original random culture.

A multiplicity of balanced growth patterns must be allowed with any pattern capable of unlimited cyclic repetition regarded as balanced. Schaechter, Maaløe and Kjeldgaard<sup>4,5</sup> have demonstrated such a multiplicity in the adaptation of *Salmonella* to varying conditions of, for example, temperature, nutrition and cell concentration with concurrent changes in cell composition and growth patterns.

An example of the magnitude of effects which can result from a redistribution of cells in the life cycle is the experiment of Kim *et al.*<sup>6</sup> in which HeLa cells were treated with excess thymidine, which stopped DNA but not RNA and protein synthesis. An increase in total RNA by a factor of 1.36 was observed in the 11 h after cessation of DNA synthesis (ref. 6, Fig. 4). This could have been a result of unbalanced growth as they suggest; however, before that conclusion is reached, consideration should be given to a possible alternative, namely, that RNA synthesis was continuing only as long as permitted by biochemical balance (that is until each cell in turn reached

the boundary between  $G_1$  and  $S$ ). A calculation for the latter possibility, assuming RNA to increase linearly by a factor of 2 around the life cycle and DNA to double during the  $S$  phase, yields a factor of 1.21 for the increase of RNA relative to DNA for the thymidine-blocked "balanced" culture with all cells stopped at  $S$  or at the boundary between  $G_1$  and  $S$ . (The data used for the HeLa cell life cycle were from Puck and Steffan<sup>7</sup>.) This condition should be reached about 14 h after the thymidine block became effective. Growth in the presence of thymidine probably was unbalanced, but two-thirds of the observed increase in RNA can be accounted for by age redistribution. So large an effect should not be neglected when gross chemical composition of a culture is used as a criterion of balanced growth.

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Table 1. PERCENTAGE BINDING OF 1:2 DILUTION OF SUCROSE GRADIENT FRACTIONS (POOLED SERA)

Fraction	0.01 $\mu$ g nitrogen	0.10 $\mu$ g nitrogen	1.00 $\mu$ g nitrogen
1 (top)	0	0	0
2	22.7	13.7	5.1
3	96.7	94.3	54.3
4	94.6	92.7	45.8
5	73.6	49.5	18.8
6	62.5	23.5	7.1
7	31.8	11.2	3.5
8	9.5	3.5	1.6
9 (bottom)	4.2	3.6	1.5

characterized by radioimmuno-electrophoresis<sup>7</sup> and the Farr technique<sup>8</sup>; a detailed account of the preparation of BSA labelled with iodine-131 (BSA\*) is given elsewhere<sup>11</sup>.

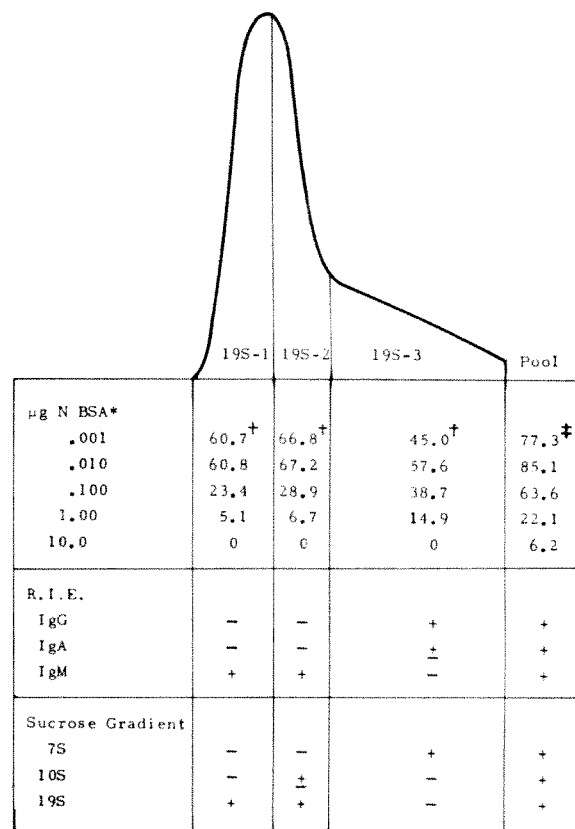
Table 1 shows the binding data of the nine sucrose gradient fractions of pooled sera using three concentrations of BSA\*. Each 0.5 ml. sucrose gradient fraction was diluted with 0.5 ml. of 20 per cent normal rabbit serum to ensure uniform ammonium sulphate precipitation. When 0.01  $\mu$ g nitrogen BSA\* was used substantial binding of BSA\* was observed in both the 7S (fractions 3 and 4) and the 19S (fractions 6-8) portions of the sucrose gradient. In contrast, little if any binding was noted in the 19S region when 1.00  $\mu$ g nitrogen BSA\* was used. To ensure that the binding activity which we observed in fractions 6-8 using 0.01  $\mu$ g nitrogen BSA\* was due to the presence of 19S antibody and not due to contaminating 7S antibody, we analysed the 19S-1, 19S-2 and 19S-3 portions of the recycled 19S 'Sephadex' peak for binding activity using five concentrations of BSA\* over a 10,000-fold range. The results are shown in Fig. 1. Positive binding of the 19S-1 and 19S-2 fractions was obtained only when from 0.001 to 0.100  $\mu$ g nitrogen BSA\* was employed (binding of 5 per cent or less was not considered a definitive result); fraction 19S-3 showed binding up to 1.00  $\mu$ g

## IMMUNOLOGY

### Sensitivity and Detection of 19S Antibody by the Farr Technique

THE detection of humoral antibody can only be adequately assessed by using techniques which are solely dependent on the primary interaction of antigen and antibody<sup>1-5</sup>. Two such primary measures of antibody detection are the Farr technique<sup>6</sup> and radioimmuno-electrophoresis<sup>7</sup>. Radioimmuno-electrophoresis is an extremely sensitive test which is dependent on the specific activity of the labelled antigen. It is, however, qualitative, whereas the Farr technique is a quantitative test to determine either the capacity of an antiserum to bind the antigen<sup>6</sup> or the amount of antibody<sup>8,9</sup> the antiserum contains. Recently, the sensitivity of the Farr technique has been questioned, especially with respect to the detection of 19S antibody<sup>10</sup>. The work reported here shows that 19S antibody can readily be detected by the Farr technique only if an appropriate concentration of antigen is used to achieve maximum sensitivity of the method.

Ten adult New Zealand White rabbits were immunized intravenously with 50 mg bovine serum albumin (BSA) either absorbed onto aluminium hydroxide or in conjunction with a heat-killed suspension of *Corynebacterium parvum* which is a powerful adjuvant<sup>11</sup>. The rabbits were bled 9 and 12 days later and the sera pooled. 20 ml. of the pooled sera was fractionated by gel filtration through 'Sephadex G-200' using a 6 x 80 cm column and 0.075 molar phosphate buffer, pH 7.0) containing 0.075 molar sodium chloride. The 19S peak was concentrated by negative pressure dialysis to 5 ml. and recycled through a 2.5 x 35 cm 'Sephadex G-200' column. The pooled sera and the ascending (19S-1) and descending portion (19S-2 and 19S-3) of the recycled 'Sephadex' 19S peak were fractionated by sucrose density gradient ultracentrifugation as previously described<sup>12</sup>. Anti-BSA antibody activity was



<sup>+</sup> Percentage binding of a 1:3 dilution (undilute with respect to neat serum).

<sup>±</sup> Percentage binding of a 1:30 dilution.

Fig. 1.

nitrogen BSA\*. Studies of binding by the sucrose gradient fractions of the 19S-1 and 19S-2 portions of the recycled 'Sephadex' peak using 0.01 µg nitrogen BSA\* revealed activity exclusively of the 19S class; 19S-3 showed binding predominantly of the 7S class. These data were supported by radioimmuno-electrophoresis of the three 19S 'Sephadex' fractions; 19S-1 and 19S-2 contained only IgM anti-BSA antibody whereas 19S-3 contained only IgG anti-BSA antibody.

We have shown here, as have others<sup>1,2,5</sup>, that 19S (IgM) antibody can be readily detected by the Farr technique. Rosenquist and Gilden<sup>13</sup>, on the other hand, were unable to detect 19S anti-BSA antibody in early, primary response chicken sera using from 1 to 10 µg BSA\*. Similarly Wei and Stavitsky<sup>10</sup> reported that the Farr technique, using 12.5 µg human serum albumin (HSA), lacked the sensitivity needed to detect 19S antibody to HSA in rabbits which could be detected by passive haemagglutination and radioimmuno-electrophoresis. In the light of the present findings it is not surprising that these authors were unable to find binding by 19S in view of the relatively large amount of antigen used. We consider that the Farr technique can be a highly sensitive test for the detection of small amounts of antibody (including IgM) provided that small concentrations of antigen, for example, 0.001–0.010 µg nitrogen BSA\*, are used. It should be emphasized that in experiments where small amounts of antibody may be present, for example, in studies of acquired immunological tolerance, it is essential to use sufficiently small amounts of antigen to ensure adequate sensitivity of the test. In view of the recent advances in the preparation of iodine-131 labelled proteins with high specific activity<sup>14</sup>, the concentrations of antigen suggested in this report should present little experimental difficulty.

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### Use of Tissue Culture to restore Immunological Competence to the Neonatally Thymectomized Mouse

MILLER<sup>1</sup> has reported a lack of development of lymphoid tissue in mice after neonatal thymectomy. These mice had a low lymphocyte count in the peripheral blood, and both spleen and lymph nodes failed to develop germinal centres and only a few plasma cells were found in them. In addition, he found that allogeneic skin which was rejected in 10 or 12 days by normal mice grafted when 5 days old survived and flourished for more than 2 months in animals thymectomized at birth. On the other hand, animals

thymectomized at birth which received a syngeneic homograft of thymus at the age of 3 weeks were able to reject allogeneic skin homografts like normal intact animals.

Although mice thymectomized at or after 3 weeks of age grow normally and have a normal life span, most strains thymectomized at birth suffer from a wasting syndrome similar to that seen in graft-versus-host reactions in *F<sub>1</sub>* hybrid mice injected with parental lymphoid cells. They grow normally for a time and then show wasting, lethargy, ruffled hair, hunched posture, diarrhoea and finally die<sup>2,3</sup>. This syndrome has been shown to be caused by infection with micro-organisms in animals unable to form antibodies<sup>4</sup>; neonatally thymectomized mice reared in a pathogen-free environment gained weight normally and did not develop the wasting syndrome.

Auerbach<sup>5</sup> showed that spleens of 13 or 14 day mouse embryos grown alone in tissue culture failed to thrive, but if they were grown in combination with embryonic thymus they produced lymphoid follicles which remained for more than 5 weeks.

Neonatally thymectomized mice implanted intraperitoneally by Osaba and Miller<sup>6</sup> at 7 days of age with 'Millipore' diffusion chambers containing embryonic or neonatal thymus tissue did not develop the wasting syndrome and could reject allogeneic skin homografts. These experiments seem to suggest that a humoral factor is made by the thymus, and that this is responsible for producing immunological competence in newborn animals. The following experiments were carried out in an attempt to determine the existence of such a factor.

Two inbred strains of mice, *A2G* and *C3H*, were used. The offspring of the originals and three subsequent generations were thymectomized within 24 h of birth and reared with their mothers until weaned at about 28 days.

Three whole thymuses from the newborn mice were explanted on the sides of each 10 × 1.3 cm test tube which was then incubated at 37° C for 1 h to ensure attachment of the thymuses to the glass surface. Growth medium (1 ml.) was then added and the tubes were incubated at 37° C at 5° to the horizontal. The growth medium was medium 199 (ref. 7) containing 0.15 per cent sodium bicarbonate and 20 per cent horse serum. To this was added 100 U of penicillin, 100 µg of streptomycin and 50 U/ml. of nystatin.

At first the effect of injecting the tissue culture fluid was studied. The medium was decanted at periods between 3 and 7 days after explantation and stored at 4° C. It was then injected in 0.3 ml. and later 0.6 ml. amounts intraperitoneally on five occasions within a period of 10 days into neonatally thymectomized mice aged between 5 and 30 days. The animals tolerated the larger amount well. Similar amounts of the unused growth medium were administered intraperitoneally to neonatally thymectomized mice as a control. Between 56 and 70 days later the cells of the spleen which produced antibody were measured.

A second series of animals were grafted at the age of 28–42 days with explants of whole syngeneic thymus grown in tissue culture for 1–9 days. Under ether anaesthetic, the explants were introduced beneath the capsule of the left kidney with an 18 s.w.g. lumbar puncture needle (personal communication from E. A. Wright). The abdominal muscle and skin were then repaired with interrupted braided silk sutures. Between 42 and 56 days later, the cells of the spleen which produce antibody were measured and the graft was examined histologically.

The antibody producing cells of the spleen were measured by the method of Jerne *et al.*<sup>8</sup>. Washed sheep erythrocytes in samples of 0.2 ml. of a 50 per cent suspension were injected intraperitoneally into the mice. Four days later, the mice were killed, the spleens were removed and a suspension of spleen cells was prepared. This was mixed with fresh sheep erythrocytes and DEAE-dextran and poured in a base of molten agar on a solid agar plate. After incubation at 37° C for 3 or 4 h, complement was added for 30 min. When it was washed away, plaques of haemolysis



surrounding each spleen cell which had produced haemolysis were counted. The total number of plaques/million spleen cells in the suspension was calculated.

Fifty-one neonatally thymectomized mice were given intraperitoneal injections of tissue culture explant fluid and forty-five were given similar injections of unused growth medium as controls. Table 1 shows the average number of plaques/ $10^6$  cells in both test and control animals of different ages. Each time an experiment was performed, a normal unthymectomized mouse was used as an additional control, and each time it produced more than 100 plaques/ $10^6$  cells.

Twenty-two neonatally thymectomized mice received grafts of thymus explants. Table 2 shows the average number of plaques/ $10^6$  spleen cells in mice given grafts of different ages in tissue culture. Again, each time an experiment was performed, an unthymectomized mouse was tested and produced more than 100 plaques/ $10^6$  cells. Fig. 1 shows a section of a neonatal thymus grown in tissue culture for 4 days and then grafted beneath the renal capsule of a 33 day old neonatally thymectomized mouse. It was allowed to grow on this site for 45 days.

Neonatally thymectomized mice have a severely impaired capacity to produce cells in their spleen which will haemolyse sheep erythrocytes<sup>9</sup>. An estimation of these "plaque forming cells" is a particularly convenient method of assessing the capacity of a thymectomized mouse to form antibodies, and has been used in the present experiment to determine the effects of tissue culture explant fluid on the immune response of such animals.

The results indicate that thymuses grown in tissue culture do not secrete a humoral substance capable of restoring the antibody forming capacity of neonatally thymectomized mice. This could be caused by degeneration and death of the explants with a consequent failure to produce the substance. But thymic explants in tissue culture for periods up to 9 days were found to be viable when transplanted under the renal capsule of syngeneic thymectomized mice. Not only were they well preserved histologically but they also led to a considerable restoration of antibody forming capacity in these animals. This agrees well with Miller's observation that thymic tissue grafts restore to neonatally thymectomized mice the capacity to effect transplantation immune reactions<sup>1</sup>. It has been shown that most of the lymphoid cells in the graft were eventually derived from invading host cells<sup>10-13</sup>, but in our experiment no attempt has been made to assess the origin of the cells of the graft.

The experiments of Osaba and Miller<sup>6</sup>, where the thymic implant placed in a 'Millipore' diffusion chamber within the peritoneal cavity of young neonatally thymectomized mice restored the immune capacity of the animals, suggest

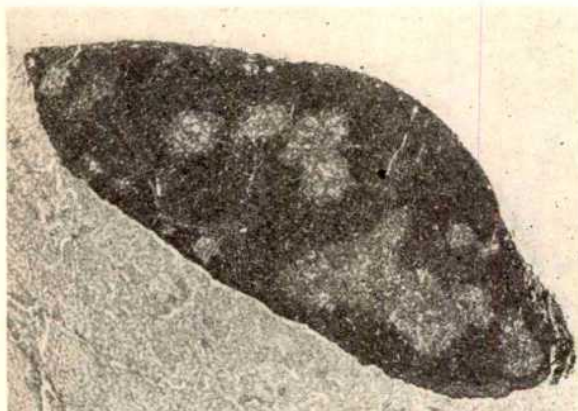


Fig. 1. Section of a neonatal thymus grown in tissue culture for 4 days and then grafted beneath the renal capsule of a 33 day old neonatally thymectomized mouse. It was allowed to grow in this site for 45 days. Stained with haematoxylin and eosin ( $\times 25$ ).

Table 1. PLAQUES IN TEST AND CONTROL ANIMALS OF DIFFERENT AGES AFTER INJECTION OF TISSUE CULTURE EXPLANT FLUID

Age during which fluid was given	No. of test animals	Average No. of plaques/ $10^6$ cells	No. of control animals	Average No. of plaques/ $10^6$ cells
5-15 days	19	15	18	14
15-20 days	15	13	11	10
15-25 days	10	10	9	9
20-30 days	7	10	7	10
Total No. of animals	51	12	45	11

Neonatally thymectomized mice were given intraperitoneal injections of tissue culture explant fluid. The controls were given injections of unused growth medium. The number of antibody producing cells was calculated. Unthymectomized mice tested at the same time showed more than 100 plaques/ $10^6$  spleen cells.

Table 2. PLAQUES IN MICE AFTER GRAFTING OF THYMUS EXPLANTS

No. of days of explant in tissue culture before grafting	No. of animals	Average No. of plaques/ $10^6$ spleen cells
1	3	75
2	2	81
3	5	70
4	4	75
5	2	80
6	1	75
7	2	80
8	1	75
9	2	70
Total No. of animals	22	75

Neonatally thymectomized mice were grafted with thymuses grown in tissue culture for 1-9 days. They were killed 42-56 days later, and good grafts were present beneath the renal capsule. The number of antibody producing cells was calculated. Unthymectomized mice tested at the same time showed more than 100 plaques/ $10^6$  spleen cells.

that a humoral mechanism may be important in the establishment of a functioning immunological system. The failure to find such a diffusible factor in tissue culture fluids of thymic explants would appear to negate this possibility. It can, however, be argued that thymic explants in tissue culture medium are surviving in a foreign environment, and that their metabolic processes cannot be equated with those of the thymus in a living animal. Nevertheless, thymic extracts have so far failed to restore antibody forming capacity in thymectomized mice, and it may well be that the interaction between thymus and immature lymphocytes takes place at a more intimate cellular level.

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## PATHOLOGY

### Lymphoid Cells in Hodgkin's Disease

THERE is now considerable evidence that there are in animals tumour specific antigens of a transplantation type, that the host reacts against its own tumour and that this reaction is in some cases immunological, mediated by lymphocytes and similar to the delayed hypersensitivity reaction and homograft rejection<sup>1</sup>. The situation in man

is not so clear because of the difficulty of research. We therefore decided to study the morphology and DNA synthesis of lymphocytes in Hodgkin's disease for two reasons. First, in this disease cellular immunity is depressed more than in any other human malignant disease; and second, lymphocytic and/or histiocytic proliferation is associated with localized disease and a relatively good prognosis, whereas lymphocytic depletion is associated with a poor prognosis and a generalized disease<sup>2,3</sup>.

Lymphoid cells were obtained from defibrinated blood using finely divided iron particles and methyl cellulose<sup>4</sup>. The preparations contained 98-100 per cent lymphoid cells, the phagocytic monocytes and granulocytes having been removed.

In fourteen out of eighteen untreated patients with Hodgkin's disease there was a definite increase in the number of large lymphoid cells (Fig. 1), which were approximately 20 $\mu$  in diameter, with prominent nucleoli, deeply basophilic cytoplasm, and a well developed Golgi zone, and which on electron microscopy had large numbers of ribosomes arranged in rosettes, but little endoplasmic reticulum. Similar cells are found in the efferent lymph nodes draining the sites of antigenic stimulation in experimental animals<sup>5,6</sup>. In addition there is also an increased number of medium-sized lymphocytes with basophilic cytoplasm, an increased ribosomal content and, in some, well developed endoplasmic reticulum.

DNA synthesis in the lymphoid cells was assessed by measuring, in a liquid scintillation counter, the amount of tritiated thymidine taken up by the cells after incubation for 30 min, using the technique of Cooper<sup>7</sup>. The cells were not stimulated by phytohaemagglutinin or any other agent. The results, shown in Fig. 2, are expressed as c.p.m./million cells. In fifteen of the seventeen patients studied, the DNA synthesis was greater than the upper limit of normal controls.

As expected, autoradiographs have shown that the large lymphoid cells constitute nearly all the labelled cells and smaller lymphocytes were only occasionally labelled. Kuper *et al.*<sup>8</sup> also noted increased uptake of tritiated thymidine in the mononuclear cells in three patients with Hodgkin's disease, but did not state whether these were lymphoid cells.

These changes in the lymphoid cell population may be seen in all stages of Hodgkin's disease and in the presence of normal or low haemoglobin, serum folate levels and total lymphocyte counts. In addition there is no apparent

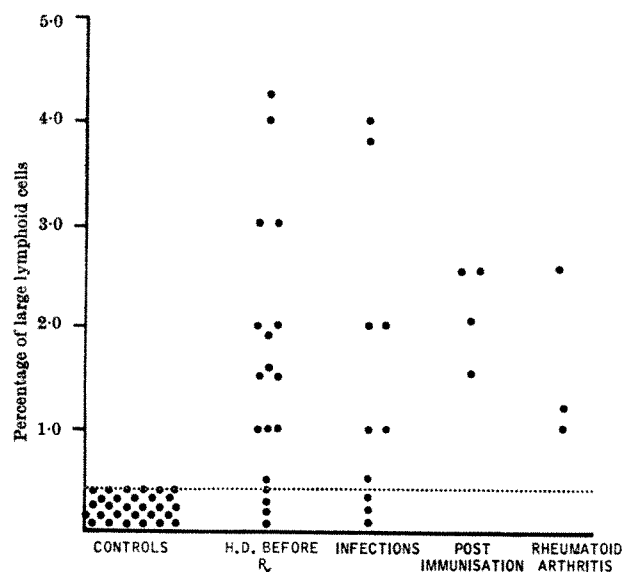


Fig. 1. Percentage of large lymphoid cells in peripheral blood lymphocyte preparations.

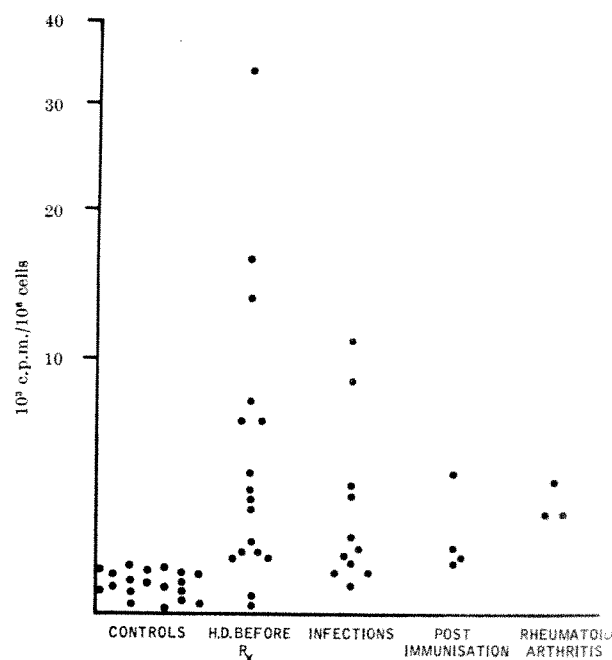


Fig. 2. DNA synthesis in peripheral blood lymphoid cells.

correlation with the ability to give delayed hypersensitivity reactions to tuberculin and streptokinase.

One possible explanation for these findings is that the lymphocytes in Hodgkin's disease are involved in an "immunological" reaction against the disease, and we therefore studied the lymphoid cells in the peripheral blood of patients with a variety of infections, normal subjects after immunization and patients with rheumatoid arthritis. The results are shown in Figs. 1 and 2.

We excluded those bacterial, viral and rickettsial infections in which labelled mononuclear cells had previously been described<sup>9-11</sup>. Only some of the patients with bacterial infections had an increased number of large lymphoid cells and they were suffering from a staphylococcal pneumonia and empyema, bronchopneumonia and acute pyelonephritis. Two laboratory workers with common colds, and four volunteers, who were injected with antigens (killed poliovaccine and tetanus toxoid intramuscularly, and tuberculin and streptokinase intradermally) also showed changes in morphology and DNA synthesis of the same type as that found in Hodgkin's disease. Following immunization, more than a four-fold rise was found in the numbers of large lymphoid cells synthesizing DNA, and up to 1.1 per cent of the cells were labelled using autoradiographic techniques. This rise was followed by a subsequent return to normal levels.

Increased numbers of peripheral blood lymphoid cells synthesizing DNA have been described in patients with auto-immune diseases<sup>12</sup>, and the three patients with rheumatoid arthritis whom we studied showed morphological changes indistinguishable from those in Hodgkin's disease.

These results support the theory that the changes in the lymphocytes in Hodgkin's disease could represent an immunological reaction, but other possibilities exist. For example, it is known that malignant cells in the peripheral blood actively synthesize DNA. This is unlikely in our cases because we were unable to find any reticulum or Reed-Sternberg cells in the lymphoid cell preparations, and because it is improbable that the large lymphoid cells are the malignant cells in Hodgkin's disease.

Another possibility is that, because of disorganization of the normal lymph node architecture, increased numbers of immature lymphoid cells appear in the peripheral blood



just as, with infiltration of the bone marrow, primitive bone marrow cells appear in the blood causing a leucoerythroblastic anaemia. The patients we have studied with carcinomatous infiltration of the lymph nodes, however, have usually had normal lymphoid cell populations in the peripheral blood. We are now extending the study to include more patients with carcinoma and reticulum cell sarcoma.

Undoubtedly, the most attractive hypothesis, which leads to the greatest amount of further research, is that the changes in Hodgkin's disease represent an immunological reaction. Whether this is directed against tumour specific antigens and hence the disease, or against some undiscovered subclinical infection, remains to be proved.

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## BIOCHEMISTRY

### Pentitols and the Mechanism of Insulin Release

ALTHOUGH glucose was for a long time considered the most important physiological agent for stimulating release of the insulin from the islets of Langerhans, there have recently been reports that the five carbon polyhydric alcohol xylitol is also active in causing insulin secretion, either in whole animals<sup>1</sup> or in perfused pancreas preparations<sup>2</sup>. The substance is believed to be metabolized in mammalian tissues through the pentose phosphate pathway<sup>3</sup>. Because this pathway seems to be important in the release of insulin<sup>4,5</sup>, the effects of pentitols and pentoses on insulin secretion have been examined in greater detail, using isolated rat islets of Langerhans.

Islets of Langerhans were isolated from pancreas taken from male albino Wistar rats which had been fasted overnight. The islets were obtained free from acinar tissue using collagenase as in methods already described<sup>6,7</sup>. The separated islets were incubated at 37° C for 30 min in a bicarbonate buffered medium, containing 2.5 mmolar glucose. After the pre-incubation period, the islets were divided into groups of ten, and incubated for a further 30 min in medium containing albumin, together with either glucose, pentose or pentitol. The medium was then sampled for determination of its insulin content by immunoassay<sup>8</sup>.

The results of incubations are shown in Table 1. It can be seen that, in addition to xylitol, ribitol and ribose markedly stimulate the release of insulin. Ribitol and ribose are as effective as glucose, although xylitol is more effective; it has not previously been shown that ribitol stimulates insulin release. It has also been demonstrated

that the polyhydric alcohols arabitol, sorbitol and mannitol and the pentoses, xylose and arabinose, are without effect on insulin secretion<sup>9</sup>. None of these other polyhydric alcohols or pentoses are metabolized through the pentose phosphate pathway in mammalian tissues<sup>3</sup>. On the other hand, the three compounds ribose, ribitol and xylitol are all known to be readily metabolized through this pathway<sup>3</sup> and all promote insulin release.

Table 1. EFFECTS OF GLUCOSE, XYLITOL, RIBITOL AND RIBOSE ON INSULIN RELEASE FROM ISOLATED RAT ISLETS

Substance in medium	Concentration (mmolar)	Insulin release (mean $\pm$ S.E.M.) $\mu$ U insulin/ten islets/min
Glucose	2.5	19 $\pm$ 0.9 (40)
	20	37 $\pm$ 1.1 (40)
Xylitol	2.5	16 $\pm$ 1.6 (20)
	20	45 $\pm$ 1.6 (20)
Ribitol	2.5	18 $\pm$ 1.1 (20)
	20	38 $\pm$ 2.0 (20)
Ribose	2.5	18 $\pm$ 1.1 (20)
	20	41 $\pm$ 1.6 (20)

Numbers of observations are in parentheses.

Evidence about the mechanism of release by pentoses and hexoses has been provided from a study of the effects of adrenaline on insulin release. This is already known to inhibit the secretion of insulin caused by glucose both *in vivo*<sup>10</sup> and *in vitro*<sup>11</sup>. The effects of low concentrations of adrenaline on insulin release from isolated islets in the presence of xylitol are shown in Table 2. It can be seen that adrenaline is a potent inhibitor of insulin release caused by xylitol, as well as of release caused by glucose. This suggests that both glucose and xylitol are effective through a common adrenaline sensitive pathway.

Table 2. THE EFFECTS OF ADRENALINE (200  $\mu$ g/ML.) ON INSULIN RELEASE FROM ISOLATED RAT ISLETS IN THE PRESENCE OF GLUCOSE AND XYLITOL

Substance in medium	Insulin release (mean $\pm$ S.E.M.) $\mu$ U insulin/ten islets/min	
	Without adrenaline	With adrenaline
Glucose (20 mmolar)	40 $\pm$ 1.8 (15)	23 $\pm$ 1.3 (15)
Xylitol (20 mmolar)	46 $\pm$ 1.8 (15)	22 $\pm$ 1.5 (15)

Numbers of observations are in parentheses.

We do not know the complete biochemical sequence of events by which hexose sugars release insulin, although it seems likely that in order to stimulate release, hexoses have first to be metabolized to pentose phosphates.

Further evidence for this has been obtained by studying the effects of 2-deoxy-glucose on insulin secretion in this system. Equimolar concentrations of this substance inhibit the effects of glucose in stimulating insulin release but not those of xylitol. Because 2-deoxy-glucose is known to inhibit phosphohexoseisomerase<sup>12</sup> and glucose-6-phosphate dehydrogenase<sup>13</sup>, this indicates that xylitol is not being metabolized to glucose-6-phosphate, but rather that it is converted to another pentose derivative more directly concerned with insulin release.

The results shown in this communication strongly suggest a direct involvement of the pentose phosphate pathway in insulin release caused by sugars. On this basis, the mechanism of insulin release due to glucose may be as follows: glucose is first metabolized to a pentose by way of the pentose phosphate pathway, and some further metabolite of this affects the release of insulin. There is evidence that the later stages of the release mechanism may involve the synthesis and breakdown of cyclic 3' 5'-AMP (refs. 14 and 15). It may be that a pentose, or some derivative of a pentose, regulates the concentration of this substance intracellularly.

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### Loss of Neural Inductive Capacity of the Chick Primary Organizer by Treatment with Histone and its Restoration by Follicle-stimulating Hormone

THERE is some evidence that histones inhibit synthesis of DNA-dependent RNAs and thereby regulate genetic activity<sup>1-3</sup>. If this is so they should influence embryonic development by interfering with the differential expression of genetic activity. The effects of calf thymus histone fractions on the development of the chick embryos seem to depend on the stage chosen for treatment while those of poly-L-lysine do not<sup>4,5</sup>. Such effects are presumably caused by histones complexing with cytoplasmic molecules such as those concerned in embryonic inductions which appear in a sequential order during development<sup>6</sup>.

The primary organizer of a chick primitive-streak embryo induces formation of secondary neural tissue when it is placed between the epiblast and hypoblast of a host embryo<sup>7</sup>. This phenomenon obviously consists of a derepression of specific genes, the derepression being produced by the inductor molecules derived from the organizer graft. We have therefore chosen this process of neural induction as an experimental system and have studied the effects of the F1-histone fraction to elicit information on the possible interaction between the inductor molecules and the histones.

The organizer pieces (only the Hensen's nodes were actually used) were excised from primitive-streak embryos and placed in histone solution (2.5 mg/ml.) for 3 h, washed for 1 h in Pannett-Compton (PC) saline and transplanted into host primitive-streak embryos. Control grafts were placed in the same saline for 4 h and then transplanted.

The capacity of the grafts to form neural induction has been expressed as the "induction index" which reflects both the frequency and quality of neural inductions (see note in Table 1).

It will be seen from Table 1 that after a 3 h treatment with histone, grafts failed to produce inductions (Fig. 1). Reduction of histone treatment to 2 h resulted in a slight retention of the inductive capacity (Fig. 2). This may indicate that the loss of the inductive capacity is due to the histone complexing with the inducing molecules found in the organizer graft.

Table 1. INDUCTIVE CAPACITY OF HENSEN'S NODE GRAFTS AFTER HISTONE AND FSH TREATMENTS

Experiment	Histone treatment (h)	FSH treatment (h)	No. of grafts	No.	Inductions	A	B	C	D	Induction index
1 C	—	—	7	6	2	1	8	—	—	2.44
E	3	—	16	—	—	—	—	—	—	0
2 C	3	—	17	—	—	—	—	—	—	0
E 1	3	1	5	1	—	1	—	—	—	0.6
E 2	3	2	13	6	3	—	2	1	—	1.31
3 C	2	—	17	6	1	1	3	1	—	0.82
E 1	2	1	8	3	2	—	1	—	—	1.25
E 2	2	2	6	6	3	1	2	—	—	2.8
E 3	2	3	13	11	1	4	5	1	—	2.1

The induction index is arrived at as follows: The inductions produced are classified as A, B, C, D, based on their quality and scored 4, 3, 2, 1 respectively. The total points are divided by the number of grafts (including those which failed to induce neural tissue). The induction index therefore takes into account the frequency as well as the quality of inductions.

Histones are said to be highly toxic. But organizer grafts treated with histone seemed to be in a healthy state despite the use of large amounts of histones (Fig. 3). They did not, however, differentiate into neural tubes, while the control grafts did (Fig. 4).

The effects of poly-L-lysine and lysozyme were studied in similar experiments in order to see if the effects of histone were specific. The induction index of pieces of Hensen's node was 2.8 after 3 h of treatment with poly-L-lysine. Even a long treatment, up to 24 h, had no effect. The grafts, however, showed an abnormal cytological picture with most of the cells undergoing cytolysis. It is

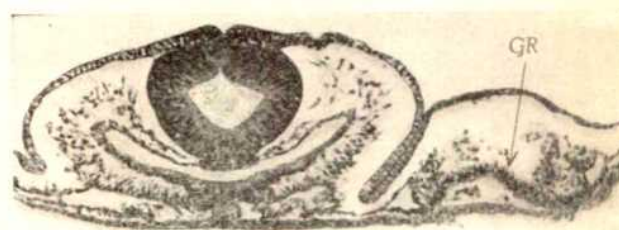


Fig. 1. Graft treated with histone 3 h illustrates the loss of neural inductive capacity. GR, graft.

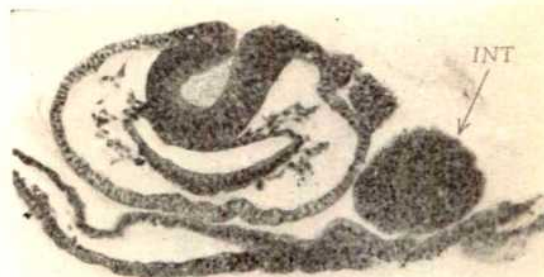


Fig. 2. Graft treated with histone 2 h showing induction of a lump of neural tissue (INT).

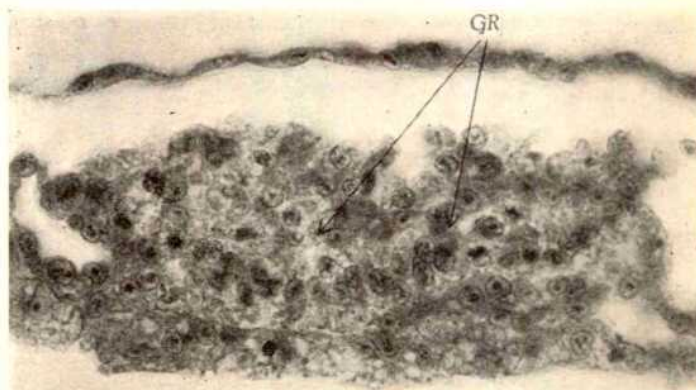


Fig. 3. Graft treated with histone 3 h to show the state of graft cells (GR).



possible that the inductive capacity of the grafts treated with polylysine may not have been their primary effect but may be caused by influences released from the cytolyzing cells. This point was checked using post-nodal pieces of primitive-streak which normally possess little inductive power<sup>9</sup>. In the controls of this series *PN-1* pieces (those pieces of primitive-streak from 0.8 mm behind the Hensen's node) had an induction index of 0.25. Treatment of these pieces with poly-L-lysine did not cause any significant increase in the induction index (see Table 2). Incidentally, this fact tallies with our earlier observation that the morphological effects exerted by histones on chick embryos *in vitro* could not be produced by poly-L-lysine<sup>4,5</sup>. Neither did lysozyme seem to have any inhibitory effect on the inductive capacity of the Hensen's node. The histone effects therefore appear to be specific.

It may be of some interest that we have been able to restore, by using follicle-stimulating hormone (FSH), the inductive capacity which had been lost by a previous treatment with histones (Figs. 5 and 6).

The grafts were treated with histone solution as described earlier and some of them were then transferred to a solution of FSH (NIH-FSH-S2) for 1, 2 or 3 h, washed for 0.5 h and grafted into host embryos as usual. The controls were left in PC saline until grafting. Various combinations of histone and FSH treatments were used. All procedures of treatment and washing were carried out at 37° C. The results (Table 1) show that increase of FSH treatment increased the capacity of the grafts to induce neuralizations. In both sets of experiments (3 h and 2 h treatment with histone), the increase in induction index was proportional to the duration of FSH treatment.

It would be necessary at this stage to say why FSH was chosen to restore the lost inductive capacity of the Hensen's node grafts. Experiments by us have so far shown that FSH has the capacity to support and induce morphogenesis<sup>8-13</sup>.

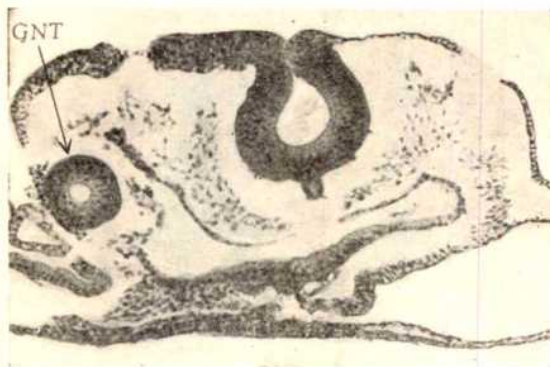


Fig. 4. The normal differentiation of untreated Hensen's node grafts into neural tube (GNT).

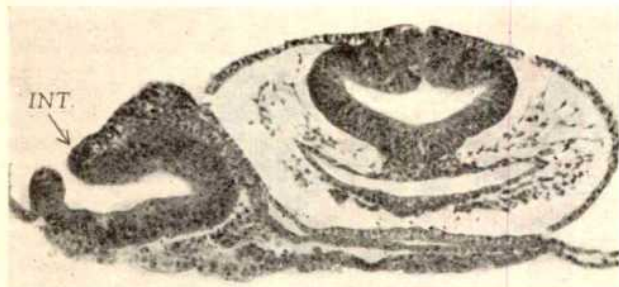


Fig. 5. FSH (3 h) induction (INT) after 2 h of treatment with histone.

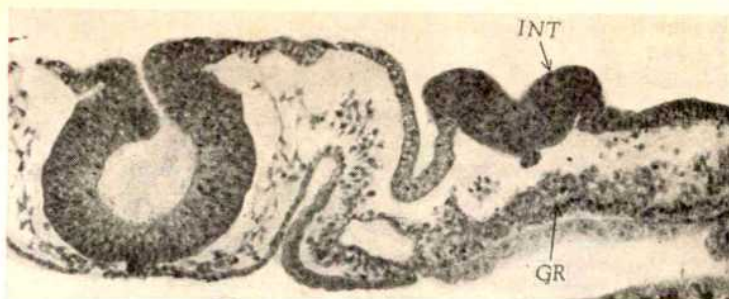


Fig. 6. FSH (3 h) induction (INT) after 2 h of treatment with histone. GR is the graft.

Table 2. EFFECTS OF POLY-L-LYSINE AND LYSOZYME ON THE INDUCTIVE CAPACITY OF *HN* AND *PN-1* GRAFTS

Inhibitor	Graft	Dura- tion of treatment	No. of grafts	Induc- tions	A	B	C	D	Induc- tion index
Poly-L-lysine (2.5 mg/ml.)	<i>HN</i>	3 h	15	15	4	6	3	2	2.8
		24 h	4	3	1	2	—	—	2.5
	<i>PN-1</i>	3 h	7	3	—	—	—	3	0.42
No inhibitor (control)	<i>PN-1</i>		8	2	—	—	—	2	0.25
Lysozyme (2.5 mg/ml.)	<i>HN</i>	3 h	18	18	13	4	—	1	3.6

*HN*, Hensen's node; *PN-1*, primitive-streak pieces 0.8 mm behind *HN*.

From the present experiments, it seems likely that histones complex with the inductor molecules to be found in the inducing system and cause a loss of the neural inductive capacity and that as FSH is put into the system this capacity increases. This could be caused by a direct inductive effect exerted by the FSH molecules or to a release of the natural inductor by a shift in the equilibrium leading to a breakdown of the inductor-histone complex after increase of the concentration of FSH.

These observations seem to show clearly the morphogenetic effects of FSH and also suggest that the FSH molecules may be similar to the inductive molecules. This is a very interesting possibility because ovary is the target organ of FSH and it is plausible that FSH could be localized in the developing ovum and take part in subsequent morphogenesis. Incidentally, these experiments can also be interpreted as suggesting that in primary embryonic induction the inducing stimulus acts in a genotropic way, that is, by impinging directly on the DNA and derepressing the genes.

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## Reaction of Ethyl Isocyanide with Haemocyanin

MANY compounds have been tested for their ability to react with the oxygen-binding site of haemocyanin and it has been found that thiourea<sup>1</sup> and thiocyanate<sup>2</sup> cause the expulsion of oxygen from *Helix pomatia* haemocyanin with a consequent decrease in the absorption of the copper bands at 346 and 570 m $\mu$ . This effect was almost completely reversed by dialysis or by treatment with an anion exchange resin. A report on the exchange reaction of ethyl isocyanide with oxygen in oxyhaemoglobin<sup>3</sup> encouraged us to investigate the effect of this compound on haemocyanin. Ethyl isocyanide does not remove copper from haemocyanin, but it does cause a decrease in the copper bands, which presumably indicates the expulsion of oxygen.

Haemocyanin was obtained from the haemolymph of the whelk, *Murex trunculus*, either by the method of Bannister *et al.*<sup>4</sup>, or by passing the haemolymph through a column (80  $\times$  2.4 cm) of 'Sephadex G-200' equilibrated with 0.05 molar *tris*-hydrochloric acid, pH 7.0, containing 0.1 molar potassium chloride. Ethyl isocyanide was synthesized by the method of Jackson and McKusick<sup>5</sup>. Copper was estimated by the method of Peterson and Bollier<sup>6</sup>, and protein was estimated from the absorbance at 280 m $\mu$  in 0.1 molar borate buffer, pH 9.2 at which pH the error caused by light scattering is minimal<sup>7</sup>.

The addition of ethyl isocyanide in concentrations from 0.5 to 50 mmolar to the haemocyanin caused a very rapid decrease in the copper bands at 346 and 570 m $\mu$ . The reaction was almost complete in less than 1 min at room temperature, and thereafter there was a slow drop in absorbance during a period of hours. Fig. 1 shows the relationship between concentration of ethyl isocyanide and absorbance at 346 m $\mu$  after 3 min (that is when the reaction is at least 95 per cent complete). The slow decrease in absorbance over longer periods may be partly caused by the fact that ethyl isocyanide is gradually hydrolysed in aqueous solution with the production of cyanide, which removes the copper from haemocyanin.

That ethyl isocyanide does not remove the copper atoms from haemocyanin is illustrated by the following experiment. To 3 ml. of haemocyanin (34.5 mg) in 0.1 molar phosphate buffer, pH 7.5, was added 0.05 ml. ethyl isocyanide (0.7 mmoles). The absorbance at 570 m $\mu$  was noted continuously for 10 min, during which time it dropped by 44 per cent. The solution was then passed through a column (40  $\times$  2.4 cm) of 'Sephadex G-25' equilibrated with 0.1 molar *tris*-hydrochloric acid, pH 8.3. The protein-containing fractions of the eluate were

pooled and analysed for copper in duplicate. The original haemocyanin solution was analysed for copper at the same time. Aliquots of the column effluent and of the original haemocyanin were diluted in 0.1 molar borate buffer, pH 9.2, and their absorption spectra were recorded in a Beckman 'DB' spectrophotometer. The copper content of the original haemocyanin was 0.240 per cent, and that of the haemocyanin treated with ethyl isocyanide was 0.250 per cent. From the absorption spectra, the ratios  $E_{346m\mu}/E_{280m\mu}$  and  $E_{570m\mu}/E_{280m\mu}$  were calculated. The values of these ratios were, for haemocyanin, 0.282 and 0.022; and for ethyl isocyanide-treated haemocyanin, 0.263 and 0.019, respectively. It was possible to regenerate the copper bands simply by bubbling oxygen through the mixture of ethyl isocyanide and haemocyanin. Ethyl isocyanide was added to haemocyanin in 0.1 molar phosphate buffer at pH 7.5. After 5 min the absorbance at 346 m $\mu$  had decreased to 45 per cent of its original value. Pure oxygen was then bubbled through the mixture for 10 min. After 5 min the absorbance had risen to 76 per cent, and after 10 min to 80 per cent of its original value.

The exchange reaction of ethyl isocyanide with oxygen in oxyhaemocyanin should prove useful in studies of the mode of binding of oxygen by haemocyanin. Ethyl isocyanide displaces the oxygen more effectively than thiourea and thiocyanate, and the reaction is easily reversible. Rombauts and Lontie<sup>1,2</sup> found that at the greatest concentrations of thiourea and thiocyanate they used, only about 10 per cent and 23 per cent respectively of the original absorbance of the copper bands remained. We have found that in optimal conditions (0.5 mg/ml. of haemocyanin in 0.1 molar borate buffer, pH 9.2; molar ratio of ethyl isocyanide to copper, 4,000) the absorbance of haemocyanin at 346 m $\mu$  can be reduced to less than 1 per cent its original value by treatment with ethyl isocyanide.

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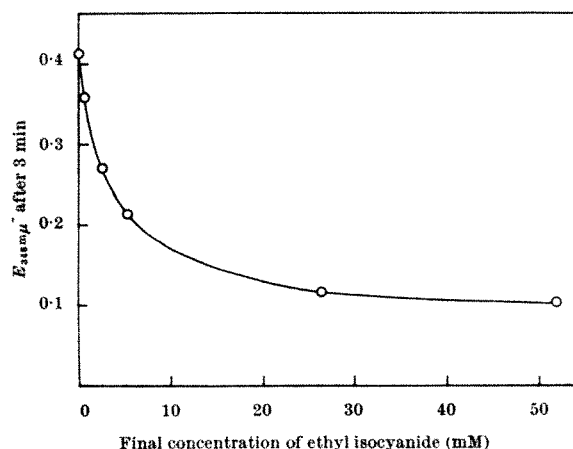


Fig. 1. Relationship between final concentration of ethyl isocyanide and absorbance of *Murex trunculus* haemocyanin at 346 m $\mu$  after 3 min. To 2.5 ml. of haemocyanin in 0.1 molar borate buffer, pH 9.2 (protein concentration, 1 mg/ml.), was added ethyl isocyanide as an emulsion in the same buffer, to the final concentration indicated. The absorbance at 346 m $\mu$  was noted continuously in a 1 cm cell. The emulsion of ethyl isocyanide also absorbed at 346 m $\mu$ , and so the same amount of ethyl isocyanide was added to 2.5 ml. of borate buffer in the reference cuvette.

## Uptake of Selenite labelled with Selenium-75 by Human Leucocytes *in vitro*

PREVIOUS work (reviewed in ref. 1) has shown that after the injection of selenite labelled with selenium-75 ( $^{75}\text{SeO}_3^{--}$ ) into animals radioactivity is incorporated into the proteins of various tissues. Recent studies in our laboratory<sup>2</sup> have demonstrated that in humans selenite is rapidly taken up by the liver, kidneys and by malignant tumours. Analyses of both normal and tumour tissues have indicated that the selenium is bound to tissue protein by a covalent linkage. We have investigated the mechanism by which inorganic selenium is taken up by human cells.

Plasma rich in leucocytes was obtained from normal human venous blood by the method of Moorhead *et al.*<sup>3</sup> using phytohaemagglutinin. Sterility was maintained throughout the procedure. To 4 ml. of this plasma was added 8 ml. of tissue culture medium, TC-199 (Difco)<sup>4</sup>, and 1 ml. of 0.9 per cent sodium chloride con-



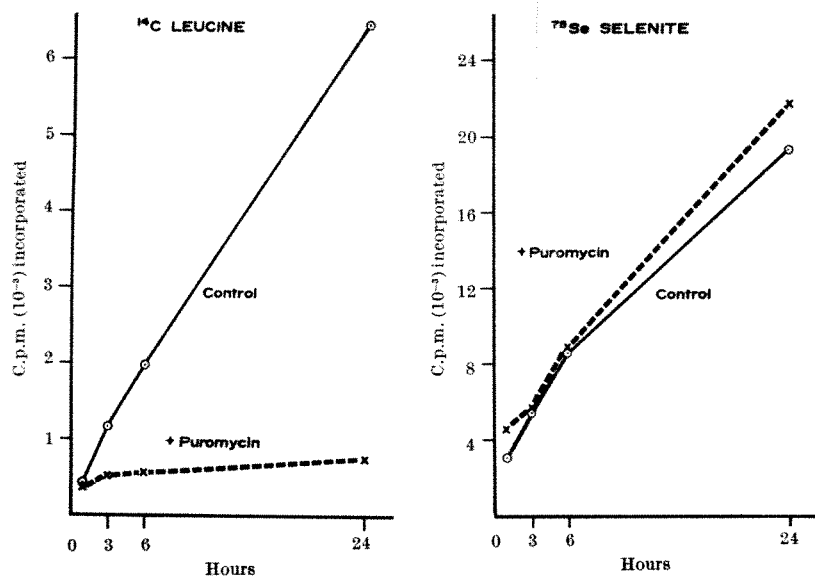


Fig. 1. The effect of puromycin on the incorporation of labelled leucine (left) and on the uptake of labelled selenite (right) by human leucocytes *in vitro*. Puromycin (3.6  $\mu\text{g}/\text{ml}$ ) was added to the cell suspensions 10 min before addition of the radioactive tracers.

taining either 1  $\mu\text{c}$ . of labelled sodium selenite (0.03  $\mu\text{g}$  of selenium) or 5  $\mu\text{c}$ . of carbon-14 uniformly labelled L-leucine (0.02  $\mu\text{moles}$ ). Within 0.5 min of the addition of radioactivity two samples of 2 ml. were removed and the cells were separated by centrifugation. Four additional 2 ml. samples were transferred to sterile siliconized tubes, stoppered with cotton-wool. Tubes were left undisturbed in an incubator at 37° C for periods from 1 to 24 h. After incubation leucocytes were separated by centrifugation and washed twice with 3 ml. of 0.9 per cent sodium chloride. The washed cells were lysed in 0.1 molar sodium hydroxide for assay of radioactivity and determination of protein concentration.

The time course of incorporation of labelled leucine and selenium and the effect of puromycin on each are shown in Fig. 1. Puromycin (3.6  $\mu\text{g}/\text{ml}$ ) markedly inhibited the incorporation of labelled leucine, but at the same concentration had virtually no effect on the uptake of selenium. These results indicate that the incorporation of selenium is not dependent on protein synthesis.

Table 1. EFFECT OF INHIBITORS ON LABELLED SELENITE INCORPORATION BY LEUCOCYTES

Inhibitor	Concentration (mmolar)	Duration of incubation		
		Initial (0.5 min)	1 h	4 h
None	—	17,028	23,025	43,302
None	—	17,912	25,324	42,064
NaF	10.0	38,877	20,562	13,701
NaF	0.2	31,397	28,010	34,145
Iodoacetamide	10.0	468	150	195
Iodoacetamide	0.2	5,442	4,164	9,737
KCN	1.0	3,906	—	2,828
PCMB	0.5	1,158	5,261	8,842
PCMB	0.01	6,130	11,580	18,522

Inhibitors were added to the cell suspension before the addition of labelled selenite.

The effects of other inhibitors upon the uptake of selenite by leucocytes are shown in Table 1. The results suggest that two processes are involved: an almost immediate binding and a more gradual incorporation continuing for several hours. The rapidity of the initial binding indicates that enzymes are not involved. From the inhibition by iodoacetamide and *p*-chloromercuribenzoate (PCMB) sulphhydryl groups seem to be involved. The reaction may be an exchange of  $\text{SeO}_3^{2-}$  for  $-\text{SH}$  or an addition of  $\text{SeO}_3^{2-}$  to  $\text{RSH}$  yielding  $\text{RS}-\text{SeH}$  (ref. 5). The inhibition by cyanide of initial binding of selenium may also be explained by

postulating an interaction with sulphhydryl groups because cyanide reacts with cysteine residues of protein<sup>6</sup>. In a separate experiment (not included in Table 1), addition of PCMB (0.5 mmolar) to the medium after 4 h of incubation resulted in an immediate discharge of 33 per cent of the radioactive selenium already incorporated into the leucocytes.

Enhancement of the initial binding of selenium by fluoride is not readily explained. In any case more than half of the selenium bound initially was lost from the cells during continued incubation in 10 mmolar sodium fluoride (Table 1).

The second process, the slower accumulation of selenium, is clearly dependent on metabolic activity because cyanide, fluoride and iodoacetamide inhibit the process. The effect of the latter two inhibitors suggests that normal glycolysis is essential. The nature of the reactions by which selenite is incorporated into cells is unknown, but presumably a series of reduction reactions is involved<sup>5</sup>. McConnell and Roth<sup>7</sup> in their studies of the incorporation

of selenite into rat liver ribosomes *in vitro* observed that the addition of cell sap and ATP stimulated, while cyanide inhibited, uptake. Their findings, together with ours reported here, support the idea that an energy dependent process is involved in the uptake of selenite.

In addition, these results indicate that the incorporation of selenite by intact leucocytes is a complex phenomenon consisting of at least two processes which differ both in respect to rate and in susceptibility toward inhibition.

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### Molecular Weight Isoenzymes of Arginine Kinase in the Mollusca and their Association with Muscle Function

CREATINE kinase from various species has always been found to have a molecular weight of about 80,000 (ref. 1) and to be composed of two subunits<sup>2-4</sup>. The subunits are of two types, one forming the enzyme (*BB*) found in brain and the other forming the enzyme (*MM*) found in the white fibres of skeletal muscle. A third, hybrid enzyme composed of one each of the two subunits (*BM*) is found in heart muscle and the red fibres of skeletal muscle<sup>3</sup>. Thus,

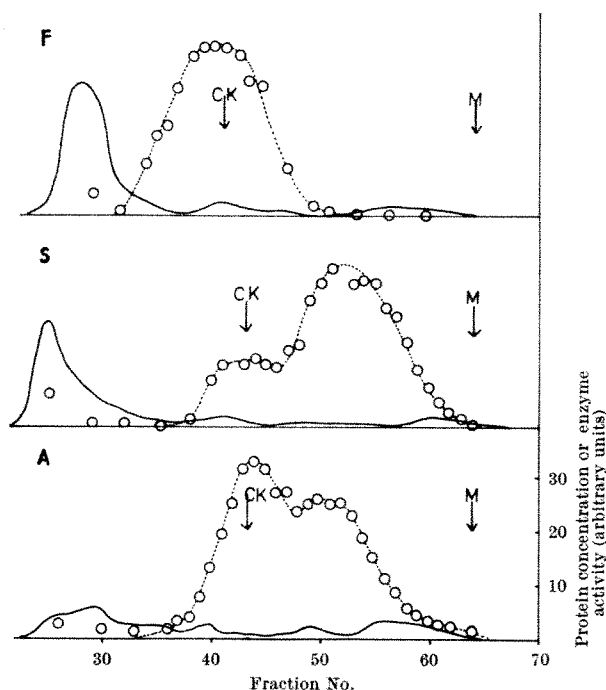


Fig. 1. 'Sephadex G-100' column elution patterns of muscle extracts of *Cardium edule*. F, Foot muscle; S, siphon muscle; A, adductor muscle. —, Protein concentration measured at 253 m $\mu$ ; ○---○, arginine kinase activity. The relative elution positions of proteins of known molecular weight are shown in the diagram; CK, rabbit creatine kinase; M, horse myoglobin.

in vertebrates, a particular enzyme type appears to be associated with the function of a particular organ or cell type.

The corresponding enzyme in invertebrates, arginine kinase, was originally thought to exist only as a monomer of molecular weight about 40,000 (refs. 5–7). More recently arginine kinases with a molecular weight of about 80,000 have been found among the annelids<sup>8</sup> and echinoderms<sup>1,8</sup>. By comparison with creatine kinase these enzymes have also been assumed to be dimers although this still remains to be formally demonstrated. The two types of arginine kinase appeared to be representative of particular species. Multiple forms of the monomer arginine kinase have been demonstrated by starch gel electrophoresis<sup>9</sup>, but no attempt was made to correlate these with a particular function.

The present communication reports the discovery in some molluscs of the 80,000 molecular weight arginine kinase together with the 40,000 molecular weight enzyme. The distribution of the two enzymes in different muscle tissues has been investigated in two species and appears to correlate with muscle function.

Animals were killed by cooling to approximately  $-10^{\circ}\text{C}$ , the appropriate tissues dissected out and extracted by grinding with *N,N*-di(2-hydroxyethyl) glycine<sup>10</sup> buffer, pH 8.0 and 1.0.005 containing 0.3723 g of disodium EDTA and 0.3735 g of *N,N*-di(2-hydroxyethyl)glycine (bicine) titrated to pH 8.0 with concentrated sodium hydroxide free of carbon dioxide and made up to a final volume of 1 l. The cell debris was removed by centrifugation and the extract concentrated by vacuum dialysis against the same buffer. Molecular weight determinations of the arginine kinases were carried out on 'Sephadex G-100' as previously described<sup>1</sup> and enzyme activity in the fractions determined by assay for acid labile phosphate<sup>9</sup> after incubation with ATP, magnesium ions and arginine.

All the extracts were found to be without kinase activity towards creatine, glycoeyamine or taurocyamine. The distribution of the two types of arginine kinase in the muscles of several eulamellibranch molluscs is shown in

Table 1, which shows that both enzymes always occur in the adductor and siphon muscles of the animals investigated and occasionally in the foot as well. The concentrations of the two enzymes, however, vary considerably in the different tissues. The enzyme of molecular weight 40,000 predominates in the siphon; both enzymes occur in almost equal amounts in the adductor; the foot contains only the 80,000 molecular weight enzyme or in addition a trace of the 40,000 molecular weight enzyme. The relative proportions of the two enzymes in *Cardium edule* are shown in Fig. 1 and a similar distribution was obtained with *Tellina tenuis*. The proportions of the two enzymes were not affected by the presence or absence of EDTA or by lowering the pH of the buffer to 6.5, and the application of low concentrations of foot extract to the column did not result in the production of any 40,000 molecular weight enzyme. Hence there is no evidence that the 80,000 molecular weight enzyme is an artefact produced by association of two monomer enzyme molecules. Furthermore, it was found that the two enzyme types differed in stability; the 40,000 molecular weight enzyme was completely inactivated by storage for 2 months at  $-15^{\circ}\text{C}$  although the 80,000 molecular weight enzyme was scarcely affected. Activity of the monomer could be largely restored by treatment with 1 mmolar dithiothreitol (Cleland's reagent).

Table 1. DISTRIBUTION OF ARGININE KINASE ISOENZYMES AMONG SOME EULAMELLIBRANCH MOLLUSCS

Order and species	Muscle type	Arginine kinase	
		80,000 mol. wt.	40,000 mol. wt.
Heterodonta			
<i>Cardium edule</i>	Adductors	+	+
	Siphon	+	+
	Foot	+	—
<i>Tellina tenuis</i>	Adductors	+	+
	Siphon	+	+
	Foot	+	—
<i>Venus casina</i>	Foot	+	Trace
<i>Venerupis pullastra</i>	Adductors	+	+
<i>Laevocardium crassum</i>	Foot	+	Trace
Anapoda			
<i>Barnea parva</i>	Foot	+	Trace

Starch gel electrophoresis of muscle pooled from twenty animals (Fig. 2) revealed that only siphon and adductors contained the most electropositive component. This is assumed to correspond to the 40,000 molecular weight

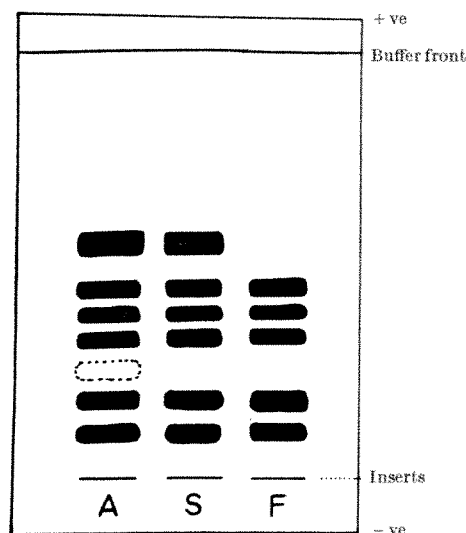


Fig. 2. Starch gel electrophoresis of muscle extracts of *Cardium edule*. The positions of arginine kinase activity are shown by solid bars, slight activity is indicated by dotted area. A, Adductor muscle; S, siphon muscle; F, foot muscle. Extracts, buffers and the starch gel were prepared containing 1 mmolar Cleland's reagent to minimize the possibility of thiol oxidation artefacts.



enzyme and has a mobility similar to that of other known monomer arginine kinases<sup>9</sup>. The 80,000 molecular weight arginine kinase is represented by five or six components, possibly reflecting the allelic variants of one or two structural genes.

Although we have not been able to distinguish the vertebrate equivalent of smooth and striated muscle in these molluscs it is interesting to compare the distribution of enzyme type with muscle function. The siphon muscle has a principally tonic function—keeping the pair of siphons extended while the animal is active under water. The adductors have a tonic function, keeping the shell valves closed under adverse conditions, but are also capable of short fast phasic contractions to close the shell valves rapidly. The foot has almost exclusively short bursts of rapid activity for burrowing in mud or sand. The distribution of the monomer arginine kinase is associated with the tonic function similar to the smooth muscle in vertebrates while the 80,000 molecular weight enzyme is associated with the active "striated type" of muscle function.

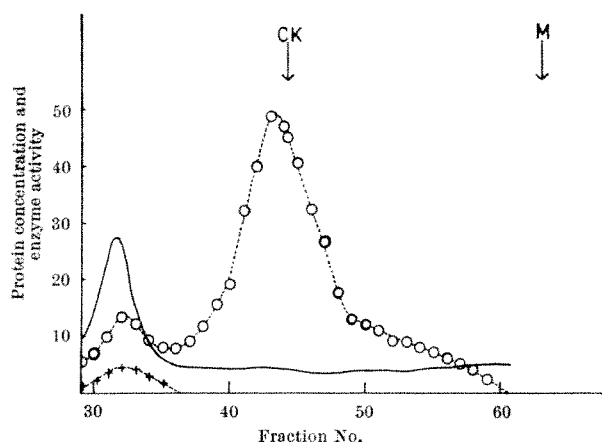


Fig. 3. 'Sephadex G-100' column elution patterns of extracts of foot muscle of *Barnea parva*. —, Protein concentration measured at 253 m $\mu$ ; ○ — ○, arginine kinase activity; + · · · +, ATPase activity. The elution positions of standard proteins, rabbit creatine kinase (CK) and horse myoglobin (M), are marked as arrows.

This type of distribution would appear logical if the less highly organized smooth type of muscle evolved first and the more rapid type of muscle activity involving a dimeric arginine kinase was a relatively more recent evolutionary adaptation. This interpretation suggests that the arginine kinase is actually involved in the contractile machinery of the muscle. Evidence has been presented that creatine kinase binds to muscle myosin<sup>11</sup>, which supports this suggestion. We have found that variable amounts of arginine kinase sometimes appear just behind the break-through peak during 'Sephadex' chromatography if EDTA is omitted from the initial extraction medium. As is shown for *Barnea parva* (Fig. 3) this coincides with a peak of ATPase activity. No ATPase activity is found associated with the principal enzyme peaks. Czok and Bücher<sup>12</sup> reported that a medium containing EDTA was most efficient for extracting creatine kinase from rabbit muscle. It seems that arginine kinase behaves in a similar way and that in both cases metal ions mediate the binding of the enzyme to myosin. Because the myosin molecule is itself a dimeric molecule with two ATPase sites, the myosin-kinase interaction could be the selective advantage which has resulted in the evolution of a dimeric arginine kinase from a monomer enzyme as part of a more functionally efficient contractile mechanism. From studies of the kinases in other phyla<sup>1</sup> it is probable that this evolutionary event has occurred on more than one occasion.

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## BIOLOGY

### Danger of Chlorinated Hydrocarbon Insecticides in Birds' Eggs

THERE has been considerable concern about the presence of chlorinated hydrocarbon insecticides in wild birds' eggs. The toxicology of chlorinated hydrocarbon insecticides has been evaluated<sup>1-3</sup>, using hens' eggs, and emphasis has usually been placed on the effects on hatching. In the experiments of Dunachie and Fletcher, hatchability was not affected even by very large doses (for example, 200 p.p.m. of dieldrin).

We have tried to evaluate the danger from these chemicals by measuring the concentration of dieldrin in the blood of embryos and in chicks hatching from eggs treated with this compound before incubation. Chlorinated hydrocarbon insecticides were injected into the yolk of White Leghorn eggs by the method of McLaughlin *et al.*<sup>4</sup>; all doses were given in 0.1 ml. of propylene glycol. In a first series of experiments, the dispersion of dieldrin in the yolk was measured at various intervals after injection. For this purpose each egg was dosed with 40  $\mu$ g of dieldrin. After each time interval the eggs were frozen at  $-15^{\circ}$  C for 1 or 2 days, after which the yolk was taken out and divided into segments while still frozen, as shown in Fig. 1. A central disk was cut out with a knife, and three circular segments were punched from the disk with a cork-borer (A, B and C in Fig. 1) which were analysed separately for dieldrin by gas liquid chromatography. Cleaning was by a dimethylformamide partition process<sup>5</sup>, followed by percolation of the extract over an activated 'Florisil' column. Indian ink was added as a marker to five of the eggs and showed that the top of the injection needle had entered segment A of each egg. The dis-

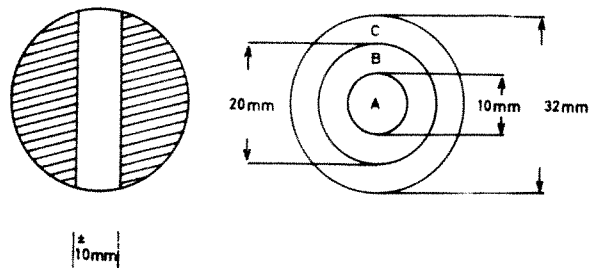


Fig. 1. Division of the yolk for the distribution study. A disk was cut from the frozen yolk with a knife (left side). Three circular segments were then punched from the disk with a cork-borer (right side).

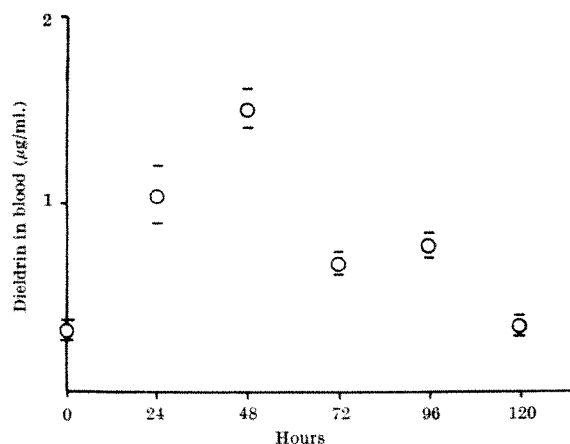


Fig. 2. Concentrations of dieldrin in the blood in normally fed chicks which hatched from eggs treated with 240 µg of dieldrin before incubation (five chicks/value).

tribution of dieldrin in the three segments at different times after treatment is shown in Table 1. For comparison, similar analyses were made of an egg from a duck which had been treated with dieldrin and of two hens' eggs in which the natural DDE background was estimated. In addition, the yolk sac of one egg which had been incubated for 14 days was divided arbitrarily into three parts, and these were analysed separately. Equal concentrations were found, which indicates that during incubation the injected dieldrin (before incubation) is distributed throughout the yolk. Distribution is nearly complete after 5 days of incubation and from this time the injected eggs can be compared with eggs which received their dose of dieldrin from the birds which laid them.

In a second experiment, the concentration of dieldrin was measured in the yolk, blood and tissues of 14 day old embryos and chicks 0-6 h after hatching. Eggs were each dosed with 40 µg of dieldrin. For analysis of blood a rapid and accurate method, making use of gas liquid chromatography, devised by Richardson *et al.*<sup>9</sup>, was used. The method consists of extracting the blood sample with acetone by percolation over a column of silica gel and shaking the extract with an aqueous solution of sodium sulphate after the addition of 1-5 ml. of hexane to the acetone extract. From the hexane phase, samples can be applied directly to the gas chromatograph (Aerograph A-610-C). Embryonic blood was obtained from a blood vessel close to the egg membrane by means of heparinized capillaries of known volume. Chick blood was obtained by decapitation.

Table 2 shows that the concentration of dieldrin in the blood of the 0-6 h old chick is little greater than that in the 14 day old embryo, which indicates that the absorption process of the yolk sac during the last week of development does not cause an important increase in the concentration of dieldrin in the blood. The increase in the tissue concentration of dieldrin indicates that the dieldrin which is released from the yolk has been deposited in the tissues of the embryo.

Table 1. RELATIVE CONCENTRATION OF DIELDRIN IN THE THREE SEGMENTS FROM THE YOLK

	No. of eggs	Segment A	Segment B	Segment C
2 h after injection, eggs not incubated	2	100	2.8	0.4
120 h after injection, eggs not incubated	10	100	4.7	0.5
110 h after injection, eggs incubated	2	100	65.5	71.6
Egg of a duck treated with dieldrin	1	100	98.8	99.1
Normally occurring DDE background (0.14-0.17 p.p.m.)	2	100	103.0	99.7

The concentration segment A has been given the value 100. The averages for each group are given.

Table 2. DIELDRIN RESIDUES IN THE BLOOD, THE YOLK AND THE BODY OF EMBRYOS AND CHICKS DEVELOPING FROM EGGS WITH 40 µg OF DIELDRIN BEFORE INCUBATION

	No.	Dieldrin concentration In blood (µg/ml.)	In yolk (p.p.m.)	In whole animal minus yolk (p.p.m.)
14 day old embryo	10	0.028 ± 0.005	1.70 ± 0.27	0.11 ± 0.02
0-6 h chick	10	0.038 ± 0.006	1.44 ± 0.31	0.42 ± 0.16

Values are ± standard deviation.

In another experiment, the blood concentrations are measured in normally fed chicks during their first 5 days of life (Fig. 2). In this case each egg had been dosed before incubation with 240 µg of dieldrin. During the first 48 h after hatching the concentration of dieldrin in the blood increases considerably to a maximum of about 1.5 µg/ml. After the third day the concentration begins to decrease. At 120 h the initial concentration was reached again. Most bird species, including the chicken, do not eat during their first 24-48 h, and so absorption of the remaining yolk during this period obviously results in a strongly increased concentration in the blood. As soon as they start to eat and body weight increases, the blood concentration of dieldrin decreases. To test the effect of starvation beyond the first day, blood was taken from starved chicks at 0, 24, 48 and 72 h after hatching. Doses ranged from 240 to 960 µg to each egg. The results of this experiment are given in Fig. 3.

In these circumstances the concentration of dieldrin in the blood continues to increase after the second day. When a dose of 960 µg to each egg (about 17 p.p.m.) was given before incubation the chicks showed convulsions between 58 and 96 h after hatching. At that time a mean (critical) concentration in the blood of 11.5 µg/ml. ± 2.27 (S.D.) (fourteen chicks) was measured. Untreated chicks starved for the same length of time (or even 24 h longer) were in good health and readily started feeding afterwards. Fig. 4 shows a linear relation between the dose and the concentration of dieldrin in the blood in the 0 h chick.

We conclude that absorption of the yolk after hatching may cause poisoning of young birds by insecticides at a concentration of the residue which does not affect the rate of hatching. Our results explain the 100 per cent mortality immediately after hatching, observed by Dunachie and Fletcher in chicks which hatched from eggs

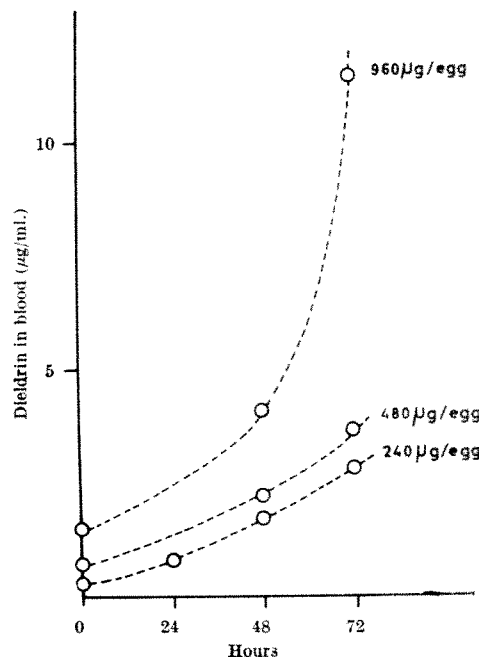


Fig. 3. Concentrations of dieldrin in the blood in starved chicks which hatched from eggs dosed with three different doses before incubation (5-14 chicks/value).



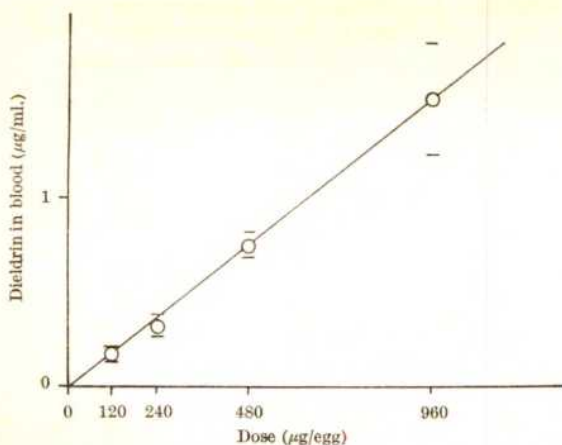


Fig. 4. The relation between blood concentration of dieldrin and dose for a chick at 0 h. Mean values and standard deviations are given (five chicks/value).

dosed with 100 p.p.m. of aldrin. These authors usually destroyed the chicks shortly after incubation. It may be assumed that several of the doses of the chlorinated hydrocarbon insecticides, which failed to produce any influence on hatchability or only caused a slight decrease in the rate of hatching, would have proved to be lethal if the chicks had been kept alive longer. In experiments of this kind it is therefore advisable, as McLaughlin mentioned<sup>4</sup>, to observe the chicks for a longer period after hatching. In trout fry mortality induced by DDT after absorption of the yolk sac has been reported<sup>6,7</sup>.

It is well known that in nature recently hatched birds are often deprived of food for long periods, for example, by unfavourable weather conditions. Their natural adaptation is the ability to live on the yolk for a few more days. The fast absorption of the yolk which follows may be hazardous when the birds have been exposed to toxic and persistent chemicals in the environment.

In 1965 we observed a large mortality among recently hatched sandwich terns in a colony in the Netherlands. The animals showed the same kind of convulsions as the chicks which hatched from the eggs dosed with 960 µg of dieldrin. Tissue analyses showed us that insecticides were the chief cause of death (Koeman *et al.*<sup>8</sup>) at that time.

We are grateful to Mr Richardson for discussion and advice on the method of blood analysis which was used. We also thank Professor C. Romijn, director of the Institute of Veterinary Physiology, for the use of the incubator and Mr D. van Dijk for his help. Eggs were supplied by the Zootechnical Institute of the University of Utrecht.

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## Respiration of Air by the Primitive Fish *Polypterus senegalus*

THE primitive actinopterygian fish *Polypterus senegalus* Cuvier inhabits shallow and swampy waters throughout tropical Africa, often in conditions of severe oxygen deficiency, and its adaptation to this type of habitat includes the possession of spiracles and a double swim bladder or lung which has long been assumed to act as an accessory respiratory organ. Conflicting views have, however, been expressed regarding the use of spiracles in connexion with the respiration of air. Budgett<sup>1</sup> stated that the spiracles are used to take in and expire air from the swim bladders; but Boulenger<sup>2</sup> concluded that there was little evidence to show that *Polypterus* comes to the surface to breathe. More recently, it has been shown that the spiracles are used to inhale air which is passed to the lungs<sup>3</sup>. Air is inspired both in conditions of low oxygen concentration and during periods of excessive activity.

The investigation reported here has been carried out to obtain further information regarding the histology and physiology of aerial respiration in this species. Fish were injected with coloured rubber latex, and examination of the lungs revealed that they are highly vascular (Figs. 1 and 2). Transverse sections through the lungs showed an outer muscular coat of two layers of oblique and circular muscles, beneath which lies a thinner layer of loose connective tissue, containing branches from the pulmonary veins and arteries. The lungs are lined with a

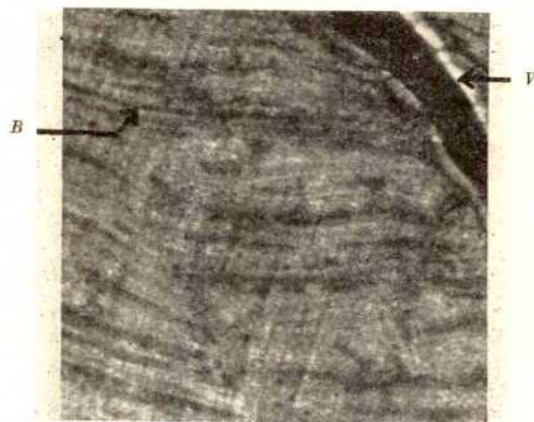


Fig. 1. The vascular epithelial lining of the lung. V, Branch of the pulmonary vein; B, branchlet of the pulmonary vein. ( $\times 60$ .)

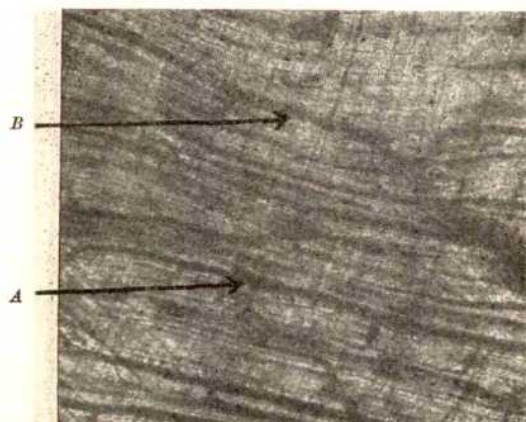


Fig. 2. Enlarged area of the epithelial lining of the lung. A, Branchlet of the pulmonary artery; B, branchlet of the pulmonary vein. ( $\times 270$ .)



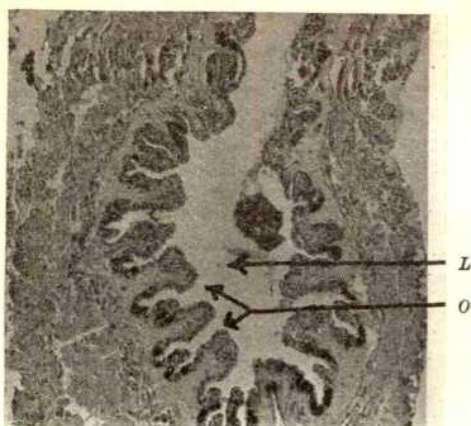


Fig. 3. Cross section of the right lung. L, Lumen; O, alveoli. ( $\times 60$ .)

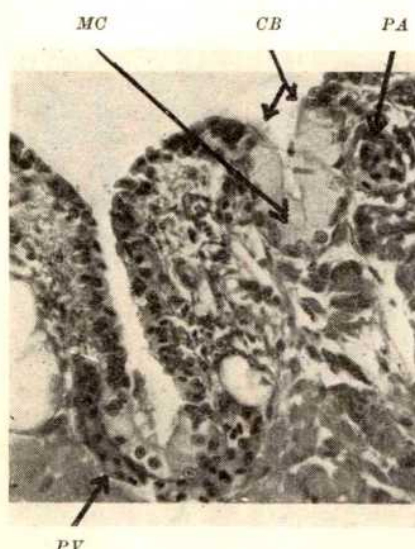


Fig. 4. Enlargement of a cross section of the right lung showing the epithelial lining. CB, Ciliated band; MC, mucous cell; PA, pulmonary artery; PV, pulmonary vein. ( $\times 270$ .)

highly vascular layer which is thrown into alveoli (Fig. 3). The epithelium of the lung is richly supplied with capillaries, among which are regular ciliated mucous bands running along the longitudinal axes of the lung (Fig. 4).

It therefore seems that the vascular lining of the lung cavity is involved in gaseous exchange. The ciliated bands secrete mucus which is distributed over the respiratory surface by ciliary action and assists in gaseous exchange. Both these features are characteristic of a respiratory epithelium. *Polypterus* makes use of its pulmonary respiratory mechanism when branchial respiration is insufficient to satisfy its oxygen requirements. Blood from the pulmonary arteries is oxygenated in the lung, if not already saturated on its passage through the gills.

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### Serological Evidence for Speciation in the Grey Kangaroo, *Macropus giganteus* Shaw 1790 (Marsupialia : Macropodidae)

THE taxonomy of grey kangaroos (*Macropus giganteus* and "subspecies") is at present poorly understood; the number of species recognized by currently accepted authorities ranges from one<sup>1</sup> to four<sup>2</sup>. As part of a broad taxonomic study we collected the blood sera of 839 animals from more than forty localities throughout the range of the grey kangaroos and measured antigenic and electrophoretic variations in the serum proteins. We hoped to find differences, characteristic of particular groups of animals, which would correlate with geographical or taxonomic groups, and which might be useful in determining the degree of mixing and interbreeding between such groups. The details of these studies will be published elsewhere; here we wish to summarize the results as they relate to the problem of speciation in grey kangaroos.

Examination of all sera by starch-gel electrophoresis<sup>3</sup> revealed a polymorphism of the iron-binding protein (transferrin) similar to that demonstrated by Cooper and Sharman<sup>4</sup> in the red kangaroo, *Macropus rufus*. The hypothesis that this is a simple genetic polymorphism controlled by two co-dominant alleles allowing the production of three recognizable phenotypes is not contradicted by the considerable amount of data obtained by breeding grey kangaroos in enclosures at Canberra. All populations sampled in Western Australia, South Australia, Kangaroo Island and central New South Wales as well as a single individual from western Victoria, possess both alleles; all members of populations sampled in eastern Victoria, eastern and northern New South Wales and south-eastern Queensland, as well as a single individual from Tasmania, were found to be homozygous for *Tf<sup>A</sup>*, the allele controlling the production of the fast moving variant and its associated minor components. Thus two groups of populations can be distinguished on the basis of the presence or absence of one allele (*Tf<sup>B</sup>*) in this simple monogenic system. Because the observed characteristics of the serum proteins correlate with the geographical distribution of the populations, we refer to these groups as Eastern and Western.

The sera were also tested by double-diffusion<sup>5</sup>, using antisera produced in grey kangaroos by injecting members of the Eastern group with sera from individuals of the Western group, and vice versa. Injection of Western group animals with sera from other Western greys provoked no antibody response; we did not immunize Eastern greys with sera from other Eastern animals. The results of these tests showed that each group possesses characteristic antigens. The antisera react with two or more proteins, and we therefore assume that the antigenic differences between the two groups have a polygenic basis. Moreover, these differences have been shown to relate to different proteins from the transferrins.

While Eastern greys reacted only with antiserum against Eastern animals, and Western greys only with anti-Western sera, sera from five Eastern  $\times$  Western hybrids bred in captivity reacted with antisera against both Eastern and Western greys. One of these hybrids, a male, was backcrossed with an Eastern group female, and the serum of the resulting offspring reacted like that of an Eastern group animal.

Significant exceptions to the generalization that Western group grey kangaroo populations could be distinguished on the basis of the presence of *Tf<sup>B</sup>* were obtained from data derived from animals in two New South Wales populations which included greys possessing this allele. Three lines of evidence suggest that at Mt. Hope, central New South Wales, both kinds of greys were present but did not interbreed. (1) Of seventy-five animals from Mt. Hope most reacted serologically as Western group greys, but fourteen reacted like Eastern greys. None showed hybrid characteristics. (2) The fourteen Eastern greys



were also homozygous for *Tf*<sup>4</sup>. (3) Seven of the fourteen were pouch young. Although their mothers were not bled, their serological reactions and homozygosity suggest that these females were Eastern greys which bred with males of their own group.

Similarly, of forty-two animals taken at Balranald in the Riverina district of New South Wales, four were Eastern group with respect to antigens and were also homozygous for *Tf*<sup>4</sup>. One of these four was an adult male and the other three comprised a breeding female with her pouch young and one young-at-foot. Clearly this Eastern group female must have bred with Eastern group males in spite of her association with a predominantly Western group population.

Thus although it is possible with limited success to cross the two kinds of greys in captivity, our results suggest that hybridization does not occur in the field. The occurrence of sympatry without hybridization is good evidence for the existence of two species of grey kangaroos. Furthermore, other differences between the two kinds have been demonstrated. Thus gestation periods determined to date at Canberra are characteristic of each group: Eastern group has a mean gestation period of  $36.7 \pm 0.30$  days ( $n=10$ ); Western group,  $29.5 \pm 0.35$  days ( $n=20$ ); and the hybrid cross,  $34.4 \pm 0.16$  days ( $n=16$ ). In addition, the two species are distinguished by a difference in coat colour. Our records show that both colour morphs have been sympatric at Mt. Hope since observations of the population began in 1962, so that temporary or recent immigration of Eastern greys into the area cannot be the explanation for the observed lack of interbreeding.

Recently we were able to investigate the sera of eleven greys, kindly obtained for us by Mr G. Blackman (Queensland Department of Primary Industries) from the Townsville area, about 300 miles south of Cooktown, the type locality of *Macropus giganteus* Shaw 1790. These animals were all Eastern and similar in appearance to greys from Cooktown. Accordingly, consideration should be given to the application of the specific name *giganteus* to the Eastern species, including the Tasmanian grey kangaroo. The Western grey then becomes *Macropus fuliginosus* (Desmarest 1817) because the Kangaroo Island form was first named of this group of subspecies.

This work was carried out while one of us (J. A. W. K.) held, consecutively, a Fulbright Student Fellowship and a National Science Foundation Graduate Fellowship; in addition, it was supported by funds made available by the CSIRO and by the University of Western Australia Research Grants Committee. We thank Professor G. B. Sharman, Dr W. D. L. Ride and Mr John Calaby for their continued encouragement and advice.

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### Multiple Budding on Carrot Embryos arising in Tissue Culture

STEWART *et al.*<sup>1</sup> reported the development in culture of carrot embryos which appeared normal and were able to continue development through all stages of the normal

carrot life cycle. Waris<sup>2</sup>, on the other hand, has reported that embryos from the seeds of *Oenanthe aquatica* L., in the presence of 0.1 per cent glycine, enter an abnormal developmental cycle in which nodules form on the embryo, then break off to form further embryos; most of the embryos were of abnormal structure. We have observed carrot embryoids of abnormal structure which were developed in tissue culture; they budded off other embryoids instead of continuing their development towards plantlets.

The carrot used was *Daucus carota* L., Carters 'Nantes Early Horn'. Sterile explants were taken from the storage root with a cork-borer. On a basal medium with 3 per cent sucrose, 15 per cent coconut milk, and 0.05 mg/l. 2,4-dichlorophenoxyacetic acid added, the tissue proliferated to form a callus which ranged in colour from orange to greenish yellow. One such culture was sub-cultured into eleven tubes of the same basal medium with the addition of 3 per cent sucrose, 1 g/l. yeast extract, 200 mg/l. casein hydrolysate, 0.5 mg/l. 1-naphthalene-acetic acid and 0.1 mg/l. of kinetin.

Three cultures produced only callus. Five cultures developed callus and roots, but in two of these the roots only appeared after more than three months. Three cultures developed embryoids in which the root development was absent or weak. True cotyledons were usually absent and there was instead a lobed leafy structure (Fig. 1). The further development of the embryoids consisted of swelling and budding off daughter embryoids. Fig. 2 shows such a rootless, mis-shapen embryoid with some buds.

The abnormal budding embryos of Waris seem to have been occasioned by the amino-acid imbalance in his culture medium and it is possible that, in our experiments, the casein hydrolysate and yeast extract may also have introduced an amino-acid imbalance, responsible for the



Fig. 1. ( $\times 8$ .)

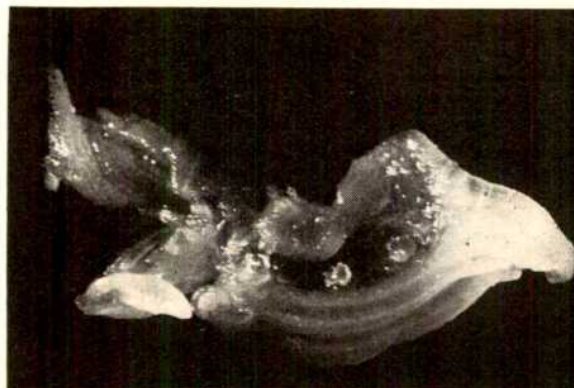


Fig. 2. ( $\times 8$ .)

budding. The observations thus provide a link between the formation of embryoids in culture and multiple budding. The variability of the response most probably reflects the heterogeneous nature of the original explants.

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## PHYSIOLOGY

### Inversion of the Fahraeus-Lindqvist Phenomenon in Blood Flow through Capillaries of Diminishing Radius

THE flow of blood in the microcirculation is of great physiological and clinical importance but, because of its complexity, the mechanism is not fully understood. Fahraeus and Lindqvist<sup>1</sup> first observed an apparent decrease of blood viscosity when it flowed through capillaries of decreasing diameters. Their work was later confirmed<sup>2-6</sup> and the reality of the Fahraeus-Lindqvist phenomenon was accepted. Its explanation, often attempted<sup>2,7-9</sup>, relied chiefly on the concepts of an axial concentration of red cells and/or on the effect of a plasmatic "lubricating" layer along the capillary walls.

Nearly all investigations, however, were carried out at capillary diameters considerably greater than  $100\mu$ , within the normal range of haematocrits. I have considered two problems: (a) whether the Fahraeus-Lindqvist phenomenon is true for a range of capillary diameters nearer to the physiological ranges observed in microcirculation; and (b) whether it is true at high haematocrits. The Fahraeus-Lindqvist phenomenon is shown to occur with capillary radii of as little as  $5\mu$ , but this phenomenon is reversed and the apparent blood viscosity increases with a further decrease in the capillary radius.

To facilitate investigation in capillaries of radii as small as  $2.5\mu$ , while retaining manageable flow times, capillary viscometers of the U-type were constructed with the usual cylindrical capillary replaced by a slit formed by two parallel glass plates. The plates were 0.75-1 in. thick and polished to optical flatness, the tolerance being few wavelengths of light only. Plates were separated by means of thin polythene strips greased with silicone grease, and clamped together by 2 in. "cramps" (Fig. 1). The glass plates used were of different sizes, depending on the radius (half-gap) required. Large slits were formed by plates  $18 \times 8$  cm, while small slits were formed by plates  $3 \times 21$  cm.

Samples of blood were obtained from human donors or from giant Queensland toads and amphiumas ("Congo eels"). Blood samples from human donors were obtained by venipuncture and collected in plastic bags containing acid citrate dextrose. Blood from toads was obtained by insertion of a needle into the aorta of the toad after the thorax was opened. Blood from a dozen toads was required for a single experiment. Amphiumas were about 3 ft. long and weighed 500-550 g. The chest was incised and, after location of the aorta, blood was collected and anticoagulated in the usual way. Haematocrit determinations were made by means of a microhaematocrit Hawksley centrifuge. Platelets were removed by repeated centrifugation and by siphoning off of the buffy coat. Blood was examined in a plastic capillary under the microscope to check for aggregation of platelets.

Platelet aggregates can clog large capillaries (larger than  $100\mu$ ) and so great care was taken to eliminate them; only siliconized equipment was used. Nevertheless, plate-

let aggregates were found in some blood samples, and such samples were discarded.

Blood pH was checked with a 'Radiometer pH-Meter'. Microcapillary slit-viscometers were calibrated using distilled water delivered through a syringe equipped with a 'Millipore' filter. All viscosity data are given as viscosity relative to water.

An effect of the capillary radius (half-gap) on the relative viscosity of human blood at 49 per cent haematocrit, toad blood at 49.5 per cent and 95 per cent haematocrits, and amphiuma blood at 29 per cent haematocrit, is illustrated by Figs. 2 and 3. Viscosity was tested at pressure gradients of 10 and 20 cm of water/cm of length of capillary.

Blood viscosity decreased as the radius of the capillary decreased, down to  $6-7\mu$ , and thus the Fahraeus-Lindqvist phenomenon is clearly present. There is, however, a sharp increase in the viscosity of both blood and packed cells when the radius of the capillary decreases to less than  $5\mu$ .

It should be clearly stated here that the red cells of humans are the smallest (about  $85\mu^3$ ), the toads' cells having about ten times more corpuscular volume, while the red cells of amphiuma are relative giants of more than  $11,000\mu^3$ . The latter cells are flat ellipsoids measuring (mean)  $68.6 \times 42.5\mu$ . It is therefore interesting to see that the behaviour of these cells, of such different dimensions, is remarkably similar.

Figs. 4 and 5 show that the reversal of the pattern of flow is not caused by a permanent clogging but by the rheological properties of blood. It can be seen that flow depends on the pressure gradient, and that even at very low pressure gradients such flow continues. Tests carried out earlier by means of the rotational viscometers<sup>10,11</sup> show that this effect is caused by the rheology of the cell interior.

From these results I draw the following tentative conclusions. (a) The Fahraeus-Lindqvist phenomenon can

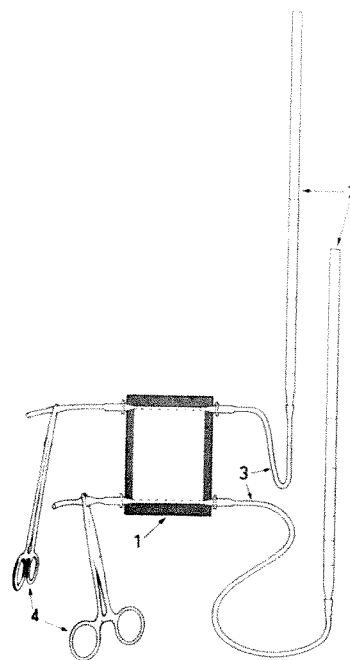


Fig. 1. A diagrammatic representation of a parallel-plate (slit) microcapillary viscometer. 1, Glass plates; 2, pipettes; 3, polythene tubing leading from pipettes to the 2 mm bore channels in the glass plates; 4, arterial clips used to clamp polythene tubing joined on the left side of the glass plates. The shaded area on the glass plates corresponds to the polythene seals and demarcates the outline of the capillary proper. The direction of flow is from the top pipette, through the slit-capillary, to the lower pipette. Tubing closed by arterial clamps is filled with the same blood sample, but is inactive during the actual operation of viscometer; it is used to facilitate cleaning of instrument.



be seen in blood of humans, toads and amphiumas in slit capillaries for a range of capillary radii; (b) the phenomenon can be seen in the packed red cells of haematocrits up to 98 per cent; (c) the critical radius, at which a transition from the Fahraeus-Lindqvist pattern to an inverse pattern (inverse phenomenon) takes place, lies between 5 and  $7\mu$  in the conditions of my investigation and in the absence of platelet aggregates; (d) the critical radius is probably not affected by haematocrit; and (e) the critical radius will be greatly affected by the presence of platelet aggregates, and this phenomenon could be caused by the greater internal viscosity of the platelets than the red cells.

This work was supported by the National Heart Foundation of Australia.

*Note added in proof.* Recent experiments indicate that the tonicity of saline, and the temperature, might also affect the critical radius. In the packed human red cells

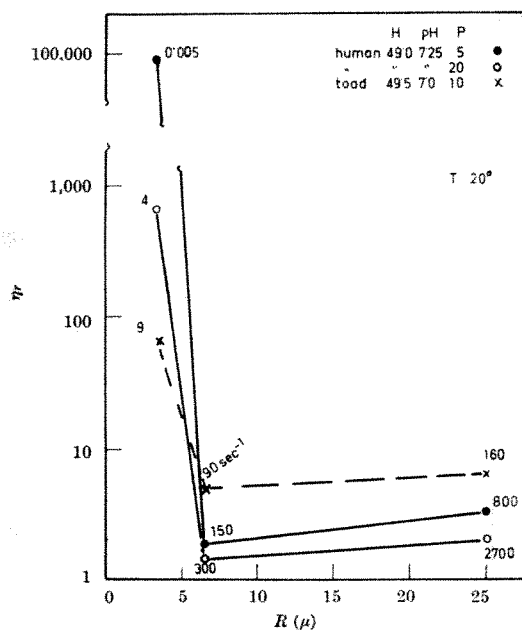


Fig. 2. The Fahraeus-Lindqvist phenomenon and the inverse phenomenon in blood of humans and toads. Viscosity of blood, relative to water, is plotted against the capillary radius (half-gap) in  $\mu$ . Viscosity is given on a logarithmic scale. Pressure gradients of 5, 10, and 20 cm of water/cm of length of capillary were used. Numbers along the curves indicate the nominal shear rate at the wall, in reciprocal seconds.  $T$  = Temperature.

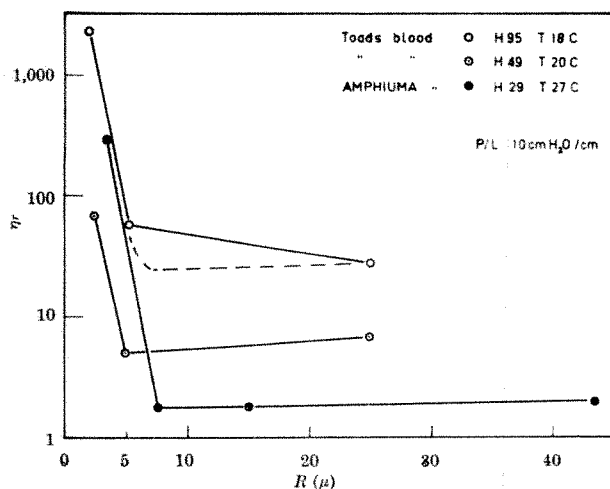


Fig. 3. The Fahraeus-Lindqvist phenomenon and the inverse phenomenon in blood of toads and amphiuma. Viscosity, relative to water, is given on a logarithmic scale and plotted against the capillary radius (half-gap) in  $\mu$ . The dotted line (open circles) indicates a probable, if speculative, outline for the true position of the curve.  $T$  = Temperature.

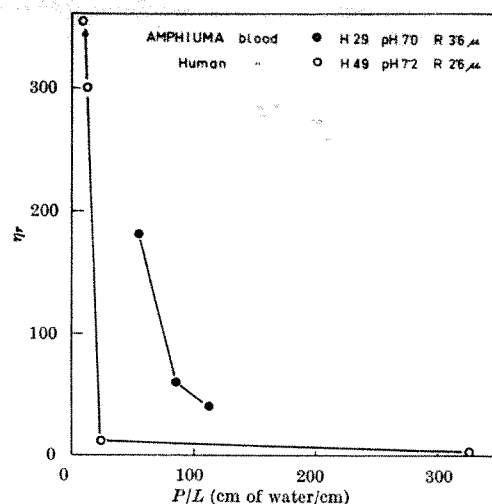


Fig. 4. Viscosity-pressure curves for human and amphiuma blood used in tests illustrated by Fig. 3. Viscosity, relative to water, is plotted against the pressure gradient in cm of water/cm of length of capillary. The top left experimental point, indicated by an arrow, corresponds to  $\eta_r = 2,000$ .  $H$ , Haematocrit;  $R$ , radius of capillary in  $\mu$ .

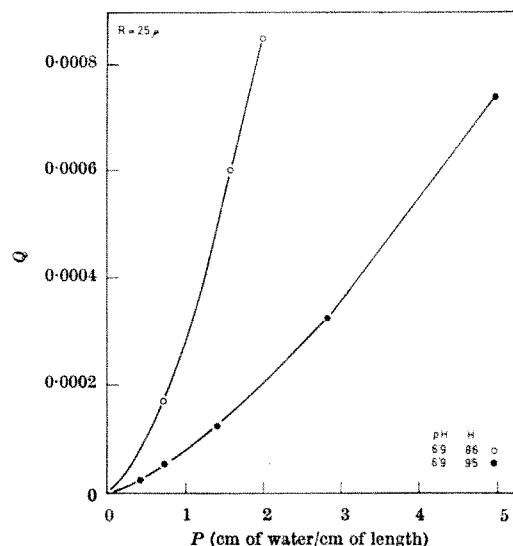


Fig. 5. Pressure-flow curves for packed toad red cells. Volume,  $Q$ , in ml/sec, is plotted against the pressure gradient,  $P$ , in cm of water/cm of length of capillary.  $H$ , Haematocrit;  $R$ , radius in  $\mu$ . The curves obtained curve gently towards zero.

(in the absence of platelet aggregates) of haematocrit of 84 per cent and tonicity of 80 per cent, the critical radius was found to be  $10\mu$  when tested at  $37^\circ\text{C}$  and  $16\mu$  when tested at  $20^\circ\text{C}$ ; under the isotonic conditions, the same red cells (at the same haematocrit) showed the critical radius of  $6\mu$  at  $20^\circ\text{C}$  and  $4\mu$  at  $37^\circ\text{C}$ .

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## BOOK REVIEWS

## CHEMICALS FROM OIL

## The Petroleum Chemicals Industry

By Richard Frank Goldstein and A. Lawrence Waddams. Third, revised and enlarged edition. (Spon's General and Industrial Chemistry Series.) Pp. xiv + 523. (London: E. and F. N. Spon, Ltd., 1967.) 120s. net.

THE transformations of the hydrocarbons contained in petroleum and natural gas are now the main processes in the chemical industry, in so far as carbon compounds are concerned. Whilst animal and vegetable natural products, such as the fats and waxes, are still important, and coal is still more so, yet the trend to the oil source is unmistakable. Mineral oil, already in a dominant position, is conquering more and more territory at an accelerating rate of progress. A surprising example of this is the extent to which the newer plants for the production of town and industrial gas are based on oil rather than on coal.

A petrochemicals factory has a general aspect similar to that of a petroleum refinery; a conglomeration of tall, relatively narrow towers, pipes, and boilers. The reason is that the operations are similar; petroleum refining involves tailoring the crude oil by fractionation, treatments by heat alone, or over catalysts and by use of physical methods of separation. A number of chemical changes are brought about by synthesis or degradation.

The marvellous alchemy of petrochemical productions approaches nearer and nearer the ideal simplicity cum complexity of the refinery. As progress is made, more and more of the processes can be operated continuously on a very large scale, and apart from catalysts the chemicals introduced are only air and water. Temperature and pressure are the chief variables.

As one illustration, among many possible, phenol (carbolic acid) used to be made (probably still is made in some localities) from coal-tar benzene (benzole) by reaction with sulphuric acid followed by treatment of the product with caustic soda and isolation after dilution with water and acidification. All this involved considerable handling. Nowadays, there are two processes dependent on oxidation of hydrocarbons, available from petroleum. They are summarized as: benzene + propylene → isopropylbenzene, which +  $O_2$  → hydroperoxide → phenol + acetone, and toluene by oxidation → benzoic acid → copper salt, and by oxidation → phenol.

The earlier editions of this book have gained an enviable reputation as the best available account of the processes used in the petrochemicals industry, and this new and considerably revised and augmented edition will be esteemed still more highly. In fact, it can safely be claimed to be the most complete and authoritative general account of the subject that is anywhere available.

The coverage extends to early 1966 and hence a number of quite new topics have been introduced. In addition, the older material has been carefully sifted and many new developments have been noted, and yet, by careful attention to what is really essential, the size of the book has been kept within such limits that it may be read with pleasure and not used exclusively as a work of reference. On the other hand, it does function successfully in this latter capacity and will be frequently consulted, especially by technologists and planners.

The fundamental basis of the book is a description of the chemistry of processes used in the industry and of the

conditions in which they may be carried out. The chemical engineering involved is not treated except for some very general implications; a few flow-diagrams are included. A chapter on chemical by-products from petroleum refining lies a little outside those based on the main chemical classification, and so does another dealing with economics and statistics.

This book, because it illuminates a whole, relatively new field of chemistry, should be studied by advanced students and research workers in universities. It will be found indispensable to those actually working in the petrochemicals industry.

R. ROBINSON

## INTRODUCING LUNAR GEOLOGY

## The Craters of the Moon

An Observational Approach. By Patrick Moore and Peter J. Cattermole. Pp. 160 + 16 plates. (London: Lutterworth Press, 1967.) 35s. net.

THIS book presents the results of the interpretation, by an astronomer and a geologist, of observations of the lunar surface. It is the latest addition to the discordant serious literature in this field, for the authors conclude that virtually all features of the surface result from endogenic processes. This is not too surprising; since Baldwin, in his book *The Face of the Moon*, established both the modern form of the impact theory of lunar surface formation and the current wave of controversy, interest in this problem has been growing, and the importance of internal activity is at last being widely recognized by all lunar specialists. The essentially new suggestion here is that the origin, as well as the subsequent shaping, of most of the surface features is caused by volcanic activity.

After a brief enumeration of the main types of surface feature the authors give a historical summary of the theories of crater origin. In order to justify their view that internal processes predominate they summarize, in the next two chapters, the geological principles and types of terrestrial volcanic structure to which they need to refer; here it is, perhaps, a pity that references to standard texts on geological principles are not more in evidence.

Turning again to the Moon, a short chapter is devoted to an interesting discussion of processes neither volcanic nor meteoritic which have been advanced to explain various features. There follows a concerted and well planned attack on the weak points of the impact theory, together with a discussion of the development of volcanic theories which lead the authors to the conclusion that no violent processes can be involved for the formation of the larger lunar features, and that some kind of relatively gentle internal activity must be postulated. This idea is justified by the comparison of selected lunar features with corresponding terrestrial ones, and good analogies are found in most cases, an exception being that of the "bowl" craters; here the authors prefer to think in vague terms of "... a type of vulcanism rather different from any that we have encountered on Earth", rather than attribute these craters to any other source.

Next, an indication of the lunar surface materials that may be present assuming volcanic processes is given; here also more references might usefully have been given to sources of information on the techniques of investigating the lunar surface by measuring its physical properties from the Earth.

After a consideration of the possibility that there is still some activity on the surface, the now less controversial subjects of lunar tectonics, deformations and the grid system are presented; this part of the book also describes the particular properties of the mare floors and mountainous regions. A chapter on the ray systems deals first with the inconsistencies of impact theories and then



suggests a mechanism involving the development of small craters along lines of weakness. Finally, the main conclusions are summarized.

The authors leave the reader in no doubt as to the bias of their views, and in fairness it must be stressed that, if the most important references given are consulted (for example, those given at the end of Chapter six), a fair cross section of opinion will be encountered. Despite the specific points mentioned here, in general the book is very well documented, particularly as it deals with so many individual types of feature. It represents a readable and wide ranging introduction to this field for both the amateur astronomer interested in the interpretation of observations and the scientist wishing to get up to date with lunar geology.

L. WILSON

## VACCINE, WITH CARE

### The Hazards of Immunization

(Based on University of London Heath Clark Lectures, 1966.) By Graham Wilson. Pp. x+324. (London: The Athlone Press, University of London, 1967. Distributed by Constable and Co. Ltd.) 45s. net.

THERE are few who have the depth of knowledge or even the courage to attempt to present the hazards of immunization. Many of the data have appeared over a long period in many journals and reports, but to assemble these facts in an interesting, balanced, and unbiased account is most difficult. Sir Graham Wilson has succeeded in his task and the medical world is the richer for this book based on four Heath Clark lectures given at the University of London in November 1966.

The hazards have been divided into (a) accidents occurring during production and administration of the vaccines and (b) complications arising in the vaccinees.

The accidents are concerned mainly in association with new prophylactics and they are dealt with in seven chapters on faulty production. Of the accidents considered under the title "Inherent Toxicity", by far the greatest number have occurred as a result of inadequate toxoiding of toxin, or the use of toxin-antitoxin mixtures now considered to be dangerous and discontinued. Also considered under this heading are the failures to kill pathogenic viruses and the use of a living virus inadequately attenuated. The conclusions drawn from the findings of the accident enquiries are of great value, and it is interesting to note that, weight for weight, man is at least as susceptible as a guinea-pig to diphtheria toxin. Equally important is the observation that diphtheria toxin must remain in tissues for a long time, where it may not be neutralized by antitoxins, and late symptoms may develop. Examples are given of the appearance of a foreign toxin in an antitoxin, the use of an incorrect culture for vaccine production, and the contamination of the final product with both bacteria and viruses. It is some comfort, however, to know that such accidents occurred before the modern methods of production and control became effective.

Having dealt with the production of vaccines, Sir Graham considers the accidents that occur during reconstitution and administration. These largely result from the use of contaminated equipment, much of which is eliminated by the use of sterile packed and disposable apparatus. In spite of all these modern developments, however, as recently as 1966 local abscesses caused by *Streptococcus pyogenes* type 12 occurred in one clinic after giving sterile DTP vaccine. The same streptococcus was isolated from the nose and throat of the doctor and nurse attending the clinic as well as from the scissors used to open the sterile packs.

Allergic manifestations occurring in the vaccinees are a much more difficult problem and no fewer than seven chapters are devoted to this aspect. Local allergy, serum sickness, encephalomyelitis, neuroparalysis and general

anaphylaxis occurring after the administration of prophylactics are all mentioned. The abnormal sensitivity of the patient has not been overlooked and skin complications of smallpox vaccine, local or generalized tuberculosis, occurring after BCG or vole bacillus vaccines or tuberculin, as well as complex reactions to other vaccines, are recorded.

Sir Graham has been particularly careful to draw attention to the potential dangers of all vaccination procedures and to put them in their right perspective. In this respect the dangers associated with immunization, using both established and new vaccines, are discussed and the need for continuous surveillance is emphasized.

There is no doubt that all physicians will wish to read this book, which should also find a place in the permanent records of all libraries. It is a unique collection of data superbly assembled.

F. T. PERKINS

## CANCER PREVENTION

### Prevention of Cancer—Pointers from Epidemiology

By Richard Doll. (The Rock Carling Fellowship, 1967.) Pp. 143. (London: The Nuffield Provincial Hospitals Trust, 1967.) 7s. 6d. net.

SINCE the first investigation by Hirsch in 1883, much information has been accumulating about the distribution of cancer in human communities in different geographical areas and with different occupations. From the information which we have already, maps can be constructed to show the relative incidence of a particular type of cancer; a map in this book illustrates clearly that cancer of the oesophagus is about two hundred times more frequent in the northern regions of the Caspian than in Holland; another shows a gradient in the incidence of stomach cancer, high in Russia, decreasing in Europe, the eastern states of the USA, but rising again in the western regions.

The subject of cancer epidemiology is the study of the relationship between the environment and the incidence of the disease. The task is not to establish only a relationship between cause and effect, but to test by various methods whether the relationship is a true one, and whether the suspected agent is directly or indirectly implicated in the causation of cancer. Can statistical evidence alone be acceptable as definite proof of a cause-effect relationship; and how far can the experimental data obtained in animals be extrapolated to man? These problems are discussed with great objectivity in the book being reviewed. Dr Doll, besides putting forward definite conclusions, draws attention to the difficulties which may arise in collecting and analysing relevant information and describes some of the pitfalls of epidemiological studies. Dr Doll is one of those to whom we owe the critical application of epidemiological principles to the incidence of leukaemia and lung cancer—revealing some causes of both of these malignant diseases. Because of his experience and scientific knowledge he is particularly well suited to argue the value of studies which concern the possible relationship between cancer incidence and environmental agents. Some of these agents (radiation, cigarette smoking, asbestos, benzene,  $\beta$ -naphthylamine) and the way by which information relating to them has been evaluated are well described. Attention has been paid to the often voiced question: why does one person develop cancer while another exposed to the same environmental hazard escapes such a fate? Is there a genetically determined susceptibility? Doll examines the evidence available from twin studies and states that in the aetiology of common types of cancer the environment is more important than the genetic component.

Doll's book merits serious consideration by all who are concerned with cancer and human well-being. According to him almost 40 per cent of cancer deaths in men and 10 per cent in women can be prevented because we know the cause. We are still ignorant of the cellular mechanism which transforms a normal cell into a cancer cell,

and no effective measure can be devised to reverse the process, so that our chance of reducing the incidence of cancer lies in removing the agent from the environment which causes it. Epidemiological studies spotlight some of these agents, and no doubt more could be identified if information was available.

Doll puts in a plea for a record-linkage system which, by covering the whole community, could provide the needed information to reveal other carcinogenic agents which then could be removed from man's environment. Doll's excellent book should be read by those, in government or industry, who have the power to act.

P. C. KOLLER

## LIVELY EMBRYOS

### Éléments d'Embryologie Causale

By J. Fautrez. (Collection d'Enseignement Biologique.) Pp. 302. (Paris: Gauthier-Villars, 1967.) 32 francs.

To many students of biology and medicine embryology means the growth and development of boredom. Microscope slides showing sections at different "levels" of particular "stages" of selected "types" are drawn, labelled and forgotten. A few plastic models and a pickled foetus or two may be thrown in for good measure, and then teacher and students alike pass with relief to something else.

Professor Fautrez gets right away from this atmosphere. He writes in a clear and lively style, and from start to finish his embryos live. He assumes that the reader knows some elementary general biology, including the rudiments of embryo anatomy, and sets out to introduce him to causal embryology. The overall plan of the book follows the conventional sequence, beginning with the gametes and fertilization, and ending with the development and co-ordination of the main organ systems. But always Fautrez asks why each embryonic structure should develop where and when it does, and how it is that the embryo is able to survive each phase of its development long enough to grow into the next.

Particularly attractive is his use of experimental evidence. The book is not intended to be an advanced treatise, and although sufficient experiments are quoted to keep the reader in touch with the excitements and frustrations of embryological research, the main argument is never swamped with experimental detail. On some topics the experimental evidence appears conclusive, but where there is uncertainty or conflict the rival theories are carefully explained. There is a nice mixture of examples from recent and older work and from a wide range of animal species. Obviously a book of this size cannot include all the experimental evidence that is relevant, and there must be an element of arbitrariness in the choice that is made; but Fautrez's aim has been to write a good book on embryology, not to administer divine justice to embryologists, and he has been admirably successful.

The deficiencies of the book are small compared with its merits. It is adequately illustrated with photographs and diagrams which, with one or two exceptions, are clear and informative; but it might have been helpful if the reader were referred to them in the text instead of being left to stumble on them by accident. The chapter on embryonic membranes is disappointing; it gives a poor idea of the intense activity of these remarkable structures and contains several misleading statements, for example that the cleidoic eggs of vertebrates are surrounded by an "impermeable" shell. There is also a curious omission from the chapters on gametogenesis of any mention of chiasmata formation and crossing-over; it is implied that the only important function of the second meiotic division is to halve the amount of DNA.

It is unfortunate that the language barrier will restrict the number of English-speaking students who can make use of this book. But it can be strongly recommended to students of all ages who either read French easily or who wish to improve painlessly both their biology and their French.

D. A. T. NEW

## KEEPING UP WITH PHYSIOLOGY

### Annual Review of Physiology

Vol. 29. Edited by Victor E. Hall in association with Arthur C. Giese and Ralph R. Sonnenschein. Pp. vii + 652. (Palo Alto, California: Annual Reviews, Inc., 1967.) \$8.50.

THERE are a number of ways in which the physiologist can keep abreast of the ever increasing volume of literature which according to MEDLARS amounts to some 120,000 papers in medicine and allied sciences each year. The conventional abstracting and indexing services are useful for obtaining information about specific topics, but for a more general coverage recourse may be had to *Physiological Reviews*, which covers a small number of subjects in great depth, and the *Annual Review of Physiology*, which gives a broad coverage.

The twenty-ninth volume of the *Annual Review of Physiology* covers the literature up to May 1966 in a wide field of physiology. Information about the nervous system occupies about a third of the volume and covers growth and differentiation of nerve cells, electrophysiology of the cell, afferent mechanisms, higher functions, hearing, vision in *Limulus* and a welcome review of motor mechanisms. Cardiovascular, respiratory and endocrine physiology (adenohypophysis, reproduction, parathyroid and calcitonin) are well represented. For the general physiologist there is much of interest about transport through biological membranes, metabolism, and invertebrate excretory organs. Gastric physiologists will be happy with the review of gastric secretion, and a chapter on lymphatics and lymphoid tissue contains useful information about the thymus and antibody responses.

The ability of the physiologist with special interests to obtain a list of relevant literature without difficulty raises the question as to the type of reader this annual review is now designed to interest. For the specialist there is no alternative but to read the literature himself. The ideal readers would presumably be teachers of physiology who, devoting their interests to special topics, may have difficulty in keeping themselves generally informed. For such readers this annual review can only be of interest if clear ideas of the advances and problems are obtained. This need is met by a number of articles in which the authors have deliberately limited the scope of their review. The articles by Gaze on growth and differentiation, Bernstein on respiration, Guillemin on the adenohypophysis, Arnaud, Tenenhouse and Rasmussen on parathyroid, and Wolbarsht and Yeandle on visual processes in *Limulus* are particularly clear and critical. These reviewers have added interest to their articles by referring to the authors of important papers by names rather than by a series of reference numbers.

On the other hand, a number of authors introduce their reviews by apologizing for their inability to cover the entire accumulation of literature in their specific field. They certainly cover an enormous amount and the thorough way in which the literature has been collected and subdivided can only lead to admiration. Such reviews, while being useful, are a non-critical compilation of the literature. It is difficult for a reviewer to be critical of work in a subject allied to his own but outside his immediate interests.

The scope of the reviews has been of concern to the editors who saw the first flicker of the publication

explosion when the first volume was produced in 1939. The preface to this volume advised authors "to attempt a critical appraisal of the contemporary field". The present editorial policy of further restricting the coverage of any field should lead to a better description of recent work. There seems little point, anyway, in reviewing a field in which there has been no significant advance. The problem seems to be defined by R. V. Short in his entirely enjoyable review on reproduction. He mentions that the *Bibliography of Reproduction* lists 10,000 articles, of which he is aware of 5 per cent and influenced by about 1 per cent. With the use of 195 references he gives the reader an outstanding account of reproductive physiology. If more chapters were written in this vein it may be that the *Annual Review of Physiology* would find its way into more personal libraries in addition to university libraries, where it still justly remains one of the pillars of the physiological establishment.

D. MENDEL

## QUANTUM MERUIT

### Vision and the Eye

By M. H. Pirenne. Second edition. Pp. xvi + 224 + 16 plates. (London: Chapman and Hall, Ltd., 1967.) 50s. net.

ONE of the enchanting aspects of the polymorphous subject of vision is that it can accommodate both the cantabrian crank and the oxonian omnivore. Dr Pirenne's far-flung interests, his erudition, and profound understanding, all characteristic of the latter species, have made him singularly suitable for the authorship of this academic treatise. And by "academic" I wish to convey the original notion of the word as depicted by Raphael in the Vatican. We are led from pin-hole camera photography (in connexion with which Fig. 1.15 needs some thought to grasp it) to the splendid illusions of baroque turmoil; from the eyes of insects to the anatomy of the brain; from some unpublished experiments due to Hartline to the directional properties of the retina. Dr Pirenne's vigorous analytical approach, followed, it should be noted, practically without any algebra at all, will be familiar to the readers of the first edition. The chapter on the nervous response to light is somewhat extended in comparison with its antecedent and concentrates on the properties of the lateral eye of the horseshoe crab. While there are people who might wish for details of recordings obtained from the eyes of higher vertebrates, if not primates, a great deal is to be said for the grasshopper's approach, which subsists in sampling tit-bits in various places.

Yet puzzles remain. There is no hint in this finely produced book why the Stiles-Crawford effect, "which cannot be described here" (last edition, page 11), can now be described. The section on binocular vision makes no mention of one of the most dramatic discoveries in the whole of this subject, namely the demonstration by Julesz that contours may arise from stereopsis rather than, as Helmholtz contended, that the contrary should be true. While Dr Pirenne wisely refrains from a discussion of the relation between visual pigment regeneration and the time-course of adaptation to darkness, his faith in some results bearing on the alchemy of human colour vision is simply endearing. But the principal puzzle relates to the inordinately large number of pages reserved for the role that the quantum and probability play in vision. The demonstration of this role is due in no small measure to the experimental work done twenty-six years ago by Pirenne in conjunction with Hecht and Shlaer. That this important contribution has received its due desert is also attributable to Dr Pirenne's many writings on the subject. Does he believe that any one in his right mind could ever challenge the basis of the experiments? Then why omit important new material and repeat the story with the detail which was necessary in 1948 but seems redundant in 1967?

Perhaps only those who have shouted in the wilderness know the answer; maybe only those whose shouts have been in vain will understand.

R. A. WEALE

## AFRICAN FISHES

### Freshwater Fishes of Southern Africa

By R. A. Jubb. Pp. vii + 248 + 57 plates. (Cape Town and Amsterdam: A. A. Balkema, 1967.) R. 12.50.

MR JUBB aims at a wide audience, in particular the naturalist, the angler and the fishery biologist. They are well served by this, the first book of its kind to give comprehensive coverage to the freshwater fishes of southern Africa, taken in this context to be that vast area stretching from the Zambezi and Cunene rivers in the north to the multitude of smaller rivers in the Cape Province of South Africa.

Although not strictly a taxonomic revision (the author, too modestly, calls it a "pictorial guide"), this book is clearly based on many years of detailed and critical systematic research, backed by much fieldwork and consequently a first-hand knowledge of the fishes' ecology. Thus *Freshwater Fishes of Southern Africa* is bound to find a place also on the professional ichthyologist's bench.

The text is divided into four principal parts, of which the longest is the systematic section dealing with the 157 indigenous species of southern Africa. With few exceptions, there is for each species a description and figure (usually a photograph, but sometimes a coloured drawing as well). The brief specific description gives live coloration and diagnostic anatomical characters distinguishing the fish from related species in the area. Distributional data, vernacular names, remarks on feeding habits and other ecological information, and often short accounts of intra-specific variability in diagnostic characters are also included.

Here I must compliment the author's wife, Hilda Jubb, on the excellent figures which she has prepared. Most of these are skilfully retouched photographs in which, without creating any imbalance or obscurity, she has emphasized the main diagnostic characters and yet retained the essential character of the fish as a whole. Opinions differ on the value of photographs as illustrations for taxonomic papers (I for one favour line-drawings); Mrs Jubb's expertise has certainly reduced my opposition, and amply justified the use of photographs in a work of this nature. In addition, Mrs Jubb has added to the value and aesthetic appeal of the book by providing more than fifty original coloured drawings.

Because the illustrations are a vital part of this work (especially in its role as a pictorial guide for amateurs) it is regrettable that there is often poor spatial correspondence between text and relevant figure. This arrangement is the more regrettable because most users of the book will have wet or dirty fingers, and the illustrations can only be found by leafing through the pages, because no figure reference is given with the species description.

Following the section on indigenous fishes there is a shorter but equally comprehensive one on introduced exotic freshwater fishes, and native euryhaline species found in fresh waters. The introduced species (all of European or north American origin) are carefully documented from the historical as well as from the ecological and distributional points of view.

A semi-pictorial key serves as an introduction to both systematic sections. This key is simple to use, and avoids the common pitfalls of many "popular" keys because of the author's skilful avoidance of too finely divided dichotomies. Instead, the user is led, in most cases, to a group of similar species and must then refer to the fuller descriptions to make his final identification. Frequent use of simple outline drawings further helps to prevent the novice from straying far down the wrong branches.

The key ends a series of wide-ranging introductory chapters. In these Mr Jubb discusses, among others, such diverse topics as fossil fishes in southern Africa, the physiological and hydrobiological characteristics and history of the region, and the classification of fishes. There is also an introduction to fish anatomy with particular reference to characters used in the systematic section. Some of these chapters are much better than others; the main faults stem from the inevitable compression needed if so many different fields are to be covered at all. I believe that Mr Jubb made the right decision in attempting this task. Too often the general reader is left in ignorance of the complex historical and environmental factors that have influenced, and still influence, the fishes which are his prime interest.

A map with the main rivers of southern Africa clearly labelled would be of great value to the reader unfamiliar with the country (the map provided shows only the most important drainage basins and is inadequate for detailed work), but the situation is alleviated by the excellent gazetteer of principal river systems (would that more authors should follow this practice). I would also criticize some of the imprecise definitions and minor inaccuracies in the anatomical section, particularly because these slips are likely to mislead the non-zoologist. For example, the circumorbital bones of a tigerfish are labelled "operculum" (Fig. 8), the premaxilla is labelled "maxillary" (Fig. 26), and the figure showing tooth-bearing bones in the jaws is rather imprecise. It must be emphasized, however, that these are but slight and easily rectified imperfections in an otherwise detailed and simply written guide to a complex subject.

Also included in the introductory section is an annotated check-list of southern African freshwater fishes. Synonymies are included, and so is information on the distribution of species (including indigenous species transferred into drainage basins to which they were not endemic). More detailed distribution lists are provided in a separate series of tables dealing with each of the main drainage systems. Taken together, these various analyses form an extremely valuable and unique compilation of data otherwise scattered through many sources, some now inaccurate and outdated.

The fourth, and final, section of the book deals with fishing methods, fish farming, angling, and the use of certain species in home aquaria and garden ponds. Much of this material is beyond my competence, but I would draw attention to the subsection on primitive fishing methods. Here, Mr Jubb has brought together much historical material and several unique photographs, all of which serve to illustrate and record a rapidly vanishing aspect of African culture. Taking his subject even further back in time, Mr Jubb also reviews the few but fascinating and somewhat enigmatic rock paintings from South Africa in which fishing scenes are depicted.

*Freshwater Fishes of Southern Africa* is a valuable contribution to the literature on African fishes, and its appearance will be welcomed by professional and amateur naturalists in many fields. Its place in the permanent literature of southern African natural history is assured; but, if future editions are to have the wide and popular use that the author's efforts and high standards so richly deserve, the publishers will have to bring down the price to a more realistic level.

P. H. GREENWOOD

## SOME PROTEIN TECHNIQUES

### Techniques in Protein Chemistry

By J. Leggett Bailey. Second, revised and expanded edition. Pp. xiv + 406. (Amsterdam, London and New York: Elsevier Publishing Company, 1967.) 110s.

THE mere fact that so specialized a book as this has required reprinting after two years and a second edition

after five demonstrates clearly, by the hard test of sales, that it is of real value. There can be no doubt that the second edition will be just as useful and successful as the first.

The title of the book is a little misleading because it is not by any means an account of all the techniques used in protein chemistry. It is, rather, a detailed account of a selection of these techniques, notably those employing chromatographic procedures and useful for sequence studies on proteins. The first three chapters deal with the chromatographic and electrophoretic separation of amino-acids and peptides and the next four with methods used for sequence determination in proteins. There is then a chapter on dialysis and gel filtration (surely a rather ill-assorted pair), followed by two chapters on the column chromatography and zone electrophoresis of proteins. A short chapter on miscellaneous analytical methods is followed by a chapter, added in this edition, on the synthesis of simple peptides. I doubt whether this last chapter, good though it is, is of real relevance to the theme of the book; biochemists might well find it best to persuade their organic chemist colleagues to undertake any such syntheses for them.

The revision carried out for this second edition has been very thorough. About one-third of the thousand or so literature references are to papers published since 1960; very few relate to papers published since 1964. The book itself, despite pruning of older material, has also been expanded by about one third. There is, however, still some duplication and some further pruning might have been carried out with advantage; thus, for example, nearly all protein chemists nowadays use automatic amino-acid analysers and much of the third chapter seems irrelevant to current practice.

The book is well and clearly written. I found few errors, but there is a misleading one on page 23 ("nitrate" for "nitrite") and something has gone very wrong with the equations for the Akabori hydrazinolysis reaction (page 226).

These shortcomings are relatively minor blemishes on an excellent book. This is a work of real character and it is clear from every page that the author really knows what he is talking about. In his preface he hopes that the book will be "something handy to have in the laboratory"; it is much more than this, being an essential tool for everyone working in, or even only on the fringes of, the field of protein sequences. We shall all be looking for a third edition in a few years.

H. N. RYDON

## ACETYLENE COMES INTO ITS OWN

### Acetylene

Its Properties, Manufacture and Uses. Vol. 2. By S. A. Miller. Pp. xvii + 406. (London: Ernest Benn, Ltd., 1966.) 126s. net.

THE appearance of the second volume of Dr Miller's work has been eagerly awaited. Not surprisingly, the large amount of chemistry to be covered has been found to necessitate a third volume and the present part covers only simple addition across the triple bond, leading largely to the industrially important vinyl compounds and halogen derivatives. Deferred until later are all reactions in which acetylene is used as a "building block" in organic synthesis, that is, ethynylation reactions, linear and cyclic polymerizations and carbonylation.

Since the first volume, debate about the relative merits of the old giant, acetylene, compared with ethylene as the optimum starting material for large tonnage chemical intermediates has continued. Some plans to use acetylene have been changed to the use of other feedstocks, but a number of manufacturers decided to opt for naphtha based acetylene processes, sometimes to replace carbide in order to continue to use existing equipment for often



simple and certainly well-tried further chemistry. Gloomy stories circulate as a result—and it is abundantly clear that what acetylene wants is a hydrocarbon based production process that works as designed within the capital sanctioned. If this can be achieved, developments towards Dr Miller's hope, expressed in an entertaining and spirited preface, of acetylene at 3d./lb. might be possible. While acetylene's troubles are being sorted out, however, ethylene production by naphtha cracking continues to expand on an ever-increasing scale and 0.5 million tons/yr units are now being made. The odds are that ethylene will reach 2d./lb. long before acetylene reaches 3d. and the old giant will still have fallen, entirely as Dr Miller suggests, through hard economics, the most lethal sickness of all.

Apart from the basis of the chemicals dealt with in this book and the processes leading to them, a substantial part is devoted to the chemistry of the initial products, and this is, and will remain, extremely valuable. The author is clearly well informed and the book is a mine of information and obviously the result of a lot of very hard work.

The first chapter deals comprehensively with hydrogenation and halogenation. The former is important not because anyone would want to make ethylene from acetylene today, but because acetylene is a contaminant in ethylene made by naphtha cracking and selective hydrogenation to ethylene is one way of removing it. Actually, as crackers grow to the 0.5 million tons/yr scale and beyond, it becomes economically possible to recover the acetylene as such from the ethylene. Such an operation would, however, require an amendment to the Explosives Act of 1875, which hitherto has forbidden the bottling of acetylene which has at any time in its history, irrespective of proportion in any mixture, been under a pressure of more than 22 lb./in.<sup>2</sup> gauge. Ethylene is commonly distilled at about 250–300 lb./in.<sup>2</sup> pressure, and so there has been a distinct lack of flexibility about what can be done with the product. A recent Home Office draft order could make the technically possible legally possible. Some relaxation of the antediluvian regulations surrounding acetylene has been clearly needed for many years; they are or were an obvious institutional barrier hindering technological progress in the UK in marked contrast to the situation in America and Western Germany.

Halogenation is covered admirably in the book. Trichloroethylene and its chief use, metal degreasing, is extensively dealt with; yet there are signs that some authorities, especially in America, are unhappy about its toxicological properties. Possibly later editions will require considerable expansion of the section on the up and coming methylchloroform, which is less toxic than trichloroethylene and finds application in, for example, cold degreasing. Room might also be found for the more exotic trifluorodichloroethane used in degreasing of electronic parts.

A whole chapter deals comprehensively with hydrohalogenation, chiefly the production, properties and polymerization of vinyl chloride and the properties of the polymer. The bibliography of this section is particularly good.

The third chapter on acetaldehyde and acrylonitrile is from the production point of view strictly historical. Again, the properties and reactions part is thorough and well done.

Vinyl ethers receive a whole chapter and the current situation is well covered and documented. Unfortunately, the price of these materials militates against their use and little progress can be expected until some new cost breakthrough is achieved.

Vinyl esters, and particularly vinyl acetate, are fully described. Interest is at a maximum, because in this area acetylene has most promise as a continuing starting material, especially if it is coupled with the cheap acetic

acid from naphtha oxidation. Several ethylene based processes to vinyl acetate are now being developed, but the degree of sophistication in the oxidation step is likely to be at a maximum, whereas the acetylene route is conversely relatively simple. The whole chapter is very informative, although copolymers certainly merit more than a page. Thus, on pages 288 and 289, the 'Elvax' type of vinyl acetate-ethylene copolymers, which are (like ICI Alkathene VIG 501, etc.) modified polyethylenes, are lumped together indiscriminately with the emulsion copolymers like 'Aircoflex' which contain only minor amounts of ethylene. The two types of product are quite different in composition, process of polymerization and end uses. Some 80 per cent of the emulsions made in the UK, USA and Germany are copolymers and their introduction has considerably expanded the field of usefulness of this type of material. The recent commissioning of a 20,000 tons/yr of vinyl "versatate" plant by Shell underlines the importance of these "other" vinyl esters, all of which are indisputably acetylene derivatives.

The final chapter deals with "other vinylation"; curiously vinylation on sulphur (in the contents list) of nitrogen and on carbon, silicon and tin. Vinyl carbazole and vinyl pyrrolidone, the two main products of interest from Reppe's pioneering work in this field, are dealt with in detail.

This is an admirable book and I look forward to the third volume. As usual, it is beautifully produced, which adds to the enjoyment of reading it. H. W. B. REED

## DRUGS AND THE BRAIN

### Pharmacology and Physiology of the Reticular Formation

Edited by A. V. Valdmán. (Progress in Brain Research, Vol. 20.) Pp. viii + 339. (Amsterdam, London and New York: Elsevier Publishing Company, 1967.) 125s.

THIS book is a collection of papers, each dealing with a specific aspect of research on the central nervous system. The authors are all research workers in the department of pharmacology at the University of Leningrad, and so it is really an up to date account of the research in that department. In that respect it represents a sequel to the two previous volumes mentioned in the preface and published respectively in 1958 and 1961. It would, however, be incorrect to give the impression that the authors refer only to their own work or to Russian publications. The world literature is extensively reviewed and there are thirty-six pages of references at the end of the text, although not many references are to papers published after 1960, which is surprising in a book published in 1967.

The book is edited by A. V. Valdmán, who is also the author of the first chapter on the "Pharmacology of the Brain" and co-author in three other contributions. This first chapter in fact comprises almost one-third of the text and is probably the most important part of the book. It is an extensive review of the state of knowledge of the actions of drugs on the central nervous system up to 1960. For example, the actions of narcotics on electrophysiological phenomena, ranging from the electrocorticogram and sensory-evoked potentials to the activity of single neurones, are discussed. There is an extensive account of the actions of analgesics, particularly morphine, and it is suggested that the analgesic action of this substance may be related to an interference with the balance of noradrenaline metabolism. Other topics treated in this chapter are sympathomimetic drugs, tranquillizers, cholinergic drugs and stimulants.

Other chapters deal with effects of drugs on behavioural responses to electrical stimulation of the hypothalamus, a detailed description of some anatomical aspects of the reticular formation, and contributions to pharmacological analyses of the pontine respiratory and vasomotor centres.

A chapter on synaptic transmission by A. I. Shapovalov is largely devoted to a consideration of events at the neuromuscular junction, although acetylcholine does not appear until the seventh page. The work of Katz, Eccles, Koelle and others is discussed in some detail. Similarly, the chapter on central inhibition is largely devoted to a discussion of the ideas of Eccles and his associates.

One might think that a chapter on the pharmacology of smooth muscle would be out of place in a book with this title, but, coming where it does, it is not inappropriate.

The book makes interesting reading, but it is more suitable for the expert in this field rather than students. Even so, those who are not familiar with Russian drug names may find themselves lost from time to time.

P. B. BRADLEY

## OBITUARIES

### A. A. Lombard

ADRIAN LOMBARD, who died on July 13, had been the director of engineering of Rolls-Royce, Ltd., since 1958. In this position he had full responsibility for the design, development and supporting research on all the company's aero engines. When he became chief engineer at the age of 39 he was, in fact, only the third man in the history of the company to take on these responsibilities, his predecessors being Mr A. G. Elliott and Sir Henry Royce. Like them, he had worked on the design of motor cars, having started his engineering career with the Rover Company. He was in the small design team that worked on the Whittle jet engine, which Rover started to develop in 1940, and he continued as chief designer when Rolls-Royce took over the Rover work in 1943. From then onwards he was directly involved with a continuing line of Rolls-Royce jet engines, including the pure jet Derwent, Nene and Avon; the turbo-prop Clyde and Tyne; the Conway, which was the first by-pass engine in production, and the Spey by-pass engines; a family of vertical take-off engines and others still under development.

Lom, as he was called throughout the industry, was a dedicated man with the single-minded purpose that the engines his company produced should be the best in the world. He would never accept second place. He drove his large team of well qualified men by example, enthusiasm, humour, sometimes sarcasm and always a sheer disbelief that improvement was impossible. He enjoyed argument—on specific cases rather than general issues—and had a phenomenal memory which enabled him to discomfort any opponent who had changed his ground since the last encounter. His own academic qualifications were slight, but he was intensely interested in any scientific work that affected a design and with an intuition uninhibited by preconceived theories could always make a penetrating comment or novel suggestion in any discussion. At the same time he was a firm believer in the absolute necessity for a far sighted programme of research on problems related to the gas turbine, particularly on the materials side, and insisted on adequate resources being provided for it. In fact, whenever a new idea came up, such as the air cooling of turbine blades in the early days of the gas turbine or more recently the use of carbon fibre reinforcement of plastics, he personally followed the progress of its development right through to the production stage.

Lombard's work did not end at home with jet engine design. He went around the world selling the engines on their technical merits and feeding back the requirements of the operators to his own team. In this dual role he had continually to make that most difficult of all design compromises, the balance between a performance that is easily achieved but not attractive to the user and that

which is extremely attractive but unachievable in an economic time scale. His subordinates were continually dismayed by the promises that he had made abroad on their behalf, but his determination ensured that those promises were finally honoured. As a result of his wide travelling and of the many important lectures he delivered to aeronautical and engineering societies in a number of countries, he was internationally recognized and honoured, being appointed C.B.E. this year and receiving the James Clayton prize of the Institution of Mechanical Engineers jointly with Dr S. G. Hooker.

The application of science to technological progress owes much to men of Lombard's calibre: it is sad there are so few of them.

S. L. B.

### Dr A. W. Gledhill

DR ALAN W. GLEDHILL died suddenly on July 19, 1967, while attending an international veterinary congress in Paris; he was 57. He was born at Southend-on-Sea and was educated at Bedford School and Trinity College, Cambridge, where he studied mathematics. On choosing a career in veterinary medicine, he studied further at the Royal (Dick) Veterinary College, Edinburgh, and the Royal Veterinary College in London. From 1935 to 1939 he served as veterinary officer in Uganda, being particularly concerned with the control of epizootic diseases.

He returned to Britain in 1939 to work with F. Blake-more at the Institute of Animal Pathology in Cambridge. Here he was concerned with one of the first isolations of swine influenza virus in Britain. His main interest, however, was in *Erysipelothrix rhusiopathiae*, the bacterium causing swine erysipelas, and his interest continued after he left Cambridge. He made important contributions to knowledge of its antigenic constitution and the application of this knowledge to production of a vaccine.

In 1947 he was appointed veterinary officer to the National Institute for Medical Research, Mill Hill, in succession to R. E. Glover. He co-operated with the team working there in WHO's World Influenza Centre. In 1951 came a discovery which affected all his subsequent work. Infant mice in the breeding stock at Mill Hill were dying of a hepatitis which was apparently due to a hitherto unknown virus. Unexpectedly, however, its activity could be inhibited by tetracyclines. It was shown that its effects were due to the synergistic action of two agents, one stable, one more labile, each relatively harmless by itself. The labile agent proved to be the blood-parasite, *Eperythrozoon coccoides*, and it was this which was susceptible to antibiotics. In much of this work Gledhill co-operated with Dr J. S. F. Niven, Dr G. W. A. Dick and myself.

These discoveries initiated a series of studies on non-specific factors affecting susceptibility and resistance. A number of agents were examined for their mutual enhancing or sparing activity: bacterial endotoxins, *Eperythrozoon*, tuberculosis and the viruses of mouse hepatitis, infectious ectromelia, mouse leukaemia and lymphocytic choriomeningitis. The results were complex; much depended on timing and dosage.

All this work had a bearing on latent infections, particularly in mice, and the confusing effect of these for laboratory workers. Gledhill was accordingly keenly interested in the possibility of maintaining stocks of "specific-pathogen-free" animals. He had been appointed head of an Animal Division at the National Institute for Medical Research and was particularly concerned in planning accommodation for laboratory animals which would ensure a supply of really clean stock.

Gledhill was an attractive colleague with an original mind, always enthusiastic, never happier than when wrestling with a problem complex enough to deter all but a really devoted seeker after truth.

C. H. ANDREWES

## University News:

London

THE title of professor of naval architecture has been conferred on Mr L. J. Rydill in respect of his post at University College.

Salford

THE university and the Shirley Institute are planning to undertake co-operative research projects which will be both of good academic research character and also provide results of direct significance to technical and industrial processes. One of the first projects selected deals with the use of high-energy irradiation to initiate grafting of polymers on to fibres, films and fabrics.

## Appointments

THE Hon. J. J. Astor has been appointed chairman of the Agricultural Research Council in succession to the Duke of Northumberland, who is to relinquish the position on June 30, 1968.

## Announcements

THE current list of reprints available from the Ministry of Technology's Forest Products Research Laboratory has now been published and is available on request from the Director, Forest Products Research Laboratory, Princes Risborough, Aylesbury, Bucks.

**CORRIGENDUM.** In the article "Surface Topography of Ice Sheets" by G. de Q. Robin, which appears on page 1029 of this issue of *Nature*, the first symbol of equation 2 on page 1030 should be the Greek letter rho and not alpha.

# CORRESPONDENCE

## Computers for Scientific Research

SIR,—During the past few years a revolution has occurred in the pattern of spending needed to support basic scientific research. In practically every scientific field, as in technology and business, computers have now become a necessity without which it is often impossible to keep in the front line. It is my purpose to point out that there is evidence of a serious failure by the British Government to provide adequate funds to keep up with this revolution and to suggest that a major revision of policy on expenditure on scientific research is needed in order to build up the computing power needed in all branches of scientific research.

Minimum expenditure on computers for scientific research in this country recommended for the current year by the Flowers committee was about £4.5 million, with a further £1 million for installation and running costs. This was expected to rise to a total of about £8 million by 1969. This sort of money has to be scaled against a total budget for all fields of research provided through the Department of Education and Science to the research councils and the University Grants Committee of about £100 million in the current year.

Unfortunately, even before the computer investment programme recommended by the Flowers committee was initiated, the rate of investment in computing facilities was cut back to a total of £3 million a year for the first three years (1966–69). Moreover, the implementation of this reduced programme of spending was severely delayed despite the emphasis of the Flowers committee report on the need to make up for the very late start in Britain in

getting under way on investment in computers for basic scientific research. There is no doubt that this cut-back and delay on the minimum rate of investment in computers is bound to have serious repercussions on progress in Britain in practically every field of scientific research.

It is instructive to contrast the Government's investment policy on this very basic and essential general research facility with the policy on investment in the largest single item, in a sense a luxury item, in our scientific budget: high energy nuclear physics. Britain is spending £12 million on high energy physics in the current year. And there are signs that there will be strong pressures to increase this. For instance, the mammoth high energy machine recently proposed for CERN will cost in the region of £150 million, of which Britain's share would be in the region of £33 million (see also *Nature*, 214, 1283; 1967). The contrast between investment in computers and that in high energy physics is the more striking in that an increasing proportion of the high energy physics budget is being spent on providing computing facilities exclusively for high energy physics research. An example of this is the recent purchase by the Science Research Council of two large (by British standards) American computers, one sited in Oxford and one in London, specifically for the purpose of processing data from high energy physics experiments. These computers, at a cost of £0.25 million each, are each more powerful (though with slightly less peripheral equipment) than most of the computers at present serving the needs of entire universities in Britain (if they are lucky enough to have one at all). For instance, the University of Sussex was only able to spend about half the cost of one of these computers on the computer they recently installed to serve the needs of the entire university.

One of the problems facing scientists in other fields is that they have no direct way of applying pressure for support for computing needs. High energy physics, on the other hand, by the very nature of the large funds already committed to it, is in a strong position to get still further support for its computing needs: if you are spending £12 million a year to gather data, then it is eminently reasonable to spend a further £0.5 million to process some of it. But the frustration of scientists starved of computer access who see thousands of pounds worth of computer time going unused during the development period of these specialized computer experiments is not to be under-estimated. The expenditure of these large sums when essential needs are being under-supported should not be allowed to continue without question.

What is to be done about this? The high energy physics lobby is very well organized. Individual computer users are not. As presently organized, university computer policy is organized from on high, and computer time is simply handed out when available. One possibility might be to make all computer users pay for computer time with money awarded to them in the form of research contracts as is the practice in the United States. This would both encourage economy and enable individual users to put pressure on the Government directly on the basis of the merits of their research proposals. It would also allow individual scientists to go to commercial companies for facilities, such as on line programming, which are not at present provided by university computer services.

Whatever happens, it is vital that the training of our scientists and the morale of research workers in all fields of basic research should not be undermined through an unwillingness to change established patterns of research spending. Britain's future depends on our ability to face the computer age fairly and squarely.

Yours sincerely,

S. DONIACH

Department of Physics,  
Imperial College,  
London.

## FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

## Sunday, September 3—Wednesday, September 6

BRITISH ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE (at Leeds)—Continuation of 129th Annual Meeting.

## Sunday, September 3

At 10.30 a.m.—OFFICIAL SERVICE IN LEEDS PARISH CHURCH. Preacher: The Most Reverend and Right Honourable F. D. Coggan, P.C., D.D., Lord Archbishop of York.

## Monday, September 4

At 10 a.m.—Dame Kathleen Lonsdale, F.R.S.: "Physics and Ageing" (Presidential Address, Section A).

At 10 a.m.—Mr N. L. Falcon, F.R.S.: "The Geology of the North-East Margin of the Arabian Shield" (Presidential Address, Section C).

At 10 a.m.—Dr A. T. Lucas: "Felling and Napping in Early Ireland" (Presidential Address, Section H).

At 11.30 a.m.—Dr D. E. Broadbent: "Aspects of Human Decision Making" (Presidential Address, Section J).

At 11.30 a.m.—Prof. T. A. Bennet-Clark, C.B.E., F.R.S.: "The Role of Plant Sciences in the Modern World" (Presidential Address, Section K).

At 2.30 p.m.—Dr B. J. Mason, F.R.S.: "Thunderstorms and Lightning" (Young People's Lecture).

At 8 p.m.—Mr D. R. Pilbeam: "Human Origins" (Evening Discourse).

## Tuesday, September 5

At 11.30 a.m.—Dr J. A. Gray: "The Physiological Basis of Personality" (Lister Lecture).

At 2.30 p.m.—Prof. C. S. Whewell: "The World of Fibres" (Young People's Lecture).

## Friday, September 8

BRITISH SOCIETY OF AUDIOLOGY (at the Royal Society of Medicine, 1 Wimpole Street, London, W.1), at 5 p.m.—Inaugural Meeting. Dr J. D. Hood: Inaugural Address. Dr I. C. Whitfield: "Centrifugal Control of Threshold Responses to Auditory Stimuli at the Level of the Cochlear Nucleus"; Mr A. Boothroyd: "Developments in Speech Audiometry".\*

INSTITUTE OF MATHEMATICS AND ITS APPLICATIONS (at the (proposed) University of Dundee—Tower Building, Dundee), at 10.15 a.m.—Symposium on "Some Recent Applications of Pure Mathematics". Speakers: Mr N. Lawrie, Dr A. Page and Dr R. Delbourgo.\*

## Sunday, September 10—Tuesday, September 12

BOTANICAL SOCIETY OF THE BRITISH ISLES, in association with the LINNEAN SOCIETY OF LONDON (at the Hartley Botanical Laboratories, the University of Liverpool)—Conference on "Modern Methods in Plant Taxonomy".

## Monday, September 11

INSTITUTION OF MECHANICAL ENGINEERS, AUTOMOBILE DIVISION (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Mr R. C. Teasel and Mr R. D. Miller: "Characteristics of New Ignition Systems to Improve Engine Performance".

## Monday, September 11—Friday, September 15

INSTITUTION OF MECHANICAL ENGINEERS, APPLIED MECHANICS GROUP (at Imperial College of Science and Technology, London, S.W.7)—Conference on "High Pressure Engineering".

## APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

RESEARCH ASSISTANT to direct a research unit working on human colonic physiology in conjunction with surgical staff—The House Governor, The London Hospital, Whitechapel, London, E.1 (September 9).

RESEARCH ASSISTANT (with the degree of B.Sc. (Ordinary) or an H.N.C. (Chemistry) in the DEPARTMENT OF CHEMISTRY to join a team engaged in co-polymerization studies under Dr G. G. Cameron—The Secretary, The University, Aberdeen (September 11).

LECTURER or ASSISTANT LECTURER in SOCIAL STATISTICS in the DEPARTMENT OF SOCIOLOGY, SOCIAL STUDIES AND ECONOMICS—The Secretary, Bedford College (University of London), Regent's Park, London, N.W.1 (September 12).

DEMONSTRATOR or SENIOR DEMONSTRATOR in the DEPARTMENT OF PURE MATHEMATICS—The Registrar, The University, Liverpool, quoting Ref. RV/158 (September 15).

JUNIOR RESEARCH FELLOW (with a good honours degree in physics and research interests in solid state physics) in PHYSICS—The Registrar, The University, Sheffield (September 15).

SENIOR LECTURER in BIOCHEMISTRY; a SENIOR LECTURER/LECTURER in ENGINEERING; a SENIOR LECTURER in MATHEMATICS; a LECTURER in GEOLOGY; a LECTURER in GEOGRAPHY, and a LECTURER in PSYCHOLOGY at the University College of Townsville, University of Queensland—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 22).

LECTURER (with research interests in the field of cell biology or embryology and preferably some proficiency in biochemical techniques) in ZOOLOGY in

St. Salvator's College—The Secretary, University of St. Andrews, College Gate, St. Andrews, Fife, Scotland (September 23).

RESEARCH FELLOW in ORGANIC CHEMISTRY to join a group working on the chemistry of orthoquinones—Professor J. M. Tedder, Chemistry Department, The University, Dundee, Scotland (September 23).

RESEARCH ASSISTANTS (with experience in electronics, experimental psychology, electro-physiology, or computer techniques) in the DEPARTMENT OF COMMUNICATION for a programme of basic research into perceptual and communication problems of the blind and deaf—The Registrar (N), The University, Keele, Staffordshire (September 25).

ASSISTANT LECTURER or LECTURER in CHEMISTRY (the appointment may be made in any branch of physical chemistry)—The Registrar, The University, Sheffield (September 30).

LECTURERS/ASSISTANT LECTURERS (3) in CIVIL ENGINEERING at the University of Hong Kong—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Hong Kong and London, September 30).

RESEARCH FELLOW (M.B., Ch.B., or B.Sc. with honours in pharmacology, physiology or biochemistry) to join a group engaged in research on the mode of action of narcotic analgesic drugs—Dr H. W. Kosterlitz, Marischal College, The University, Aberdeen (September 30).

SENIOR LECTURERS or LECTURERS (with qualifications in either pure or applied mathematics) in MATHEMATICS at the University of Canterbury, Christchurch, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, October 9).

PROFESSORIAL FELLOW, SENIOR FELLOW, FELLOW, SENIOR RESEARCH FELLOW and a RESEARCH FELLOW in the DEPARTMENT OF ASTRONOMY, Institute of Advanced Studies, Australian National University—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 20).

ENTOMOLOGIST (with a good honours degree in zoology with special emphasis on entomology and an interest in and aptitude for taxonomic research) for work on hymenoptera in the COMMONWEALTH INSTITUTE OF ENTOMOLOGY identification service at the British Museum (Natural History)—The Secretary, Commonwealth Agricultural Bureaux, Farnham House, Farnham Royal, near Slough, Bucks (October 31).

SENIOR LECTURER (medically qualified with a higher degree in pathology and experience in teaching and routine diagnostic work) in the DEPARTMENT OF PATHOLOGY (Austin Hospital), University of Melbourne, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 31).

GEOCHEMIST (with evidence of research ability at the postgraduate level) in the CHEMISTRY DIVISION, DEPARTMENT OF SCIENTIFIC AND INDUSTRIAL RESEARCH, Lower Hutt, New Zealand, to join a group doing research on the geochemistry of natural hydrothermal systems in New Zealand—The High Commissioner for New Zealand, New Zealand House, Haymarket, London, S.W.1, quoting Ref. B 13/15/57/2543 (November 30).

JOHN INNES CHAIR OF GENETICS; and JOHN INNES CHAIR OF APPLIED GENETICS at the School of Biological Sciences and John Innes Institute—The Registrar, University of East Anglia, Earlham Hall, Norwich, NOR. 88C (November 30).

ASSISTANT PROFESSOR or ASSOCIATE PROFESSOR (interested in cardiovascular pharmacology or biochemical pharmacology) in the DEPARTMENT OF PHARMACOLOGY—Professor G. B. Frank, Department of Pharmacology, University of Alberta, Edmonton, Alberta, Canada.

CHEMIST or BIOCHEMIST (graduate) to co-operate in a research programme to study the physical characteristics and mechanism of action of insulin—The Registrar, University of York, Heslington, York.

GRADUATE ASSISTANT in CHEMISTRY to work under the direction of Dr S. T. Reid on the synthesis of heterocyclic compounds of potential biological interest—The Registrar, The University, Canterbury, Kent, quoting Ref. A73.

J. R. MCKENZIE RESEARCH FELLOW (preferably Ph.D. or M.Sc. with experience in the lipid carbohydrate metabolic field) in BIOCHEMISTRY at the Wellington Hospital Medical Unit, New Zealand, undertaking clinical research in the lipid field and major epidemiological cardiovascular surveys among Polynesians in the Pacific and New Zealand in 1968–69—Professor W. J. H. Butterfield, Department of Medicine, Guy's Hospital, London, S.E.1.

LECTURER (with good academic qualifications and capable of supervising research students for the Ph.D. degree) in CHEMICAL ENGINEERING—The Registrar, University of Salford, Salford 5, quoting Ref. CE/3.

LECTURERS in GEOGRAPHY at the University College of Rhodesia—The Inter-University Council, 33 Bedford Place, London, W.C.1.

PHYSICIST or CHEMICAL ENGINEER (with a first- or upper-second-class honours degree in physics or the equivalent, and preferably some experience of research in the application of physical methods to the properties of materials, especially foods) in the BIOENGINEERING SECTION, to work as a member of a team concerned with technological problems in improving the efficiency and output of the British meat industry—The Secretary, Meat Research Institute, Agricultural Research Council, Langford, near Bristol, Somerset, quoting Ref. BMI.

POSTDOCTORAL RESEARCH BIOCHEMIST to study the effect of anti-inflammatory drugs on protein and nucleic acid biosynthesis—Professor M. J. H. Smith, Department of Biochemical Pharmacology, King's College Hospital Medical School, London, S.E.5.

POSTDOCTORAL RESEARCH WORKER to work in association with Dr R. A. Beatty on problems of animal gamete genetics—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh, 8.

RESEARCH ASSISTANT (science graduate preferably with experience in cytogenetics) in CYTOGENETICS—Professor W. Davidson, King's College Hospital, Denmark Hill, London, S.E.5.

RESEARCH FELLOW (with a Ph.D. degree or equivalent research experience) at the University College of Townsville to undertake full-time research on the structure and metabolism of polysaccharides of tropical pasture legumes—The Secretary-General, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1; and The Warden, University College of Townsville, P.O. Box 999, Townsville, Queensland, Australia.

TEACHING FELLOWS in the FACULTY OF SCIENCE, University of Otago, Dunedin, New Zealand—The Secretary-General, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1, or the Registrar, University of Otago.

TECHNICIAN or JUNIOR TECHNICIAN in the BIOCHEMISTRY DEPARTMENT—The Staff Officer, University of Surrey, Battersea Park Road, London, S.W.11.

TECHNICIAN (preferably with previous experience) in the DEPARTMENT OF OBSTETRICS and GYNACOLOGY for research work in cytology—The Clerk to the Governors, St. Bartholomew's Hospital, London, E.C.1, quoting Ref. ASC/1158.



## REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

### Great Britain and Ireland

- Bulletin of the British Museum (Natural History). Entomology. Vol. 21, No. 1: The Indo-Australian Species of the *Ulor*-Group of *Apanetes* Förster (Hymenoptera: Braconidae). By G. E. J. Nixon. Pp. 1-34. 15s. Catalogue of Fossil Hominids. Part 1: Africa. Edited by Kenneth Page Oakley and Bernard Grant Campbell. Pp. xv+128. 45s. (London: British Museum (Natural History), 1967.) [137]
- The Theory of Natural Immunity by Reference to the Saline Oxygen Level of the Blood and Plasma—The Effect of the pH Value. By A. J. Cullinane. Pp. 24. (Binnegar, Wareham, Dorset: A. J. Cullinane, The Wareham Ball Clay Co., 1967.) [137]
- British Welding Research Association. Twenty-second Annual Report 1966. Pp. 58. (Abingdon Hall, Cambridge: British Welding Research Association, 1967.) [147]
- Natural Environment Research Council. National Institute of Oceanography. Collected Reprints, Vol. 14. Reprints Nos. 559-595. (Wormley, Godalming: National Institute of Oceanography, 1966.) [147]
- Philosophical Transactions of the Royal Society of London. Series A: Mathematical and Physical Sciences, No. 1124, Vol. 262 (13 July 1967): A Discussion on Orbital Analysis. Organized by D. G. King-Hele, F.R.S. Pp. 1-202+plates 1-5. (London: The Royal Society, 1967.) 80s.; \$12. [147]
- Office of Health Economics. Pharmaceutical Research: The Case for Growth in Britain. Pp. 32. (London: Office of Health Economics, 1967.) 2s. 6d. [147]
- Department of Education and Science and the British Council. Scientific Research in British Universities and Colleges, 1966-67. Vol. 1: Physical Sciences. Pp. xx+473. 40s. net. Vol. 2: Social Sciences (Including Government Department and Other Institutions). Pp. xxvii+272. 32s. 6d. net. Vol. 3: Biological Sciences. Pp. xx+445. 40s. net. (London: H.M. Stationery Office, 1967.) [147]
- Cotton Research Corporation. Progress Reports from Experiment Stations, Season 1965-66. Republic of the Sudan. Pp. 62. 2s. 6d. Uganda. Pp. 66. 2s. 6d. Western Nigeria. Pp. 27. 2s. 6d. (London: Cotton Research Corporation, 1967.) [147]
- Overseas Development Institute. Annual Report 1967. Pp. 33. Pledged to Development: a Study of International Consortia and the Strategy of Aid. By John White. Pp. 235. 30s. (London: Overseas Development Institute, 1967.) [147]

### Other Countries

- Institut Royal des Sciences Naturelles de Belgique. Bulletin. Tome 42, No. 29: A propos de "*Anthrax collaris*" Wied. 1828, de "*Bibio lar*" F. 1781 et du Genre *Litorhynchus* Macq. 1840 (Bombyliidae, Diptera). Par F. J. Francois. Pp. 19. Tome 42, No. 33: Etudes Hydrobiologiques sur les Eaux Saumâtres de Belgique. 8: Les Eaux de Doel et Environs dans la Région du Bas-Escaut Belge (1), (Période 1950-1951). Par Ludo Van Meel. Pp. 37. Tome 42, No. 35: A Survey of the Dragonfly Fauna of Morocco (Odonata). By M. A. Lieftinck. Pp. 63+map. Tome 42, No. 37: Contribution à l'Etude des Ephéméroptères du Surinam. Par Georges Demoulin. Pp. 22. Tome 42, No. 38: Sur la Formule Dentaire de Deux Primates du Landenien Continental Belge. Par G. E. Quinet. Pp. 6. Tome 42, No. 39: On Organic Remains in Shells of Palaeozoic and Mesozoic Cephalopods (Nautiloids and Ammonoids). By Charles Grégoire. Pp. 36+54 photographs. Tome 43, No. 4: Les Hypopes Parasites des Tissus Cellulaires des Oiseaux (Hypodectidae: Sarcophagidae). Par A. Fain. Pp. 139. Tome 43, No. 10: Résultats Scientifiques de l'Expédition Pédologique Hongroise au Congo-Brazzaville. 15: Brentidae (Coleoptera-Curculionidae) (1). Par Roger Damoiseau. Pp. 14. (Bruxelles: Institut Royal des Sciences Naturelles de Belgique, 1966 et 1967.) [226]

Transactions of the American Philosophical Society. New Series, Vol. 57, Part 2: Excavations in the Tehuantepec Region, Mexico. By Matthew Wallrath. Pp. 173. (Philadelphia: The American Philosophical Society, 1967.) \$4.50. [226]

Bulletin of the American Museum of Natural History. Vol. 136, Article 1: A Review of the Rhinocerotoid Family Hyracodontidae (Perissodactyla). By Leonard B. Radinsky. Pp. 1-46+1 plate. (New York: American Museum of Natural History, 1967.) \$1.50. [236]

Population Bulletin, Vol. 23, No. 3 (June 1967): Punta del Este, 1961-1967—Early Dawn of a Demographic Awakening. Pp. 45-84. (Washington, D.C.: Population Reference Bureau, Inc., 1967.) [236]

CERN-European Organization for Nuclear Research. Status of the Project for a European 300 GeV Proton Synchrotron. July 1966. Pp. 22. ECEA-European Committee for Future Accelerators. Utilization Studies for a 300 GeV Proton Synchrotron. Vol. 1: Pp. xiii+406. Vol. 2: Pp. viii+469. Vol. 3: Pp. viii+160. (Geneva: CERN-European Organization for Nuclear Research, 1966 and 1967.) [236]

United States Department of the Interior: Geological Survey. Bulletin 1117-D: Distribution of Minor Elements in Some Coals in the Western and Southwestern Regions of the Interior Coal Province. By Peter Zubovic, Nola B. Sheffey and Taisia Stadnichenko. Pp. iv+33+plate 1. \$0.50. Bulletin 1163-D: Geology of the Taunton Quadrangle, Bristol and Plymouth Counties, Massachusetts. By Joseph H. Hartshorn. Pp. iv+67+plate 1. Bulletin 1228: Bauxite Reserves and Potential Aluminium Resources of the World. By Sam H. Patterson. Pp. vi+176+plates 1 and 2. Water Supply Paper 1819-K: Correlation and Analysis of Water-Temperature Data for Oregon Streams. By A. M. Moore. Pp. iv+33+plate 1. Water Supply Paper 1839-D: Summary of Hydrologic and Physical Properties of the Rock and Soil Materials, as Analysed by the Hydrologic Laboratory of the U.S. Geological Survey, 1948-60. By D. A. Morris and A. I. Johnson. Pp. iv+42. \$0.25. Professional Paper 384-B: Solution Studies of Chrysotile, Lizardite and Antigorite. By George T. Faust and Bartholomew S. Nagy. Pp. iii+93-105. \$0.20. Professional Paper 516-D: Geological Interpretation of Gravity and Aeromagnetic Maps of Tintic Valley and Adjacent Areas, Tooele and Juab Counties, Utah. By D. R. Mabey and H. T. Morris. Pp. iii+10+plate 1. Professional Paper 538: Geology of Epigenetic Uranium Deposits in Sandstone in the United States. By Warren I. Finch. Pp. iv+121+plates 1 and 2. Professional Paper 549-A: Physical and Chemical Description of Birch Creek, a Travertine Depositing Stream, Inyo County, California. By Keith V. Slack. Pp. iii+19+plate 1. \$0.55. Professional Paper 554-A: Minor Elements in Alluvial Magnetite from the Inner Piedmont Belt, North and South Carolina. By P. K. Theobald, Jr., W. C. Overstreet and C. E. Thompson. Pp. iii+34+plates 1 and 2. Professional Paper 573-B: The Mesozoic Pelecypods *Olapiria* Marwick and *Lupherella* Imlay, New Genus, in the United States. By Ralph W. Imlay. Pp. iii+11+plates 1 and 2. \$0.25. (Washington, D.C.: Government Printing Office, 1967.) [236]

Ontario Research Foundation. Annual Report 1966. Pp. 56. (Toronto: Ontario Research Foundation, 1967.) [37]

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Federation of Malaya. Report on Forest Administration for the year 1963. By Mohd. Alwy bin Haji Suleiman. Pp. 115. (Kuala Lumpur: Chief Conservator of Forests, Malaysia, 1966.) \$2.50. [57]

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Applicants should possess a good honours degree in science and have had experience in food science and preservation; they should also have administrative ability and a creative interest in the food industry. The Director is the chief executive officer of the Association and his duties are many sided.

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(726)

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H. J. JOHNSON,  
 Group Secretary,  
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Both projects are sponsored by the Natural Environment Research Council and are tenable for two years with possible extension to a third year.

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Inevitably and deservedly, *The New Industrial State* (Hamish Hamilton, 42s.) will be widely read. Like its predecessor, it has an apocalyptic sweep which is almost hypnotic. Once again, critics will find themselves having to agree that even though Galbraith seems to them to be wrong, he is likely to be at least half right. Yet the critics will find it easier to make away this time, chiefly because the new book is not merely intended as a sequel to *The Affluent Society* but as a more comprehensive conceptual framework within which Galbraith's observations on the condition of modern society can be accommodated. As such, *The New Industrial State* is vulnerable in two quite different ways. For one thing, it is written with the open intention of changing people's minds and not as a work of scholarship, which means that there will be endless disputes about the validity of Galbraith's assertions. It is also a kind of doomsday book. Galbraith is not only deeply pessimistic about the way that things are going, and also considers that technology is one of the most immediate dangers. That will not immediately win him everybody as a friend.

The starting point of *The New Industrial Society* is a series of paradoxical observations about the modern society, some of them familiar and all of them in some way stimulating or provoking. According to Galbraith, modern industry is neither the embodiment of the kind of capitalism which Marx described nor an extension of conventional statements about the character and the importance of free enterprise, but an institutional phenomenon in its own right. In the great industrial corporations, the commercial swashbuckling of the old entrepreneurs and the managerial interests of the stockholders have been subordinated to the interests of the salaried executives who form a self-perpetuating oligarchy. Corporations and governments are not the enemies which popular myth sometimes supposes, but back-scratching symbionts. Modern

industry is made ponderous and inflexible by its dependence on modern technology—and Galbraith has great fun with a comparison of the three months needed to put the first Ford motor car on the market in 1903 and the three years needed to market a new car sixty years later. For him, more technology means more time, more capital investment, manpower which is more specialized. One consequence of this, the argument goes, is that industries organize themselves in such a way as to insulate themselves as far as possible from ordinary commercial forces such as the vagaries of the market and the supply of capital. Strictly commercial goals are subordinated to other ends. But—paradox on paradox—the growing complication of modern industry entails increasing dependence on an increasingly influential body of scientists and academics—"the Educational and Scientific Estate"—who are by temperament and training inclined to reject the values of the juggernaut they serve. And these, says Galbraith, are precisely the people on whom society must rely for an escape from the monolithic industrial system. Galbraith only properly escapes from gloom in his chapter called "Education and Emancipation".

One of the most urgent questions raised by Galbraith's argument is that of the extent to which technology can be held responsible for the condition of modern industrial society and for its most serious defects. Plainly the charge cannot lightly be dismissed, particularly because there is no reason to think that Galbraith is one of those people for whom society took a turn for the worse when housewives gave up baking their own bread. He has nothing against motor cars as such, but he protests at the manner in which they are produced by huge mindless corporations aiming simply to produce and to survive. One immediate difficulty is, of course, that the corporations may not be as stable and unchanging as Galbraith says they are. Even the biggest among them go out of business from time to time, which is not merely a virtue by the standards of *The New Industrial State* but also something of a proof that market forces have not been nullified entirely. Indeed, the most quickly growing corporations are often the youngest and the smallest—and it is remarkable how willingly the Educational and Scientific Estate in the United States has given these enterprises its blessing. By now, the outskirts of half a dozen American university cities are ample evidence of that.

For what, then, is technology to be blamed? The fact that it now takes several years to put a new kind of motor car into service is in the last resort a consequence of trying to produce motor cars with high performance as cheaply as possible. The first



Henry Ford could move more quickly because his much smaller market could afford comparatively higher prices. To begin with, there was no need to invest huge sums of money in machinery for mass production, and no need to worry about the refined development of the components of which his motor cars were assembled. The link between modern technology and the other aspects of modern industry of which Galbraith complains is very much the same. Efficient production at low cost means planning ahead and persuades industrial corporations to insulate themselves as far as possible from the forces of the market. None of this conflicts with what Galbraith has to say, and indeed he is the first to admit that modern industry is capable enough when only the production of goods is in question. He is, however, a little mischievous, to say the least of it, when he attributes the change which has come over industry to the influence of a seemingly animate entity called technology. Society could have less of technology and therefore less of its supposedly malevolent influence if society would settle for a smaller volume of production.

There must also be doubt about the value of Galbraith's concept of the Educational and Scientific Estate—not quite the same thing as Dr D. K. Price intended a year ago with the title of his book *The Scientific Estate*. For one thing, it implies a degree of homogeneity which may be unrealistic. Worse still, it implies what may be too sharp a distinction between those with a university education and those without. As with what may be called Snow's hypothesis, any attempt like this to cut the community up into intellectually distinct pieces is likely to founder on the rocks of careful observation. But this, perhaps, may not be part of what Galbraith intended, in which case it is a pity that he has given his critics more openings than they deserve. And in the last resort, of course, Galbraith is right to pin his hope for the future—pale though it may be—in the possibility that academics and like-minded people may be able to engage so successfully in the rough and tumble of deciding what goals should be chosen for industry, government and society at large that the modern world will be a better place. To put it at its most trivial, Galbraith is right in saying that you cannot rely on the forces of the economic market to ensure that unsightly advertising hoardings will not proliferate. He is also right in thinking that the universities and similar places are best placed, by virtue of their independence, to provide the kind of influence that society would find healthy. His book will do good in this direction, for it will be widely read and it is bound to be provoking. It remains to be seen whether academics, as is their custom, will think their public duty has been done when they have read the book. It is also an open question whether the strong and sometimes over-clear lines in which Galbraith's picture has been painted will serve as a spur to persuade the Educational and Scientific Estate that it could easily win more creative influence than it has or whether, on the other hand, it will become another excuse for inactivity.

## NO OIL ON WATER

SOME details of the appalling destruction of sea birds caused by the wreck of the Torrey Canyon are reported on page 1123 of this issue in a survey of 1,223 dead birds collected on Cornish beaches during the month following the disaster. What stands out is that mortality was almost exclusively restricted to two species of offshore swimming birds—78 per cent of the sample was guillemots and 20 per cent razorbills. Other species appear to have escaped major killing. The same two species formed respectively 81 per cent and 17.5 per cent of the much larger sample of 7,849 birds taken at the Cornish cleaning stations. A summary provided by the Royal Society for the Prevention of Cruelty to Animals (see page 1119) draws attention to the very low survival rate of birds at cleaning stations. Above 450 of the 7,849 had been rehabilitated on April 1 and many of these have since died. A similar fate befell most of the 2,000 birds taken to cleaning stations in France. In other words, a little over 10,000 birds are known to have died and, given all the difficulties of recovering oil-soaked bodies, it is likely that total of anything between 30,000 and 100,000 birds were killed.

Luckily, the damage to the English sea bird populations is not likely to prove permanent. Even the breeding populations of the southern guillemot *Uria aalge albionis*, which has for the past fifty years suffered increasingly from oil pollution, are expected to recover. The populations of puffins, guillemots and razorbills on the coast of Brittany have, however, suffered very badly and may well fail to recover.

As the figures show, and as the RSPCA and the Royal Society for the Protection of Birds would be the first to admit, the magnitude of the Torrey Canyon disaster completely overwhelmed the facilities of the cleaning stations. When oiled birds are arriving in thousands, there is little hope for most of them. An oiled bird is to be cleaned and successfully rehabilitated it must be given constant and individual attention and many ornithologists believe that in emergencies like the Torrey Canyon wreck, it is better to kill birds than to prolong their suffering in vain attempts to clean them. But if attempts to save birds are to be made at all, there is an urgent need of many more cleaning centres, perhaps mobile ones, and much more research into the way of cleaning contaminated birds. Apparently some of the £60,000 collected by the Wildlife Fund, the RSPCA and the RSPB in the joint Seabird Fund appeal is earmarked for this, the £5,000 which the British Government contributed is to pay for the work done last spring—but the amount available are not sufficient for all the future work that is needed. The RSPCA alone spent £7,000 on cleaning operations after the Torrey Canyon wreck.

The figures in these reports show just part of the suffering that can result from a massive pollution of the sea, and unfortunately the Torrey Canyon disaster, though by far the worst in British waters, is by no means an isolated incident. Last year, 1,700 tons

oil were accidentally pumped from the German tanker Seestern into the River Medway—an estimated 8,000 birds were among the victims—and earlier this year a Greek tanker left behind 250 tons of oil in Milford Haven. What is being done to prevent oil pollution in the future? The shipping world is slowly accepting that the sea must not remain its largest and cheapest dustbin. An indication of this is that the Japanese Government seems to be on the verge of formally joining the Convention for the Prevention of Oil Pollution of the Sea—which does not imply that Japanese ships are responsible for most oil pollution. The Convention, which is now supported by the governments of all the major maritime powers except Japan and the USSR, designates zones throughout the world where the discharge of oil is forbidden. Of course, it has no legal powers; if a ship breaks the rules on the high seas and is caught—which is rare—it can be punished only by the country of registration. There is a tortuous legal procedure by which any member nation which spots a transgression anywhere in the world reports the evidence to the flag government which, if satisfied that the evidence is sufficient to provide a case against the ship according to its own law, should take immediate proceedings against it. If Japan, the fifth largest maritime nation, does in fact join, the Convention's moral authority, if nothing else, will be greatly strengthened.

But obviously the only effective way to stop voluntary pollution is to remove the financial incentives. This could be done by making fines imposed on ships caught discharging oil so severe that ships' masters would not dare to risk the penalties. For example, why should not the signatories to the convention agree to ban from their ports any ship known to have caused wide-scale pollution? A subcommittee of the Inter-Governmental Maritime Consultative Organization (IMCO) is to meet in London next week and will be considering, among other things, ways of increasing the penalties for oil pollution, but nobody seems to think it will go so far as to recommend such radical measures.

Deliberate pollution is much more likely to stop when the oil companies develop ways of recovering the oil previously discharged and saving themselves some money. In the past, oil tankers returning empty to the land fields have washed their tanks at sea to save a few hours sailing time and have discharged the washing—oil and seawater. A system known as 'load on top', which prevents some of this waste and pollution, has been developed by the oil companies. With 'load on top' the tanks are washed with hot sea water, and all the washings are collected into a tank to separate, when the lower aqueous phase can be discharged. The oil companies make great claims for this system and even conservationists agree that it represents a considerable improvement, but an IMCO study found that it is not entirely within the pollution limit that the convention sets. 'Load on top' is still only a compromise between the old discharge system and the use of really effective separation plants. None the less, if it really

does save money and reduce pollution as well, we can be sure it will find widespread acceptance.

Even in the unlikely event of all deliberate pollution ceasing, the risk of accidental pollution is increasing as more and more oil is transported. There will always be the spectre of a one or two hundred thousand ton tanker being wrecked. After the Torrey Canyon disaster, the British Government called for a special session of IMCO, which met in May, and which began a series of urgent studies into sea lanes, speed limits, crew training, construction of tankers and procedures for mobilizing national and international efforts to deal with pollution. Despite the protestations of the oil companies to the contrary, these studies clearly imply that the standards of safety and navigation of tankers can be greatly improved. As Torrey Canyon showed, to save a few hours sailing time some masters seem prepared to cut safety margins.

IMCO has also set in motion research into ways of dispersing oil without destroying marine flora and fauna—one of the problems of the Torrey Canyon disaster was that no one knew the toxic effects of the detergents used. The results of the IMCO studies are to be presented in 1968 and, if nothing else, they should lead to sensible speed limits and better defined sea lanes. The problem, however, as with all other international agreements, will be one of enforcement. Tradition apart, it is difficult to understand why shipping should not be as closely regulated as aircraft. May not the concept of untrammelled freedom of the high seas outlive its usefulness?

Apart from trying to prevent accidents, IMCO is also tackling the more important job of devising procedures for accidents. Delays in burning the Torrey Canyon, wrecked outside territorial waters, and the subsequent events leading to the melodrama in Singapore, where her sister ship was arrested by the British Government and only released on £3 million security, revealed the complete lack of internationally accepted procedures for obtaining adequate compensation for people and governments which suffer directly from pollution and for allowing a government to destroy a potential pollution hazard. Groups in the various member nations of IMCO are investigating the legal responsibilities of shipowners and masters whose vessels, though wrecked on the high seas, damage the livelihoods of an adjacent country. It is essential that some sensible code of practice for third party insurance be devised (*Nature*, 214, 1; 1967). They are also studying the extent to which a state threatened by a wreck outside its territorial waters can protect itself even if that necessitates destroying the vessel and cargo and so affects the interests of shipowners, salvage companies, insurers and even flag governments. The maritime nations must reach some firm agreement on this quickly and accept the inevitable but probably small increase in insurance costs. It is inconceivable that when the next loaded tanker is wrecked, it should be allowed to spew out thousands of tons of oil while there are protracted legal discussions between the parties involved.

## STRENGTH THROUGH MISERY?

THE British Association seems to have had a successful meeting this year, and there is nobody who will be sorry. A great many people with puritan inclinations will, however, be delighted to observe that adversity seems to have brought out the best in the association. It is several years now since the association suffered its chronic penury in silence. Instead, it has taken to complaining that the British Government does not reward it properly for doing what is in the last resort a public service—the provision of a lecture service for young people. More recently still, the association has been dropping hints that if the Government does not pay up, the public service will have to go by the board. But this quite proper concern with the health and otherwise of the balance sheet seems to have spurred the association to a wider consideration of the foundations of its continued existence. One way and another, it seems to have resolved that if the world will not give it a living, it must make one for itself. That is a healthy state of mind.

These, however, are only the beginnings of change. The association is still bound in many ways to the traditions of another century. The annual meeting remains partly—not entirely—an anachronism. It covers too much ground and is too didactic. Triviality is being chased away, but there is still some way to go. Nobody is quite sure what purposes are served by the meeting, although most of those who participate enjoy themselves. There is a danger that the indiscriminate encouragement of good works will persuade the association into the encouragement of mediocrity, which implies that there should be some kind of hurdle, however innocuous, to improve the quality of what young people are invited to exhibit in what is called the science fair. There is also a case for a deliberate erosion of the sense of comradeship which sometimes divides the old hands from those who have only recently joined the association, which implies that there might be great profit in some kind of system under which certain officers of the association were elected—one man, one vote—by card-carrying members. As things are, the association must be unique among modern institutions in not offering those who pay to belong any sense, as of right, of influencing the course of events. Anyway, throw the presidency open to election? That would kill a dozen birds with the same stone. In its attempts to import real live public issues into its proceedings, the association has not been entirely successful—which is no reason for going back to platitudes. There is a great deal that the association could do as a lightning conductor for public controversy.

But is it really wise to let everything depend on the success of the annual meeting? That is a question the association has been asking itself for several years. The answer is no, which is why the association has been trying to diversify its activities. Its lecture

service for young people (and adults) has been a moderate but not an outstanding or even an indispensable success, but this now seems threatened by the lack of money. This is inevitable. The association cannot hope to win its reputation by spotting tasks that other people should already have undertaken and then assure its survival by being paid to carry them out. Rather, it must expect to live by its wits, starting up new projects and then handing them over to others to finish off. This is why, in the long run, it will be best if the association can struggle back towards a conception of its public function much more like the one it used to carry out a century ago, when it used to startle its contemporaries with perceptive and authoritative studies of urgent problems inseparable from or occasioned by the growth of science. The association could do worse than become a writer and distributor of tracts. The association would have to learn somehow to escape from the convention of good manner which at present inhibits it, but it would discover that independence is cheap and exhilarating as well.

## COSMOLOGY TO PLAY WITH

THE discovery in the past few years of the cosmic black-body radiation may not be a final proof that or kind of cosmology is preferable to another, for it still not beyond the bounds of possibility that the advocates of steady-state theories will somehow be able to turn the tables on their rivals, but it has already proved to be a powerful stimulus to productive speculation. The calculation by Dr K. C. Jacobs which appears on page 1156 is one valuable illustration how curiosity has been fired by the recognition that the universe appears to be filled with microwave radiation corresponding to a temperature of about  $3^\circ$ . Jacobs sets out to construct a realistic model of universe which, starting from a dense concentration, largely dominated by radiation for the first 2,000 years of its existence. Thereafter, matter and radiation are not strongly in interaction with each other. To begin with, of course, the outward movement of the matter of which his model is composed is rapid but rapidly decelerating. As time goes on, the deceleration itself diminishes. This sequence of events is a more plausible version of the kind of model obtained when a universe dominated by radiation is joined discontinuously to one dominated by the presence of matter. The numbers in the model which Jacobs has constructed are immediately of importance than the possibility that the universe in which there is a transition for a rapidly decelerating to a less rapidly decelerating universe might account for the way in which the red-shifts of quasars seem to be bunched around certain values. In other words, there is a possibility that models like these may yet be put to some kind of test.

## NEWS AND VIEWS

### Graceful Decline

THE National Coal Board seems to be presiding successfully over the gradual contraction of the coal industry in Britain. The board's annual report for the year ending on March 31, 1967, shows a small profit of £0.3 million, after the payment to the Government of £28.2 million interest, which is a tiny proportion of the total cost of the board's operations—£890 million in the year just past. The production of coal in 1966-67 amounted to 173 million tons, a decline of 0.3 per cent in the year. The labour force in British collieries continues, however, to decline even more quickly, which implies an increase of productivity amounting to 1.4 per cent in the year. The board says in its report that it was able financially to break even only because of an increase of output and of productivity towards the end of the year. At the beginning the rate of loss of miners from the industry was so great—amounting to 1,000 or so a week—that the efficient operation of the collieries was impeded.

The report will as always be scanned carefully for evidence of the directions in which British fuel policy is evolving. The National Coal Board seems now conciled to a further decline in the scale of its operations under the combined pressures of competition from natural gas and nuclear power. The report, however, repeats the board's well known view that the exploitation of new sources of fuel should be "so regulated as to secure the greatest advantage to the economy as a whole, now and in the immediate future".

It is particularly outspoken about the building of clear power stations. "Nuclear stations cost huge sums in capital, and account needs to be taken of the cost to which the excess capital expenditure could be put if it were not sunk in these stations. To the extent that the basic technology remains unproved, the justification for spending these large sums of capital must be open to question. And if the second generation stations are but a step along the road to the development of the fast breeder reactor stations, it would not be logical to make this step no larger or more expensive than it needs to be. The mistake of an over-zealous experiment which was made in the first nuclear programme ought not to be repeated."

In practice, the National Coal Board seems to be doing well in its resistance to over-rapid contraction. Earlier this year it was agreed that coal production should amount to 155 million tons a year at the end of the decade—15 million tons more than the natural demand and for coal estimated by the Ministry of Power. Before the publication of the annual report, the board also announced a month long halt in the process of shutting down collieries as part of its strategy of entraining production where coal can be won profitably. Although this agreement between the board and the British Government is prompted by a wish to limit or at least to restrain a further increase of

unemployment, the long-term result could well be still more coal in 1970. Much will depend on how resolutely the Government can hold to its cheap fuel policy.

### New Faces on the Front Bench

THE changes in the British Government which Mr Wilson set in motion at the end of August now seem to have been completed. At the Department of Education and Science, the duties of the two Ministers of State have been decided; Mrs Shirley Williams will be responsible for the universities and the research councils, while Miss Alice Bacon will look after other problems, schools and teacher supply among them. There will be widespread interest in universities and research councils over Mrs Williams's appointment; although she has only been a member of parliament since 1964, a considerable reputation has preceded her to the department.

At the Ministry of Technology, an appointment has been made to replace Mr Edmund Dell, who was transferred to the Department of Economic Affairs as a Joint Under Secretary. Mr Gerald Fowler takes over Mr Dell's old job as Joint Parliamentary Secretary at the Ministry of Technology. Mr Fowler has spent most of his career at the University of Oxford as a lecturer, but spent a year at the University of Lancaster before his election as MP for The Wrekin in 1966.

### BA at Leeds

THE 129th meeting of the British Association for the Advancement of Science seems to have been a success. This year the association visited Leeds for the fourth time, and the meetings were held in the buildings of the University of Leeds. As usual the association was catholic in its choice of subjects, and the 3,200 delegates who turned up could listen to dissertations on subjects as diverse as the changing face of West Yorkshire, or the control of crime. In general it seemed that the specialized section meetings were more successful than the attempts to involve a larger public by symposia on subjects such as science policy or the reasons why children do not choose science as a career. The best discussion of the public role of scientists, for instance, was to be found in the section devoted to the sociology of science.

The BA is showing some talent in adjusting to reduced circumstances. Last year it made a small profit, apparently by cutting its coat to fit its cloth, after appeals to the Department of Education and Science for more money had gone unheard. Despite this, its organization, particularly at the grass roots, is ill defined to say the least of it, and even those involved find it hard to explain. The current financial squeeze for the BA, which everybody hopes will be only a temporary phenomenon, offers an opportunity for reorganization which the association would be unwise to miss.

### Saudi Arabia and Telescopes

LAST week Dr Abdul-Aziz Khowaiter, the vice-rector of Riyadh University, Saudi Arabia, put forward a tentative proposal for the construction of an optical



telescope near Riyadh. The maintenance and operation of the telescope would be undertaken by British scientists, for the first few years at least. The proposal was prompted by a BBC overseas broadcast, "Science and Industry", in which Sir Bernard Lovell said that no new telescopes had been built for eight years. In October, Sir Richard Woolley, the Astronomer Royal, is visiting Riyadh as a guest of the vice-rector to look into the possibilities of the project. Until the new university at Jeddah opens, the University of Riyadh is the only one in Saudi Arabia with science faculties. A department of astronomy was set up this year, under the direction of Mr Robert Seeds, a British lecturer.

## The More we are Together

THREE bodies which represent chemists in Britain are contemplating amalgamation. The Chemical Society, the Royal Institute of Chemistry and the Society of Chemical Industry have agreed that an independent investigation should be undertaken with a view to making recommendations about amalgamation. Sir Eric Bingen, a former Deputy Chairman of Imperial Chemical Industries, Ltd., is to carry out the investigation. His first task will be to assess the work done by each of the societies, and to work out ways in which the essential activities of each may be maintained within an organization incorporating all three. His report will be complete by the end of the year, and each of the societies will be taking it with them to their annual conferences in the spring of 1968.

The idea of amalgamation has been talked about for many years (see *Nature*, **214**, 335; 1967). Casting envious eyes at the American Chemical Society, previous presidents of the British societies have felt that British chemists might be better represented by a larger society on the American model. The functions of the American Chemical Society are in Britain carried out by half a dozen different societies, none of which alone can truly be said to represent chemists. The Royal Institute and the Chemical Society already have some joint activities; together they publish *Chemistry in Britain*, and their annual conference next year in Dublin will be a joint affair. At the local level, the three bodies hold joint meetings, and it is not uncommon for officials to overlap between the societies, holding local office in one society one year, and in another the next. With a combined society of 40,000 chemists, it is felt, better representation and perhaps administrative savings would be possible.

The latest move towards amalgamation seems to have been the joint inspiration of the three presidents. Sir Harry Melville, President of the Chemical Society, sparked it off in a recent speech, and since then has met Mr N. A. Iliff, President of the SCI, and Mr L. H. Williams, President of the RIC, for talks. Sir Eric Bingen was selected for the task of bringing the three societies together because of his success in an equally delicate operation, organizing the amalgamation of the Association of British Chemical Manufacturers and the Association of Chemical and Allied Employers into the Chemical Industries Association. How Sir Eric will work is not clear, but he has already had talks with the three organizations. The three presidents are playing their cards close to their chests, and saying nothing.

## Paper Productivity

WHILE the United States engages in doctorate production (*Nature*, **215**, 805; 1967), India seems to concern itself with the "productivity of publications". This is one of the section headings in a report issued by the Indian Council of Scientific and Industrial Research in its "Occasional Papers Series", entitled *Scientific Research in India; an Analysis of Publications*, by Dr B. V. Rangarao. It appears that 10 or 12 scientist years are required to produce one Indian paper. By far the biggest output of papers came from the universities, with 3,800 out of the 9,800 published in all of India in 1965. Top of the paper production league is the University of Calcutta, with 279 papers, closely followed by Agra University (260). The report does not actually name the unproductive laboratories; it merely observes that "though there are a large number of research units in the country, the activity is serious only in a small number of them", and warns that "it may be worth studying the *per capita* publication in different organizations".

Medicine is the subject of most Indian papers indeed, of the 64 papers from the University of Patna 44 were in medicine. By contrast, workers on electricity, thermoelectricity and electric lights are completely uncommunicative. The report bemoans this but gives no account of how many people there are either to produce the papers or to read them. Team work, Dr Rangarao continues, is of the essence; most papers published in the advanced countries are by two joint authors, but in India the rule still seems to be one man one paper. "Even in the technological universities, where team work should be easy to organize the average authorship is only 1.6. This aspect requires some critical analysis . . ."

The report is somewhat at a loss to explain the low output of scientific papers in India. The thirteen most prolific universities were neither the oldest nor were they better endowed. The best explanation seen therefore, to be that Indian scientists are uninterested in interdisciplinary activity and prefer to work alone. Cure this, and paper productivity and average authorship will no doubt rise.

## Tropical Aid

IT seems that the appointment of Dr P. C. Spens as director of the Tropical Products Institute from March 1967 has been a very good thing for all concerned. The Tropical Research Institute aims to provide sophisticated research which developing countries cannot afford, and technological development work aid their industrialization programmes. A radical organization took place during 1966, so that an emphasis could be placed on food science and technology, industrial economics, industrial engineering and process development. Signs of change are also seen in the annual report which was published last week. Instead of giving incomplete progress reports on the year's work for all the current projects, as in the past, the report now includes short monographs that are complete in themselves, on a few selected ones.

The first of these deals with the report on production of protein products from oilseeds for the relief of malnutrition. This report has been written as a guide

those who have to cope with malnutrition problems and indicates sources of protein for dietary improvement as well as production methods which might be suitable for particular local conditions. Product distribution is considered to be a very important part of any scheme of this nature. Prospects in the market for bananas, and costings for orange squash production have been subjects for reports. The natural products chemical research division reports on three of its current projects. The fungus growing on mouldy corn has been found to cause disease in pigs and cattle that eat it, and the toxins produced by *Penicillium rubrum* are thought to be the cause. The mycotoxins section of the institute has been working on these toxins and has found, in experiments with mice, that damage is caused to the liver. Work on the chemistry of scirpentriol and verrucarol is also described, the interest being in reactions leading to detoxication. Diacetoxyscirpenol has been implicated in the important problem of mouldy corn toxicosis. There has also been a search for reasonably simple, naturally occurring antifungal molecules which are obtainable by synthesis, but results have proved disappointing so far. Investigation is still going on into more complex molecules.

The services of the Tropical Products Institute are available to almost any country and enquiries were received from eighty-eight different countries during 1966. The work of the institute is growing, as seen in the budget figures for the last two years. For 1966-67 it was £360,000 and for the current year, 1967-68, £444,000.

## Finding Bombs

In August a panel of experts met at the International Atomic Energy Headquarters in Vienna to discuss techniques of ensuring that atomic installations are not used for military purposes. The members of the panel came from Canada, India and Japan as well as the four nuclear powers which are members of the agency. In addition, there were observers from West Germany, Czechoslovakia, the European Nuclear Energy Agency and Euratom. The British member of the panel was Dr Frank Morgan of AWRE Aldermaston; Mr D. B. B. Janisch of UKAEA Risley and Mr J. McAdam Clark of the Ministry of Technology were present as observers.

The panel discussed how to verify statements on the movement of fuels, and the production of plutonium and electricity by a reactor. Methods of non-destructive analysis of nuclear fuel, and of sealing techniques, were also considered. The panel concluded its work by commending the directions the agency's research and safeguards techniques should take.

## Immigrating Animals

The most unusual immigrant granted right of entry to Britain in 1966 may well have been the rhinoceros imported in a West German Zoo. Other rare wild animals which import licences have been granted are listed in a report published by the Advisory Committee on Animals (Restriction of Importation) Act, 1964 (MSO, 3s.). This report is the first of an annual summary of statistics of animal importation and gives numbers of licences granted in 1965 and 1966.

The Advisory Committee concentrates on conservation of wild animals and advises both the Board of Trade, which grants the licences, and the Customs, who enforce them. The animal families are classified by rarity into two groups, rare and not so rare. During 1966, 22 Anthropoid Apes (Pongidae) were refused admission. Conservationists will also be pleased to learn that in 1966 as compared to 1965, a larger number of licences was granted to only two of the 17 families mentioned. These two were opossums and marmosets. The highest figure is, surprisingly, not for Old World Monkeys (Cercopithecidae, 21,066) but for tortoises (Testudinidae). Despite a fall of 16,446 from the previous year it still stood at 320,573. The committee says that the life span of this animal must be severely reduced when it comes into Britain. As yet it does not know what effect this trade is having on the animal in its wild state in any particular area.

The rest of the report covers countries of origin of the animals and declared purpose of importation—the latter is divided into the four categories of exhibition, scientific research, pets and resale. The rhinoceros was heading for exhibition, but one of the 16 opossums was named as a pet. Despite the appearance of the wombat in the list of the rare animals, it seems that nobody has attempted to import one in the past two years. The statistics refer to the number of licences issued, not to the number of animals brought in. They are therefore in some way misleading, for not all the licences were taken up. During last year, however, the Board of Trade changed the system so that 1967 will be the first year in which the number of animals arriving will be accurately known.

## New Man at Argonne

It has been announced that Dr R. B. Duffield is to become the Director of the Argonne National Laboratory of the United States Atomic Energy Commission. He will take up his appointment in November. Immediately after receiving his doctor's degree from the University of California at Berkeley, in 1943, Dr Duffield became a member of the team of workers on the Manhattan Project at Los Alamos Laboratory. In 1946, he joined the staff of the University of Illinois where he worked for ten years, finally becoming associate professor. He then became chairman of the chemistry department of the General Atomic Division of the General Dynamics Corporation, where he directed first the design and later the operation of the high temperature gas cooled reactor at Peach Bottom, Pennsylvania. His work in connexion with this brought him on many visits to the Dragon Project at Winfrith which collaborates with the Peach Bottom team. Dr Duffield will be the first chemist, as opposed to a physicist, to direct Argonne, and there may be significance in the fact that his recent experience has been concerned with reactor research; his predecessor, Dr A. V. Crewe, returned to the University of Chicago to continue basic research.

## Exporting Science

In spite of the trouble in Nigeria and the devaluation of the Indian rupee, the British Council remains

optimistic about the future of its aid programmes in scientific education. Four of the seven scientific education officers, all of them in Commonwealth Africa, had been appointed to Nigeria. The officer in Enugu, Mr G. Howell, was able to return on leave and no equipment or buildings appear to have been damaged. Even so, the Nigerian war will undoubtedly set back the council's plans for scientific education which had been progressing splendidly; last year the number of scientific education officers equalled the number of scientific liaison officers for the first time. In India, the devaluation of the rupee has had an effect on the low priced books scheme, effectively increasing the price of British books, while American and Russian scientific literature can be bought without hard currency.

The report of the Science Advisory Committee of the British Council was, in fact, issued before both the Nigerian and Arab-Israeli wars. The council administered seventy-two scientific bursaries for three or six months research and training in Britain, and 118 scholarships were awarded, many of them in science. There is, apparently, an "untouched reservoir of scientific talent" in Latin America, Japan, Israel and certain other countries, but in the rest of the world the number of scholarships available is in proportion with the number of candidates. Under a new agreement, the number of exchange studentships available in the Soviet Union will rise from 22 to 45, but the council anticipates difficulty in filling this quota for 1968-69. Applicants for studentships must be graduates working at recognized institutions and must be competent at Russian. It would seem that most of the few people with appropriate qualifications will already have been to Russia on previous studentships. The council will soon be advertising for students for 1968, for the smaller quota for 1967 has already been filled. Of the Warsaw pact countries, only in Poland is the work of the British Council not governed by an official exchange programme. Polish students are able to travel independently to Britain and many do. Perhaps because of this, scholarship applicants from Poland are of a very high quality.

In all, 12,350 scientists visited Britain in 1966-67 under the auspices of the British Council, but only 1,370 British scientists attended courses, held scholarships or took up appointments abroad. Most of the movement of scientists took place within Europe. Only four British scientists visited non-Commonwealth Asia and only 140 came from that region to Britain. But it is interesting to see that four times as many workers came from South America as from Australia, Canada and New Zealand, and that nearly as many Britons took up scientific appointments there as in the underdeveloped parts of the Commonwealth—good news indeed for those who have felt Britain to have been too long neglectful of early ties with Latin America.

### Students' Living

THE design of living accommodation for students has been considered by the Department of Education and Science and the University Grants Committee (*Student Residence*, HMSO, 10s. 6d.). The study, issued as a Building Bulletin, explains the conclusions reached by a joint working party from the two organizations which, with the Scottish Department and the Building

Research Station, has investigated how different buildings have been designed and what they cost.

First, the different space requirements for a variety of activities are discussed. The area needed for study can be considerably reduced by using desks with fold-down fronts. The bed is the largest single item, but there are various ways of diminishing its bulk by day. In the United States, the report says, beds which fold back into the wall have been installed in some student accommodation, but this type of fitting is usually expensive. In Britain, the current philosophy seems to be to design a bed which can be used as a settee during the day, often by sliding it back into the wall and using the space underneath for storage. Several of the colleges mentioned have adopted this approach, and at the Henry Price Flats at the University of Leeds conventional sheets and bedding have been abandoned in favour of sleeping bags. Space is also needed for washing, dressing, heating appliances and finally for conviviality. The move towards small student rooms which cost less to build has meant that entertaining in student rooms is severely curtailed. One possibility is to provide separate entertainment areas for each group of student rooms. Adding up the spaces needed for different functions gives a bare minimum size of 70 sq. ft. for a study-bedroom, although in recent years the UGC has been more generous than this, providing about 110 to 140 sq. ft. per person. Alas for aesthetics, the report concludes that long, narrow rooms are cheaper to build, provide better use of space and a clearer division of function.

The report also discusses the provision of other facilities, such as communal and recreational areas. These drastically increase costs. A grant sufficient to house 200 students in a residential annexe would house only 130 if full allowance were claimed for additional facilities. For this reason the UGC and the Department of Education and Science are unwilling to approve such facilities without justification. High tower blocks come in for some criticism; they cost more to build because of the need to provide lifts and the fact that they cover a greater area for each student housed. Firmer foundations, fire precautions and the need for more durable materials can also add to the cost of tower blocks. Since the towers require more space between them, claimed savings in land costs may not be realized. Unfortunately, the demands of cost and aesthetics are rarely in step but, as the photographs in the report show, it is possible to achieve attractive compromises.

### Money for Schools

LOCAL education authorities in England and Wales are to receive a special allocation of £16 million for the building of schools in "educational priority areas". The concept of these areas was formulated in the Plowden report, but the areas are not to be formally designated; instead, local authorities have been asked in a departmental circular, to apply for grants for the reconstruction or replacement of both primary and secondary schools which are not up to standard.

This news will no doubt be very welcome, for in 1964 and 1965 far fewer new schools were begun than usual, because of economic conditions. As a result correspondingly few schools have been completed recently. Earlier this year the then Secretary of State

for Education and Science, Mr Anthony Crosland, said that the building of eighty million pounds worth of new schools was begun in 1966-67. The rate at which Britain is building new schools has therefore returned to where it stood in 1962 and 1963.

At the same time as the news of this special allocation comes details of a slight increase in the amount education authorities will be able to spend on "minor works", worth less than £20,000, which do not require permission from Whitehall. The amount set aside for this will now be £27.5 million.

## Britain by Numbers

THE latest *Abstract of Regional Statistics*, issued by the Central Statistical Office (HMSO, 10s.), covers a lot of ground. As well as the tables of population and area, fuel and power, production, construction, distribution, transport and education, there are three new tables. These cover the number of agricultural holdings, a survey of personal incomes in 1964-65, and a survey of average gross annual earnings.

Many of the tables reflect the erratic progress of the British economy in the past ten years. Unemployment, up to 2.6 per cent in 1963, was down again to 1.6 per cent in 1966—the last year for which figures are given in the abstract. Now it is up to near the 1963 peak once again. Steel production reached peaks in 1957, 1960 and 1965, but has now declined from the 1965 figure of 27 million tons to 24.3 million tons in 1966. Deep mined coal shows a steady decline since 1956, from 207 million tons, to 166.5 million tons in 1966. The profitable coalfields in Yorkshire and the East Midlands, on the other hand, show only a very slight decline. Over the ten year period, the number of miners in the pits has fallen from nearly 700 thousand to 426 thousand, while open cast mining has shown an even faster decline. Output per man-shift in coalmining has, however, increased rapidly, from 24.7 cwt. in 1956 to 36.4 cwt. in 1966.

New tables compiled by the Ministry of Social Security gave figures for annual earnings of people in civil employment in much greater detail than before. These show that the average gross earnings for men in the age range 18-64 increased from £1,033 in 1964-65 to £1,104 in 1965-66. The figures for women are far lower, £525 in 1964-65 and £558 in 1965-66. This includes married women, who tend to earn less than spinsters; the figure for unmarried women was £601 in 1964-65 and £638 in 1965-66. The earnings are also broken down by area; men in the GLC area earned an average of £1,204 in 1965-66, against the national average of £1,104. The worst paid men were those in East Anglia, with annual earnings of £1,007.

## Problem Solving

THREE British research associations—the British Cast Iron Research Association, the British Welding Research Association and the Production Engineering Research Association—have formed one of the largest known organizations for solving production problems and improving manufacturing efficiency. The organization, called the Production Advisers Consortium (PRODAC), will provide a pool of more than 1,200 engineers, metallurgists, designers, economists and

other qualified people to carry out research into all problems of production from the initial raw metal to the final marketing and distribution.

Hitherto, companies wishing to sponsor research could only use the services of the associations of which they were members, but under the new scheme the research teams of all three of the associations will be at their disposal. The research and advisory service is available on a confidential fee-paying basis to any member of one of the research associations, with the exception of a member firm which has resigned from the research association whose advice is sought. PRODAC will not replace the normal service provided by each research association to its members, for it will be concerned solely with sponsored or repayment work.

The consortium will be controlled by a consultative board consisting of Dr D. F. Galloway, director of the Production Engineering Research Association, as chairman, Mr H. Morrogh, director of the British Cast Iron Research Association, and Dr R. Weck, director of the British Welding Research Association.

## More Dead Birds

THIS Table, provided by the RSPCA, gives details of the commoner species of birds which, contaminated by oil from the Torrey Canyon, were sent to cleaning stations by April 17. Of the 443 birds listed as rehabilitated, many have since died either in captivity or on release.

Cleaning and reception station	Alive	Died	Guillemot	Razorbill	Shag	Puffin
St. Ives 1	76	3	313	62		
2	251	45				
Veryan 1	308	300	495	98	2	7
Newquay 1	153	62	175	38	1	1
2	268	406	558	112	1	3
St. Keverne	101	35	114	22		
Sennen	120	20	116	23		1
Mevagissey	20	9	28	1		
Perranporth	542	456	827	166	1	4
Scorrier	252	138	318	58	8	2
Hayle	325	154	399	78	1	1
Ruan Minor 1	16	4	18	2		
2	28	27	48	7		
Mousehole	3,351	379	2,946	717	27	23
Totals	5,811	2,038	6,355	1,384	41	42
				<i>Birds rehabilitated</i>		
Totals			335	103	3	2

## Progress in Instruments

ANOTHER year of rapid expansion in the activities of the Scientific Instrument Research Association is reported by the chairman in his annual report for 1966. The industrial income in support of group-sponsored projects has risen by 380 per cent over the past three years to £66,800. Income from subscription has increased by 23 per cent over the three year period to £72,200 and income from sponsored research has increased to a record level of £100,711. As a result, the association is negotiating with the Ministry of Technology to increase the ceiling of its grant. The total budget of the association reached the figure of £438,000.

Expenditure on industrial measurement and control projects increased from £75,000 in 1965-66 to £145,000. The association has decided to recruit staff to undertake technically oriented market research and has set up an industrial communications group to provide a broader base for its information department and to help in the task of spreading technical knowledge by every possible means, especially in automation.



One of SIRA's many projects is making thin film microcircuits by laser micromachining. A magnified pattern of the film to be removed is reproduced in a mask which is illuminated by a Q-switched pulse of radiation from a laser. The optical image of the mask is focused on to the thin film and, when the laser is fired, the areas of the film exposed to the laser radiation evaporate. Successful experiments have been carried out with nichrome, cermet, chromium, gold and aluminium. The possibility of applying this technique to graticule production is also being studied.

## Elephants Caught

AN aerial technique employing poisoned darts, shot from a crossbow by a marksman in a helicopter, has been used to capture young elephants during preliminary trials in the culling of herds in the Kruger National Park. Dr U. de V. Pienaar describes in the latest edition of the *Journal for Scientific Research in the National Parks of the Republic of South Africa* (No. 101; 1967) how a method already used successfully to catch rhinoceros in Kenya has been applied to young elephants—poisoned darts are already used to capture adults from the ground.

A helicopter first located a herd of breeding elephants, and waited until the ground party arrived. The helicopter then swooped in on the herd, usually causing it to stampede, and at a range of twenty-five to thirty yards suitable calves were shot with darts containing 1 mg of propylorvinol hydrochloride, and 7 mg of acetylpromazine maleate in a volume of 3 ml. After about 10–15 min the calves which had been hit fell to the ground, and the ground party moved in. A nylon thread was passed around the elephant's neck and this was pulled into an open crate. The antidote to the immobilizing mixture was administered to the ear-vein and the animal rose to its feet within minutes to be guided into the crate. Any mothers which attempted to rescue their young, which they often did, were kept away by the helicopter. In cases where the mother refused to leave a very young calf she was also immobilized.

After being tamed and adjusted to captivity the young elephants were shipped to America. The method proved very successful; twenty-seven young elephants, aged between 12 and 56 months, were captured in seven days of hunting. The great danger involved when breeding herds are disturbed by hunters operating on foot is avoided with the use of the helicopter, and the captures are made without damage to elephants or hunters.

## Ångstrom Ruler

from our Molecular Biology Correspondent

THE phenomenon of excitation energy transfer has found occasional use in biochemistry, notably as a method for following binding processes by sensitization or quenching of fluorescence. Despite much interest and effort, however, its application to the study of topological detail in macromolecules has for the most part given trivial and disappointing results. Promise of better things comes from work of Stryer and Haugland (*Proc. Nat. Acad. Sci. US*, **58**, 719; 1967), which should both please the spectroscopist and stimulate the biochemist.

An energy transfer system is set up when two fluorescent molecules are brought into proximity to one another; if there is large overlap between the emission spectrum of the first and the absorption spectrum of the second, and the geometry is favourable, excitation of the first molecule (the sensitizer) by irradiation at a wavelength in its absorption band leads to fluorescence from the second (the emitter). In such a system, no matter what the wavelength of excitation, fluorescence will always come from the species emitting at the lowest energy (longest wavelength). The theory of energy transfer, in terms of resonance between the transition dipoles of the two molecules, was developed twenty years ago by Förster, and predicts that the extent of transfer will vary inversely as the sixth power of the mean distance between the molecules ( $r^{-6}$  dependence). Whatever the situation in free solution there seems little doubt that excitation transfer between chromophores fixed in a macromolecule must proceed by this mechanism. Stryer and Haugland use the  $\alpha$ -naphthyl group as sensitizer and the dansyl group as emitter. These are attached at opposite ends of an oligoproline chain. A series of such sandwich molecules has been prepared, in which the length of the proline chain varies from one up to twelve residues. The advantage in using prolines as spacers is that at chain lengths greater than five a rigid helical structure (polyproline II helix) is formed, so that the separation of the two chromophores is always more or less precisely fixed. Further, the complete depolarization of the dansyl fluorescence in these compounds, when observed in a rigid glass matrix, shows that the chromophore is freely rotating; consequently there are no complications from differences in the relative orientation of the chromophores (since the efficiency of transfer depends also on the angle between the two transition dipoles).

The extent of energy transfer is determined from the excitation spectrum: the intensity of fluorescence from the dansyl group is measured with varying excitation wavelength. If there is no energy transfer, the excitation profile simply follows the absorption spectrum of the dansyl group, whereas for complete transfer it follows in effect the sum of the absorption spectra of sensitizer and emitter.

From the crystallographic dimensions of the polyproline II helix one can determine the separation of the two chromophores for each compound in the series, and it is found that the extent of energy transfer falls from 100 per cent when one proline residue separates the chromophores to 16 per cent when there are twelve prolines; moreover, it is found that the  $r^{-6}$ -law holds with remarkable precision (an exponent of 5.9 is found). This is a pleasing result, for it provides the most conclusive evidence yet in favour of the Förster theory.

Stryer and Haugland refer to these measurements of energy transfer as a function of distance as a spectroscopic ruler, and indeed the method holds out the promise of applications to biological molecules. There are many systems in which there is a possibility of attaching a sensitizer and an emitter at functional sites on a macromolecule or assemblage of molecules so as to determine their separation. Whether such an elegant approach is in practice too good to be true—at least as far as more complex systems are concerned—will remain to be seen, but there is no shortage of applications that come to mind.

# Further Education in Australia

by

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In Britain there has been a movement (frustrated by Mr Anthony Crosland's decision to establish polytechnics) to abandon the binary system of higher education. Dr Wark, who is Chairman of the Commonwealth Advisory Committee on Advanced Education, believes that the binary system should stay. What follows is derived from an address by Dr Wark to the Australia and New Zealand Association for the Advancement of Science earlier this year.

THE Government of Australia set up a committee in 1961 under the chairmanship of Sir Leslie Martin, to report on tertiary education in Australia. This committee strongly advocated the development of institutions differing in outlook from universities, to train and educate people for various professions. It was felt that these would mainly develop from existing colleges. The Commonwealth Government, and the States too, have wholeheartedly accepted this main theme of the Report. It was, indeed, time they did so. During the years 1947 to 1962, the share for the technical colleges of the total money available for tertiary education dropped from twelve to seven per cent, while their percentage of the total tertiary student pool rose from thirty-four to thirty-seven per cent. They were merely marking time.

To facilitate the development of these colleges of advanced education, the Commonwealth Advisory Committee was set up under my chairmanship. Our first report, submitted in June 1966, has been accepted almost in full by the Commonwealth and State Governments. While this report has been circulated fairly freely in educational circles in Australia, there are one or two aspects of it which I think should be emphasized today. It is insisted by the States that, while the emphasis should be on training for a particular vocation or profession, the colleges must also impart a broad education. This is essential in the present-day world, for the average student can expect to have to change his vocation at least once, and possibly many times, during the course of his working life. Our colleges must aim to produce engineers who are more than engineers and accountants who are more than accountants. Throughout life there are very limited numbers of opportunities for the determined specialist, except within the universities where he is undoubtedly needed.

I would like to feel that Australia will benefit from Great Britain's mistake, conceded now on many sides, of having converted first-class institutes of technology into universities. Fortunately, some of them seem determined to remain true to their former selves, but already others are said to be striving to emulate older universities.

I know that some Australian institutions have had the ambition to be recognized as universities, but to me it seems obvious that were this achieved, they could hardly expect to be ranked with the major universities of this country, at least for a very long time to come. I therefore greatly admire the decision of the Council of the South Australian Institute of Technology, which for many years has trained students for certain degrees of the University of Adelaide, to restrict its activities from 1970 onwards to the training of students for its own awards.

My Committee has repeatedly stressed that the students of universities and colleges will, in general, be different, but that neither should be regarded as superior or inferior. In our report we draw a comparison between the two types of institution and the two types of students. In the colleges there would be:

- (a) A greater concentration on part-time studies associated with employment, especially in scientific fields.
- (b) An emphasis on applied rather than theoretical work.
- (c) A more direct and intimate relationship with industry.

(d) Far less attention to post-graduate training and research.

(e) A primary emphasis on teaching.

Whereas in a university the subject is all-important, in industry the problem transcends the subject or discipline. The recruit to a technical company must be prepared to devote his whole attention to some problem, even though it takes him away from his original interests and into neighbouring disciplines.

Armstrong, Hill and Ross found that ninety per cent of the Ph.D. graduates in chemistry in Australia wanted in their next year to go on dealing with the problem of their last year of formal training. Ninety per cent! It is appalling. Their Ph.D. studies have evidently failed to develop a spirit of adventure, a desire to accept a challenge such as industry presents. Are the professors to blame? Are the students themselves so timid that the training money has been wasted on them? Or is industry to blame for not having sold its challenge?

In New South Wales an attempt was made to abandon the binary system of higher education, and the diploma courses of the old Sydney Technical College were absorbed into the University of New South Wales. Judging by the reaction of the employers, this experiment was no great success, and new courses at New South Wales Institute of Technology are now being established to replace the diploma courses of the Technical College.

Following the recommendations of the Martin Committee, Australian universities have been happy to support the development of the new colleges, thinking that their own problems will become intolerable if the colleges are not developed rapidly. It would be helpful, I think, if leaders of industry were to make public their views in this matter. My Committee has found them to be wholeheartedly behind the scheme, but they have not been as outspoken as we would have liked.

Because of their emphasis on teaching rather than research, colleges can at present train men and women at perhaps two-thirds the cost of university training, and probably do a better job for the type of student for which they cater. As the colleges develop specialities associated with particular industries, however, the difference in cost will lessen. Twenty years from now it seems that there will be more students in the colleges than in the universities, which will become still less committed to vocational courses and more interested in advancing scholarship.

In Australia, the forgotten man in all the main educational discussions is now the technician. I use the term technician in a general sense, to include the non-commissioned but highly skilled officers who serve in technology, commerce, public health, librarianship, music, art, and so forth. Without them, however talented the leaders might be, industry would grind to a halt.

There are two main ways of becoming a technician. One involves a two-year, tertiary level, full-time course, or its equivalent part-time. The other is via "failed diploma" or "failed B.Sc.". Each route produces worthwhile technicians, though the latter lack any specific qualification and suffer thereby, at least at the outset. I used to laugh at the old story of the Indian who cited as

a qualification, "Failed B.A."; I no longer do so. The first method will yield ten to twenty per cent of qualified men who could with advantage continue with study: at present, through close adherence to prescribed standards, we make it too difficult for them.

Other countries take much interest in the training of technicians. In a recent visit to the USA I called at the Wentworth Institute in Boston, which confines itself to technician training. This is a most impressive college—perhaps the most impressive of its type in that country. The courses are designed to produce men for industry: they do so with the co-operation of industry. Industry may itself impart a training to quite high levels; trainees lacking formal qualifications may find themselves "leg-roped" to a particular company.

At Breukelen, in Holland, I visited a commercial college where a residential course runs for two years from approximately matriculation level. The aim is to produce men with a knowledge of the techniques required for export business, upon which the livelihood of Holland depends. A thorough grounding is given in the commercial side of several European languages—English, German and French—for it is considered that unless you know the language of the country with which you are trading, and you know it to perfection, you will have difficulties, and you will lose to a competitor with a better technique. There are 150 students graduating from this residential college each year, and more than 300 positions are available for them throughout the world.

At the Hague, I visited one of thirty of Holland's senior technical colleges, where the aim is to produce a sub-professional engineer—or superior technician. Students attend college for two years after secondary schooling followed by one year's training in industry, and a final year back in the college. Post diploma night courses, taken by half the students, close the gap with the professional engineer.

At the other end of the spectrum is a very advanced commercial college, the Management Development Institute, in Geneva. This was brought into existence by

Nestlé in close co-operation with the Harvard Business School. Age of entry is between thirty and forty years. Appropriate formal education and business experience are prerequisites for admission. The course is an intensive one of nine months, and it is expected that the sponsoring company of a student will support him during this period and pay his fees. These cover about 90 per cent of the cost, and the deficit is borne by the Nestlé Company which each year provides some six or eight students out of the total of fifty. There is no government subsidy, no control except through the Harvard Business School, and the language spoken is English.

I have a feeling that more Australians should attend these two schools, and that Australia should be represented much more strongly in the queue for the students from Breukelen, many of whom emigrate. They are given no formal qualification whatever, which is perhaps the reason why we are unduly suspicious about employing them.

During my life I have come to have a very high regard for those who accept leadership in industry, commerce, government and community services; for those who make the decisions and judgments affecting us all; for the men in charge of production, of the health of the nation, of our own financial policies and international relationships. Too often they are criticized, even scoffed at, by intellectuals who could with benefit go humbly before them for a sharpening of their own outlook and a broadening of their education.

This brings me to my main conclusion: that industry has been and always will be a most important source of training for service within industry. This training, however, must be supplemented at one time or another by first-class courses, conducted within colleges or universities, by men and women with a close knowledge of their own subject and also of what industry needs.

Behind all this is the need for new discoveries, new knowledge, on which to build. These will come from fertile and adventurous minds engaged in research, whether in educational establishments or in industry.

## Testing Sonic Booms

by  
RODNEY CLARK

The British summer has been disturbed on one or two occasions by tests of aircraft at supersonic speeds. In the United States there is more experience.

IN July 1967 the United States National Sonic Boom Evaluation Office published an interim report on the sonic boom experiments at Edwards Air Force Base by Stanford Research Institute. One of three types of supersonic aircraft—the *F104*, the *B58* and the *XB70*—was flown over Edwards AFB between three and eight times a day for four years. Most of the aircraft were flown directly over the testing site at constant supersonic speed. The aircraft then slowed to turn at subsonic speed.

One series of experiments was concerned with the effect of booms on people living in houses typical of the American west, constructed mainly of wood, air conditioned, with the windows closed. Booms heard outdoors were found to be slightly less tolerable than those heard indoors, but "it is possible that sonic boom and house will interact in such a way that the interference effects on human beings are aggravated more than other externally generated noises". Particular rooms which magnified the booms did not necessarily magnify the sound of subsonic aircraft. The subjective unpleasantness of the booms increased faster than their intensity. A peak overpressure of

1.69 lb/ft<sup>2</sup> from a *B58* was considered almost intolerable by 27 per cent of the residents of Edwards, and by 40 per cent of the inhabitants of the towns of Fontana and Redlands, which were not near the flight path of supersonic aircraft. There seemed to be little difference in the response of the subjects to booms of equal overpressure caused by the three aircraft, despite the much longer duration of the "signature" of the boom of larger aircraft. When heard indoors, the boom from the *XB70* was least tolerable, but out of doors it was the *B58* that was the greatest nuisance.

It was possible to compare the subjective effects of booms and the noise of subsonic aircraft. People were more sensitive to the former, and could distinguish between booms differing by only 1 dB overpressure. According to this report, it was not possible to say which of two aircraft sounds was the loudest when they differed by less than 2 dB. 71 per cent of the residents of the area considered that sonic booms were the most intolerable noise to which they were subjected. There was, however, no indication of any effect on farm animals, perhaps, th-

report suggests, because they had become adapted to the noise.

It was confirmed that atmospheric conditions within two thousand feet of the ground contribute most towards the variability of the booms. How booms affect structures has not yet been analysed in detail, but little damage could, apparently, be definitely attributed to them. Altogether 57 complaints were received, most of them concerning glass, but investigations showed that only three panes out of more than 100,000 had definitely been cracked by supersonic aircraft. This represented a rate of one window pane broken for between 780,000 and 1,300,000 panes exposed to booms. (If this proportion seems low, it is worth remembering that more than a hundred million panes of glass might be exposed to each supersonic aircraft flying over London.) No seismic effects that might cause damage in mines were observed.

Most of the conclusions of the report are in agreement with theoretical predictions, and its authors enter into no speculation about what life will be like under the flight path of supersonic airliners. The effects of a boom could be expected to be extreme at some points on the ground if the boom was caused by an aircraft which was accelerating and turning. These conditions were avoided in most of the tests, but in one series of flights, an F106 was flown in "porpoising" flight. These movements were shown not to contribute to the features of the boom measured on the ground, but the report remarks "that a larger airplane has a sonic boom that depends relatively more on its lift, so motions of a SST (supersonic transport) in flight may still lead to significant variations in the sonic boom". Booms can be magnified several times by reflections off surfaces of courtyards, rooms, or narrow streets. This was not investigated in the experiments; the test houses were widely spaced, and although tests were carried out on a long building, a bowling alley, none of the buildings in which instruments were placed could be described as tall. It follows that even if the results give some slight indication of the effect of supersonic aircraft on small towns in the western United States, they will be of very little practical use to planners in Britain.

In Britain, however, despite the fact that Concord will fly in six months time, only eleven tests have been conducted by the Ministry of Technology. It is difficult to see how any information can have been derived about so complex a phenomenon from so small a number of experiments. In any case, it appears that few of those working on the psychological and structural effects of

supersonic aircraft actually assisted at the experiments. Owing, in part, to the ban on supersonic flights over Britain, little practical research has been done into the effect of booms on people and buildings. Although it was long ago shown, in tests with the Fairey Delta II, that resonance to booms might be hazardous<sup>1</sup>, there is at present no programme of experiments into the subject although a good deal of theoretical analysis has been done by a team under Professor E. J. Richards at the University of Southampton. The Building Research Station investigates complaints of structural damage allegedly caused by supersonic aircraft.

British psychologists have had only limited benefit from the vast number of American experimental booms, over Oklahoma City, White Sands and, now, Edwards. And the small amount of research done in Britain confirms that there may be differences between relevant conditions in Britain and America. It seems, for example, that sonic booms are less bearable in British houses than outside them (Johnson, D. R., and Robinson, D. W., *Acustica*, 18, 241; 1967).

Table 1. AIRCRAFT USED IN SONIC BOOM TESTS

	Wingspan (ft)	Length (ft)	Weight (lb)
F104(S)	22	54	31,000
B58	56	96	160,000
XB70	105	185	530,000
F106	38	71	38,000
Lightning	34	55	50,000
Supersonic transports			
Concord	84	185	330,000
Boeing 2707	174	306	675,000

The American tests, extensive though they have been, have not been comprehensive—no great, densely packed metropolis has been subjected to sonic booms day and night. And even if it were possible to draw valid conclusions from them, there is little to indicate that they would be relevant to Britain. Nevertheless, the British government has not yet said that there will be further tests, nor has it given any indication that the full results of the recent series will be published. It would seem that the Concord will be flying long before there is justification for the remark of Mr E. Dell, lately Joint Parliamentary Secretary to the Ministry of Technology, who said "We have achieved the object of informing people about the nature of a supersonic bang".

<sup>1</sup> Newbury, C. W., *Materials Research and Standards*, 4, 601 (1964).

## Birds killed in the Torrey Canyon Disaster

by

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The Torrey Canyon disaster probably killed more than 30,000 seabirds. In a sample of 1,223 dead birds of eight different species 97 per cent were guillemots and razorbills.

OIL pollution at sea has been a growing hazard to birds since the beginning of the century. Since the First World War the Royal Society for the Protection of Birds has carried out intermittent surveys of bodies on beaches to assess the damage, and, since the Second, the International

Council for Bird Preservation has played a leading part in agitation for the control of oil pollution<sup>1</sup>. While the general character of the damage has long been known, there has been less information on the origin and age of the birds affected. Following the success of thirty years of



beach patrolling in adding to the knowledge of the birds of New Zealand and their mortality<sup>2</sup>, and a preliminary survey of a pollution incident on the north-east coast of England early in 1966 which yielded new information on the composition of the wintering auk population<sup>3</sup>, when the RSPB launched a further beach survey in the winter of 1966-67 the Seabird Group also asked for more detailed information on the bodies found.

It would have been best to have whole bodies for identification, and failing these heads might have been the most useful item, for they commonly show the greatest variation with race, age, sex and season, but neither of these is easy to handle in bulk, so we asked for one wing from each bird. Only a small number, useful principally for comparative purposes, were collected along the east and south coasts of Britain during the winter months. Larger numbers were not received until the tanker Torrey Canyon was wrecked on the Seven Stones rocks off Cornwall on March 18, 1967, and the animal protection organizations agreed to send us a wing from each dead bird that they handled. We received 1,223 wings from south-west Britain during the months following the wreck

### Numbers and Species Killed

In our sample nearly 98 per cent were wings of the larger auks—guillemots and razorbills—with six other species comprising the other 2 per cent (Table 1). Although the relative proportions of guillemots and razorbills killed showed local geographic variations<sup>4</sup>, the ratio of these two species in the sample is very close to that in the much larger sample of 7,851 birds sent to the Cornish cleaning centres<sup>5</sup>. In France, however, in a small sample the ratio was reversed<sup>6</sup> with about 75 per cent razorbills and 25 per cent guillemots (Table 2).

Table 1. CLASSIFICATION OF BIRD WINGS RECEIVED AFTER THE TORREY CANYON DISASTER

Species	Number	Length (range and mean in mm)	Proportion (per cent)
Guillemot <i>Uria aalge</i>	957	(See Table 2)	78.2
Razorbill <i>Alca torda</i>	237	178-206 192.6	19.4
Shag <i>Phalacrocorax aristotelis</i>	20	255-276 163.7	1.6
Puffin <i>Fratercula arctica</i>	5	155-162 160.0	0.4
Great northern diver <i>Gavia immer</i>	1	(278: in moult)	0.1
Black-throated diver <i>Gavia arctica</i>	1	(229: in moult)	0.1
Cormorant <i>Phalacrocorax carbo</i>	1	307	0.1
Kittiwake <i>Rissa tridactyla</i>	1	308	0.1

Nearly all the birds at the Cornish cleaning centres died and so did about 2,000 sent to the French centres in Brittany. Hence the absolute minimum total kill was about 10,000 birds and after allowing for birds lost at sea and

Table 2. PROPORTIONS OF GUILLEMOTS AND RAZORBILLS FROM DIFFERENT LOCALITIES

	Guillemots		Razorbills		Total
Cornwall: early April	(No.)	(%)	(No.)	(%)	
Newquay	61	61	39	39	100
Mousehole	168	73	61	27	229
Truro (1)	173	82	39	18	212
Truro (2)	192	86	31	14	223
Truro (3)	85	94	5	6	90
Slapton	115	94	7	6	122
Miscellaneous	43	91	4	9	47
Total	837	82	186	18	1,023
Late April	68	72	28	28	96
May	52	69	23	31	75
Grand total, Britain	957	80	237	20	1,194
Perros Guirec, France <sup>6</sup>	110	22	390	78	500
Grand total	1,067	63	627	37	1,694

Note. All these places were cleansing stations for birds collected elsewhere, and the precise date and place of origin of each sample are uncertain, although there was clearly a difference in the proportion of guillemots to razorbills from place to place in Britain (possibly because razorbills died more rapidly before they were evacuated), and between Britain and France (where there were more razorbills). The proportion of razorbills also increased later in the season in Britain.

on inaccessible parts of the coast the full total is likely to be at least two or three times this figure, and could be anything up to ten times as large. Although small numbers of a few other species were killed, the species listed in Table 1 were those with an important mortality.

### Sub-species of the Guillemots

We were able to classify most of the guillemot wings into sub-species on the basis of colour and size. The upper parts of the race *U.a.albionis*, which breeds south of Central Scotland, are comparatively brown while the backs of birds breeding further north, the nominate race *U.a.aalge* from the Baltic, Scotland, Iceland and eastern North America, and the race *U.a.hyperborea* from the Barents Sea<sup>7</sup>, are darker. The two northern races can be distinguished from each other and from the southern race *U.a.albionis* by their size; *U.a.hyperborea* have larger wings than *U.a.aalge* and *U.a.albionis* although there is a wide range of overlap<sup>8</sup>.

Many of the wings we received were too dirty to assess, but the remainder after drying were compared with museum skins of adults obtained from breeding stations and wings collected on north-eastern beaches from Durham to Caithness (Table 3).

Table 3. WINGS OF GUILLEMOTS FROM THE BREEDING STATIONS AND BEACHES

Origin	No.	Wing length (range and mean in mm)	% of first year birds
Breeding stations			
England, Wales, Ireland ( <i>U.a.albionis</i> )	20	188-201	194.9
North Scotland and islands ( <i>U.a.aalge</i> )	20	193-206	200.0
Bear Island ( <i>U.a.hyperborea</i> )	7	204-216	210.0
Beaches			
Torrey Canyon, brown-backed (61%)	470	180-204	192.3
Torrey Canyon, black-backed (39%)	296	184-205, 215*	195.1
North-east coast, mainly dark	15	201-215	206.4
			(20)†

\* One exceptional bird of the race *U.a.hyperborea*.

† Ratio in 266 birds washed up in the spring of 1966 (ref. 3); only one, or 6.7 per cent, in the spring of 1967.

Table 3 shows nearly two-thirds of the birds killed in the Torrey Canyon disaster belonging to the southern race *U.a.albionis*, and most of the rest to the nominate race *U.a.aalge* from regions immediately to the north; one bird, however, was conspicuously larger than the others with a wing of 215 mm and it was probably of the Arctic race *U.a.hyperborea*. A number of birds from north-eastern English beaches were as large as this race in both 1966 (ref. 3) and 1967, and its occurrence there is further demonstrated by the recovery in Durham of a bird ringed near Murmansk<sup>9</sup>, but *U.a.hyperborea* has not been found so far south-west before. We examined the guillemot wings for evidence of the occurrence of the postulated race *U.a.spiloptera* of the Faroes, which is reported to have a heavily marked underwing<sup>10</sup>, but this type of underwing was found in approximately 10 per cent of birds of both the southern and nominate races. It has also been reported in several other populations<sup>11</sup>, so that this character alone does not seem very reliable.

A razorbill with a wing of 212 mm, which falls within the range of the northern nominate race *A.t.torda* of this species<sup>7</sup>, was seen in the Scillies during the Torrey Canyon disaster by J. L. F. Parslow, and birds of this race, ringed in the Murmansk area, have been recovered in Eas Lothian, Flint and Cornwall<sup>9</sup>, but this form was not detected in our sample of wings.

### Age Distribution

Guillemots in their first year appear to be distinguished by the pale base of the bill, the retention of faded inner primary coverts<sup>3</sup>, and the possession of pale edges to the longer under wing coverts. Only the last character, the

validity of which was confirmed by examination of a series of skins in the British Museum (Natural History) collection, was easily applicable for ageing our sample of wings. Twenty-nine per cent of the brown-backed and 48 per cent of the black-backed birds in the Torrey Canyon kill showed this first year character. In contrast, only 20 per cent of the birds examined on the north-east coast in the early spring of 1966 showed first year characters. There was also a small but consistent difference in the average size of first year and adult wings in our sample, the brown-backed averaging 192.3 and 193.7 mm and the black-backed ones 194.4 and 195.7 mm, respectively. Storer<sup>11</sup> reports a large increase in size with age in some populations, but possibly some of his young birds were autumn specimens which were not yet fully grown. Apart from the pale feather-edgings in first year birds there were no important differences in the amount of marking of the underwing between first year and adults in either the brown-backed or the black-backed populations.

About half the guillemot population fail to breed<sup>12</sup>, and pairs that do breed produce on average about 0.4 young annually<sup>13</sup>. At most therefore the proportion of yearlings at sea should not exceed the 20 per cent found off the north-east coast in the spring of 1966 (ref. 3), and indeed there was only one bird in fifteen, or 6.7 per cent, in 1967. Yet there were 29 per cent yearlings among the southern brown-backed birds in the Torrey Canyon kill, and 48 per cent among the northern black-backed ones. The explanation is probably that young birds are more migratory than adults<sup>14</sup>. While some local adults will have been killed in Cornwall, and some local yearlings may have been outside the area, they will have been replaced by immigrants including a high proportion of immatures from the larger populations to the north.

The twenty shag wings from the Torrey Canyon sample contained fully grown feathers, but showed an interesting mixture of plumages. The primary moult of this species usually begins with the inner (first) primary and progresses to the outer (eleventh) feather at the average rate of one every 22 days. The moult may occur between February and December, but mainly in the autumn, and in many shags the cycle is not completed in 1 year. In these cases the moult recommences the following year both at the previous growing-point and at the beginning again<sup>15</sup>. Consequently, it is possible to age shags by identifying the number of plumages present at any one time; the age of the Torrey Canyon birds is shown in Table 4.

Table 4. THE PLUMAGE AND AGE OF SHAGS FROM THE TORREY CANYON KILL

Plumage cycles present	Number	Cohort (note overlap)
Juvenal	1	I
Juvenal and post-juvenal	0	
Juvenal, post-juvenal and first nuptial	4	II
Post-juvenal and first nuptial	6	
Post-juvenal, first and second nuptial	2	III
Post-juvenal, first, second and third nuptial	1	
First nuptial	3	IV
Two nuptial	3	II+

It can be assumed for present purposes that the number of two and three-year-olds is 12; thus the age distribution of the present sample is first year, 1; second and third year, 12; and more than 3 years, 7. Yearlings should have been in the area at the time of the disaster<sup>16</sup>, and a full adults should have been nesting. From the mortality rates of this species<sup>15</sup> an expected age distribution can be drawn up: first year, 4; second and third year, 6; more than 3 years, 10. The difference between

what was observed and expected is significant ( $P \approx 0.01$ ). This unexpected composition of the age-groups of the shags might be explained by a different survival of past year-classes, but seems more likely to be due to differences in the extent to which the birds move around, and become susceptible to pollution, during the period when they start to prospect nest-sites. In this matter, as with others, more direct observation in the oiled areas would have been useful.

While all the other birds had fully grown flight feathers, the two divers, a great northern and a black-throated, were both in full wing-moult, with short, growing primaries. Many water-birds are known to lose their flight-feathers simultaneously<sup>17</sup>, so that they become flightless, but this usually occurs in the late summer. Only the larger divers postpone the main wing-moult until the spring<sup>18</sup>, and this has already caused a severe mortality from oil pollution on the New England coast<sup>19</sup>. It seems probable that the small part of the Cornish winter population of divers that was still in the area in March, completing the moult, was killed flightless in the water. Most of the divers would, however, have migrated north.

Oil is, potentially, an even greater danger to species like the auks when both adults and young are flightless on the water at the same time in late summer. The protracted moult of shags, however, spares them from this danger; of the shag wings we examined, most showed a two or three year moulting cycle (see Table 4) but one exceptional wing showed traces of a four year cycle. Remarkably, this bird would only renew its outer primary once in 4 years.

This survey clearly shows that the main bird mortality from the Torrey Canyon disaster involved local British populations, especially the larger auks, including disproportionately high numbers of the more migratory young birds. Some sub-adult shags, moving around in search of nest-sites, were also killed. We received only two specimens of northern birds, the great northern diver and the solitary northern auk *U. a. hyperborea*.

We are grateful for wings, a variety of assistance, and information from many organizations and individuals, including the Royal Society for the Prevention of Cruelty to Animals, John Nicholson and the Royal Society for the Protection of Birds, Messrs Robert Spencer and Henry Mayer-Gross of the British Trust for Ornithology, Messrs J. L. F. Parslow and N. R. Phillips, who later carried out breeding censuses for the Nature Conservancy, Dr C. J. F. Coombs and the Rev J. A. Beckerlegge of the Cornwall Bird-watching and Preservation Society, Dr G. M. Dunnet and Mr A. W. Diamond of the Natural History Department, Marischal College, University of Aberdeen, Messrs Tony Soper and Einar Brun, and Dr Finn Salomonsen. Dr Dunnet and Dr D. W. Snow commented on drafts of this note.

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# New Syntheses of Porphyrins and Related Tetrapyrroles

by

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Recently methods of synthesis of porphyrins have become more versatile. This makes available many new routes to biologically significant compounds.

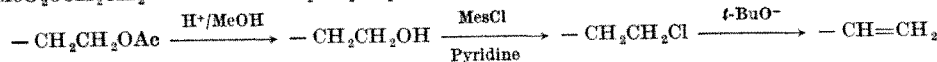
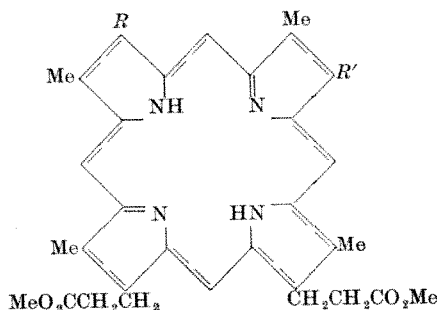
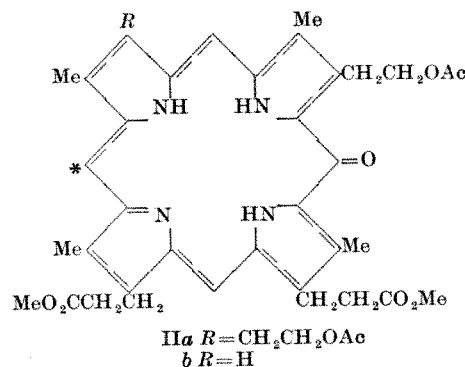
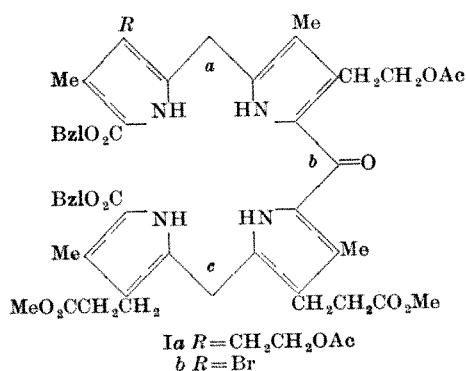
NUMEROUS porphyrins were synthesized by Hans Fischer and his co-workers<sup>1</sup>, and these classical investigations established the structure of haem and many other porphyrins of natural origin. Fischer's syntheses, however, gave poor yields in complex cases and left considerable room for improvement. A notable advance was made by MacDonald<sup>2</sup>, who developed an elegant method employing pyrromethanes, which led to syntheses of all four coproporphyrins among others, and the same principle was incorporated in Woodward's brilliant total synthesis of chlorophyll<sup>3</sup>. More recently Fischer's route has also been significantly varied and extended by Johnson and his colleagues<sup>4-6</sup>. At the same time we have been developing quite different routes to porphyrins, and these now offer considerable scope for synthesis of compounds of biological significance.

The basic tenet of our programme has been that the porphyrin macrocycle should be formed from a well defined tetrapyrrolic compound, constructed logically from four separate pyrrole derivatives. Our initial studies<sup>7</sup> produced some new methods for linking pyrrole nuclei by methylene groups, after the pattern of biosynthesis from porphobilinogen, but it was soon apparent that stepwise synthesis of tetrapyrrolic compounds with three methylene links were beyond the scope of this approach. Such compounds are prone to cleavage by acids and other electrophiles, and therefore we turned to construction of tetrapyrrolic compounds stabilized by an internuclear carbonyl group, namely, the oxobilanes (for example, I).

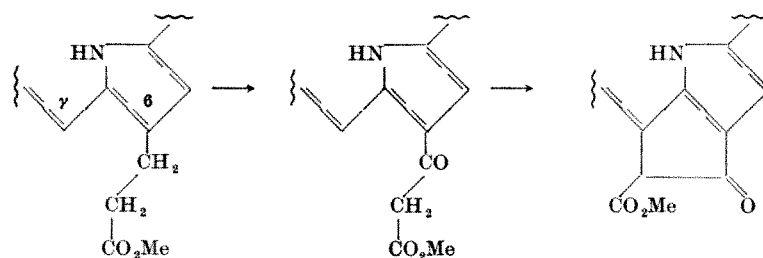
Two syntheses through oxobilanes were devised<sup>8</sup> and about twenty different porphyrins have now been prepared (ten by each method). The first method involves *a*-oxobilanes and is described elsewhere in detail<sup>9</sup>. Examples of biologically important porphyrins which have been synthesized by this method include mesoporphyrin-IX (ref. 8) and coproporphyrins-III and -IV (unpublished work of Mr J. Wass). An important improvement in the general method is the recent use of  $\alpha$ -pentachlorophenyl- $\alpha'$ -benzylpyrromethane dicarboxylic ester as intermediates; the pentachlorophenyl esters can be selectively hydrolysed (or the benzyl esters selectively hydrogenolysed) to give the pyrromethane  $\alpha$ -carboxylic acids required in the *a*-oxobilane synthesis. Protoporphyrin-IX has now been synthesized by this route and also by the *b*-oxobilane method as described below<sup>10</sup>.

The *b*-oxobilane (Ia) was constructed from pyrrole units by essentially similar methods to those described elsewhere for analogous cases, and after removal of the protective benzyl ester groups by hydrogenolysis it was cyclized to the oxophlorin (IIa) with methylorthoformate in mildly acidic conditions. The oxo-group was removed (by acetylation, reduction and re-oxidation) to give the bis-acetoxyethylporphyrin (IIIa), and modification of the acetoxyethyl side-chains as indicated schematically below then afforded protoporphyrin-IX dimethyl ester (IIIb).

Another example of the use of the *b*-oxobilane method is the synthesis<sup>11</sup> of chlorocruoroporphyrin (IIIc) dimethyl ester which is derived from the haem of *Spirographis* *spa*.



*lanzanii*<sup>12</sup>. The bromo- $\alpha$ -oxobilane (Ib) gave the oxophlorin (IIb) and thus the porphyrin (IIIc) the acetoxyethyl group of which was transformed into chloroethyl as indicated already. The formyl group was introduced at the 2-position of the chloroethylporphyrin (IIIc) and then protected as the relatively stable neopentyl glycol acetal



during elimination of hydrogen chloride. This synthesis is of more general interest because chlorocruoroporphyrin contains two of the three sensitive substituents present in haem- $\alpha$ , that is, the formyl and vinyl groups. Haem- $\alpha$  is the prosthetic group of cytochrome oxidase and because its structure is still in doubt<sup>13</sup> (especially the precise nature of the long fatty side-chain) our new methods are clearly of potential use in a synthetic proof of structure.

The foregoing synthetic work has also clarified the structure of pemttoporphyrin, a recently isolated faecal metabolite<sup>14</sup> with one of the structures (III;  $R, R' = H$ ,  $-\text{CH}=\text{CH}_2$ ) (ref. 15). One isomer (III $f$ ) was made from the intermediate (IIIe) and the other (III $g$ ) in a separate synthesis by the  $\alpha$ -oxobilane route. The nuclear magnetic resonance spectra of the two isomers (in  $\text{CDCl}_3$ ) showed considerable differences, and that of the synthetic porphyrin (III $f$ ) is identical with that of pemttoporphyrin<sup>15</sup>.

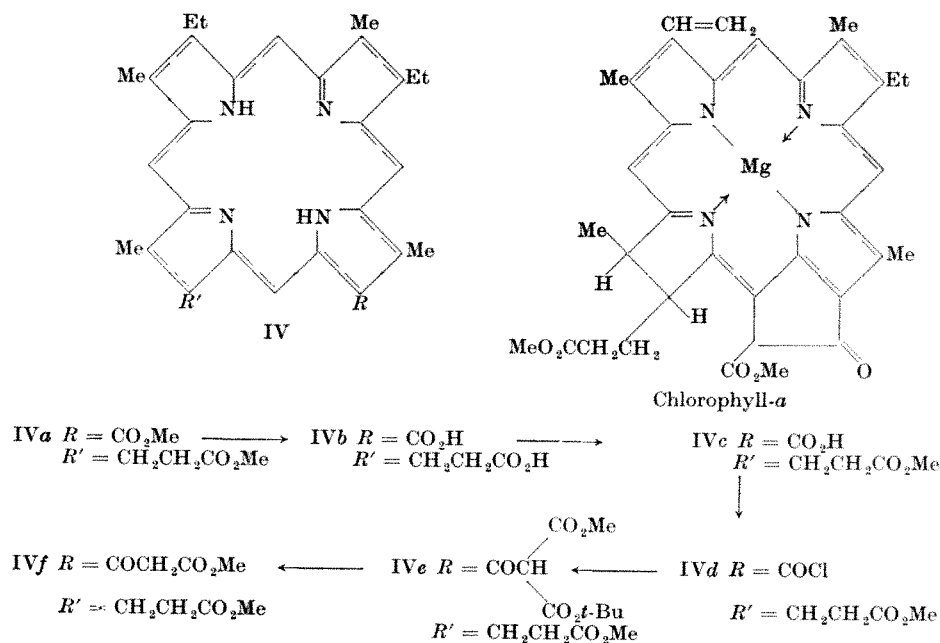
The biosynthesis of chlorophyll from protoporphyrin-IX has been studied extensively in recent years and the work of Bogorad, O. T. G. Jones and others<sup>16</sup> has revealed much of the route. Our new syntheses of protoporphyrin permit, in principle, labelling with carbon-14 at various positions for biosynthetic studies, and a valuable possibility, which we have explored, is tritiation. The oxophlorin (IIa) undergoes specific exchange with tritiated acetic acid at the *meso*-position opposite the oxo-group (\* in the formula), whereas under more strongly acidic conditions no exchange occurs owing to formation of the dication. Protoporphyrin, specifically tritiated at the  $\delta$ -position, has now been prepared from this oxophlorin.

An outstanding problem in the biosynthesis of chlorophyll is the mode of formation of the so-called isocyclic ring, and we have suggested before<sup>17</sup> that this arises by oxidation of the 6-propionate group (originally present in protoporphyrin) to a  $\beta$ -keto-ester followed by cyclization at the  $\gamma$ -position, that is

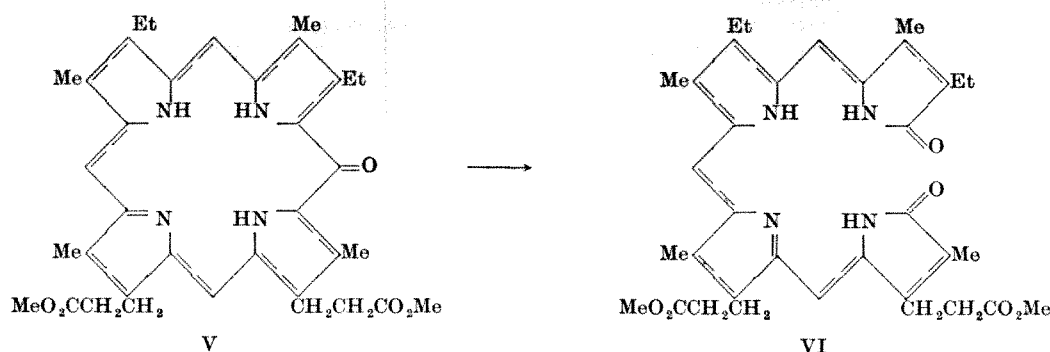
We have accordingly synthesized (unpublished work of Mr T. T. Howarth) rhodoporphyrin-XV dimethyl ester (IVa) by the  $\alpha$ -oxobilane route and elaborated the 6- $\beta$ -ketoester side-chain as shown in the scheme outlined below. The cyclization of this ester *in vitro* is currently being investigated, and synthesis of the 2,4-divinyl analogue (which would be the biosynthetic intermediate) is planned. An alternative pathway for the formation of the isocyclic ring may also be envisaged involving a 6-acrylic ester group ( $\text{CH}=\text{CH}-\text{CO}_2\text{Me}$ ) (compare chlorophyll- $c$  which has recently been shown to contain an acrylic ester side-chain at the 7-position<sup>18</sup>); such compounds should be readily accessible by a variant of our new methods.

Oxophlorins (or "oxyporphyrins" as they were originally named) have been known for nearly thirty years, and were originally prepared by non-specific oxidation of porphyrin iron complexes<sup>19-24</sup>. We prefer the name oxophlorins because extensive spectroscopic observations<sup>25</sup> have clearly shown that the *meso* oxygen function is present as an oxo-group in both the free base and the mono-cation (which is protonated on nitrogen). For purposes of nomenclature, however, it is convenient to regard oxophlorins as derivatives of the tautomeric hydroxy porphyrin.

Oxophlorins are of considerable biochemical interest because they (or rather their iron complexes) may be intermediates in the catabolism of haem to bile pigments. Indeed, the "oxyporphyrins" originally prepared<sup>19-24</sup> by Fischer and Libowitsky, and by Lemberg have been converted *in vitro* into bile pigments, and we have con-







firmed these earlier studies by showing that "β-oxymesoporphyrin-IX dimethyl ester" (V) gives glaucobilin-IX-β dimethyl ester (VI) in good overall yield. In this connexion we have recently synthesized "α-oxymesoporphyrin-IX", and the preparation of "α-oxyporphyrin-IX" is well in hand; we intend to study their conversion into bile pigments both *in vitro* and *in vivo*.

All this work has been greatly aided by extensive use of spectroscopic methods. Mass spectrometry is particularly suited to determinations with the small quantities of material available in structural studies (or in "pilot" preparations of porphyrins) and the relatively high stability of the macrocyclic porphyrin nucleus makes observation of the molecular ion nearly always possible<sup>27</sup>. A drawback of the method is that it gives very little information about the precise arrangement of substituents around the periphery of the ring, but this is to some extent remedied by careful studies of the nuclear magnetic resonance spectra<sup>28,29</sup> (as well as by biogenetic speculation).

We have also studied extensively the mass spectra of open-chain mono-, di-, tri- and tetra-pyrrolic compounds<sup>30</sup> and further work is in progress, especially on bile pigments where there is a number of unsolved structural problems. Interpretation of the spectra of urobilinoid compounds<sup>31</sup> is complicated by some unusual hydrogen transfers (or migrations), but recent work (unpublished, by Jackson, Murray, O'hEocha and Smith) has confirmed that the urobilin obtained by catalytic reduction of phycoerythrobilin<sup>32</sup> is structurally very similar to mammalian urobilins.

We hope to synthesize a number of model compounds for further mass spectrometry, both by ring opening of oxophlorins and by synthesis from some of the dipyrrolic intermediates we have used in our syntheses of oxobilanes.

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## Food and Activity in the Mole *Talpa europaea*

by

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Moles may be much less active and voracious than is commonly believed. This explains their ability to colonize infertile soils with little fauna.

THE commonly accepted view of moles is that they are "very voracious, consuming more than their own weight of food every 24 h; their whole life is an incessant hunt for food, only short intervals of repose punctuating

their nearly continuous feverish activity"<sup>1</sup>. It has been said that moles can only find enough food in habitats like deciduous woodland and old grassland which have a rich soil fauna<sup>2</sup>. Nevertheless we do find them in the

dry sandy soil of the East Anglian Breck, and at more than 600 m above sea level in the Pennines; in both these habitats soil animals, particularly earthworms, are far from abundant.

Many of those who have tried, unsuccessfully, to keep a mole in captivity have had these views on feeding and activity reinforced. A mole has been put in a box of soil; it has burrowed vigorously and continuously for a few hours and then died. Nevertheless, moles are easily kept alive and healthy for quite long periods, and with comparatively modest amounts of food.

A newly caught mole is probably starving; it may have been active for some hours and it is in a strange environment. The essential first step is to persuade it to feed. Although captive moles may be maintained on many articles of food, including liver, baby mice, mealworms and maggots, they always seem to prefer earthworms, particularly large *Lumbricus terrestris*. Newly caught moles often refuse any other type of food; if they are offered some three large worms (a large *L. terrestris* weighs about 5 g) they will probably consume these in a matter of minutes. They will then explore their surroundings and if a small box containing hay is present they will crawl in and go to sleep. They will probably sleep for at least 4 h, possibly for up to 7 h, and when they awake they will be, to all intents and purposes, domesticated and suitable subjects for further study.

Moles have been kept successfully in various types of container. Some workers have used a nest box connected to runs made of wire mesh; this has allowed behaviour to be studied over long periods<sup>3</sup>. Others, however, have found simple wooden boxes reasonably satisfactory<sup>4,5</sup>. Some have filled these with soil, but this becomes foul, and must be changed frequently. Wooden boxes, empty except for a small amount of hay or straw to make a nest, have also been used. I have used a wooden box, 60 × 35 cm, and 25 cm deep, with a wooden lid fixed over half the top, and a movable glass plate—to allow the animal to be watched—covering the rest. In the bottom I put "bulb fibre", about 2 cm deep. This contains some charcoal, which absorbs excretory products without quickly becoming foul. A small box 15 × 11 × 10 cm, with an entry hole, fixed at one end of the larger box and filled with hay, serves as a nest.

Moles do sometimes eat large quantities of food, but "more than their own weight every 24 h" is an exaggeration. I have made some experiments to determine the maximum amount of food taken. The box described was balanced so that it moved when the mole came out of the nest. This movement made an electric contact and rang a bell. Each time the bell rang, the mole was offered as many earthworms as it would eat; any not immediately devoured were removed. The total consumption in five periods of 24 h was 47, 51, 47, 54, 46 g (mean 49 g); the weight of the mole varied between 87 and 102 g during this period. This means that the food taken was just about half the body weight. However, even this is an over-estimate. The mole was frequently weighed just before and after feeding, and the gain in weight was always less than the weight of worms. Thus if 15 g of worms were taken, the gain in weight might only be about 12 g. This difference was not caused by excretion, for when the mole was fed standing on a clean piece of paper no substantial micturition or defaecation was detected. Parts of the worm, particularly the gut, which contained soil, were discarded.

Larger quantities of worms than can be eaten at once provoke an interesting reaction. Moles usually run a worm through their front feet until they reach the head end, and this is eaten first. When the mole is "full", it still bites off the head, but then it plays with the worm and usually buries it in the corner of the box. In the field caches of worms, with their heads bitten off, are sometimes found<sup>6</sup>; these are presumably worms buried by a satiated

mole. This habit of storing surplus food has caused some observers to over-estimate food consumption, because caches are easily overlooked.

Previous workers have shown that moles fed on insect larvae, which have a high calorific value compared with earthworms, can be kept alive when given 20–30 g/day<sup>7</sup>. These are probably maximum figures. I have never been able to make a captive mole take more than about 20 g of mealworms in 24 h. Incidentally when mealworms are eaten, the weight gained is generally slightly higher than the weight of the mealworms, probably because some soil is accidentally ingested at the same time. Surplus mealworms may, like earthworms, be cached, but the mole does not immobilize them and they may crawl away and escape.

Captive moles readily drink water, except when fed on an unlimited diet of earthworms, which contain at least 85 per cent of water and so presumably supply all requirements. In many natural situations, particularly under dry conditions where worms are scarce, moles must seldom have the opportunity to drink. Nevertheless, their reaction to water is so positive that drinking appears to be a frequent and normal activity. If a dish of water is placed near a thirsty mole it is soon found, and the animal drinks rapidly, taking as much as 10 ml. in under 5 min. If a mole fills its stomach with water, it may retire to its nest to sleep, but instead of remaining dormant for several hours it usually comes out again in less than an hour and will then take food as if completely unfed.

It is usual in studies of animal nutrition to note changes in body weight, and to assume that if this remains constant then an adequate diet is being given. Care must, however, be taken in interpreting gross body weights, particularly in animals like moles which can take meals which may be nearly 20 per cent of their starving weight. A starving mole may take up food (and water) and increase by as much as 15 g (for example, from 87 to 102 g). Thus only weights of animals in a similar state of repletion should be compared. Except in experiments when moles have been fed "on demand", I have compared weights of hungry animals with their stomachs empty of food, because these are basic measurements of the living tissues of the animals. A fall in the starving weight may mean a loss of reserves, as the result of tissue catabolism, or it may be the result of desiccation. In my experiments, I found only comparatively small changes in the starving weight of adult moles, whatever the diet administered. The effects of reduced intake of food were studied together with measurements of the activity of the animals.

Wild moles have been caught, fitted with radioactive cobalt tail rings, and after liberation their subterranean activity has been followed by means of a Geiger counter<sup>8</sup>. In this way it has been established that they have periods of inactivity, when they sleep in their nests, followed by periods of activity, when they travel along their burrows and dig new tunnels. This activity pattern is repeated about three times a day. There does not seem to be any regular rhythm, and the lengths of the rest and active periods are by no means constant. Thus there is no particular hour when a mole is most likely to be active or asleep. Nevertheless there are usually three periods of sleep, of between 3 and 5 hours, and three periods of activity, of roughly the same duration, every 24 h. The technique using radioactive tail rings has enabled much to be learned about mole activity, but it has limitations. The mole cannot actually be watched—lateral movement only can be detected—and its food consumption cannot be studied.

In my experiments in which the mole rang a bell when it left its nest, it was seen that a captive animal had a behaviour pattern on the same lines as had been demonstrated in the field; the mole slept for half the 24 h and usually took this sleep in three main periods. In order to study activity in more detail, and to allow the observer an unbroken night's rest, a simple actograph was con-

structed. A pen was fitted to the box, recording its movements on a revolving drum. Characteristic records are reproduced in Fig. 1. The first and last traces record behaviour when unlimited food, either earthworms or liver, was available. The second and third traces are from a starving mole. These and other records show a very striking similarity to the activity records of wild moles obtained using radioactive tail rings. They suggest that moles do not have an accurate internal "clock" and that, as the periods of activity and sleep are not always the same length, either type of behaviour may be found at any hour. They all show that long periods are spent in sleep. Most interesting, they show that starving moles are not more active than those which are fed. It appears that when a mole wakes up it has a period of activity, when it searches for food. If this is readily found the mole may go back to its nest and have a "nap", but it seems usually to come out again after perhaps half an hour, and not to enter a long (more than 3 h) sleep period until the end of a "normal" period of activity. On the other hand, if no food is found, searching goes on for some 3 h or so, and then the mole, still hungry, goes back to the nest and sleeps for at least 4 h. At the end of 24 h of starvation moles were found to have lost only about 5 g in weight, and they fully recovered this if allowed unlimited access to earthworms for a further day. I have not starved a mole for more than 24 h, but these results suggest that it might well survive for several days without food, though others have reported deaths in 48 h when feeding of captive moles was overlooked during weekends. Certainly there was no appreciable decrease in body weight when only between 5 and 10 g of food, which included worms and insect larvae, was provided in experiments lasting up to 14 days.

escaped into a paved area in my garden. There were pockets of earth, into which the animal burrowed, uprooting alpine plants and bulbs. Very little animal food was present, and over a period of a week the mole kept coming to the surface and exploring another soil pocket, coming up and down many times in one day. During this week it cannot have had a daily ration of as much as 10 g. Incidentally it still had its periods of sleep, between its largely fruitless searches for food.

These observations on feeding and activity may throw some light on the normal behaviour of wild moles. It has always been a mystery how these animals survive in soils with a poor fauna. If they can in fact exist on as little as 5 g of food, instead of 100 g or more assumed by some workers, the situation is easier to understand.

The observation that a starving mole spends much of its time asleep fits in well with field observations. It is not always realized that the mole's tunnel system is essentially a "pit-fall trap", and that digging is done to enlarge this system rather than to catch food<sup>1a</sup>. In soil with plenty of food, moles may do little or no digging for months at a time, and tunnels remain patent, used by successive generations, for many years. In poor soils, with little fauna, moles may need very much larger systems of burrows; these may be continually enlarged, and the effects on the vegetation, where annual plants easily colonize the fresh earth, will be consequently much greater. A mole, on waking, runs through its burrow system, feeding on the worms and other animals it finds. If it has been active for several hours, and, although it has only obtained a partial meal, it can find little more food, then further activity will be a waste of energy. By sleeping the mole reduces its metabolic rate, conserves its

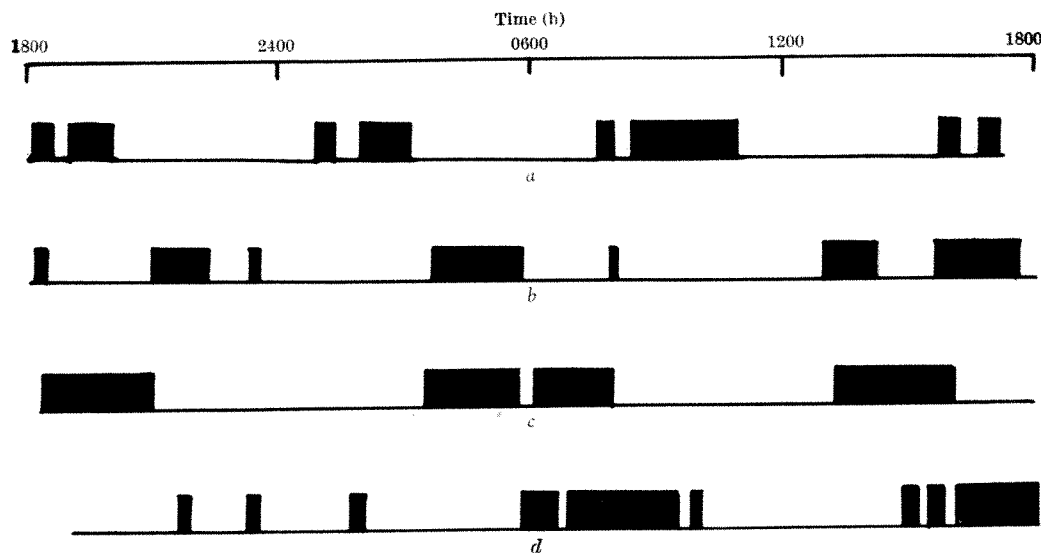


Fig. 1. Behaviour of moles under different feeding regimes. The black rectangles indicate activity, the base lines show when the animals were asleep. *a*, Unlimited earthworms; *b* and *c*, starved; *d*, unlimited liver.

Moles do not live entirely underground. Young animals, in spring and early summer, leave the maternal burrow and move overground to fresh territory. Here they may enter existing old burrow systems, or they may excavate new tunnels, but during the summer their runs are often very superficial, scarcely going below the mat of the vegetation. This is where much animal life is found, except in very dry conditions. In a severe drought they have been found living even more superficially<sup>9</sup>, apparently unable to burrow in hard earth, which must contain few worms in the superficial layers. Moles also come to the surface when burrowing conditions are difficult and food is absent. This was demonstrated when one animal

energy, and gives time for more food animals to enter the burrows to be consumed in the next round of activity.

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# Visual Pigments of Crayfish

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Crayfish eye extracts contain two visual pigments resembling in spectrum vertebrate rhodopsin and iodopsin. In these crayfish, as in primates, the "iodopsin" appears to be the red sensitive pigment of colour vision.

I HAVE found that the eyes of the common northern crayfish, *Orconectes* (then *Cambarus*) *virilis*, contain vitamin A<sub>1</sub> (retinol<sub>1</sub>) and that the destruction of its visual pigment releases retinal<sub>1</sub> (retinene<sub>1</sub>)<sup>1,2</sup>. Since 1943 not only has the name of the crayfish changed—as is usual—but so have the names of the molecules. Vitamin A<sub>1</sub> and retinene<sub>1</sub> have become retinol and retinal<sub>1</sub>; and vitamin A<sub>2</sub> and retinene<sub>2</sub> have now the formal names 3,4-dehydroretinol and retinal<sub>2</sub>. This was of some interest because, although terrestrial and marine vertebrates and several marine invertebrates characteristically possess visual pigments based on vitamin A<sub>1</sub> and retinal<sub>1</sub>, those of freshwater vertebrates are as characteristically based on vitamin A<sub>2</sub> and retinal<sub>2</sub>. No invertebrate is yet known which normally contains vitamin A<sub>2</sub>.

Dark adapted eyes of this crayfish, dried by grinding with anhydrous sodium sulphate and extracted in the dark with petroleum ether, yielded vitamin A<sub>1</sub> (Fig. 1a). Re-extraction with petroleum ether in bright light yielded

only a little more vitamin A<sub>1</sub>, apparently a residue left over from the dark extraction (Fig. 1a); although the same treatment of vertebrate retinas would have yielded a large amount of retinal<sub>1</sub>, released by the bleaching of visual pigment by the light. The extraction of crayfish eyes in light or darkness with chloroform, however, which denatures all known visual pigments, yields retinal<sub>1</sub> (Fig. 1b).

It seemed probable that the eye of the crayfish contains a visual pigment with retinal<sub>1</sub> as its chromophore, but that unlike vertebrate rhodopsin this does not yield its retinal to petroleum ether on exposure to light. This behaviour has since become familiar. A number of invertebrate rhodopsins (lobster<sup>3</sup>, *Limulus*<sup>4</sup>, grapsoid crabs<sup>5</sup>, squid<sup>6</sup>, cuttlefish and octopus<sup>7</sup>) bleach in the light only to metarhodopsins, which release retinal only very slowly and in circumstances that either denature the protein, such as here, or competitively remove it with hydroxylamine to form retinal oxime<sup>3-7</sup>.

Kennedy and Bruno found the visual sensitivity of the southern swamp crayfish, *Procambarus clarkii*, to be maximal near 570 mμ. They pointed out that this lies farther towards the red than all known rhodopsins, and resembles in spectrum the pigment of the vertebrate cone, iodopsin. They suggested that this displacement of visual sensitivity toward the red represents a general adaptation, common to both vertebrates and invertebrates, to life in relatively turbid freshwater environments, with their poor transmission of short wavelength radiation.

This observation led me to attempt to extract a visual pigment from the crayfish eye, and surprisingly my extracts contained at least two such pigments, whether taken from eyes of the swamp crayfish<sup>8</sup> or the northern crayfish from which I had earlier extracted vitamin A<sub>1</sub> and retinal<sub>1</sub> (ref. 9). This led in turn to an electrophysiological examination of the spectral sensitivities of these and other arthropod eyes<sup>10</sup>. Both crayfishes seem to possess apparatus well adapted for two colour vision, based on a blue sensitive and a red sensitive receptor system. In each of these animals the most important visual pigment extracted from the eye seems to be that of the red sensitive receptor. No visual function has yet been found for the minor pigments extracted from these eyes; nor, conversely, has the visual pigment of the blue receptor been identified in extracts.

The procedures were usually the same as those employed to isolate and extract rod and cone outer segment fragments from vertebrate retinas, on the tenuous ground that the rhabdomeres of invertebrate eyes look like the same type of structure, fortified by previous successes with squid, lobster and *Limulus*<sup>4</sup>. The structures which are isolated are composed largely of membranes, with a large content of phospholipid that makes them float in media in which other components of the eye tissues sink. With crayfish preparations large amounts of screening pigments that accompany the rhabdomere fraction must be removed. All the procedures were carried out in dim red light.

Whole eye stalks of dark adapted animals were ground in a mortar in M/15 phosphate buffer at pH 6.5. The

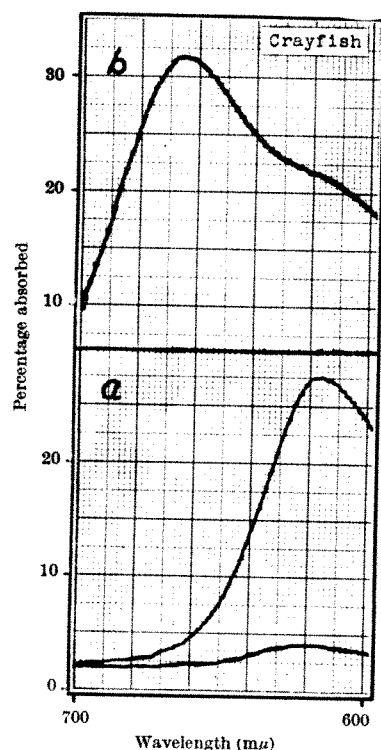


Fig. 1. Vitamin A and retinal from eyes of the common northern crayfish, *Orconectes virilis*. Absorption spectra of the blue products obtained by mixing extracts of the eyes with antimony chloride. The extraction of dark adapted eye tissues with petroleum ether yields vitamin A<sub>1</sub> alone (tall curve in a;  $\lambda_{\max}$  617 mμ; yield about 0.08 μg/eye). Re-extraction with petroleum ether in bright light yields only a little more vitamin A<sub>1</sub> (a, smaller curve). Extraction with chloroform, which denatures the visual pigment, releases retinal<sub>1</sub> (b;  $\lambda_{\max}$  664 mμ; yield about 0.09 μg/eye). All these extracts had also contained astaxanthin, which was removed by filtering in petroleum ether through calcium carbonate before performing the antimony chloride tests.





In all these properties the 510 m $\mu$  pigment strongly resembles vertebrate rhodopsin. Its yield, in terms of the absorbance at 510 m $\mu$ /100 eyes extracted into 1 ml. of digitonin solution and measured in a 1 cm layer, was 0.029.

A neutral extract of *Procambarus* eyes, exposed for 5 min to deep red light, yielded, as did the red sensitive pigment of *Orconectes*, a transient intermediate with similar properties. Recorded at 5.5–8 min after the start of irradiation, this displayed a difference spectrum, relative to the final product of bleaching, maximal at about 515 m $\mu$ , and with minima near 405 m $\mu$  and—rather unexpectedly—also in the red, near 620 m $\mu$ . Thereafter this pigment continued to bleach in the dark for at least an hour at 27° C. The difference spectrum shown in Fig. 3—the difference in absorption spectrum between the initial extract and the final product when red light produced no further change and all dark reactions were completed—has  $\lambda_{\max}$  about 556 m $\mu$ , and a minimum at about 392 m $\mu$  owing to retinal, either free or still combined with opsin in a meta-pigment. This is the chief pigment in these extracts, and in its difference spectrum, although not in its kinetics of bleaching, closely resembles vertebrate iodopsin. Its yield, in terms of the absorbance at 556 m $\mu$ /100 eyes extracted into 1 ml. of digitonin solution and measured in a 1 cm layer, was 0.059.

Subsequent exposure to yellow light, or long-continued exposure to the same red light, resulted in some further bleaching, maximal at about 525 m $\mu$ , and accompanied by a further rise in spectrum at about 390 m $\mu$  owing to retinal. The maximum bleaching in this case was only about one-third as great as that of the 556 m $\mu$  pigment, and the difference spectrum was not highly reproducible in detail. No intermediates of bleaching were apparent.

Both these crayfish have mixtures of visual pigments in their eyes, based on retinal, and yield mixed photopigment extracts. In both animals the chief component is a pigment that absorbs much further toward the red than any vertebrate rhodopsin, and resembles in this respect the vertebrate cone pigment, iodopsin. The *Procambarus* pigment, with  $\lambda_{\max}$  about 556 m $\mu$ , falls somewhat short of the spectral sensitivity maximum at about 570 m $\mu$  found by Kennedy and Bruno; and further still from the sensitivity maximum at about 575 m $\mu$  that characterizes the red receptors in what appear to be the colour vision systems of both crayfish<sup>10</sup>. This eye contains large amounts of highly coloured screening pigments that could well be responsible for displacements of sensitivity of this magnitude<sup>13</sup>. It is perhaps pertinent that the eye of *Orconectes* seems to contain much less screening pigment, and perhaps for this reason its "iodopsin" is the red receptor sensitivity more closely. I think that probably the 556 m $\mu$  pigment in *Procambarus* and the 562 m $\mu$  pigment in *Orconectes* represent the red receptor pigments of these animals. It is interesting, because of their other resemblances to vertebrate iodopsin, that the latter is the pigment of the red sensitive cones in human colour vision<sup>14</sup>.

In both crayfish I have also found blue receptors with peak of sensitivity at about 445 m $\mu$  in *Procambarus*, and 435 m $\mu$  in *Orconectes*. They seem to contribute little to the spectral sensitivities of these animals in the dark adapted state. I found no reliable evidence of photopigments with a peak near these wavelengths in extracts. It may well be that the blue sensitive pigment occurs in relatively small amounts in these eyes.

The functions of the "rhodopsins", at 510 m $\mu$  in *Orconectes* and about 525 m $\mu$  in *Procambarus*, remain problematical. Screening pigments could displace the action spectra of these pigments far enough toward the blue to account for the blue receptor sensitivities, but this would make a clumsy and very uneconomical arrangement that seems intrinsically unlikely. It would violate so what is probably a general principle in the construction of colour vision systems. For such systems to behave

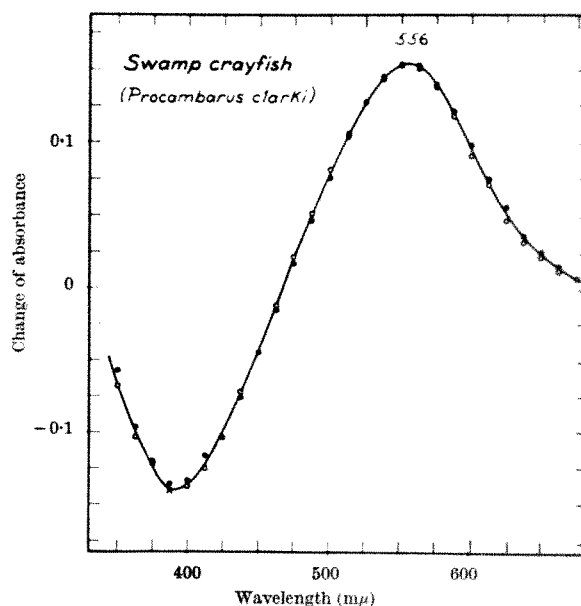


Fig. 3. Difference spectrum of the principal visual pigment in extracts of eyes of the southern swamp crayfish, *Procambarus clarkii*, pH 6.95, 27° C. Exposure to deep red light (Corning filter 2404; wavelengths longer than 625 m $\mu$ ) yielded unstable intermediates which continued to bleach in the dark for about 1 h longer. The difference in absorption between the original extract and this final product is shown. It has  $\lambda_{\max}$  about 556 m $\mu$ , and a minimum at about 392 m $\mu$  owing to the formation of retinal, perhaps still bound to opsin. After red light has no further effect, exposure of the residue to yellow light results in a little further bleaching, maximal at about 525 m $\mu$ , and yielding a little more retinal.

reasonably, their photopigments must maintain reasonably parallel kinetics. They must keep in step with one another in bleaching and regeneration. Otherwise their proportions would vary greatly with the conditions of illumination and adaptation, and it should be impossible to associate specific hues with objects in the environment<sup>15,16</sup>.

From this point of view it seems very unlikely that photopigments that bleach as differently as the crayfish "rhodopsins" and "iodopsins" could co-operate with one another in colour vision. The "rhodopsins" probably have some altogether different function. They may operate at higher or lower levels of brightness than the colour vision systems, in that case presenting a further analogy with the relations between vertebrate rhodopsins and iodopsins. It is interesting also that Bruno and Kennedy found that the caudal photoreceptor in *Procambarus*—a pair of light-sensitive neurones in the sixth abdominal ganglion—had its maximal sensitivity at about 500 m $\mu$ <sup>17</sup>, not far from the  $\lambda_{\max}$  of *Procambarus* "rhodopsin".

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# Comparative Nutrition in Pregnancy

by

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Maternal starvation reduces the viability of the offspring less in man than in other mammals. It is suggested that this is associated with the less exacting demands made on the mother by slower growing primate foetuses.

In prosperous as well as in developing countries, it is generally accepted that poverty is accompanied by a reduction in both average weights at birth and the survival rate of infants<sup>1</sup>. The relative importance of the factors which go to make up a poor socio-economic background are less well established, and there is considerable disagreement as to the role of malnutrition in this context.

## Growth Rates of the Foetus at Birth and the Nutritional Demands of Pregnancy

Experimental animals such as the dog<sup>2</sup> and sheep<sup>3</sup> show marked reductions in the size and viability of offspring when the mother is maintained on an inadequate diet. Birth weight in these animals may be reduced by 20–25 per cent, whereas in man a period of severe starvation has been reported to produce a reduction of only 5–10 per cent<sup>4</sup>. It is important to discover whether this apparent insensitivity to nutritional deprivation is due to the existence of some metabolic protective mechanism in the human mother, or to a reduced requirement associated with a rate of foetal growth much slower in relation to the mother's size than that of other species. Figures quoted by previous workers show the relatively slow rate of growth of the human foetus<sup>5</sup>, and Fig. 1 shows that this phenomenon is common to all the primates, because in general they produce smaller offspring than non-primates having the same gestation time. It would therefore be of interest to discover whether foetal growth rate and maternal weight are connected by any general relationship, and to what extent, if any, man and the other primates are exceptions to such a rule.

We have recently shown<sup>6</sup> that the weight curves during prenatal life of a number of species can be satisfactorily described as consisting of an initial "lag" phase, during which growth is very slow, followed by a period when growth conforms to a "cubic" law. This is a mode of growth to be expected of an organism to which nutrients are supplied at a rate directly proportional to its own surface area. This cubic law is expressed as the equation

$$W = a(t-t')^3 \quad (1)$$

where  $a$  is a constant related to the supply of nutrient to the foetus per unit of surface area, and  $t'$  is the length of the "lag" phase prior to the development of effective placental function. This gives an accurate prediction of foetal weight  $w$  at different gestational ages  $t$  for such diverse species as the chick, man and the cow. Differences in rates of development among the foetuses of these species can be ascribed simply to different values of  $a$  and  $t'$ , while the exponent of  $t-t'$  always assumes a value close to 3.

Such a family of parabolas has the useful property that at any given time  $t$ , and weight  $w$ , the rate of growth is proportional to  $\frac{w}{t-t'}$ . This follows from differentiating

$$\text{equation (1) when } \left(\frac{dw}{dt}\right)_{t=t} = 3a(t-t')^2$$

and combining this with the expression, also derived from (1)

$$a = \frac{w_i}{(t_i - t')^3}$$

Hence

$$\left(\frac{dw}{dt}\right)_{t=t_i} = 3 \frac{w_i}{t_i - t'}$$

In particular, at birth, when  $w = w_B$  and  $t = t_B$ ,

$$\frac{dw}{dt} = 3 \frac{w_B}{t_B - t'}$$

Values obtained for  $t'$  by Payne and Wheeler show that it is a small ( $\sim 20$  per cent) and relatively constant proportion of  $t_B$ , and so we can say that at full term the daily increment in weight is simply proportional to  $\frac{nw_B}{t_B}$

where  $n$  is the number in the litter. Mitchell<sup>7</sup> has suggested that the total products of conception may be regarded as growing in constant ratio to the size of the foetus, and hence we can regard the quantity  $\frac{nw_B}{t_B}$  as a valid index of the total metabolic and nutritional load imposed upon the mother at the end of pregnancy. In Table 1 are summarized a set of values of birth weights and gestation times of a number of mammalian species, taken from the

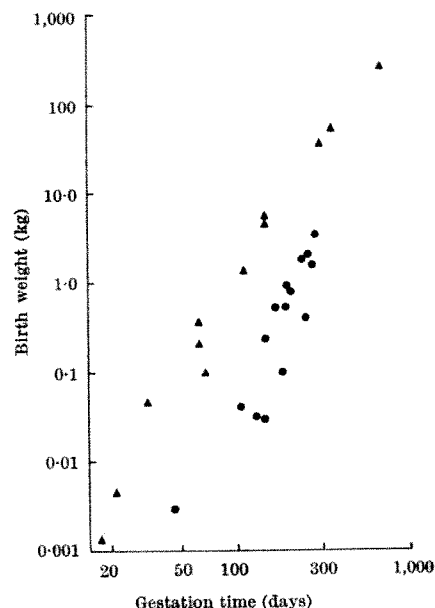


Fig. 1. Birth weights and gestation ages for primates and non-primate mammals. ●, Primates; ▲, non-primates.

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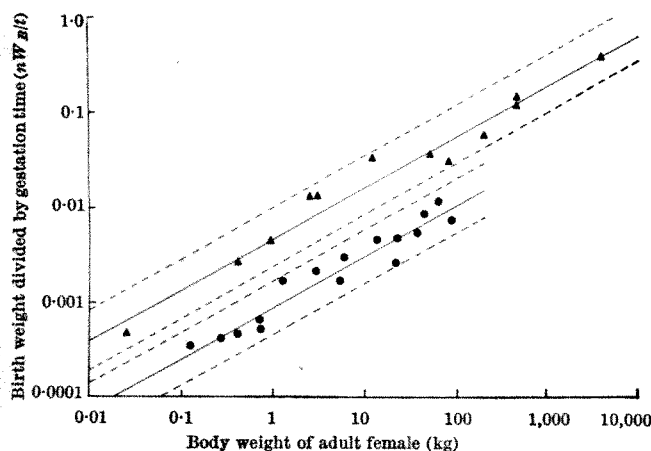


Fig. 2. The relationship between maternal weight,  $W_m$ , and the metabolic and nutritional load of pregnancy, represented by the ratio: weight of products of gestation/gestation time ( $nWB/t$ ). ●, Primates,  $nWB/t = 0.00087 W_m^{0.54}$ . ▲, Other mammals,  $nWB/t = 0.0047 W_m^{0.54}$ .

literature. Fig. 2 illustrates the relation obtained when  $nWB/t$  and maternal weight  $w_m$  are plotted on logarithmic scales. The points fall into two groups, consisting of the primates and the other mammals, and it may be seen that the stress of pregnancy imposed on a primate mother is less than that for a non-primate of similar weight. This is reflected in the values of the constants in the regression equations, which are in the ratio of 5 : 1. In the equations describing both groups, however, the exponent of  $w_m$  is the same (0.54), showing that, irrespective of species, the stress of pregnancy does not increase directly in proportion to the size of the mother, but rather in decreasing proportion as  $w_m$  increases. It might have been expected that the stress would increase in proportion to the "metabolic" body size of the mother—that is, that the exponent would be 0.73. The foetus, however, may be considered to be growing at a rate limited by the passage of nutrients across the effective placental surface. If this surface is related to the two-thirds power of placental weight, which is in turn related to the metabolic body size of the mother ( $W_M^{0.73}$ ), then the growth rate of a foetus would be proportional to  $(W_M^{0.73})^{\frac{1}{3}}$ , that is to  $W_M^{0.49}$  which is in close agreement with the observed value.

#### Growth Rate of the Foetus at Birth in Relation to Milk Composition

The low growth velocity exhibited by primates during foetal life is also characteristic of their subsequent growth. In Fig. 3, the curve representing the growth of non-primates is based on that given by Brody<sup>9</sup>. Similarly, the growth curves of three primates have been re-drawn from Schultz<sup>9</sup>, and it can be seen that these species have a much lower growth velocity during the early stages of growth. For example, at birth the rhesus monkey has a similar weight to a beagle, but subsequently takes 8 years to reach an adult weight which the dog has attained within 2 years.

In view of this, it is of interest to see if the composition of the milks of primate and non-primate species reflects differences in nutritive value compatible with these differences in growth performance. In order to relate growth performance to nutritive value it is necessary, first, to express growth rates as a proportion of metabolic body size, an index of which can be calculated as

$$\frac{W_B}{t_B} / W_B^{0.73}$$

and, second, to express the nutritive value of milk as the ratio of utilizable protein to energy content, which may be

Table 1. BIRTH WEIGHTS, GESTATION TIMES AND WEIGHTS OF ADULT FEMALES OF A NUMBER OF MAMMALIAN SPECIES\*

Species	Weight of adult female $W_m$ (kg)	No. in litter, $n$	Total weight of litter, $nW_B$ (kg)	Gestation time, $t$ (days)
Mouse ( <i>Mus musculus</i> )	0.025	7	0.0084	18
Rat ( <i>Rattus norvegicus</i> )	0.400	10	0.05	21
Guinea-pig ( <i>Cavia porcellus</i> )	0.900	3	0.3	67
Cat ( <i>Felis catus</i> )	2.5	4	0.8	63
Rabbit ( <i>Oryctolagus cuniculus</i> )	3.0	8	0.4	31
Dog ( <i>Canis familiaris</i> )	12.0	6	2.1	63
Goat ( <i>Capra hircus</i> )	50.0	1	5.3	150
Sheep ( <i>Ovis aries</i> )	80.0	1	4.5	150
Pig ( <i>Sus scrofa</i> )	200.0	5	6.5	114
Cow ( <i>Bos taurus</i> )	450.0	1	35.0	285
Horse ( <i>Equus caballus</i> )	450.0	1	50.0	340
Elephant ( <i>Elephas maximus</i> )	3,625.0	1	240.0	621
Tree shrew ( <i>Tupaia glis</i> )	0.124	2	0.017	48
Marmoset ( <i>Hapale jacchus</i> )	0.269	2	0.060	146
Tamarin ( <i>Tamarinus</i> sp.)	0.400	2	0.063	140
Galago ( <i>Galago crassicaudatus</i> )	0.712	2	0.072	115
Squirrel monkey ( <i>Saimiri sciureus</i> )	0.717	1	0.095	187
Lemur ( <i>Lemur catta</i> )	1.246	1	0.240	144
Rhesus monkey ( <i>Macaca mulatta</i> )	5.9	1	0.485	164
Gibbon ( <i>Hylobates lar</i> )	5.3	1	0.400	240
Atbara baboon ( <i>Papio doquera</i> )	13.2	1	0.807	180
Drill ( <i>Mandrillus leucophaeus</i> )	21.4	1	0.480	190
Chacma baboon ( <i>Papio ursinus</i> )	22.4	1	0.861	187
Orang-utan ( <i>Pongo</i> sp.)	36.6	1	1.590	276
Chimpanzee ( <i>Pan</i> sp.)	42.7	1	1.858	227
Gorilla ( <i>Gorilla gorilla</i> )	84.4	1	2.000	259
Man ( <i>Homo sapiens</i> )	60.0	1	3.200	280

\* This is a summary of data from about 40 sources, details of which can be supplied on request.

expressed as Net Dietary-protein Calories per cent (NDpCal%)<sup>10</sup>.

Table 2 shows such values calculated for several of the species in Table 1. The mean index of growth rate at birth for the non-primates is 107, whereas that for the two primates is 45. The composition of the milk supply reflects the varying growth requirements of the foetuses. Thus there are species differences in the proportion of energy in the milks which is derived from crude protein (protein-Calories per cent), and the distinction between primates and non-primates becomes more striking when a correction is made for the differences in protein quality of the milks. Thus the NDpCal% of non-primate milks is in the region of 12.0 and that of the two primates is close to 8.0.

This evidence provides an explanation of the apparently lower levels of intake necessary to produce damage to the human foetus. This should be taken into account in the application of the results from experimental animals to the evaluation of protein and calorie requirements of man during pregnancy. The data presented in Fig. 2 show that it is a fundamental feature of the primate order that the metabolic stress of pregnancy is related to a smaller fraction of maternal body weight than in other mammals. Slow growth and a long development period are commonly

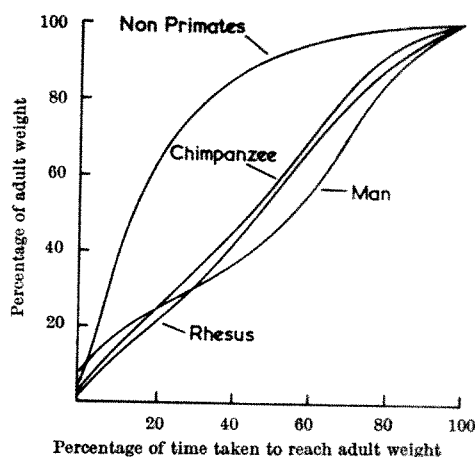


Fig. 3. Comparative growth of three primates, with a curve, taken from Brody<sup>9</sup>, representing all non-primate species.



Table 2. INDEX OF GROWTH RATE AT BIRTH AND NUTRITIVE VALUE OF MILK, FOR SEVERAL SPECIES

Species	Index of growth rate at birth	Protein value of milk	
	$\frac{W_{Birth}}{W_{9-12B}} \times 10^4$	Protein-calories per cent	NDpCal%
Rat	110	34	12.5
Guinea-pig	79	41	11.0
Rabbit	145	27	12.6
Dog	118	25	—
Pig	95	20	12.6
Cow	94	28	12.0
Orang-utan	42	9.4	7.5
Man	48	10.0	8.0

held to be characteristic only of the higher primates<sup>11</sup>, although very few examples of complete growth curves are available in the literature. The slow growth (see Fig. 1) of the fetuses of all primates suggests, however, that a long maturation period may be a common characteristic of the whole order. This observation, together with the larger

cephalization coefficients demonstrated by Stahl<sup>12</sup>, may be significant in the context of the group learning characteristics of primates.

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## Autonomy of H-2 Genes in Individual Immunocytes

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Antibody forming cells express one of two codominant alleles in the case of allotypes and perhaps of other traits connected with the formation of immunoglobulin. That this property does not apply to all genetic systems of these cells is demonstrated by the presence of both parental histocompatibility antigens in heterozygous immunocytes.

SINGLE antibody forming cells do not express all the genetic potentialities of immunoglobulin synthesis, but each one expresses some of the information available in the organism. This results in a cellular mosaic such that each of the different antibody molecules present in the circulation (characterized by their class of heavy chain; type of light chain; allotype and specificity) corresponds to a cell or cell line<sup>1-3</sup>. This phenomenon may have a selective advantage; it makes possible considerable genetic variation, and at the same time avoids the danger of mounting asymmetrical (heterologous) antibody molecules<sup>4</sup>.

The mechanism by which the phenotype of immunocytes is restricted is not known, but it seems to be acting at two different levels. There is (a) a choice of expressing one of a series of genes on the same chromosome, as in the case of three classes of mouse heavy chain, or of four human  $\gamma$ G sub-classes which are coded by closely linked genes<sup>5,6</sup>, and (b) a choice between two alleles codominant at the level of the organism, as in the case of the allotypes in man, rabbit and mouse, where each heterozygous cell expresses the allele of one or the other parent<sup>4</sup>.

While (a) as a selective activation or repression of a portion of the genome is a general if unexplained phenomenon in differentiation, (b) seems to be more peculiar: in mammals a cellular mosaic has only been found in relation to sex linked traits<sup>7,8</sup>. In the case of immunocompetent cells, we can envisage mechanisms which range from the exclusion of single alleles to the inactivation of chromosomal portions or entire chromosomes, similar to the inactivation of one of the X chromosomes of the female somatic cell<sup>9</sup>. Another possibility is that immunocytes contain one or more pairs of autosomes inherited from the same parent as a result of atypical somatic segregation<sup>10</sup>. This reasoning is based on the discovering in deer-mice (*Peromyscus*), heterozygous for a chromosomal marker, of some diploid cells with a reconstituted homozygous karyotype. These cells were found only in the spleen.

Until the chromosomes of identified antibody forming cells are analysed, the possibility of such genomic segregation giving rise to homozygous cells in the immune system cannot be tested. This explanation postulates, however, the involvement of several autosomes, because otherwise the inactivation of non-linked genes (determinants of H and L chain allotypes) in multiple heterozygotes cannot be explained.

One way to investigate the mechanism of allelic exclusion during differentiation of the antibody forming cell is to examine the phenotypic expression of other genetic markers which are unrelated to antibody formation and expressed at the cellular level. We have investigated the antigenic products of the H-2 locus and particularly the autonomy of their expression, in immunocytes of heterozygous origin. Both *in vivo* and *in vitro* experiments were carried out.

We used an *in vivo* transfer system which has been standardized in our laboratory<sup>11</sup>. Donors were *A/Sn*, *A.SW* and (*A/Sn*  $\times$  *A.SW*)  $F_1$  mice, immunized 6-8 weeks before the experiment with 5 mg of human serum albumin (HSA) in Freund's adjuvant which was injected subcutaneously. To sensitize recipients against prospective donors,  $2 \times 10^7$  spleen cells of the prospective donor's genotype were injected intraperitoneally 10 days before the transfer. This has been shown previously to be effective; the sensitized state thus obtained is highly radioresistant<sup>12</sup>. One hour before transfer the recipients were exposed to 450 r. of X-rays. A fixed dose of  $2 \times 10^7$  donor spleen cells was injected intravenously, followed by 10  $\mu$ g of human serum albumin administered intraperitoneally. This dose of antigen is optimal to elicit a secondary response from the transfer cells, while causing no primary response in an intact animal.

The recipients were bled 7, 14 and 21 days after transfer. Their serum was titrated for human serum albumin-binding antibodies by a modified Farr technique<sup>11</sup>. In

these conditions the survival of transferred donor cells was tested by determining their ability to produce a secondary response against human serum albumin. This system is very sensitive<sup>12</sup> and the parental strains used, the coisogenic lines established by Snell on an *A/Sn* background<sup>13</sup>, differ antigenically only in the products of the H-2 locus, and so it was possible to test for the presence of both parental antigens on  $F_1$  immunocompetent "precursor" cells. These cells were transferred into both cross immunized parental lines (*A/Sn* anti *A.SW* and *A.SW* anti *A/Sn*) and as a positive control for optimal growth into syngeneic  $F_1$  recipients (Table 1c). As negative controls, to check the efficiency of immunoselection, immune parental donor cells were also injected into syngeneic recipients and into specifically sensitized opposite parents (Table 1a and b). The results showed that (a) all cells were able to survive and function in syngeneic recipients, (b) the selective system was efficient, and (c) precursor cells of  $F_1$  origin were unable to function in either parental mice, where they produced less than 1/400 of the antibody made in the syngeneic recipient (the difference in log titres was greater than 2.672). Thus precursor cells seem to express their full genotype as far as transplantation antigens are concerned.

Table 1. SUMMARY OF *in vivo* EXPERIMENTS

	Donor cells	Recipients	Log ABC
(a)	<i>A/Sn</i> anti HSA	<i>A/Sn</i>	1.123 ± 0.25
	<i>A/Sn</i> anti HSA	<i>A.SW</i> anti <i>A/Sn</i>	< -1.0
(b)	<i>A.SW</i> anti HSA	<i>A.SW</i>	1.700 ± 0.30
	<i>A.SW</i> anti HSA	<i>A/Sn</i> anti <i>A.SW</i>	< -1.0
(c)	<i>A/Sn</i> × <i>A.SW</i> anti HSA	<i>A/Sn</i> × <i>A.SW</i>	1.672 ± 0.16
	<i>A/Sn</i> × <i>A.SW</i> anti HSA	<i>A.SW</i> anti <i>A/Sn</i>	< -1.0
	<i>A/Sn</i> × <i>A.SW</i> anti HSA	<i>A/Sn</i> anti <i>A.SW</i>	< -1.0

Peak titres of secondary response of  $10^7$  pre-immunized spleen cells, transferred into irradiated syngeneic or to presensitized allogeneic recipients.

The titres are expressed as mean log ABC (antigen binding capacity) of groups of 8-10 mice ± 95 per cent confidence limits.

To test the expression of H-2 antigens on the antibody forming cells themselves the cytotoxicity of selected antisera was tested against homozygous and heterozygous target cells, capable of producing zonal haemolysis of sheep red blood cells in gel. Direct (A) and indirect (B) experiments on *A/Sn* and (*A/Sn* × *A.CA*)  $F_1$  target cells were performed, using the following antisera: *A/Sn* anti *A.CA*; *A.CA* anti *A/Sn*; anti 3 from (*A.CA* × *C57BL*)  $F_1$  mice immunized with *A.SW* cells; anti 4 (contains possibly anti 10 and anti 12) from (*C3H* × *C57BL*)  $F_1$  mice immunized with *DBA/2* cells; anti 5 (contains possibly anti 28 and anti 29) from *A.CA* mice immunized against *S2Y*, a tumour of *ABY* genotype; anti 8 (contains also anti 10 and anti 13) from (*A.SW* × *C57BL*)  $F_1$  mice immunized with *DBA/2* material; anti 9 from (*A* × *A.SW*)  $F_1$  mice immunized with *A.CA* material; anti 11 (contains possibly anti 1 and anti 25) from (*DBA/2* × *C57BL*)  $F_1$  mice immunized with *C3H* cells.

In (A) experiments the test mice were injected intraperitoneally with  $4 \times 10^8$  sheep erythrocytes. Between 3 and 5 days later they were killed and suspended spleen cells were mixed with fluid agarose (final concentration 0.65 per cent in Eagle's medium) in a water bath at 42° C and sheep erythrocytes ( $2 \times 10^5$ /ml.). The average concentration of spleen cells was  $5 \times 10^5$ /ml., 0.8 ml. of the mixture was plated as a thin layer on Falcon tissue culture dishes of diameter 5 cm. The plates were incubated for 30 min at 37° C in a humid atmosphere saturated with carbon dioxide. Two variations of the remaining procedure were then followed. While the results were comparable one method was more economical on antiserum consumption, and the other gave a slightly better survival of the negative control cells.

In the first method, guinea-pig complement was added (dilution 1:15, 1 ml./plate) and the plates were reincubated for 30 min. After this period the haemolytic plaques were visible. A small quantity of antiserum or control serum (estimated as a fraction of a  $\mu$ l.) was layered on the top of each plaque by means of a fine Pasteur pipette. The plates were incubated for 20 min, after which complement was added again in a concentration of 1:5. After 30 min the complement was washed away, the plates were rinsed twice with Eagle's medium and exposed for 15 min to 1 ml. of phosphate buffered saline containing 10  $\mu$ g of fluorescein diacetate. After a final rinse they were ready to be examined at the microscope.

In the second method, the plates were first exposed to the fluorescein diacetate substrate for 15 min. Antiserum 1:2 (~20  $\mu$ l./plate) was then spread on the central part of the agarose surface with a Pasteur pipette. After 20 min of incubation 1 ml. of guinea-pig complement 1:5 was added, and the plates were incubated for 30 min. This had the double effect of developing the haemolytic plaques and of producing a cytotoxic effect on the nucleated cells which had been attacked by the antiserum.

The principle of the fluorochromatic cytotoxic test has been described by Celada and Rotman<sup>14</sup>. The capacity of uptake and retention of the hydrolysed substrate depends on the integrity of the cell membrane<sup>15</sup>. Living cells exhibit intense diffuse intracellular fluorescence. Damage to the cell membrane by, for example, antibody and complement leads to rapid loss of fluorescence by rapid diffusion of the small fluorescein molecule. In the present test, the single plaque forming cells, located in the geometrical centre of the zone of haemolysis, were examined in a darkfield microscope under blue light; if they exhibited fluorescence they were scored as "living", and if they failed to do so they were scored as "dead".

Where the identification of the central cell was uncertain because of clumping or of presence of several cells in the central area, plaques were not scored. These plaques amounted to about 20 per cent of the total examined.

The first column of Table 2 shows the results obtained by the fluorochromasia test. Specific antisera directed against the whole H-2 complex, or against certain H-2 factors only, killed a large percentage of the plaque forming cells in every case, both when *A/Sn* and when *A/Sn* × *A.CA* cells were used as targets. Whenever a direct comparison was done, there was no significant difference between the susceptibility of parental and  $F_1$  cells to the same antiserum. All oligofactorial antisera which were tested on  $F_1$  cells only (anti 3, anti 4, anti 5 and anti 9) gave a very high specific killing (59, 75, 91 and 87 per cent respectively).

The indirect (B) experiment was done as follows. Target spleen cell suspensions (0.3 ml.), derived from mice pre-immunized with sheep red blood cells, were incubated for 15 min in small tubes with 0.02 ml. of antiserum. Complement (1:4, 0.1 ml.) was added to the mixture and the tubes were reincubated for 30 min. They were then centrifuged, the supernatant discarded and the pellet resuspended in 0.1 ml. of Eagle's medium. These suspensions were plated by the usual technique described by Jerne<sup>16</sup>. The number of haemolytic plaques obtained with the suspensions treated with antiserum was expressed as a percentage of the number found with a suspension treated with the corresponding control serum.

The second column of Table 2 shows the results of this test. The cytotoxic effect of the reciprocal anti *A/Sn* and anti *A.CA* antisera as well as of the oligospecific sera was at least as high against heterozygous as against homozygous *A/Sn* cells.

Comparison of the first and second columns of Table 2, that is, of the direct and indirect cytotoxicity test on antibody forming cells, shows that the results are superimposable both quantitatively and qualitatively, and therefore

Table 2

1: Fluorochromasia assay on PFC					2: Inhibition of plaque formation						3: Cytotoxicity on lymphoid cells			
Target cells: A/Sn					(a) 15% serum			(b) 40% serum						
Sera	Living PFC	Dead PFC	% Survival	% Specific killing	No. of plaques	% Living	% Specific killing	No. of plaques	% Living	% Specific killing	Living cells	Dead cells	% Survival	% Specific killing
Normal serum	11	2	85	0	—	—	—	—	—	—	50	17	75	0
A anti A.CA	6	1	86	0	69 49	100	0	42 46	100	0	50	23	68	0
														100
A.CA anti A	3	15	16	80	15 20	29	71	4 3 5	9	91	0	50	0	97
Anti 3	—	—	—	—	—	—	—	17 13	15	65	1	50	2	85
Anti 4	—	—	—	—	—	—	—	—	—	—	7	55	11	85
Anti 5	—	—	—	—	—	—	—	3 5	9	91	0	46	0	100
Anti 8	—	—	—	—	—	—	—	32	73	27	1	53	2	97
Anti 9	—	—	—	—	—	—	—	53	120	—	—	—	—	—
Anti 11	2	20	9	89	—	—	—	11 9	12	88	0	50	0	100
Target cells: A × A.CA														
Normal serum	27	6	88	0	273 243	100	0	57 64	100	0	46	23	68	0
A anti A.CA	5	60	13	84	120 133 130 116	48.5	51.5	14 3	24	76	10	44	19	72
A.CA anti A	2	59	4	96	35 55	17.5	82.5	4 6	8	92	1	53	2	97
Anti 3	4	8	33	59	—	—	—	18 22	20	67	2	52	4	94
Anti 4	2	8	20	75	—	—	—	9 12	18.3	81.7	26	26	50	27
Anti 5	1	14	7	91	—	—	—	—	—	—	2	51	4	94
Anti 8	—	—	—	—	—	—	—	55 18	60	40	3	46	6	91
Anti 9	1	9	10	87	—	—	—	4 9	11	89	—	—	—	—
Anti 11	2	10	17	79	—	—	—	4 8	10	90	0	53	0	100

The "% specific killing" was calculated by considering the fraction of dead PFC found in the negative controls (averaging 16 per cent) as non-specific, and correcting the % killing in the experimental assays accordingly.

corroborate each other. Comparison of these combined data with the titres obtained by testing with the same antisera against lymph node suspensions in the ordinary cytotoxic test (third column, Table 2) shows also a general agreement. This means that cells actually producing antibodies are as sensitive to cytotoxicity as the lymphoid cell population as a whole.

The present experiments have shown that the adoptive transfer of an immune response by  $F_1$  hybrid cells to each of the coisogenic parental lines is totally inhibited by the homograft reaction. Plaque forming cells of  $F_1$  hybrid origin were killed *in vitro* by antisera against each parent's H-2 antigens, and at the same rate as the corresponding parental cells.

This means that all  $F_1$  memory cells, that is, the sensitized precursors of the antibody forming population, express H-2 factors of both parental strains and can therefore be recognized as foreign by either one, and that this is also true for cells engaged in antibody production. This second conclusion was not implicit in the first one, because there is no definite evidence that memory cells and antibody forming cells are identical, although antibody producers seem to derive from memory cells through mitosis and differentiation.

The fact that a cell of  $A/Sn \times A.CA$   $F_1$  genotype is killed both by anti A/Sn and by anti A.CA antibodies does not prove that all factors of the H-2<sup>a</sup> and the H-2<sup>f</sup>

complex are expressed on its surface; the presence of part of them would probably suffice to cause a high susceptibility to antibody and complement. It follows that the expression of antigens may still be restricted to part of the antigens of both parental types. This reasoning is more difficult to defend, however, in the case where the PFC-inhibition test was carried out at a low serum concentration (see Table 2, column 2a): the homozygous cells should be differentially favoured in these conditions, but this was not the case. It is even more improbable when the results obtained with oligospecific antisera are considered.

We tentatively conclude that both parental genes are active in the  $F_1$  immunocytes also at the single factor level, with the reservation that the "monospecific" sera might contain antibodies directed against some other weak H-2 factor (for example, 10, 13, 25, 28, 29) in addition to the one which appears on their label.

These conclusions have an indirect bearing on the mechanism of the phenotypic restriction operating at the level of immunoglobulin synthesis in single cells. They exclude some of the proposed or possible mechanisms for this phenomenon, for example, that the antibody forming cell is, or behaves like, a completely hemizygous cell, or that it carries several chromosomes inactivated at random. They are instead compatible with the hypothesis that allelic exclusion or, conversely, selective activa-



tion affects loci concerned with antibody synthesis in a specific way, while the other genes of the same cell enjoy normal expression.

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## Steroid Hormones in Sick-cell Disease

by

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Progesterone, testosterone and nor-androstenolone can inhibit the development of sickling in sickle cell disease, which they seem to do by stabilizing the membrane of the erythrocyte. This could be medically useful.

THE clinical features of sickle cell anaemia fall into two classes, those arising from the short survival of the erythrocyte, comparable in many respects with the features of other haemolytic anaemias, and those caused by local obstruction of the micro-circulation from impacted deformed sickle cells, with consequent formation of infarcts. The sickling infarcts may occur at various sites, notably the spleen, parts of the skeleton, the lungs and the central nervous system, and produce painful and disabling effects.

When the structural defect of the haemoglobin molecule in sickle cell anaemia was recognized, after the demonstration by Pauling and his colleagues<sup>1</sup> of a difference in electrophoretic mobility of the abnormal haemoglobin S as compared with normal haemoglobin A, and the later definition of the molecular abnormality in haemoglobin S as replacement of a glutamic acid residue in the sixth position in the  $\beta$ -chain by a valine residue<sup>2,3</sup>, there was great interest in determining how this change actually resulted in disease, and hope that means might be found to counteract the process and achieve an effective treatment.

There seems little doubt that the sickling deformation of the erythrocytes results from intracorporeal formation of long, unidirectional haemoglobin crystals or tactoids, which can be observed microscopically in affected cells before sickling. Perutz and Mitchison showed that deoxygenated haemoglobin S is more insoluble than deoxygenated solutions of haemoglobin A (ref. 4), and Harris demonstrated tactoid formation in deoxygenated solutions of haemoglobin S (ref. 5). Certain hydrocarbons (such as methane, ethane and propane) reverse the process of gel formation and from a consideration of the mechanism of this effect Murayama<sup>6</sup> proposed that hydrophobic bonding between the N-terminal valine and the valine in the sixth position of haemoglobin S was responsible for the sickling process. Satisfactory models for the sickling phenomenon could be constructed on this basis. Efforts to reverse the sickling process *in vitro* and *in vivo* include those of Lewis<sup>7,8</sup>, who made several attempts to inhibit sickling with promazine alone or in combination with dapsone, and reported a prolongation of red cell survival in patients suffering from sickle cell anaemia and given promazine.

Neither of these agents has been shown to inhibit sickling consistently *in vitro*, and there has never been a demonstration of inhibition of sickling after *in vivo* administration of any agent. Kosower *et al.*<sup>9</sup> have recently used the N-carboxy-anhydride of glutamic acid at pH 10 to increase the solubility of deoxyhaemoglobin S *in vitro* and are working to find suitable conditions for bringing about similar "protein transformations" *in vivo*.

During an investigation of possible differences in the mitotic cycle of bone marrow cells treated with testosterone as compared with those of untreated marrow an effect of testosterone on the sickling process was found. Two prescription bottles with 6 ml. each of a growth medium consisting of 20 per cent T.C. 199, 15 per cent horse serum, 15 per cent pooled AB serum and 50 per cent Hanks solution were inoculated with bone marrow aspirated from a sickle cell haemoglobin carrier. One bottle had 1  $\mu$ g/ml. of testosterone propionate added to it and both contained 0.5  $\mu$ c./ml. of tritiated thymidine. The first set of Romanowsky stained smears made from the untreated bottle at 3 h showed that a large proportion of the erythrocytes were needle-shaped (Fig. 1), whereas those from the bottle treated with

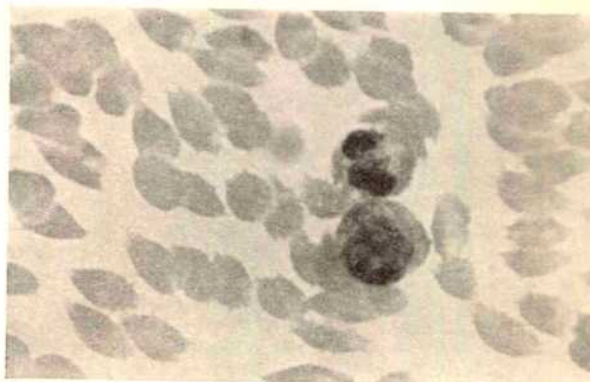


Fig. 1. Smear of 3 h marrow culture, incubated without testosterone, from a subject with Hb AS; lanceolate erythrocytes are conspicuous.



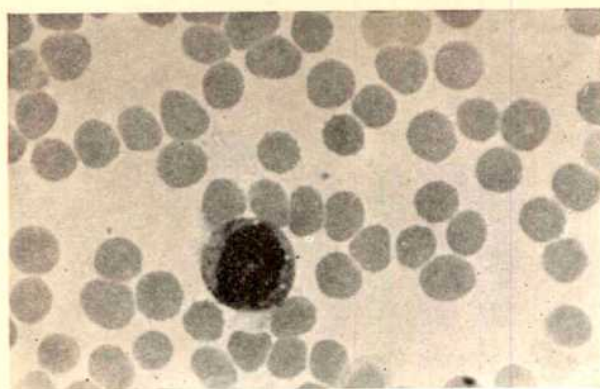


Fig. 2. Preparation similar to that in Fig. 1, except that testosterone was added to the culture medium; the erythrocytes remain normocytic.

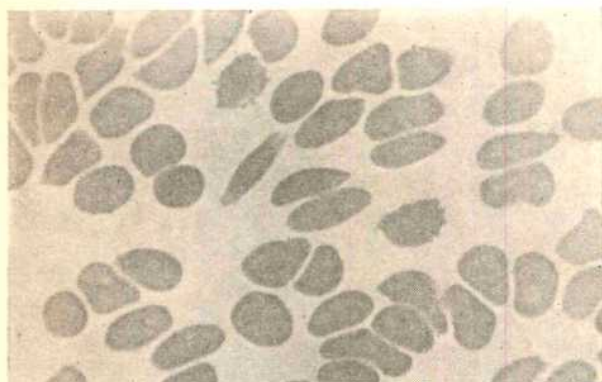


Fig. 3. Smear made from peripheral blood of a subject with Hb AS after 36 h incubation without testosterone. Many erythrocytes are deformed.

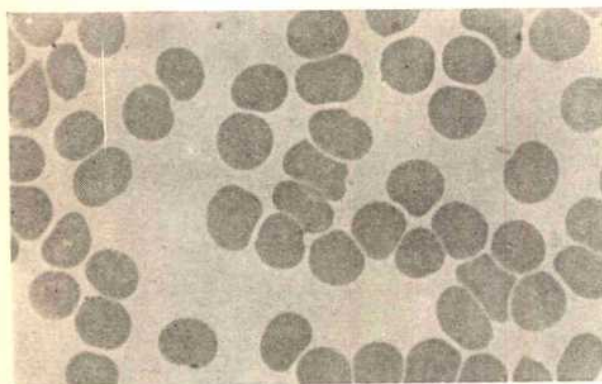


Fig. 4. Preparation similar to that in Fig. 3, except that testosterone was added to the incubate; the erythrocytes remain normocytic.

testosterone retained a round shape (Fig. 2). The only difference between the two bottles was the presence of testosterone in one and not in the other, and so it seemed possible that the oxygen content of the environment was lowered in the bottles, causing a sickling tendency, which was somehow counteracted by the presence of testosterone in the treated bottle. Similar samples were obtained from each bottle, spun down and the cells subjected to the sickling test<sup>10</sup> (in which the oxygen in the environment is removed with 2 per cent sodium metabisulphite). While there was 100 per cent sickling within a few minutes in the untreated preparation, less than 5 per cent of the treated preparation of cells sickled after 1 h. Smears were made from the two culture bottles at intervals and the difference observed here persisted for 51 h. The culture experiments

were repeated using blood instead of the marrow. The same effect of added testosterone was observed, although it took 36 h for sickling to appear in the control culture. This was presumably because peripheral blood cells metabolize less actively than marrow cells and require more time to develop acidotic conditions (needed for sickling to take place) in the culture (Figs. 3 and 4).

Sickle cell suspensions in 2 per cent sodium metabisulphite (using haemoglobin S fingerprick blood) were then made in wells formed by sealing coverslips to both sides of a drilled microscope slide. After complete sickling had been observed, 1  $\mu$ g/ml. of testosterone, primolut (17-OH progesterone), durabolin (nor-androstenedione phenyl propionate), prednisolone, oestrone and T.C. 199 respectively were added to different preparations. Identical operations were carried out on a sample of haemoglobin A blood. Reversal of sickling was obtained in the preparations treated with progesterone, testosterone and nor-androstenedione, and was maximal (virtually 100 per cent) at 24 h. The cells remained sickled in the preparations containing prednisolone, oestrone and T.C. 199. The haemoglobin A preparations showed no obvious changes during the whole procedure (Figs. 5, 6, 7 and 8).

An experiment was then carried out to determine whether the *in vitro* effects of testosterone and progesterone were paralleled by *in vivo* effects. Ten milligrams of testosterone propionate in oil was injected intramuscularly into a male sickle cell carrier; 24 h afterwards the sodium metabisulphite test resulted in prompt sickling in erythrocytes obtained from the subject, but 4 h later the same procedure produced less than 5 per cent sickling in several preparations. This effect persisted for 2 days, after which the sodium metabisulphite test again became promptly positive. Two weeks later, the whole experiment was repeated in the same subject, when complete inhibition

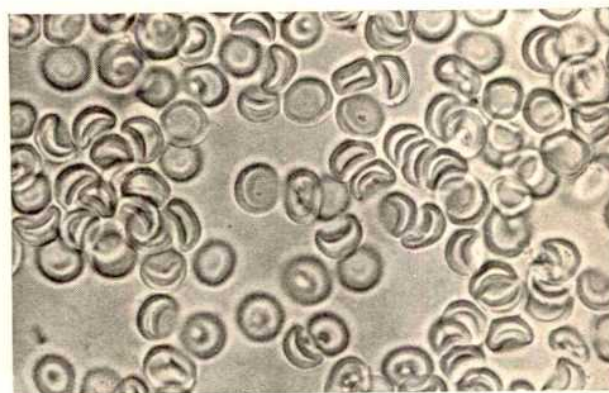


Fig. 5. Phase contrast photograph showing sickling produced by sodium metabisulphite; erythrocytes from a subject with Hb AS. Addition of T.C. 199 produced no change.

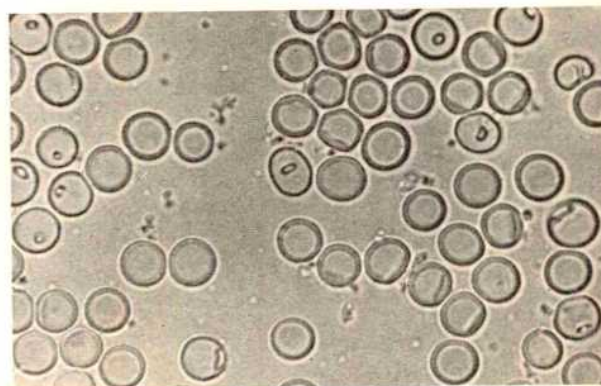


Fig. 6. A similar preparation to that in Fig. 5, but showing reversal of sickling after the addition of progesterone.



was first observed 48 h after the injection. This seems to indicate some variation in the rates of absorption of this oily steroid preparation from an intramuscular site of injection.

The investigations of the sickling phenomenon after administration of testosterone and progesterone were next repeated using patients with sickle cell disease. Primolut (17-hydroxy progesterone) in a dose of 12 mg was injected intramuscularly into a 41 yr old female with sickle cell haemoglobin C disease and sodium metabisulphite tests were carried out at intervals on fingerprick blood. Two 4 yr old males with sickle cell disease were investigated similarly, using 5 mg of testosterone propionate in place of the progesterone preparation. The results are shown in Table 1.

Table 1

Patients		Percentage inhibition of sickling			
		2 days	3 days	4 days	7 days
1 Female, aged 41	Hb SC	50-70	—	95	—
2 Male, aged 4	Hb SS	70	95	—	—
3 Male, aged 4	Hb SS	70	95	—	80

Osmotic fragility tests (using buffered saline solutions)<sup>11</sup> were carried out on blood from patient 3 in Table 1, his mother (Hb AS), a male carrier (Hb AS) and his daughter (Hb AS), two normal controls and a patient with congenital spherocytosis. The results are shown in Fig. 9. It would appear that the parenteral administration of testosterone did not affect the osmotic fragility of the red cells.

Radioactive chromium (chromium-51) studies were also carried out on patient 3 before and during parenteral administration of testosterone. The method used was that described by Dacie and Lewis<sup>11</sup>, the cells being washed twice with 1.2 normal saline. Administration of 5 mg of testosterone before and 5 mg during the study of red cell

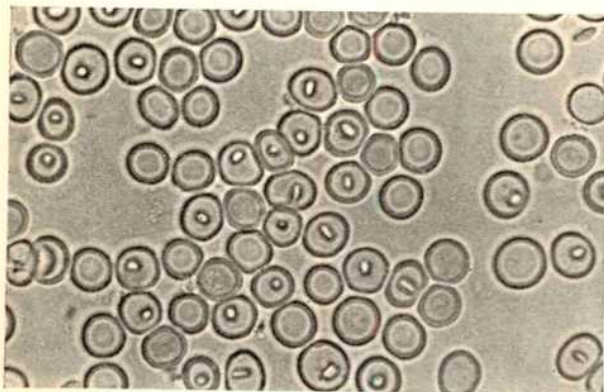


Fig. 7. A similar preparation to that in Fig. 5, but showing reversal of sickling after the addition of testosterone.

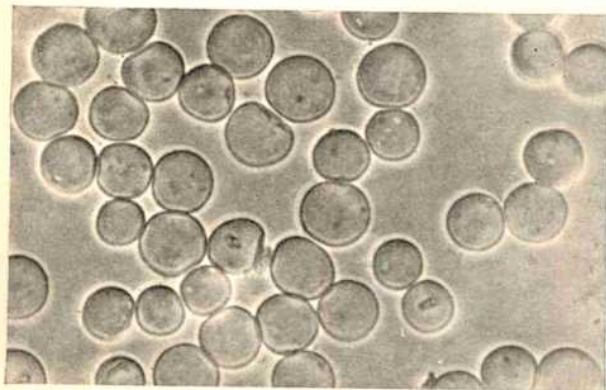


Fig. 8. A control preparation, treated similarly to that shown in Fig. 7, but using erythrocytes from a normal subject (Hb AA).

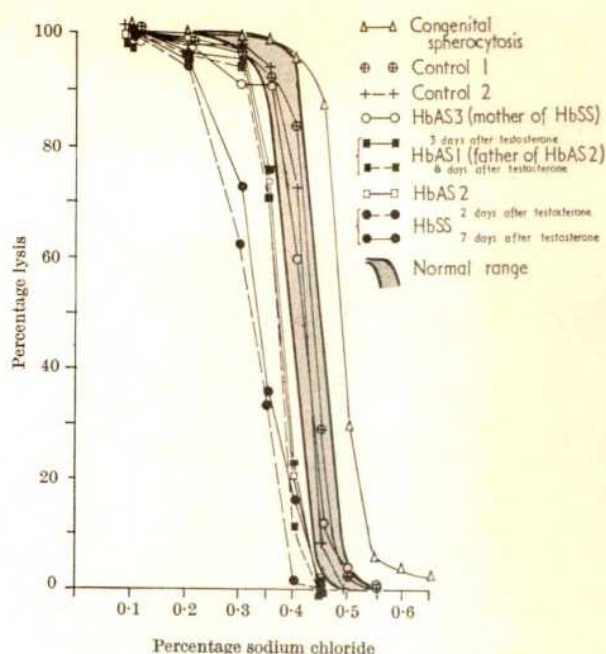


Fig. 9. Erythrocyte osmotic fragility curves from subjects with Hb SS, Hb AS or Hb AA, as described in the text.

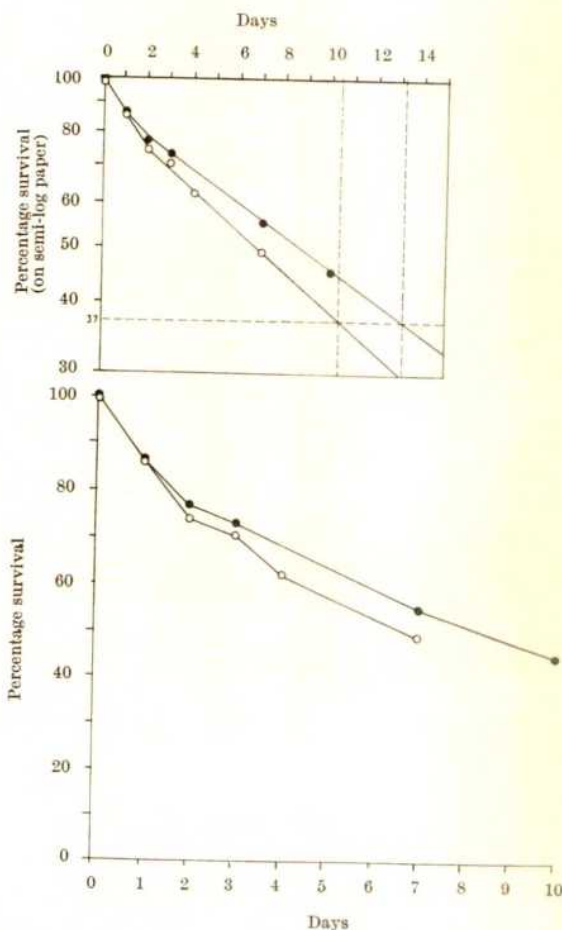


Fig. 10. Chromium-51 erythrocyte survival curves of Hb SS subject before and after treatment with testosterone. ●, Before treatment with testosterone (5 mg testosterone given after the chromium-51 count at 3 days); ○, after treatment with testosterone (5 mg testosterone given 3 days before start of counts and 5 mg given after chromium-51 counts at 2 days). Mean cell life: 13.1 days (before), 10.4 days (after). Chromium-51  $T_{1/2}$ : 8.5 days (before), 6.8 days (after).

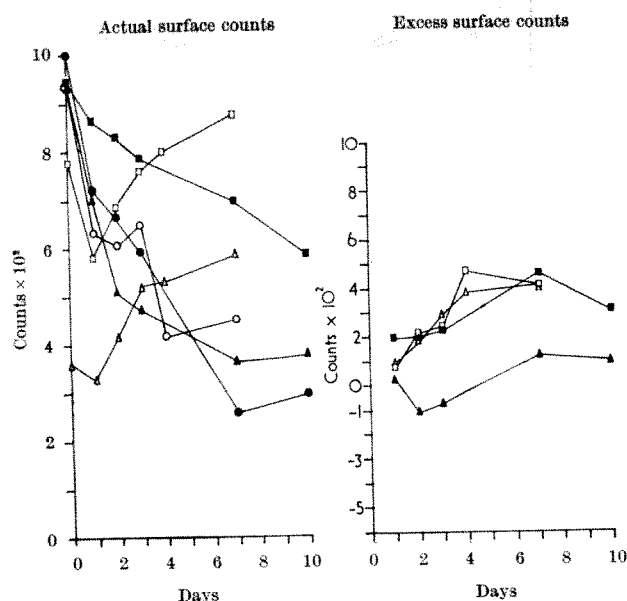


Fig. 11. External scintillation counts of chromium-51 during erythrocyte survival studies on a subject with Hb SS before and after treatment with testosterone. Before testosterone: ●, heart; ▲, spleen; ■, liver. After testosterone: ○, heart; △, spleen; □, liver.

survival decreased survival slightly (Fig. 10). External scintillation counting showed that administration of testosterone was followed by some degree of erythrocyte sequestration in the spleen in addition to the sequestration observed in the liver before administration of testosterone (Fig. 11).

It seems clear that there is an effect of testosterone and similar steroids on the sickling process *in vitro* and *in vivo*, although this effect did not enhance erythrocyte survival in the single case so far investigated in this respect. The mechanism of the action of testosterone remains to be elucidated. The sequence of events observed in the experiments demonstrating reversal of sickling shows similarities to the changes noticed when small defects were caused in the membrane of the sickle cell erythrocyte after micro-incision by a laser beam<sup>12</sup>. In addition, the time required for the testosterone to produce the effect in the first experiment with the bone marrow is probably too short for any important intracellular change to have taken place. This would suggest that the effect of this

group of steroids might be on the membrane, and the work of De Venuto<sup>13</sup> suggests that *in vitro* progesterone probably only gets adsorbed on the membrane. Furthermore, crystal like appearances were observed in the wet preparations of sickled cells treated with testosterone and progesterone under phase-contrast (Figs. 6 and 7), which suggests that the crystals of haemoglobin S are still being formed, but they fail to deform the membrane. Subject to the results of future investigation, it seems likely therefore that the effect of these steroids is to stabilize the erythrocyte membrane.

Blood transfusions and administration of alkali and supportive measures currently form the standard treatment for sickle cell disease, and have indeed greatly contributed to decrease in morbidity. None of the alternative procedures proposed, including chronic carbon monoxide poisoning<sup>14</sup>, oxidation to methaemoglobin S (ref. 15), or combined promazine and dapsone therapy<sup>7,8</sup>, has been shown to alter the general course of the disease. While the experiments reported here do not suggest that treatment with testosterone or progesterone would affect the haemolytic component, there is a possibility worth clinical exploration, that sickling crises might be halted by prompt administration of the appropriate steroid or perhaps prevented by low-dosage maintenance therapy.

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## Metabolism of Labelled Carcinogenic Hydrocarbons in Rats

by

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Polynuclear hydrocarbons given by stomach tube induce mammary tumours. Their absorption, distribution and excretion have been studied. Prolonged retention in ovaries and adrenals may reflect the fact that both these organs produce steroid hormones which stimulate mammary growth.

WE have been studying the influence of the pituitary gland on growth<sup>1-4</sup> and on various organs and tissues of the body<sup>5,6</sup>. Tissues studied have included hormone-dependent mammary tumours induced in rats by the intragastric administration of 3-methylcholanthrene and

7,12-dimethylbenz[a]anthracene<sup>7-10</sup>, methods of experimental tumour production pioneered by Huggins<sup>11-13</sup>. Little is known about the fate of these compounds when given by this route, and so we decided to study the absorption, distribution and excretion of four radioactively

labelled polynuclear hydrocarbons, of which three are potent carcinogens and the fourth non-carcinogenic.

Sixty-one young virgin female Sprague-Dawley rats and nine older lactating animals were used. They were maintained on Diet 41B (MRC or Oxoid) and given water freely. The polynuclear hydrocarbons used were: 3-methylcholanthrene (3-MC), 7,12-dimethylbenz[*a*]anthracene (DMBA) and dibenz[*a,h*]anthracene (DahA), which are all carcinogenic, and dibenz[*a,c*]anthracene (DacA) which is closely related but non-carcinogenic. All were labelled either with 14-carbon (specific activity 1–10 mc./mmole) or with a general tritium label (specific activity 0.1–5.0 c./mmole). In each animal a single dose from 5 µg to 2 mg of one of the polynuclear hydrocarbons, dissolved in 0.5 ml. of sesame oil (R. C. Treatt, London), was administered by stomach tube, after food had been withheld for 18 h. This dose is too small to induce mammary tumours, and because we wished also to learn whether the carcinogen is concentrated in tumour tissue, nine of the rats were given 10 mg of 3-MC labelled with tritium three times a week for 7 weeks. This routine reliably induces mammary tumours<sup>14–16</sup>.

Five series of experiments were undertaken: in the first and second experiments, the rats were left intact so that there was no interference with the natural processes of absorption, distribution and excretion of the hydrocarbon, and in the third, fourth and fifth experiments, indwelling catheters were inserted for the collection of various body fluids.

In the first series of experiments, thirty-four rats were kept for periods varying from a few hours to several months after the administration of the hydrocarbon. These animals included the lactating rats and the rats which had been given a tumour-inducing dose of carcinogen. All the rats were finally anaesthetized with ether, the lactating rats were milked and all animals were heparinized and killed by bleeding out. The blood was immediately centrifuged and the separated plasma was frozen. At autopsy, samples were taken in duplicate from most of the tissues of the body, one sample being quickly frozen for assay of the radioactivity and the other fixed in buffered formal-calcium for embedding in paraffin wax and sectioning for autoradiography.

In the second series of experiments, five rats were kept in metabolism cages and urine and faeces were collected for 4 days after administration of the hydrocarbon. At autopsy, blood and tissues were taken as in the first series.

In the third series of experiments, twenty rats were anaesthetized with ether and one end of a polythene catheter, filled with heparinized saline and plugged, was passed into the inferior vena cava by way of the femoral vein, the other end being brought out through the skin of the back. While the rat was still anaesthetized the hydrocarbon was given by stomach tube. The animal was then placed in a restraining cage<sup>17</sup> that allowed normal eating and drinking but did not permit the eating of faeces. After recovery from the anaesthetic, blood samples were taken at regular intervals. The experiments lasted for 1 to 4 days. The procedures at autopsy were as described for the first series of experiments.

In the fourth series of experiments, six rats were used. The procedures were similar to those in the third series, except that in addition to the venous catheter a polythene catheter was inserted into the cisterna chyli (Fig. 1) and exteriorized for the collection of lymph from the gastro-intestinal tract<sup>18</sup> and an indwelling urethral catheter was also inserted. Lymph and urine samples were collected serially in small polythene vials, which were changed at regular intervals when blood samples were also taken. The faeces were collected. Autopsies were carried out as described above.

In the fifth series of experiments, five rats were used. The procedures were similar to those in the fourth series except that, instead of lymph, bile was collected from a catheter placed in the common bile duct. (To maintain a

normal rate of bile secretion in a rat with a bile fistula, 50 mg of sodium glycodeoxycholate was given in aqueous solution at the same time as the hydrocarbon.)

Radioactive assays were carried out with a liquid scintillation counter. Tissues or fluids containing protein were extracted with chloroform-methanol (1:1) and faeces with hot ethanol. Fluids free of protein were assayed directly. Additional samples or extracts were subjected to thin-layer chromatography on silica gel in benzene, and the chromatograms were scanned by means of an 'Actigraph Model III' radiochromatogram scanner (Nuclear Chicago) and by liquid scintillation counting of the material eluted from separate segments.

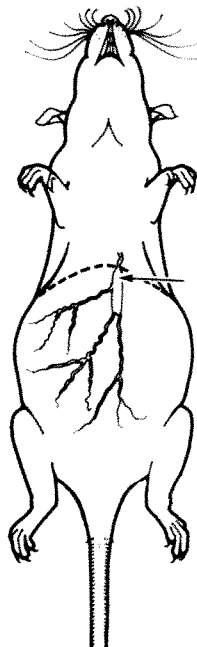


Fig. 1. Diagram to show site (arrow) where catheter was inserted into cisterna chyli for the collection of gastro-intestinal lymph.

The results of the experiments are considered in three stages. During the first stage, lasting as long as 24 h, much radioactive material was found in the intestinal contents. The amount of radioactive material in the blood plasma rose steadily after administration of the dose of the hydrocarbon, the time of the peak level varying in different animals from 4 to 24 h (Fig. 2). Radioactivity appeared in the gastro-intestinal lymph within about 1 h of administration of the dose (Fig. 3) and throughout the first 24 h the concentration of radioactivity in the lymph continued to be considerably greater than that in the blood plasma (Fig. 4). The lymph was visibly milky during the time when it showed a high concentration of radioactivity. Most of the radioactivity was present in the fat droplets of the lymph, and chromatography suggested that the material was largely unchanged hydrocarbon. The total amount of lymph collected during the first 24 h usually contained radioactive material amounting to about 5 per cent of the dose administered. The bile (Fig. 5) and the urine first showed the presence of radioactive material about 1 h after administration, the concentration in both rising for some hours; the amount in the urine was always less than that in the bile. When the concentration of radioactive material in the blood plasma was near its peak value a similar concentration was present in most of the tissues examined. Tissues, however, such as the liver, kidney, body fat and brown fat, contained rather more radioactive material, while other



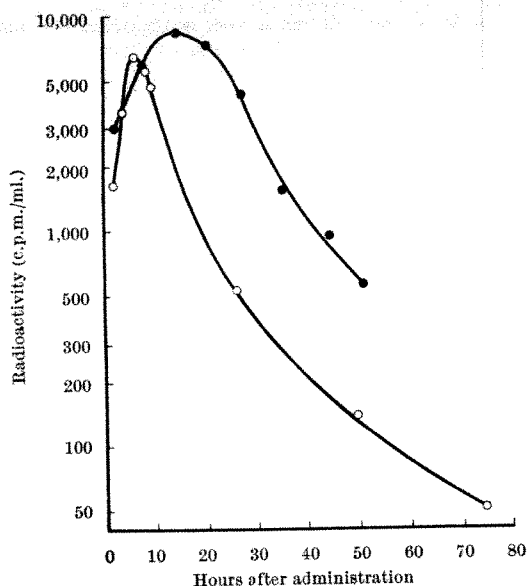


Fig. 2. Radioactivity in successive samples of blood plasma of two rats during the first 3 days after intragastric administration of two different polynuclear hydrocarbons, labelled with carbon-14. ●, DMBA; ○, DahA.

tissues, particularly the brain, muscle and salivary gland, contained rather less than the blood (Fig. 6a). At this time the total radioactive material contained in the tissues of the body was approximately 10 per cent of the dose that had been given.

The second stage in the metabolism of the polynuclear hydrocarbons lasted a further 2 or 3 days. The level of radioactivity in the blood plasma decreased rapidly to about 1 per cent of the peak value (Fig. 2). The levels in the lymph and urine also decreased sharply. The amount of radioactivity in the bile, however, tended to continue at a high level for somewhat longer (Fig. 5). Chromatography showed that much of the material extracted from

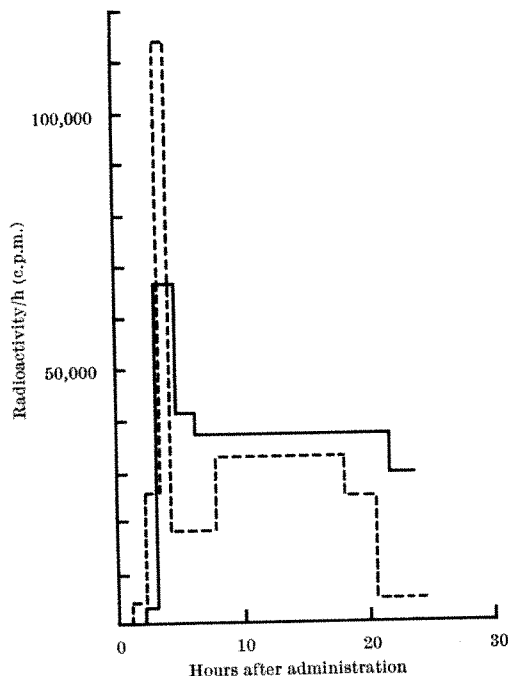


Fig. 3. Radioactivity/h in serial samples of gastro-intestinal lymph, collected from two rats during the first 24 h after they had been given 8-4 µc. of DahA labelled with carbon-14 in oil by stomach tube.

the plasma, urine and bile was no longer in the form of unchanged hydrocarbons. The faeces contained a considerable quantity of radioactive material. The amount of radioactivity in the tissues decreased during this period, but the various tissues did not all lose radioactivity at the same rate. In the liver and kidneys the loss was relatively rapid, whereas in the adrenals, ovaries and the group of mesenteric lymph nodes through which the greater part of the intestinal lymph passes (Fig. 1) it was relatively slow. Thus at the end of this period, that is, at about 3-4 days, the pattern of distribution of radioactive material in the various tissues had altered considerably (Fig. 6c). The highest concentrations were now present in the adrenals, the body fat, the mesenteric lymph nodes and, in some animals, the ovaries. The brain and muscle still contained the least radioactivity. The milk

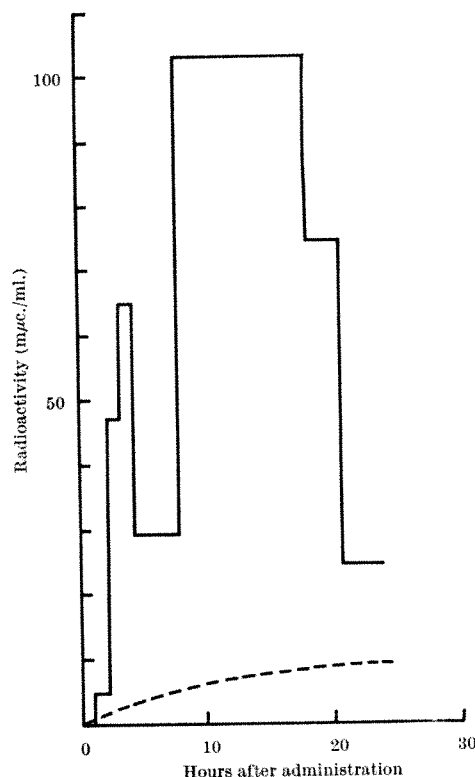


Fig. 4. Concentration of radioactive material in serial samples of lymph (—) and of blood plasma (---) collected from a rat given DahA (dose as in Fig. 3).

of the lactating rats was examined 3-7 days after administration of the labelled hydrocarbon and radioactive material was found to be present, chiefly in the butter fat.

The third stage in the metabolic process was studied for several months more. From about 2 weeks after administration of the dose only very small amounts of radioactivity could be detected in the blood plasma (the only body fluid examined during this prolonged period). The concentration of radioactivity in the tissues showed a steady decline, but even at the longest survival times the adrenals, the mesenteric lymph nodes and, in some animals, the body fat and the ovaries still retained higher concentrations of radioactivity than the other tissues. The concentration of radioactive material remaining in the body fat after weeks or months was variable; sometimes it was of the same order as that in the adrenals (Fig. 6e, f) and sometimes it was much less. Normal mammary glands, freed from fat (Fig. 6c), and mammary tumours (Fig. 6f) examined up to 3 months after administration of the radioactive

hydrocarbon, did not show a concentration of radioactivity such as that in the adrenals and body fat, the level being no higher than that found in the other tissues.

During the first two stages of absorption there were some differences in the distribution of radioactive material in the tissues of animals from which the gastro-intestinal lymph or the bile had been collected and in the tissues of those animals from which these fluids were not taken. In the animals from which the lymph had been taken the tissues contained relatively low concentrations except for the liver (Fig. 6b). In animals from which the bile had been collected the tissues usually contained considerably more than the usual amount of radioactive material. The reason for this larger amount may be that the exogenous bile salt given with the dose of polynuclear hydrocarbon in an attempt to compensate for the loss of endogenous bile salts through the bile catheter had facilitated the absorption of the polynuclear hydrocarbon from the gut.

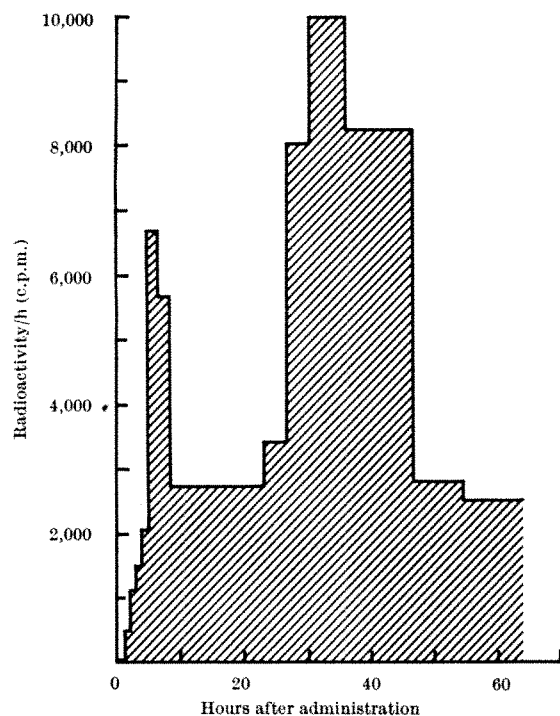


Fig. 5. Radioactivity/h in serial samples of bile collected from a rat during the first 64 h after administration of DahA (dose as in Fig. 3).

The general picture of absorption and excretion was similar for all four polynuclear hydrocarbons, with only minor differences, for example, DMBA seemed to be less rapidly absorbed than were the other three compounds. The findings were substantially the same whether  $^{14}\text{C}$ - or  $^3\text{H}$ -labelled polynuclear hydrocarbons were used, except that some 20–30 per cent of the isotopic label separated from the material labelled with tritium within a few hours after the dose had been given. The separated tritium was detected in the form of water in the urine, in which it was excreted relatively slowly. It was also excreted in the breath, as subsequent experiments have shown.

This study shows that induction of mammary tumours by intragastric administration of carcinogenic hydrocarbons is extremely wasteful of carcinogen, because most of the dose is rapidly excreted from the body in the faeces and urine, and also shows what happens to the small residue which is retained in the body. The first stage in the absorption of hydrocarbon from the gastro-intestinal tract is its entry into the lymphatics of the gut, and presumably, because the carcinogen is dissolved in oil, its transport through the intestinal epithelium is similar to

that of other fatty substances that are absorbed from the intestine. From the lymph the carcinogen, in solution in chylomicrons, is carried into the blood stream which then distributes it to all the tissues of the body. The extent to which the different tissues take up the carcinogen and the periods for which they retain it are variable. At an early stage after administration relatively large quantities of the carcinogen are held for a short period by both the kidney and the liver. From the kidney some of the carcinogen is excreted in the urine, and from the liver it is excreted in the bile. Polycyclic hydrocarbons given intravenously are excreted rapidly in the bile as conjugates of their phenolic derivatives, which undergo hydrolysis

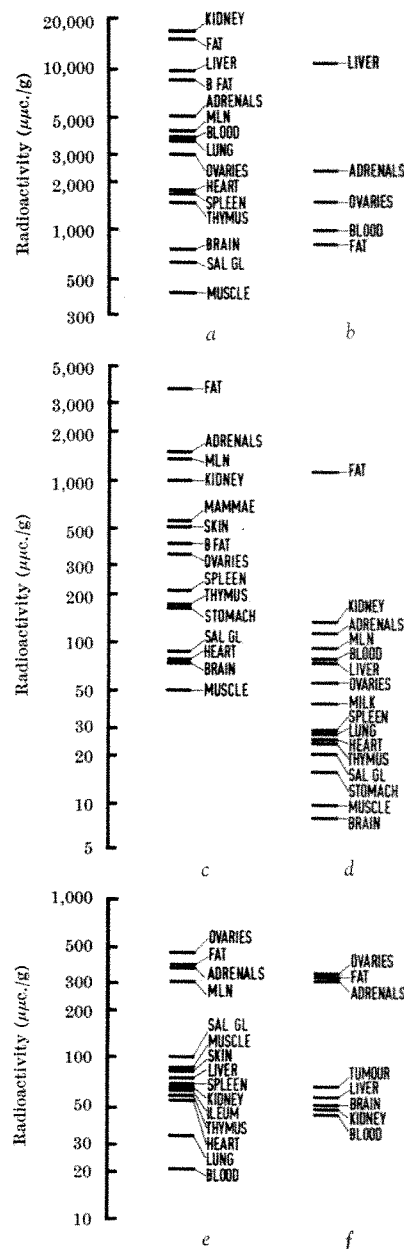


Fig. 6. Concentration of radioactivity in tissues and blood plasma taken at autopsy from six rats at various times after intragastric administration of labelled carcinogenic polynuclear hydrocarbons. The tissues listed here were those taken routinely from all animals in this study. (BP, Brown fat; MLN, mesenteric lymph nodes; SAL GL, salivary gland.) The values for the different animals should not be compared because the radioactivity of the carcinogen given was not the same in each rat. (a) Nine hours after  $^{14}\text{C}$ -DMBA. (b) Twenty-four hours after  $^{14}\text{C}$ -DahA (the gastro-intestinal lymph had been collected from this rat). (c) Twenty-four hours after  $^{14}\text{C}$ -DMBA. (d) Seven days after  $^{14}\text{C}$ -DMBA (lactating rat). (e) Twenty-two days after  $^3\text{H}$ -3-MC. (f) Eighty days after last of multiple doses of  $^3\text{H}$ -3-MC (tumour-inducing dosage).

in the gut, and appear in the faeces as free phenols<sup>19-21</sup>. Our work suggests that after absorption of polycyclic hydrocarbons from the gut they are rapidly excreted in the bile, while probably only very little of the metabolites thus excreted is reabsorbed.

The body fat also takes up much of the absorbed carcinogen at an early stage, but this tissue, unlike the liver and kidney, often retains it for a long period (Fig. 6). The adrenals, and usually also the ovaries, take up the carcinogen in relatively large quantities and elimination from these organs is slow. The variable uptake by the ovaries was probably caused by the carcinogen having been given to the rats at different phases in their oestrous cycles. It is interesting that the brain, although it has such a large content of lipid, is one of the tissues which takes up very little of the carcinogen (Fig. 6). A large proportion of the lipids of the brain, however, consists of polar lipids (for example phospholipids) and it may well be that it is these polar lipids and/or the blood-brain barrier which prevent any substantial uptake of the carcinogen by the neural tissue.

The retention of polynuclear hydrocarbons in the body fat probably has a special significance in relation to the production of mammary tumours by carcinogenic hydrocarbons. The mammary glands (and indeed the mammary tumours) did not show any appreciable concentration of radioactive material, but these glands are embedded in fatty tissue and all body fat takes up a high concentration of the carcinogen and often retains it for a long period. This store of carcinogen in the fat adjacent to the mammary tissue may thus act continuously on the mammary glands in the manner of a locally applied carcinogen. The prolonged retention of carcinogenic hydrocarbons by the adrenals and to a variable extent by the ovaries may also be significant in the induction of mammary tumours. Both these organs produce steroid hormones which promote

mammary growth and steroid hormones are known to have a strong chemical resemblance to carcinogenic hydrocarbons such as DMBA. The retention of carcinogenic hydrocarbons in the adrenals and the ovaries suggests a physiological as well as a chemical parallel between these substances. If this were so it could explain why these carcinogenic hydrocarbons act selectively on tissue which is a target organ for the steroid hormones of the adrenals and the ovaries.

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## Ancient Oyster and Bay Scallop Shells from Sable Island

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Radiocarbon dating suggests that warm water oysters and bay scallops migrated northwards during and after the climatic thermal maximum. The bay scallops may have arrived too late to reach areas in Canada which are now favourable for them.

SABLE ISLAND is a low, sandy shoal, about 21 miles long and less than 1 mile wide, and is located 100 miles south-east of Canso, Nova Scotia, near the edge of the Continental Shelf (Fig. 1). The presence on the beaches of "drift shells" which do not occur alive near Sable Island has long been known<sup>1</sup>. Investigations of the significance of these shells with respect to the postglacial marine zoo-

geography of eastern Canada and the post-Pleistocene history of Sable Island have just begun<sup>2</sup>.

Neither oysters (*Crassostrea virginica* (Gmelin)) nor bay scallops (*Aequipecten irradians sablensis* Clarke) now live near the island, but shells of these and of some other species which may also be locally extinct are often found on the beaches and the surrounding Sable Island Bank<sup>3</sup>.

The ages of oyster and bay scallop shells washed on to the beaches and of a sample of peat dredged from 1 mile south-east of Sable Island from a depth of 10 m have been determined by the radiocarbon method. The ages are shown in Table 1.

Additional oyster shells collected on North Beach near West Light and on South Beach near Wallace Lake were examined for characters indicative of ecology and for growth characteristics. Most specimens were found to have numerous deep and wide chalky deposits between

One of the North Beach shells is infected by blister worm (*Polydora*) and another, together with a specimen from South Beach, seems to have been attacked by boring sponge (*Cliona*). The *Polydora* infestations, at least, almost certainly occurred while the oysters were living. Although *Polydora* often infests in salinities low enough to produce the chalky deposits noted here, *Cliona* is only associated with higher salinities. All these observations suggest that the individuals involved come from widely varying and widely separate environments. Finally, the shell ridges

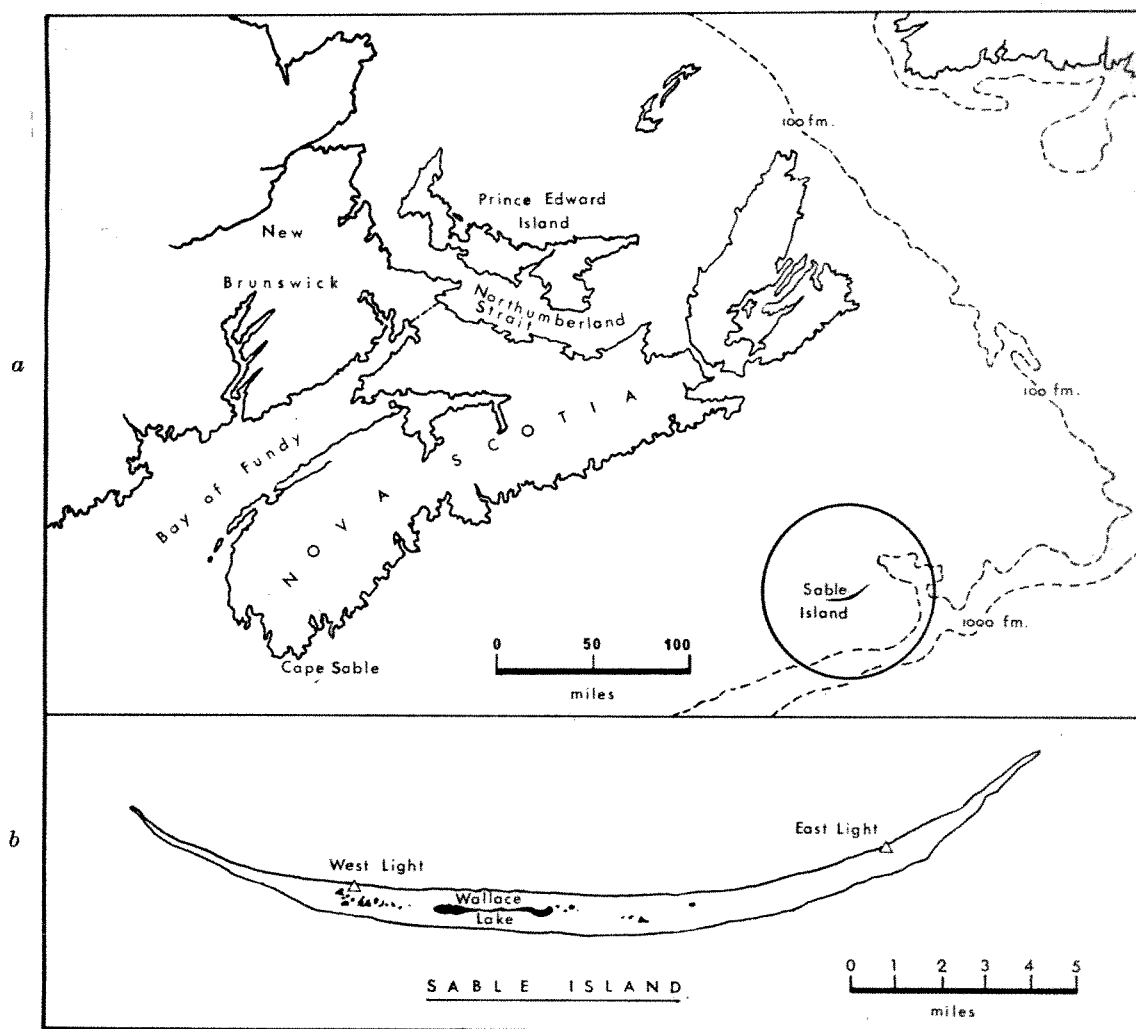


Fig. 1. (a) Location of Sable Island and of other areas mentioned in the text. (b) Map of Sable Island with ponds and lakes shown in black.

thin layers of calcite-ostracum. This is characteristic of a brackish-water habitat<sup>4,5</sup>. Similar oysters now live in Bras d'Or Lake, Cape Breton Island, Nova Scotia, and at the head of saltwater-freshwater estuaries. The specimens from North Beach also show considerable variation in shell shape. Some have thin shells with large ratios of length : width, twisted lips, and left valves with little "cupping"—characteristics of oysters growing in clumps on soft substratum. Others have thicker shells with small ratios of length : width and deeply cupped left valves—characteristics of oysters growing on firm substratum. Both soft and firm substrata often occur, however, in close proximity in oyster habitats.

imply that the oysters grew quite rapidly—as fast as, or perhaps a little faster than, oysters now living in Malpeque Bay, Prince Edward Island. Accurate estimations of shell growth and life span from shell ridges in unknown oyster stocks are not yet possible.

The Sable Island bay scallop shells have been described before<sup>2</sup>. Growth rates, deduced from growth annuli, seem to have been moderate but less than those for the five populations of *Aequipecten irradians irradians* or the eleven populations of *A. i. concentricus*<sup>2</sup>. *A. i. sablensis* represents a distinct sub-species apparently endemic to Sable Island, and so detailed comparisons of that with other bay scallop populations cannot be made with confidence. It is never-



Table 1

No.	Sample No.	Radiocarbon age (years before 1950)	Sable Island location	Remarks
1	Oyster, GSC-635	5,650 ± 140 (inner) 5,320 ± 140 (outer)	North Beach near West Light	Inner and outer layers dated separately—shell not blackened
2	Oyster	4,710 ± 120	Beach flat, S.-S.E. shore of Wallace Lake	
3	Oyster, GSC-634	3,630 ± 130 (black) 3,540 ± 140 (white)	North Beach near West Light	Shell layers alternately blackened and not blackened—layers separated and similar layers combined for dating
4	Bay scallop, GSC-699	1,800 ± 125	North Beach near West Light	Shells not blackened
5	Bay scallop	1,432 ± 125 (ref. 2)	North Beach, unspecified location	Shells not blackened
6	Peat, GAK-748	6,800 ± 150 (ref. 7)	1 mile S.E. of Sable Island, depth 10 m	

theless probable that, like other day scallops, *A. i. sablensis* lived in a bay of medium to high salinity or in a lagoon. Paradoxically *A. i. sablensis* seems to be more closely related to the more southern *A. i. concentricus* (New Jersey to South Carolina, Florida to Texas) than to the more northern *A. i. irradians* (Massachusetts to New Jersey), but even the latter sub-species occurs no closer than 500 miles west-south-west of Sable Island today.

The dates of the oyster shells (about 5,670–3,560 yr B.P.) compare reasonably well with the dates of 6,850 ± 100 yr B.P. and 7,335 ± 105 yr B.P.<sup>6</sup> for ancient oysters collected in Northumberland Strait from 37 and 22 m respectively. The two latter dates are in excellent agreement with the date 6,800 ± 150 yr B.P. determined for the offshore peat<sup>7</sup>. (The plant species which comprise the peat are now being identified by Dr J. Terasmae of the Geological Survey of Canada and their significance will be discussed later.)

On the basis of a sample of salt-marsh peat collected from a depth of 59 m on George's Bank and dated by the radiocarbon method as 11,000 ± 350 yr B.P. Emery *et al.*<sup>8</sup> have deduced that much of George's Bank was subaerially exposed and heavily vegetated about 11,000 yr ago. There has been a steady rise of sea level along the coasts of New England and the Mid-Atlantic States<sup>9,10</sup>. According to the charts presented by Merrill *et al.*<sup>9</sup>, at about 6,800 yr B.P. the ocean level was probably about 10 m lower in those regions than it is at present.

The peat date from the Sable Island sample indicates that about 6,800 yr ago sea level on Sable Island Bank in the vicinity of Sable Island was probably at least 10 m lower than today. This, in turn, implies that the innermost Nova Scotian Shelf and shallow areas near Cape Sable also may have been exposed at that time. The Sable Island peat and oyster dates also correspond well with the climatic thermal maximum (or post-glacial hypsithermal warm period) in eastern Canada<sup>10</sup>. Many warm water marine animals found south of Cape Cod also flourish in the Northumberland Strait region and as small, isolated populations elsewhere in eastern Canada<sup>11</sup>. The dates indicate that oysters, at least, had spread northward to Northumberland Strait by the beginning of the hypsithermal and were at Sable Island about the same time or shortly thereafter.

Bay scallops do not now occur live in eastern Canada. The radiocarbon dates for Sable Island specimens (about 1,820 to 1,450 yr B.P.) correspond to a warming period in eastern Canada when the land temperature was about the same as it is today<sup>9</sup>. This period occurred after a cold period, the onset of which had marked the end of the climatic thermal maximum<sup>9</sup>. The presence of bay scallops at Sable Island at this time agrees well with the concept of an extended and therefore warmer Sable Island where the oceanic influence would temper winter conditions. It also indicates the existence of other suitable habitats between Massachusetts and Sable Island, probably as bays and lagoons along the mainland, before or during that time<sup>10</sup>. Sable Island populations are taxonomically distinct, and this implies that they were isolated for a substantial period there or that the founding stock was itself distinct from other mainland populations and has left no fossils elsewhere. Bay scallops probably migrated north to Sable Island after the hypsithermal warm period and further

migration to the Northumberland Strait region was effectively blocked by cold water or severe winter conditions.

Finally, the dated presence of oysters and scallops at Sable Island reveals much about the island itself. During the period 5,670–3,560 yr B.P. a lagoon or bay must have existed at Sable Island, perhaps in the vicinity of the present large brackish Wallace Lake. Specimens of *Mya arenaria* also attest to the presence of a low energy environment during that time. These have been found in dark brown organic-rich silty sand beneath 3 m of sand on the south shore of Sable Island. These clams lie just above the buried peat horizon. This information combined with the date derived from the peat suggests the existence of a bay with a partially muddy substrate on what is now the southern margin of the island. It suggests that Sable Island was then somewhat wider than it is now. The morphology of many of the Sable Island oysters and their dates, together with the other evidence, imply that a bay or series of bays existed there from about, 6,800 to about 3,600 yr B.P. Within historic time a long, narrow inland lagoon is known to have existed and to have disappeared during the nineteenth century<sup>12</sup>. Today the island is composed only of sand; sand and gravel; and mixtures of sand, gravel and shell. The coasts are high energy environments of continuously moving sand and changing bars<sup>14</sup>.

Whether the oysters disappeared because of disease<sup>14</sup>, climatic worsening, or elevation of sea-level<sup>9</sup> (or other invasion of the habitat by cold oceanic water) is unknown, but the two latter possibilities acting together appear most likely. It seems certain that from 1,450 to 1,820 yr B.P. a bay or lagoon also existed in which bay scallops thrived. It probably was not the same lagoon that supported the oysters earlier but another lagoon, perhaps in the present location of Wallace Lake. Wallace Lake is at present very variable. Its size and salinity fluctuate widely because of periodic freshening during isolation from the sea (a fresh water wedge over salt water underlies the island) and subsequent winter invasion by the sea. Scallops would not have been able to survive freshening of their habitat or gross invasion by cold oceanic water during the summer, but which of these or other reasons are responsible for their extinction is unknown.

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# Mineral Deposits on the Moon

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Professor Mueller uses evidence from many sources to reconstruct mineral assemblages on the Moon. His findings indicate that the Moon's resources may well be worth exploiting if only the cost of space travel can be reduced.

THIS article is based on the interpretation of recent rocket photography of the Moon and on certain comparisons and extrapolations made on the basis of the properties and theories of genesis of terrestrial deposits which are described in recent textbooks and review articles of economic geology (see, for example, refs. 1-3). Interpretation of rocket photographs from the Moon has led me to the conclusion<sup>4</sup> that it is unlikely that a high proportion of the meteorites known from collections are derived from the Moon. The theory of asteroidal origin seems to be more probable. It appears that the mineral deposit pattern of a given celestial body may depend on its mass, and it is therefore possible that the pattern of mineral deposits for the Moon is in many respects intermediate between that of the Earth and that indicated for the bodies with the size of asteroids (smaller than the Moon) from which the meteorites may be derived.

My principal aim is to assess the extent to which it is possible to reconstruct the mineral assemblage of a given celestial body. This assessment may be tested in the near future in the course of manned lunar landings and explorations. Each genetical class of mineral deposits which might be expected to be found is briefly considered.

**Magmatic segregates.** The gravitational segregation of deposits of refractory minerals from the molten igneous rocks within the Earth's crust is a relatively rare phenomenon. This rarity may explain the lack of any magmatically segregated material among the small number of the meteorites which crystallized from a melt, namely, the achondrites. In view of the low albedo, and therefore the probable basic character of much lunar rock, deposits produced by magmatic segregation—in particular magnetite, pyrite and chromite—are likely to occur, so that the prospects of finding high grade deposits of these minerals would seem to be good. Because of lower rate of erosion, however, dissection of major intrusions cannot occur and the less intensive tectonics and the lower gravitational field of the Moon may be factors adverse to the formation of gravitational segregates. For these reasons outcrops of high pressure, high temperature minerals such as diamonds of the Kimberley type are unlikely to be found. There is an intriguing probability of magmatic segregates within lava flows on the Moon. The existence of this type of deposit has not been conclusively proved on Earth, although I have found indications that haematite-magnetite bodies of El Laco, Northern Chile, were generated in this way. It would seem likely that on the Moon there would be increased chances of encountering such effusive, magmatic, segregates for the following reasons: there is presumably a greater abundance of basic rocks which contain the highest proportion of the refractory minerals and according to the interpretation of lunar photography in general there are a far greater number of individual lava flows preserved on the Moon than on the Earth.

**Fumarole deposits.** The abundance of fumarole and hydrothermal products on the surface of the Moon would primarily depend on the ratio between the number of craters produced by impact and volcanism, because only volcanism would substantially contribute towards the

accumulation of magmatic condensates. On Earth today the ratio volume of impact/volume of volcanic rocks is in the order of  $10^{-8}$  or less. The lower intensity of volcanic activity (due to the smaller mass of the Moon) on the one hand, and the preservation of archaic impact craters on the other hand, may drastically increase the impactite/volcanite ratio on the Moon. I think, however, that it is unlikely that these factors would be responsible for an increase in the ratio of eight orders of magnitude, bringing the ratio in question to unity. It therefore seems likely that the surface of the Moon is rich in fumarole products.

A full discussion of the problem of interpretation of lunar craters<sup>5</sup> is outside the scope of this paper. It should be stressed, however, that most of the terrestrial craters show positive mean elevation, because lava has been added from deep sources in the course of volcanic activity. The rare maar type crater of Nilahue, Southern Chile, has zero mean elevation<sup>6</sup> because all the material was removed as ejecta by gas explosions. The few known or inferred impact craters on Earth also reveal zero mean elevation because under the usual impact velocities the volume of the impacting object is negligible when compared to the volume of rock displaced by the impact. Craters and domes of well defined positive elevation appear on wide angle frame 213 of Orbiter (November 25, 1966), and this fact strongly supports the volcanic origin of at least some of the lunar craters.

On the Earth the fumarole deposits are of more or less transitional character because the sulphur of such deposits becomes oxidized in the atmosphere, and most of the saline condensates are washed away by precipitation into the oceans or into desert basins. Many of the magnesium and potassium ions of these exhalation products of the Earth are later absorbed on the floors of the oceans so that evaporites, which form during the drying out of the marine lagoons, are relatively depleted of these metals, and are therefore enriched in the salts sodium and calcium, which are not so readily absorbed.

On the Moon there is no atmospheric transport and the resulting "fumarole-evaporite" type of deposit must therefore retain its magnesium. Other factors which may contribute to a relatively high percentage of magnesium salts of these lunar deposits would be the alkali poor, basic character of the lunar magma, and the elimination of free sulphur (and possibly also alkalis) through solar radiation and the solar wind, particularly within the older deposits.

The foregoing considerations lead us to the prediction that the surface of the Moon is exceptionally rich in fumarole and evaporite deposits and that the volume of these is comparable (after making allowance for the smaller surface and lower rate of erosion on the Moon) to the total volume of the salts which are concentrated within the terrestrial evaporites, oceans and fumaroles. Such deposits (which may reach masses in the order of several hundred million tons) would have a surface zone affected by solar radiation and the solar wind in which  $MgO$ ,  $MgSO_4$ ,  $MgCO_3$ ,  $MgCl_2$  and some iron and calcium salts would be intermixed with volcanic ejecta, meteorites

and cosmic dust. There is a possibility that with increasing depth in such deposits, the percentage of the relatively more volatile alkali salts, sulphur and hydrocarbons would tend to increase.

It is interesting to note that Kuiper<sup>7</sup> interpreted the white, snow like substance on the peak of the Alphonsus crater (shown on Ranger VII photographs) as a fumarole product which traversed a tuff like material of pyroclastics; he compared this with a white mixture of gypsum and calcium oxide which covered the slopes of Laimanca Volcano, Hawaii, after an eruption. The Orgueil and other type I carbonaceous chondrites show considerable petrological similarities to terrestrial tuffs impregnated with fumarole products<sup>8</sup>. The amount of  $\text{MgSO}_4$ , approximately 15 per cent, which is present in these stones is significant in view of the foregoing considerations and furnishes further indications that magnesium salts may be expected as important constituents of the lunar deposits.

**Hydrothermal deposits.** The lunar pegmatites are expected to be chiefly of basic type, for it is likely that the Moon, because of its low gravitational field, does not have a segregated granitic continental crust. This would favour pegmatites enriched in rare earths, but the economically more important constituents of terrestrial pegmatites, associated with granites—lithium, beryllium, boron and vanadium—may be absent or very subordinate.

As regards the relatively lower temperature, deep-seated hydrothermal deposits, the Moon is expected to be essentially an oceanic type of mineralogical province. This means that the mineral veins may contain volatile condensates characteristic of the terrestrial basic rock, namely copper and iron. Deposits of mercury, bismuth, antimony, gold, lead, zinc, arsenic, barium and phosphorus are associated with all the terrestrial igneous rocks and they may therefore be present in relatively smaller quantities on the Moon. The typical granitic volatiles, that is, molybdenum, tungsten, uranium and fluoride, may, however, be practically absent.

Lack of deep erosion may render the hydrothermal deposits of deep-seated origin relatively rare, and the exposure of types close to the surface more common. These latter contain the sulphides of the heavy metals and some elementary sulphur. Such deposits in question might have little or no thermal zoning, and would have a simple mineralogy of impure ores and gangues, because their cooling and crystallization occurred too rapidly to produce large and well formed crystals of their minor constituents.

Because these deposits will be close to the surface there appear to be good prospects for the presence of arsenic, mercury, antimony and boron. It would seem likely that solar radiation and wind would cause the formation of a surface zone which is depleted in the hydrothermal minerals of extreme volatility, such as yellow sulphur and red  $\text{HgS}$  and  $\text{As}_2\text{S}_3$ . This possible scarcity of coloured minerals may render difficult the visual detection of hydrothermal deposits from coloured photographs, or black-and-white photographs made through different filters. On the photographs made by Rangers VII, VIII and IX, numerous (up to several kilometres long) lodes appear, most of which are of a lighter shade than the surrounding ground. The light colour favours the possibility of fissure fillings of close to the surface fumarole type which have a composition typical of fumarole deposits—rich in earth-alkali and alkali salts with sulphur. The presence of gaping fissures on other photographs may be accounted for either by displacements without the ascent of condensable volatiles (this occurs in the case of non-mineralized faults of the Earth) or the preferential erosion of highly volatile material, possibly sulphur and hydrocarbons which filled the fissures.

I have proposed<sup>8</sup> that the carbonaceous chondrites in general closely resemble terrestrial pyroclastics impregnated with hydrothermal products, and in the type I stones features suggestive of terrestrial fumarole condi-

tions appear as well. According to the computation of data from the up to date literature, the redistribution ratios, that is, p.p.m. in carbonaceous chondrites + p.p.m. in mean chondrites of some of the hitherto determined minor elements are as shown in Table 1.

Table 1

Element	Redistribution ratio
Mercury	220
Bismuth	52
Carbon	19
Lead	17
Silver	16
Zinc	3.9
Copper	2.5
Vanadium	1.9
Sulphur	1.7
Rare earths	1.6
Uranium	1.1

I am at present engaged in statistical work on the terrestrial hydrothermal deposits which indicates that the foregoing redistribution ratios are usually very similar to those between the mean of hydrothermal veins in the terrestrial oceanic provinces and that of the Earth's crust. The only really significant difference is the higher value for sulphur and the lower value for carbon in the terrestrial hydrothermal deposits. Such difference may be explained by the possibility that under the relatively oxidizing conditions within the crust of a comparatively smaller celestial body, sulphur would escape in the free state, and carbon would tend to persist as graphite, amorphous carbon and higher molecular organic substances. Under the relatively more reducing conditions which prevail within the crust of a body the size of the Earth the carbon tends to escape as low molecular hydrocarbons, and a higher proportion of sulphur would be retained as sulphides of iron and other heavy metals. We may therefore expect that the mean sulphur carbon ratio of the lunar hydrothermal deposits would be lower than the value of approximately 10 for the Earth, but higher than that of 1.5 for the carbonaceous meteorites.

**Sedimentary and metamorphic deposits.** No sign of sediment or metamorphosed sediment has been detected among the 2,000 or so meteorites in our hands and it seems unlikely that a relatively small celestial body can retain an atmosphere and hydrosphere for a time sufficient to produce appreciable amounts of sediments. The absence of clear indications of sedimentary rocks on the hitherto published rocket photographs of the Moon suggests that mineral deposits of sedimentary origin may be absent or very subordinate on the Moon. These include secondary concentrations of gold and platinum, minerals of zirconium, thorium, the rare earths and iron and sands, clays, bauxite, limestone, dolomite, chert, phosphorite, garnet and corundum.

**Carbonaceous deposits.** It appears that the Moon may have more carbon in its crust than the Earth has. Reconstruction of how lunar carbon is distributed is difficult because it is not easy to assess the relative importance of several factors.

I have suggested<sup>9</sup> that the mean oxygen/hydrogen ratio will increase and the hydrogen/carbon ratio of a carbonaceous complex will decrease with decreasing mass of a given celestial body. The presence of organisms of terrestrial type would increase the organic carbon and hydrogen/carbon ratio. As the presence of abundant life on the Moon seems unlikely at any stage of its history, it seems probable that the Moon's carbosphere contains more of the Moon's total carbon than Earth but in a more carbonized form (chiefly as graphite and graphitite). The highly aromatic and oxygenated nature of average lunar rock makes it unlikely that craters on the Moon would be filled with tar like hydrocarbons as envisaged by Wilson<sup>10</sup>.

On Earth the concentration of the carbonaceous complex into relatively pure and extensive deposits of coals is principally due to the abundance of living organisms. With the absence or more subordinate character of this

Table 2

Volume and grade compared to similar terrestrial deposits	Magmatic segreg.	Fumarole-evaporite	Hydrothermal	Sedimentary	Carbonaceous	Impactite
Superior	Chromium	MgSO <sub>4</sub>	Arsenic, antimony, selenium, bismuth, mercury	—	Graphite, graphitite	Iron, cobalt, nickel, platinum metals, diamonds
Equal	Iron	CaSO <sub>4</sub> , CaCO <sub>3</sub>	Copper, zinc, silver, cadmium, manganese, gold	—	Abioliths	—
Inferior	—	Sulphur, NaCl, KCl	Lead, BaSO <sub>4</sub> , phosphate	—	—	—
Very inferior or non-existent	Diamonds	—	Lithium, beryllium, boron, titanium, vanadium, niobium, molybdenum, tin, rare earths, tantalum, uranium, CaF <sub>2</sub>	Aluminium, manganese, iron, titanium, zirconium, tin, platinum metals, gold, clays, phosphate, CaCO <sub>3</sub> , SiO <sub>2</sub> , corundum, garnet	Coals, oil, asphalts, gas	—

biological factor on the Moon there may be fewer high grade organic deposits, although this trend may be partially or wholly counterbalanced by the higher overall percentage of carbonaceous matter and therefore the improved probabilities of the occasional concentration of carbonaceous matter through hydrothermal and volcanic processes. It is possible that the individual deposits have an evaporated or carbonized surface zone which may be underlain by a zone which is enriched with the products of distillation from the surface; this lower zone should also have a large hydrogen/carbon ratio.

Photographs from Surveyor spacecraft show that the lunar matter which was disturbed through the impact of one of the legs of the spacecraft was of a darker shade than the undisturbed powdery matter. This suggests either the efflorescences of salts on the surface or the leaching out of dark carbonaceous material from the surface through solar radiation. The second alternative would imply a lunar carbonaceous complex, which may originate from lunar dust, cosmic dust and carbonaceous meteorites.

**Impactites.** Because of the lack of an appreciable atmosphere the "impactites" on the surface of the Moon must be of greater importance than on the Earth. The abundance of nickel iron alloy present on the lunar surface is difficult to assess, but it may prove to be considerable. It is possible that after the impact of larger bodies secondary volcanism may bring some of the nickel iron from deeply buried impactites to the surface. Because impact on the Moon would liberate a considerable density of energy, minerals may be transformed by impact to high pressure forms unknown on Earth. The crust of the Moon may not contain diamonds but the meteoritic debris on its surface may provide a considerable source of industrial diamonds. In this connexion it may be noted that the impact of the siderites shocked by the Canon Diablo meteorite contains some 0.1 per cent of diamonds. This represents an approximate concentration 10<sup>4</sup> greater than in the richest hitherto known terrestrial concentrations from South Africa.

The systematic study of mineral deposits on the Moon should yield, in the foreseeable future, data of theoretical interest on the processes of crystallization of magmas

under gravitational fields of differing intensities and the comparison of redistribution of volatiles within celestial bodies of the mass of the Moon and the mass of the Earth. The effects of differing types of atmospheres and radiations on the zones of mineral deposits close to the surface could be studied as well.

The economic utility of the mineral deposits within the more distant future may primarily depend on how much the cost of trips to the Moon can be reduced, particularly through the advent of the atomic rocket. With the gradual exhaustion of the small number of top grade terrestrial deposits our mining techniques and metallurgy tend to become more and more adapted toward the exploration of lower grade and more extensive deposits. It may be that a considerable number of top grade deposits, including those of platinum metals, will be found on the surface of the Moon. Another potential economic advantage may be that the lunar deposits may, to some extent, compensate the terrestrial ones because of the different conditions which prevailed in the course of their genesis. For example, evaporite deposits on Earth contain chiefly sodium chloride while the deposits of the Moon may be predominantly magnesian.

Table 2 compares the suggested volume and grade of possible lunar deposits of the more important metals and non-metallic minerals, with their terrestrial genetical equivalents or quasi-equivalents.

I thank Dr Sidney W. Fox for fruitful conversations on the subject. This work was supported by the US National Aeronautics and Space Administration.

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## Ice Front in the North Sea

by  
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Study of an arcuate belt of deeps with its apex to the north of Aberdeen leads to the suggestion that at the last glacial maximum Norwegian ice failed to cross the Norwegian Trench not far south of Bergen. This left an ice free area reaching across the North Sea to Scotland.

I AM constructing a bathymetric map of the continental shelf to the north of Britain. The area covered lies between 56° N., the continental slope to the west of Orkney and to the west and north of Shetland, and the western edge of the Norwegian trench. So far I have obtained about

20,000 miles of echo trace covering the area in a moderately uniform network.

Examination of these traces reveals that many of them cross deeps of a fairly well defined type, some examples of which are shown in Fig. 1. The profiles have been



redrawn from various echo traces to a common horizontal and vertical scale. The horizontal scale is based on Mercator's projection and so varies slightly from example to example. The vertical exaggeration is about  $\times 150$  and no correction has been made for error due to interaction between the slope of the sea floor and the conical form of the transmission beam of the echo sounder. With this vertical exaggeration, deeps of the type shown are easily distinguished from all other submarine valleys and show no gradation towards them. The characteristic feature of these deeps is the low width to depth ratio. The V-shaped cross-section is, in part at least, due to the uncorrected error mentioned. Another characteristic is that they are usually closed at either end with no sign of even a shallow channel like continuation.

Although echo traces from the whole of the continental shelf north of  $56^\circ$  N. and Cape Wrath were examined, these deeps were found only within the area of Fig. 2, that is to the east of Orkney, Moray Firth and Aberdeen. Within this area nearly all the deeps are concentrated in a narrow strongly arcuate belt with its apex to the north of Aberdeen. Deeply lying outside the belt are relatively small. Furthermore, all the deeps crossed by more than one echo trace (thus fixing their orientation) lie more or less parallel to radii of the arcuate belt. Many of the deeps crossed by only one echo trace may also have this orientation because they are found chiefly where echo traces run parallel to the belt.

Some of the deeps in Fig. 2 are well known by name—for example, Devil's Hole, Buchan Deep and the Fladen Ground Deep—but only the last appears to have been surveyed before the oil and gas exploration campaign. Similar but smaller deeps of the same type occur to the south of  $56^\circ$  N., and some have been surveyed. The characteristic cross section and closed ends have been described and various hypotheses offered to explain them<sup>1</sup>. At present, the most widely held view is that they formed under the front of an ice sheet as siphons through which melt water escaped under pressure.

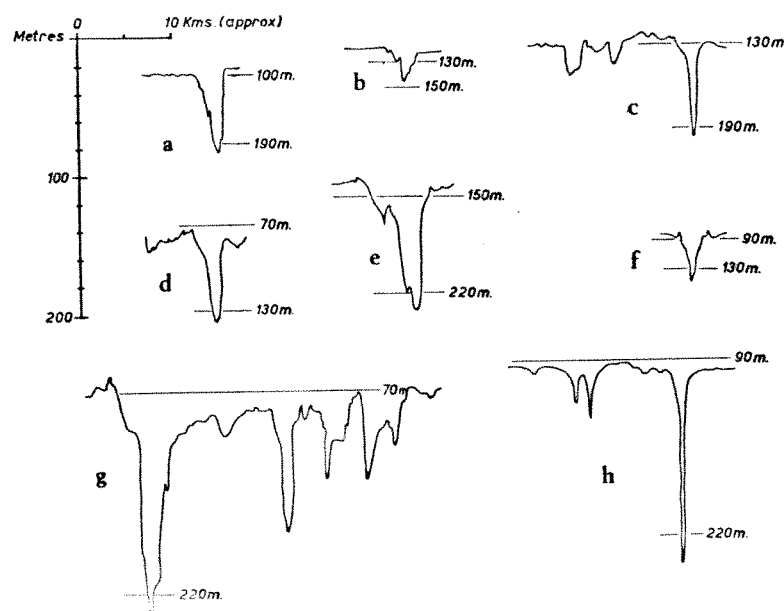


Fig. 1. Uncorrected echo trace profiles of deeps. Vertical exaggeration  $\times 150$ .

- a,  $59^\circ 20' \text{ N.}$  b,  $59^\circ 29' \text{ N.}$  c,  $58^\circ 10' \text{ N.}$  d,  $57^\circ 50' \text{ N.}$   
 $00^\circ 35' \text{ E.}$   $02^\circ 00' \text{ E.}$   $00^\circ 50' \text{ E.}$   $01^\circ 45' \text{ W.}$   
e,  $59^\circ 10' \text{ N.}$  Fladen Ground Deep f,  $58^\circ 27' \text{ N.}$   
 $00^\circ 25' \text{ W.}$   $01^\circ 30' \text{ W.}$   
g,  $57^\circ 50' \text{ N.}$  Buchan Deep h,  $56^\circ 46' \text{ N.}$  Devil's Hole  
 $01^\circ 55' \text{ W.}$   $00^\circ 56' \text{ E.}$

If this hypothesis is correct, then the arcuate belt of deeps in the northern North Sea marks an ice front at some time in the past. The arrangement of the deeps in a belt and their radial orientation supports the ice front hypothesis. There is, however, nothing in what is at present known about the arrangement or the form of the deeps to show whether the ice free area lay inside or outside the arcuate belt.

In all the echo traces examined, only three showed penetration of the sea floor. Echo traces from two different ships showed penetration inside the arcuate belt (Fig. 2). In another echo trace made by one of the ships, penetration is shown in the belt and to the north. Although both ships cruised widely over the shelf they found no penetration elsewhere. A section of echo trace showing penetration of the sea floor, and redrawn to the same horizontal and vertical scales used in Fig. 1, is shown in Fig. 3.

The echo traces show that within the arcuate belt of deeps, in the deepest part, lies a pool of sediments deposited on a rough surface so as to level it. If the speed of sound in the sediments is about the same as in the overlying seawater, the sedimentary layer is about 18 m thick. In places the sediments fill minor deeps and are too thick to be completely penetrated by the echo sounder. Minor pools of sediment occur in the belt and to the north. In Fig. 2 a boundary has been drawn to the main pool, but this boundary is unreliable and is based only on unpublished bathymetric data.

Sediments transparent to echo sounder transmissions are usually mud. Chart No. 112 of the Deutsches Hydrographisches Institut reports a sea floor of mud in many places within the area delimited in Fig. 2 and very rarely outside it.

It is possible that the arcuate belt of deeps marks an ice front with the ice free area inside the arc. The water draining out of the ice deposited the suspended mud on the floor of the ice free area. An ice free area like this reaching across the North Sea to Scotland has not been predicted in any published reconstructions of Pleistocene ice sheets. The alternative, an ice tongue reaching across to Scotland with ice free areas to the north and south, would require the late sediments to have been deposited after the disappearance of the ice from the area. Thus there would be no known source for the late sediments, and no known reason for their present location and their absence from the rest of the North Sea.

The question arises as to whether the sediments were deposited on land or under water. They seem to have been deposited by distribution over the floor of the basin and not by settling from above, though this could have been done by subaerial streams or submarine gravity currents. If the topography of the area was exactly as it is at present, then there would have been at least 70 m of water because of a barrier to the east separating the region from the Norwegian trench. Tilting of the Earth's surface under the weight of the Scandinavian ice sheet could have lowered or even removed this barrier, allowing the melt water to escape if the ice bridged and did not entirely fill the Norwegian trench.

No ice free area such as that proposed has been predicted from glacial evidence on land, so it is not easy to relate this episode to glacial episodes known on land. It is clear that the ice front along the belt of deeps was part of a

major ice sheet and that no sheet formed since has been so extensive. The ice sheets formed in the various readvances recognizable in Scotland<sup>2</sup> were not of comparable size. It is possible that the deeps mark the ice front at or soon after the last glacial maximum. If before this and after the last interglacial, the ice sheet had completely covered the area, it would have had to melt away from most of the North Sea to be able to return and take up the position indicated by the deeps, for preferential melting of the ice sheet in this part of the North Sea area seems most unlikely. Alternatively, if as has been argued<sup>3</sup> no very extensive glaciation occurred in the last maximum the deeps must mark the ice front at the maximum before the last interglacial period.

If the arcuate belt marks the front of the last major ice sheet to occupy the area, it is of interest to study the evidence of the last major glaciation on the adjacent land masses. The nearest land to the ice free area, and lying partly within it, is the part of north-east Scotland between Aberdeen and the Moray Firth. This area has a complex glacial history<sup>3</sup>. It has suffered a number of glaciations and re-advances, but its most striking feature is "moraineless Buchan" (Fig. 4). The Buchan area is not free of moraine, but in it the moraine is so strongly weathered that it has been proposed that it belongs to a previous glacial maximum, was weathered in the last interglacial period and during the last glacial maximum was free of ice but suffered much frost shattering<sup>3</sup>. It

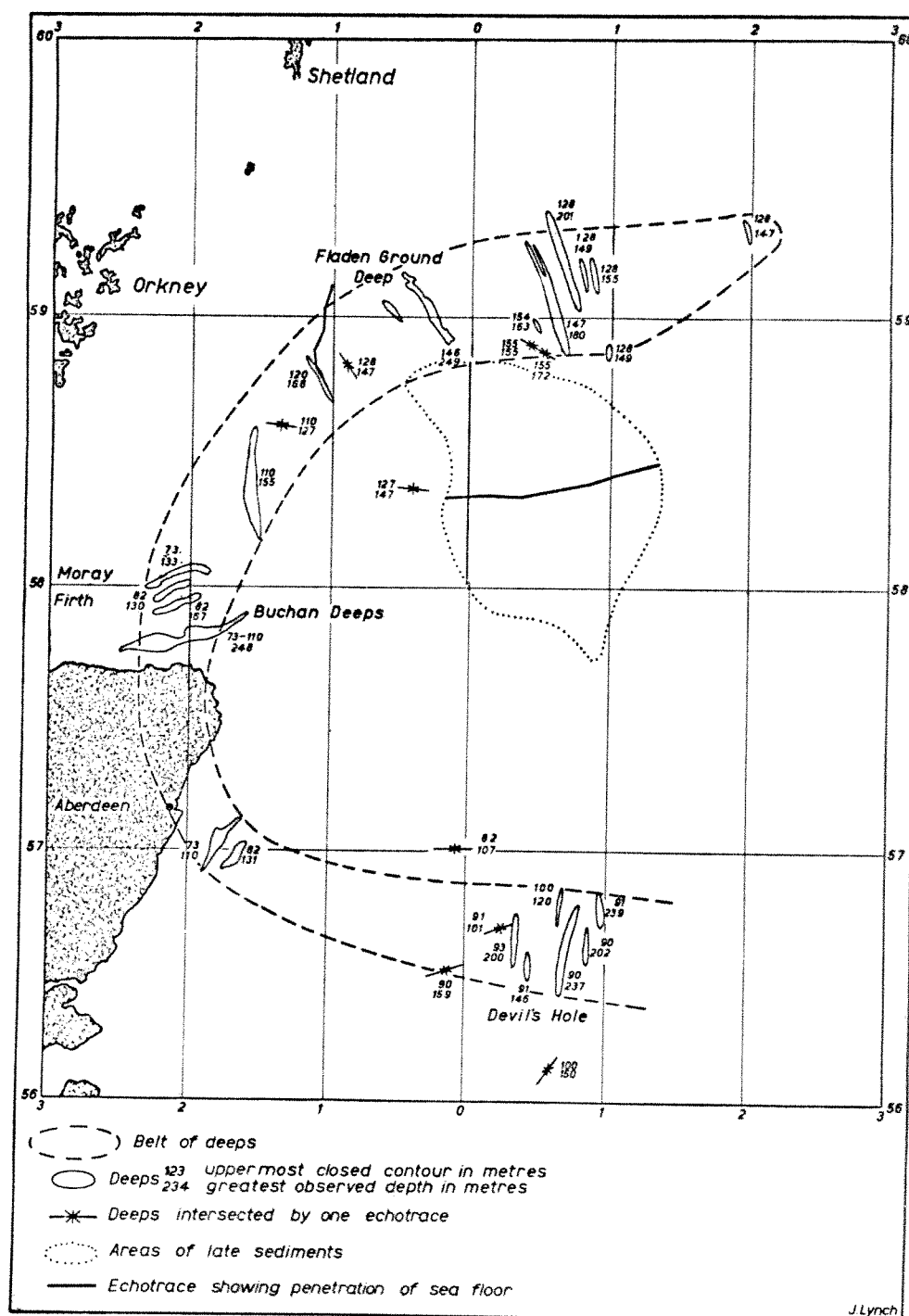


Fig. 2. Distribution of deeps and areas of late sedimentation in the northern North Sea.

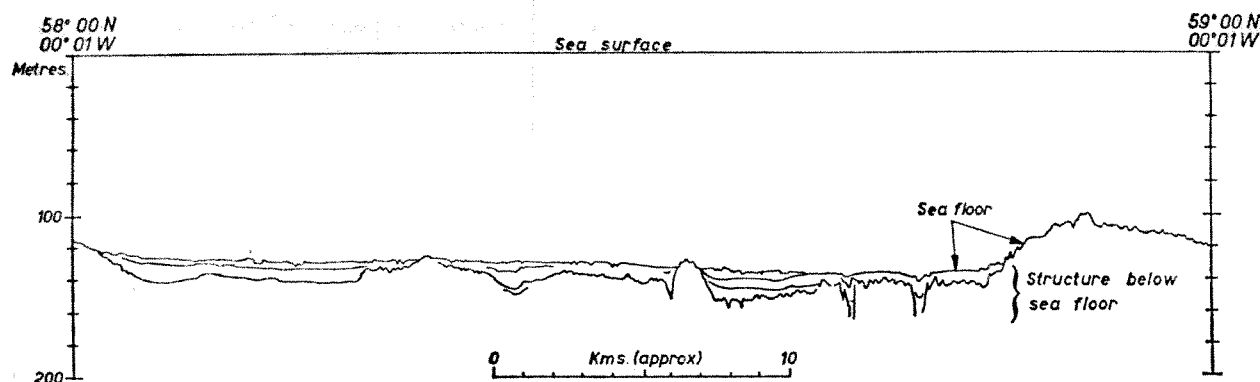


Fig. 3. Echo trace made by R.V. Ernest Holt in 1964 showing penetration of the sea floor near the Fladen Ground Deep. Vertical exaggeration,  $\times 150$ .

requires only a relatively small extension to the south-east of the ice free area delimited by the belt of deeps to include "moraineless Buchan" and that extension would be on high ground which would tend to deflect ice flowing from the west.

The directions of ice flow determined from ice moulding, striae and erratics in Shetland are shown in Fig. 4 (unpublished work by Flinn and students). Contrary to Peach and Horne<sup>4</sup> the last major glaciation of Shetland was by a local ice cap<sup>5,6</sup>, but some of their evidence for ice sweeping over Shetland from the east is correct. In the extreme south some erratics have been carried over the watershed from east to west, but on the east side all the evidence is of ice moving to the east. In the extreme north (Unst) there is strong evidence of ice moving north-westwards and less

evidence of ice moving eastwards. In east central Shetland a very strong stream of ice flowed eastwards and then gradually swung north as it got further east. This deflection was probably due to pressure of Norwegian ice. The ice crossing Unst from the east may have been local ice or Norwegian ice.

A similar deflection in northern Scotland is believed to have occurred in the last glacial maximum<sup>7,8</sup>. Pressure of Scandinavian ice in the Moray Firth and to the north caused local Scottish ice flowing eastward into the Moray Firth to swing round and eventually flow north-westwards across Caithness and Orkney.

All this evidence is assembled in Fig. 4. It is proposed that in the last glacial maximum, Norwegian ice failed to cross the Norwegian Trench not far south of Bergen. Ice crossing to the north of this point flowed westwards and south-westwards across the shelf leaving a front along the northern arm of the arcuate belt of deeps. In the Shetland area, Norwegian ice was deflected to the north and south by a local ice cap but possibly overwhelmed the extremities in the early stages. In the area east of Orkney, the ice came into contact with Scottish ice and both sheets were deflected so as to flow north-westwards across Caithness and Orkney. Ice filled the Moray Firth but did not quite cover the north-east tip of Scotland. To the south of Aberdeen, Scottish ice flowed out into the North Sea leaving a front from near Aberdeen to the Devil's Holes. From there the front may have continued south to the Dogger Bank area where the front of the ice at its maximum extent in the last glacial maximum has been recognized<sup>9</sup>.

During the bathymetric survey no submarine moraines have been recognized either as topographic features or as stony grounds<sup>10</sup>.

I thank Dr J. B. Hersey of Woods Hole Oceanographic Institute, who supplied copies of echo traces made by R.V. Chain in 1963; Mr A. J. Lee of the Fisheries Laboratory, Ministry of Agriculture, Fisheries and Food, Lowestoft, who supplied echo traces made by R.V. Ernest Holt in 1963 and 1964; and NERC (and DSIR) for providing a grant which enabled me to process the echo traces.

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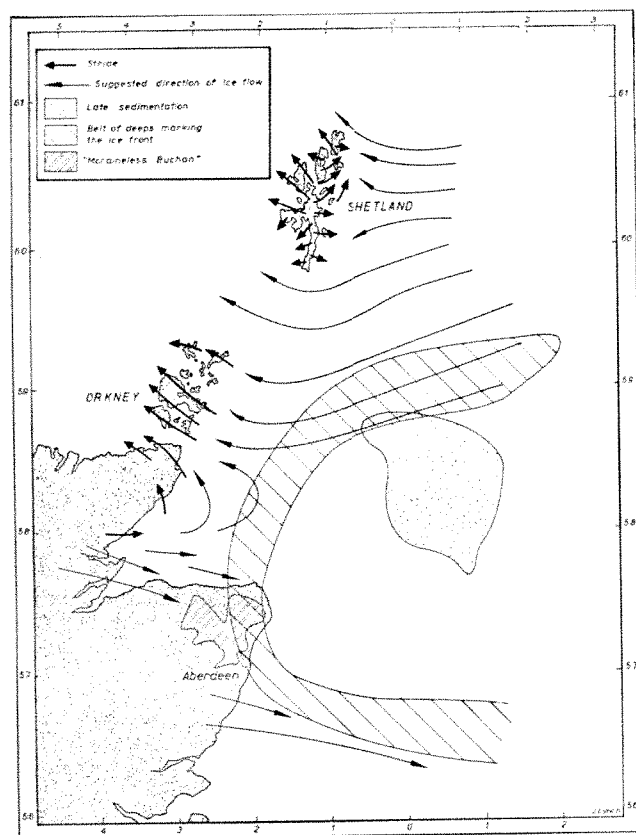


Fig. 4. Proposed distributions and directions of flow of ice sheets in the northern North Sea during the last glacial maximum.

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## LETTERS TO THE EDITOR

## ASTRONOMY

## Fluctuations in the Primordial Fireball

ONE of the overwhelming difficulties of realistic cosmological models is the inadequacy of Einstein's gravitational theory to explain the process of galaxy formation<sup>1-6</sup>. A means of evading this problem has been to postulate an initial spectrum of primordial fluctuations<sup>7</sup>. The interpretation of the recently discovered 3° K microwave background as being of cosmological origin<sup>8,9</sup> implies that fluctuations may not condense out of the expanding universe until an epoch when matter and radiation have decoupled<sup>4</sup>, at a temperature  $T_D$  of the order of 4,000° K. The question may then be posed: would fluctuations in the primordial fireball survive to an epoch when galaxy formation is possible?

Misner<sup>10</sup> has recently pointed out that fluctuations ranging in size from a photon mean free path up to the event horizon will be damped out by neutrino viscosity during the  $10^{10}$ – $10^{11}$  °K epochs. At  $10^{10}$  °K, however, the event horizon contains only  $10^{-4} M_\odot$  in a small amplitude ( $\delta\rho/\rho \ll 1$ ) fluctuation. The purpose of this communication is to demonstrate that a considerably more significant upper limit may be obtained by considering the effects of radiative diffusion on opaque fluctuations at subsequent epochs.

Over the temperature range  $10^{10}$  °K  $> T > T_D$ , the maximum mass  $M_T$  contained in a transparent fluctuation (that is, of scale  $\sim (\kappa\rho)^{-1}$ ) is

$$\frac{4}{3} \pi \kappa^{-3} \rho^{-2} \simeq 2.3 \times 10^{-29} \rho_0^{-2} T^{-6} M_\odot$$

where  $\kappa$  is the opacity, assumed to be due to Thomson scattering by free electrons, and  $\rho_0$  is the mean density of matter at the present epoch. In deriving this expression, we have assumed that the present value of the background radiation temperature is 3° K, and have used the relation  $\rho T^{-3} = \text{constant}$ . We consider initial fluctuations  $> 10^{-4} M_\odot$ , so that at some epoch subsequent to  $T = 10^{10}$  °K these fluctuations are encompassed within the event horizon. Indeed, the number of baryons corresponding to a galaxy cluster of  $\sim 10^{15} M_\odot$  is first contained within the event horizon at an epoch when  $T \simeq 10^{15} \rho_0^{1/3}$  °K. At this temperature,  $M_T \simeq 10 M_\odot$ , so that fluctuations of galactic dimensions are highly opaque.

We treat the damping of opaque fluctuations by radiative diffusion in the Newtonian approximation (an adequate approximation provided  $aT^4 \ll \rho c^2$ , where  $a$  is the radiation density constant, and equals  $7.6 \times 10^{-15}$  erg cm<sup>-3</sup> deg<sup>-4</sup>). The relevant equations are the equation of continuity

$$\frac{\partial \rho}{\partial t} + \nabla \cdot (\rho \mathbf{u}) = 0$$

the equation of motion

$$\frac{d\mathbf{u}}{dt} = \nabla \varphi - \frac{1}{\rho} \nabla p$$

Poisson's equation

$$\nabla^2 \varphi = -4\pi G \rho$$

the energy equation

$$\rho \frac{d}{dt} \left( 3 \frac{kT}{m_p} + \frac{aT^4}{\rho} \right) - \frac{p}{\rho} \frac{d\rho}{dt} = \frac{c}{3} \nabla \cdot \left\{ \frac{1}{\kappa \rho} (\nabla aT^4) \right\}$$

and the equation of state

$$p = \frac{2\rho kT}{m_p} + \frac{1}{3} aT^4$$

This set of equations is linearized about the zeroth order solution (corresponding to an isotropic, homogeneous, expanding cosmological model of arbitrary curvature)

$$\mathbf{u} = \mathbf{r} \frac{\dot{R}}{R}, \quad \rho R^3 = \text{constant}, \quad \frac{\ddot{R}}{R} = -\frac{4\pi\rho G}{3}, \quad TR = \text{constant}$$

where  $R = R(t)$ ,  $\rho = \rho(t)$ . If we assume in addition that the perturbation may be expanded in plane waves of the order of  $\exp(i\mathbf{n} \cdot \mathbf{r})$  and that the heat capacity of the radiation greatly exceeds that of the matter, we obtain

$$\frac{\partial^2 s}{\partial t^2} + 2 \frac{\dot{R}}{R} \frac{\partial s}{\partial t} - \left( 4\pi G \rho - \frac{kT}{m_p} \frac{4\pi^2}{\lambda^2} \right) s = -\frac{4aT^3}{3\rho} \frac{4\pi^2}{\lambda^2} T_1 \quad (1)$$

and

$$\left( \frac{\partial}{\partial t} + \frac{c}{3\kappa\rho} \frac{4\pi^2}{\lambda^2} \right) \frac{T_1}{T} = 1/3 \frac{\partial s}{\partial t} \quad (2)$$

where  $T_1$  is the temperature perturbation,  $s$  is the relative density perturbation ( $= \rho_1/\rho$ ) and  $\lambda = 2\pi n/R$  is the co-moving wavelength. For the optically thin case ( $T_1/T \ll 1$ ) the usual Jeans criterion is recovered<sup>2,4</sup>; that is, for instability we must have

$$\lambda > \left( \frac{2\pi kT}{m_p G \rho} \right)^{1/2}$$

In the optically thick limit, however, it can readily be shown by an asymptotic treatment of equations (1) and (2) that the dominant mode in  $s$  is damped on a time-scale

$$t_d \sim 5 \frac{\kappa \rho}{c} \frac{\lambda^2}{4\pi^2}$$

We shall apply this result to two models; one with  $\rho_0 = 3 \times 10^{-29}$  g/cm<sup>3</sup>, corresponding to an Einstein-de Sitter universe, and the other with  $\rho_0 \simeq 10^{-30}$  g/cm<sup>3</sup>, corresponding to an open universe of mean density consistent with observation<sup>11</sup>.

For an Einstein-de Sitter universe, the expansion time is approximately given by  $t \simeq 1.6 \times 10^{18} T^{-3/2}$  sec, leading to

$$t_d/t = 2.2 \times 10^{-9} M_\lambda^{2/3} T^{5/2} \rho_0^{1/3}$$

where  $M_\lambda = 4/3 \pi \rho \lambda^3$  is the mass contained in a small ( $\rho/\rho_0 \ll 1$ ) fluctuation. It follows that opaque fluctuations containing up to about  $10^{12} M_\odot$  are damped (that is,  $t_d/t \ll 1$ ). In other words, any primordial fluctuation of cosmological significance must be at least of proper diameter  $\lambda$  (where  $M_\lambda \simeq 10^{12} M_\odot$ ) at the decoupling epoch, when galaxy formation may occur. Now the angular diameter subtended by a fluctuation of proper diameter  $\lambda$  is  $H_0 \lambda z_D / 2c$ , where  $z_D$  is the value of the red-shift at decoupling, and  $H_0$  is the present value of Hubble's constant. We know that  $1 + z_D \simeq T_D/3 \simeq 1.33 \times 10^3$ , and taking  $H_0 = 100$  km/sec/Mpc, we find that the angular diameter of a primordial fluctuation must be about half of a minute of arc.

In the case of an open universe, the expansion proceeds approximately linearly with time, and one has the relation  $t \simeq 1.2 \times 10^{18} T^{-1}$  sec. Therefore

$$t_d/t = 2.9 \times 10^{-9} M_\lambda^{2/3} T^{3/2} \rho_0^{1/3}$$

and it follows that the critical mass for a fluctuation to survive into an epoch when galaxy formation is possible is about  $3 \times 10^{13} M_\odot$ . One may then show\* that this corresponds to an angular diameter of at least one minute of arc.

\* For large  $z$ , the angular diameter of a source of proper diameter  $\lambda$  in an open cosmological model (with zero cosmological constant and  $p \simeq 0$ ) is given approximately by the expression

$$\frac{z}{c} \frac{\lambda \sqrt{8\pi G \rho_0}}{3} \frac{1}{\sinh \left\{ \frac{2}{H_0} \sqrt{\frac{8\pi G}{3} \rho_0} \ln \sqrt{\frac{2}{\sigma_0}} \right\}}, \quad \text{where } \sigma_0 = \frac{4\pi G \rho_0}{3H_0^2}$$



Measurement of the isotropy of the 3° K cosmic microwave background sets an upper limit on the scale and amplitude of fluctuations at the epoch when matter and radiation decouple. Indeed, angular variation in 3° K background is due essentially to intrinsic variations in the decoupling temperature, provided that no significant density fluctuations occur in the universe on a scale much greater than 1 Mpcsec<sup>6</sup>.

A recent observation<sup>12</sup> on the isotropy of the 3° K background radiation at a frequency of 10,690 Mc/s implies that the background radiation over a right ascension interval of about 4 h is structureless to less than 0.02 per cent on an angular scale of about 1°. Angular resolution of one minute of arc or better, however, is required in order to detect primordial inhomogeneities. When observations at this resolution are forthcoming, it should be possible to answer the question of whether or not galaxies may have formed from fluctuations present *ab initio*. Dr J. P. Wright has suggested to me that another critical factor in interpreting the anisotropy limit will be the mean separation distance between coherent fluctuations at decoupling. A linearized treatment does not allow such considerations; however, our calculation sets an upper limit to the scale of anisotropy expected from primordial fluctuations.

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### Friedmann Cosmological Model with both Radiation and Matter

THE discovery<sup>1</sup> and investigation<sup>2,3</sup> of the cosmic black-body radiation have opened the door to fairly reliable calculations of such evolutionary phenomena as the temperature-density history of the universe, primordial element formation<sup>4</sup>, expansion anisotropy<sup>5</sup> and galaxy formation<sup>6</sup>. The isotropy of this radiation<sup>3,5</sup> supports the current practice of idealizing the universe as spatially homogeneous and isotropic and as filled with pressureless matter and isotropic radiation.

In this standard model of the universe the radiation dominates for roughly the first 2,000 years of evolution, and the matter predominates thereafter. Until now, such cosmological models have been studied numerically or by joining a radiation universe,  $p = \rho/3$ , to a matter universe,  $p = 0$ , smoothly at some particular time. (I choose throughout that  $c = G = 1$ .) I am aware of only one analytical solution to the Einstein field equations for a universe filled with radiation and matter, and we shall see that this solution<sup>7</sup> is physically unsatisfactory.

This communication presents a new<sup>8</sup> analytical solution to the cosmological model containing both radiation and matter. The assumptions of this model are: (1) spatial homogeneity and isotropy with the flat Friedmann line element

$$ds^2 = dt^2 - R^2(t) [dx^2 + dy^2 + dz^2] \quad (1)$$

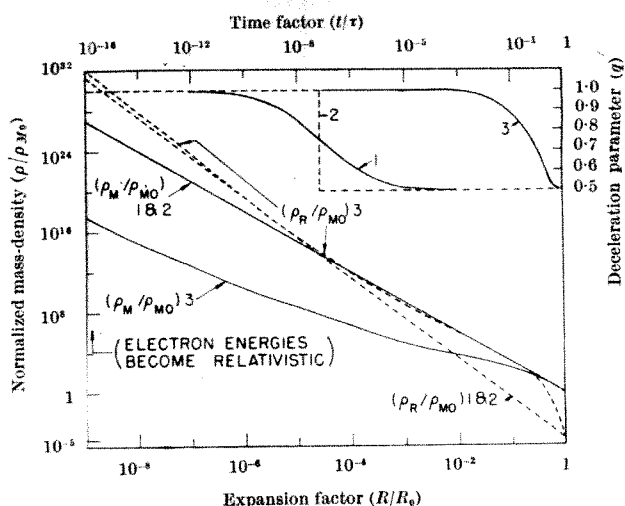


Fig. 1. Comparing the observables,  $\rho_M/\rho_{M0}$ , matter mass-density,  $\rho_R/\rho_{M0}$ , radiation mass-density, and  $q$ , deceleration parameter in the new solution (1), in the standard radiation-universe-joined-to-matter-universe solution (2), and in McIntosh's solution (3). The normalizing factors are  $\rho_{M0} = 2.3 \times 10^{-29}$  g cm<sup>-3</sup> and  $\tau = 5.9 \times 10^8$  years.

(2) a stress-energy with both pressureless matter, of mass-density  $\rho_M$ , and isotropic radiation, with pressure  $p_R = \rho_R/3$ ; and (3) independent, adiabatic expansion of the radiation and matter with negligible interchange of energy between them

$$\rho_M = \rho_{M0} (R/R_0)^{-3}, \quad \rho_R = \rho_{R0} (R/R_0)^{-4} \quad (2)$$

A subscript zero denotes the value of a quantity today. If the universe is spatially homogeneous, isotropic and Euclidean this model should be an excellent approximation from about 20 sec after creation, when the thermal electron energies become non-relativistic, until today. Under these assumptions the Einstein field equations, with zero cosmological constant, become

$$3 (\dot{R}/R)^2 = 8\pi (\rho_M + \rho_R) \quad (3)$$

$$2 (\ddot{R}/R) + (\dot{R}/R)^2 = -8\pi p_R$$

The chief result of this communication is the following analytical solution to equations (2) and (3)

$$t/\tau = [(R/R_0) - 2S_0] [(R/R_0) + S_0]^{\frac{1}{2}} + 2S_0^{\frac{3}{2}} \quad (4)$$

where

$$\tau \equiv (6\pi \rho_{M0})^{-\frac{1}{2}}, \quad S_0 \equiv \rho_{R0}/\rho_{M0} \quad (5)$$

The Hubble expansion rate and deceleration parameter are

$$H \equiv (\dot{R}/R) = (2/3\tau) (R/R_0)^{-2} [(R/R_0) + S_0]^{\frac{1}{2}} \quad (6)$$

$$q \equiv -\ddot{R}/(RH^2) = \frac{1}{2} [(R/R_0) + 2S_0] [(R/R_0) + S_0]^{-1} \quad (7)$$

and the total mass-density and pressure are

$$\rho_T \equiv \rho_M + \rho_R = \rho_{M0} (R/R_0)^{-4} [(R/R_0) + S_0] \quad (8)$$

$$p_T \equiv p_R = \rho_{M0} (S_0/3) (R/R_0)^{-4} \quad (9)$$

The observed Hubble expansion rate today<sup>9</sup>,  $H_0^{-1} = (8.8 \pm 1.6) \times 10^9$  years, and the observed temperature,  $3.0 \pm 0.5$  °K, of the cosmic black-body radiation determine the constants

$$\begin{aligned}\tau &= (5.9 \pm 1.0) \times 10^9 \text{ yrs} \\ S_0 &= \left(2.9 + \frac{5.4}{-1.8}\right) \times 10^{-2} \\ \rho_{M_0} &= (2.3 \pm 0.8) \times 10^{-23} \text{ g cm}^{-3} \\ \rho_{R_0} &= \left(6.8 + \frac{5.6}{-3.3}\right) \times 10^{-24} \text{ g cm}^{-3}\end{aligned}$$

In Fig. 1 we compare this new solution (1) with (2) the standard solution, in which a matter universe is joined smoothly, both  $R(t)$  and  $H(t)$  continuous, to a radiation universe when  $\rho_R \equiv \rho_M$

$$R/R_0 = \left\{ \begin{aligned} & (16 S_0/9)^{1/3} (t/\tau)^{1/3} \\ & [(t/\tau) + \frac{1}{4} S_0^2]^{1/3} \end{aligned} \right\} \text{ for } (t/\tau) \begin{matrix} \leq \frac{3}{4} S_0^2 \\ > \frac{3}{4} S_0^2 \end{matrix} \quad (10)$$

and (3) the solution of McIntosh<sup>8</sup> derived from

$$\log R(t) = 2 \int [3t + \beta^{-1} (1 - e^{-\beta t})]^{-1} dt + \text{constant} \quad (11)$$

The quantities  $H/H_0$  and  $\rho_M/\rho_{M_0}$  are essentially identical in all three models. The differences between McIntosh's model and the other two are most apparent in the observables  $\rho_M/\rho_{M_0}$ ,  $\rho_R/\rho_{M_0}$ , and  $q$ . Note that McIntosh's model enters the matter universe about a quarter billion years after creation, while the other two models become dominated by matter after only 2,000 years of evolution. During the period of cosmic nucleosynthesis, when  $R/R_0 \approx 10^{-8}$ , the matter density in McIntosh's model is  $10^9$  times smaller than in the other models.

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## Nine Million Wavelength Baseline Interferometer Measurements of 3C 273B

OBSERVATIONS on the angular dimensions of the variable radio source 3C 273B by Palmer *et al.*<sup>1</sup> using effective baselines of up to 2 million wavelengths have reduced the upper limit on its angular size to 0.025 sec of arc at a wavelength of 6 cm. A recent observation using a trans-Canadian baseline indicates that the source is unresolved at an operating frequency of 600 Mc/s and an effective baseline of 6 million wavelengths.

Predicted values of the angular dimensions of radio sources are based on Le Roux's model<sup>2</sup>. It is desirable to test the applicability of this model to variable sources exhibiting flat spectra in view of the cosmological implications which may be drawn from their dimensions.

In January 1967 we attempted to operate two of the NASA Deep Space Stations in Australia as an intensity interferometer<sup>3</sup> of exceptionally high resolution. The two stations were located at Island Lagoon, 12 miles south of Woomera and at Tidbinbilla, about 35 miles from Canberra. This gave a baseline of more than  $9 \times 10^6$  wavelengths at our operating frequency of 2,295 Mc/s. Signals from source 3C 273 were recorded in analogue

form together with appropriate timing codes derived from rubidium frequency standards. This attempt failed because of difficulties associated with subsequent analogue to digital conversion.

In early June the experiment was repeated, but this time the data were recorded in digital form at the two stations using analogue to digital converters controlled by SDS920 computers. The sampling rate (1 kc/s) was controlled by rubidium frequency standards at both stations, and sampling began at predetermined times. It is not possible to synchronize starting times accurately because of a small time difference between the station clocks that control the computers. These computers were programmed so as to sample for 96 sec every 2 min. For the remaining 24 sec the computers provided antenna pointing error information.

The pairs of tapes from the station computers were correlated in Adelaide, using the University of Adelaide computer (CDC 6400). The correlation coefficient  $\rho_r$  was computed, where

$$\rho_r = \frac{\sum_{N=r}^N X_N \cdot Y_{(N-r)}}{\left[ \sum_{N=r}^N X_N^2 \right]^{1/2} \left[ \sum_{N=r}^N Y_{(N-r)}^2 \right]^{1/2}}$$

$\rho_r$  is thus the correlation coefficient for a slip of  $r$  data points between  $X$  and  $Y$  and the data samples from the two stations.  $\rho_r$  corresponds to  $C(1, t)$  or  $\rho^2(1)$  of ref. 3.

The signal to noise ratio at the output of the square law detectors was extremely low ( $< 0.06$ ), so that the maximum possible value of  $\rho_r$  was  $4 \times 10^{-3}$ , assuming that 3C 273B has a strength of 25 flux units.

The correlogram in Fig. 1 required  $3.16 \times 10^6$  data samples from each station, which corresponds to nearly an hour of observing time. During this time the effective baseline varied between  $7.8 \times 10^6$  and  $9.1 \times 10^6$  wavelengths because of the rotation of the Earth. The observed peak value of the correlation coefficient ( $2.5 \times 10^{-3}$ ) is considerably less than its predicted maximum value ( $4 \times 10^{-3}$ ) for an unresolved source.

Except at the zenith, there is a difference in time of arrival of the signals at the two stations. This difference in arrival time is not constant. It changes with the orientation of the baseline at a rate of up to 1 msec/h when the source is near the zenith. This causes a time slip between the two sets of data which varies at the same rate. The sampling rate is one sample/msec so that continuous compensation for this slip is not possible without interpolation between samples. This was not done and we estimate the penalty to have been a 20 per cent reduction in the correlation peak.

In Fig. 1 time slips due to changing baseline orientation between observations were removed by slipping the sets of data by appropriate amounts. If the clocks at the two stations had been in synchronism, the correlation peak would have coincided with the  $Y$  axis. The difference between the clocks in milliseconds is equal to the displacement of the peak from the  $Y$  axis. In this instance it is about 2.5 data points, that is, 2.5 msec.

In addition to variations in time slip during observations, the correlation peak is very sensitive to signal strength changes such as those resulting from antenna pointing errors. A fall of 1 dB in signal strength at both antennas degrades the correlation peak by 4 dB.

The post detection bandwidth (400 c/s) was dictated by the availability of suitable filters, and the ratio of sampling speed to bandwidth is uncomfortably low. Under such conditions the correlation peak contains few points, and the determination of its amplitude is difficult. Faster sampling rates may be available in future.

There is some evidence of reduced correlation when the effective baseline exceeds  $8 \times 10^6$  wavelengths but, in view of the known sources of correlation loss, the observed

peak value of  $(2.5 \times 10^{-3})$  is not inconsistent with a totally unresolved source, that is, a source with an angular dimension less than 0.008 sec of arc.

In general, it was necessary to combine the results of a number of observations in order to produce a correlation peak that rose significantly above the noise. One exception was a 15 min ( $88 \times 10^4$  samples) observation at  $7.8 \times 10^6$  wavelengths. This is shown in Fig. 2. It is one of the results used in Fig. 1, and was apparently a period in which antenna pointing errors were negligible.

The direction of the measured dimension across the source varies with its hour angle. Measured from the axis of the Earth in a clockwise sense, this direction lay exclusively in the second quadrant. The results quoted here refer to orientations of the measured dimension between  $108^\circ$  and  $110^\circ$ .

The system we have used is applicable to any pair of stations in the Deep Space Network. At the moment these stations have duplexers, etc., in their antenna feeds. This causes a signal loss of at least 3 dB, or a reduction in the correlation peak of 12 dB.

The position of the correlation peak gives the difference between the clocks of the two stations. This provides a new method of comparing widely separated clocks. The accuracy of the measurement depends on the post detection bandwidth and the sampling speed. For example, a

20 kc/s post detection bandwidth with a 100 kc/s sampling rate should provide an accuracy of at least 10  $\mu$ sec.

Nearly an order of magnitude improvement in the resolution afforded by the Australian-based Deep Space Station pair can be obtained by using two or more DSS stations on different continents. Thus identical procedures may be applied to any such pair to ascertain whether the angular dimensions are compatible with the period of variation and the red-shift of the source.

We thank the staffs of both stations for their help, particularly A. J. Legg of DSS 41, who wrote the programme for the station computers. We also thank J. N. Weadon of the University of Adelaide for writing the correlation programme for the CDC 6400. The use of the stations was by kind permission of the US National Aeronautics and Space Administration and the Jet Propulsion Laboratories.

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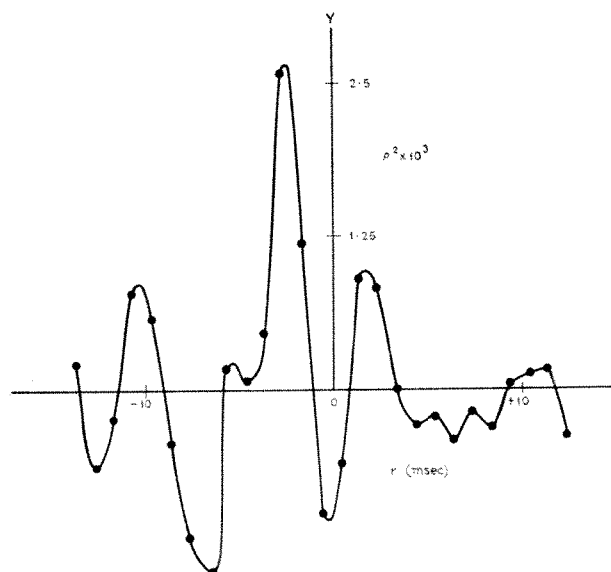


Fig. 1.

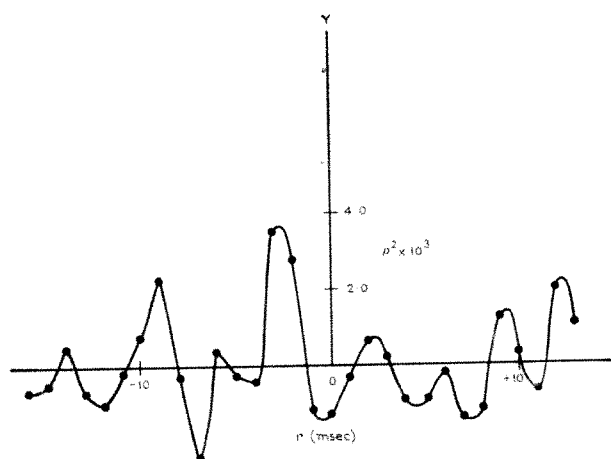


Fig. 2.

### NGC 5189 and the CRUX X-ray Source

Of the objects in the southern hemisphere which are generally classified as planetary nebulae<sup>1</sup> undoubtedly the two with the most strikingly anomalous forms are NGC 6302 (refs. 2, 3) and NGC 5189 (refs. 4, 5). Attention has been drawn by Minkowski and Johnson to the fact that NGC 6302 lies close to the position of the cosmic X-ray source SCO XR-2. It is interesting therefore that NGC 5189 ( $13.5h-65^\circ.7$ ) lies in the direction of the X-ray source CRUX recently discovered by Harries *et al.*<sup>6</sup>. The uncertainty in the position of this source is considerable (especially in declination), two measures giving  $13.7h-62^\circ$  and  $13.5h-66^\circ$ . Thus clearly no positive identification can yet be claimed. Nevertheless, in view of the small number of known X-ray sources it seems quite remarkable to find even approximate agreement in positions between two of them and two such highly peculiar objects as NGC 5189 and NGC 6302 unless there is indeed some physical connexion.

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### PLANETARY SCIENCE

#### Molecular Fluorescence accompanying the Twilight Injection of Triethylborane into the Upper Atmosphere

A RECENT note<sup>1</sup> described the yellow-green fluorescence which resulted from the injection of triethylborane into the upper atmosphere. At that time, the radiating species which produced this fluorescence was not identified, and

a spectrum has since been obtained from a similar release of triethylborane.

Originally, a boron compound was chosen in an attempt to produce boron monoxide in the upper atmosphere. The attempt to form boron monoxide had a dual purpose: to determine temperature from the fluorescence spectra and compare this with temperature obtained from aluminium oxide; and to obtain a material with a molecular weight more nearly equal to the average molecular weight of the atmosphere. The latter would allow more reliable diffusion data to be obtained than from a release of trimethyl aluminium.

Because no spectra were obtained in the first experiment<sup>1</sup>, the experiment was repeated. The object of the second experiment was to identify the radiating species from the molecular fluorescence spectrum, and at the same time to obtain data on the upper atmospheric wind from the motion of the released material.

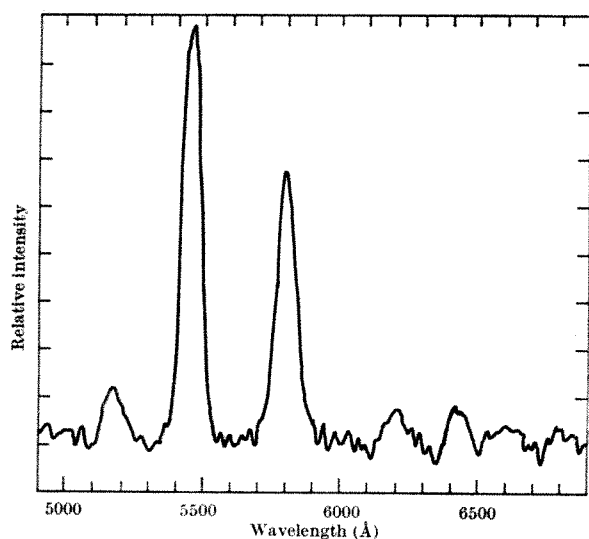


Fig. 1. Spectrum obtained from the sunlit release of triethylborane at height between 90 km and 178 km.

A two-stage Nike-Cajun rocket system was used to disperse 6.2 kg of triethylborane above the Sandia Corporation launch site at Kauai, Hawaii. Payload characteristics including orifice diameters and nitrogen pressure were the same as reported in ref. 1. The rocket was launched on June 2, 1967, at 1947 Hawaii standard time or on June 3 at 0547 GMT. Liquid triethylborane was continuously ejected, beginning at 90 km on the ascent and continuing through apogee at 178 km. The depression angle of the Sun at launch was  $\approx 7^\circ$  and the shadow of the Earth was at 30 km above the site.

Low resolution spectra were obtained using a photographic spectrograph with an effective aperture of  $f/0.87$  and a reciprocal linear dispersion of 208 Å/mm. Spectra were recorded on calibrated Eastman Kodak spectroscopic plates. A spectrum obtained from the sunlit release of triethylborane is shown in Fig. 1. The observed bands have been identified as the  $A^2\pi \rightarrow ^2\pi$  transition of boron oxide.

The most pronounced difference between this spectrum and published low dispersion spectral data<sup>2</sup> on boron oxide is the absence of the band at 6025 Å.

The molecular fluorescence spectrum obtained from a sunlit release of triethylboron has been identified as due to boron oxide. This molecule is not suitable for temperature measurement and is no better than aluminium oxide for obtaining diffusion data.

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## PHYSICS

### Barrier to Coalescence in Stabilized Emulsions

ALTHOUGH emulsions of immiscible liquids are thermodynamically unstable, it is well known that the rate of coalescence may be reduced by adding small quantities of a third component (stabilizer) which is tensioactive. Many theories have tried to explain emulsion stability but none has been perfectly general. The most general theory is based on the stabilization of thin liquid films during approach of droplets as a result of the Gibbs-Marangoni effect<sup>1</sup>. This stabilizing effect may operate during the early stages of emulsion formation. Electrical repulsion must be a barrier to coalescence in emulsions stabilized by ionized detergents<sup>2</sup>, but many emulsions of high stability may be prepared in which no electrical potential barrier is believed to be present. The purely hypothetical "solvation barriers"<sup>3</sup> cannot explain the stability of water/oil emulsions where the oil is a paraffin, for example, in which no orientation at the interfaces would be expected. A barrier resulting from the high viscosity of the stabilizing film<sup>4</sup> cannot account for the high stability of emulsions stabilized by monolayers which do not increase the interfacial viscosity. The difficulty of flow of the continuous medium from thin films between approaching droplets results in a hydrodynamic barrier which would not be expected to be influenced very greatly by the presence of adsorbed monolayers. These latter two mechanisms do not offer an explanation of emulsion type.

If we consider the change in free energy of a system of two droplets during the process of coalescence in the presence of a third adsorbed component, we see immediately a mechanism which will tend to produce a barrier to coalescence. The coalescence of two droplets with no adsorbed substance at their interface proceeds with a decrease in free energy because the product  $\gamma A$  decreases as a result of the decrease in interfacial area ( $A$ ), the interfacial tension ( $\gamma$ ) remaining constant. With an adsorbed third substance, however, the interfacial tension may change if the rate of decrease of interfacial area is fast in comparison with the rate of desorption of the third component. It will produce a temporary displacement of the adsorption equilibrium. The chemical potential of the stabilizer in the interface is raised as a result of the compression, and it will attempt to return to its equilibrium interfacial pressure ( $\pi$ ), which it can only do by desorption or increasing the interfacial area. The compression of the monolayer in this manner produces an elastic restoring mechanism which tends to oppose coalescence.

When two droplets of radius  $0.1\mu$  coalesce, there is a decrease in interfacial area of the order of  $10^{-10}$  cm<sup>2</sup>. If the average increase of  $\pi$  during coalescence is 1 dyne/cm, the work required for compressing the monolayer ( $\int \pi dA$ ) is of the order of  $10^4 kT$ . It might appear that this mechanism would produce emulsions of greater stability and that the size of the barrier would increase with increasing size of droplets. It is, however, probable that the transition complex<sup>5</sup> corresponds only to an initial critical stage in the compression. Once a sufficiently large opening is made in the droplets, the coalescence



will proceed spontaneously. Moreover, there are two factors which will tend to lower the energy barrier as the droplet size is increased. First, for equal rates of compression ( $dA/dt$ ), the rate of increase of  $\pi$ , ( $d\pi/dt$ ), will be less for a large interface than a small one, because  $d\pi/dA$  is inversely proportional to  $A$  (the compressibility  $1/A \, dA/d\pi$  being constant). Second, the rate of decrease of interfacial area ( $dA/dt$ ) would be expected to be less for two large drops coalescing if we consider the average kinetic energy of the droplets ( $\frac{1}{2}mv^2$ ) to be constant, thus allowing more time for desorption. We conclude that for a given system, there will be an optimum droplet size which will produce a maximum barrier to coalescence. This is generally confirmed in practice for the mean particle size usually becomes stabilized during formation of emulsions.

An empirical rule which appears to be very general for the prediction of emulsion type is that the phase in which the stabilizer is more soluble becomes the continuous phase<sup>6</sup>. Thus, on the basis of the present model, when two droplets collide in a system in which the stabilizer is soluble in the dispersed phase, the adsorbed stabilizer near the point of collision is free to desorb and diffuse away into the droplets whereupon coalescence may occur more easily. On the other hand, when the stabilizer is soluble in the continuous phase, the adsorbed stabilizer near the point of collision is prevented from desorbing and diffusing away because of the displacement of the diffusion medium in this region (see Fig. 1). It must therefore remain in the interface, tending to give rise to a sudden increase in the interfacial pressure of the adsorbed monolayer.

The choice of stabilizers is usually made by a trial and error procedure, but on the basis of the model it is possible to predict fairly accurately the efficiency of a particular stabilizer (see, however, Davies, J. T., *Proc. 2nd Int. Cong. Surface Activity*, 1, 426 (London, 1957)). In principle, it is possible to map the energy barrier to coalescence for a given system. For this, we require to know the time rate of change of the interfacial area during coalescence; the equilibrium adsorption isotherm ( $\gamma$ - $C$ ) and  $\pi$ - $A$  relationships of the monolayer substance; and the rate of desorption of the monolayer as a function of  $\pi$  and time.

Although the energy barrier must be assessed for the particular system studied, some general predictions may be made on the basis of the model.

In order to produce high stability, a stabilizer should have a high free energy of adsorption. This is because for equal increments in  $\pi$  above its equilibrium value, a stabilizer with a high free energy of adsorption will

produce smaller concentration gradients near the surface than a stabilizer with a low free energy of adsorption. For high stability, the monolayer of the stabilizer should have as low a compressibility as possible. Any factor which reduces the rate of desorption of the monolayer should increase the stability. Hindered desorption has previously been suggested to be important in stabilizing of emulsions<sup>7</sup>. Thus high viscosity of the continuous phase would contribute to an increase in the size of the coalescence barrier as well as increasing the stability by reducing the frequency of collision of droplets. Strong agitation of the emulsion would contribute to lower stability because it would tend to eliminate the diffusion barrier to desorption. In the case of ionized stabilizers, the rate of desorption may be considerably reduced by addition of non-ionic surface active agents which lower the electrical potential and therefore the chemical potential of the stabilizer in the interface. Thus the efficiency of alkyl sulphates as stabilizers is greatly increased by the presence of alcohols and other non-ionic surfactants<sup>8</sup>.

It should prove interesting to compare values of the energy barrier calculated on the basis of this model with values determined experimentally from emulsion stability measurements. Unfortunately, few reliable experimental values are available<sup>9</sup>.

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## Temperature of a Moving Body

LANDSBERG<sup>1,2</sup> has raised the question of the correct definition of temperature in relativistic thermodynamics. He advances<sup>3</sup> a general case against the orthodox transformation of temperature by considering the case of two systems, possessing equal proper temperatures,  $T_0$ , and in relative motion. Then observers moving with the two systems will each judge the temperature of the other system to be lower than that of the system with which he is moving. If the usual relation exists between temperature difference and heat flow, both proper temperatures should fall, a conclusion Landsberg finds unacceptable. The argument is not, however, completely convincing, and Williams<sup>4</sup> has pointed out that the direction of energy flow between two systems is, in general, dependent on the frame of reference in which the flow is observed.

Landsberg's example can be examined in the following manner. Observations made in the C.M. frame of system A show that the moving system B has a temperature of  $T_0/\beta < T_0$ , according to the orthodox transformation. Hence in this frame the system A should lose energy, and its temperature should fall, while system B should gain energy and its temperature should rise to  $T' > T_0/\beta$ . It follows then that the proper temperature of B should rise to  $\beta T' > T_0$ , unless the factor  $\beta$  has decreased to such an extent that  $\beta < T_0/T'$ . Landsberg does not examine this possibility. If, however, one requires that each system

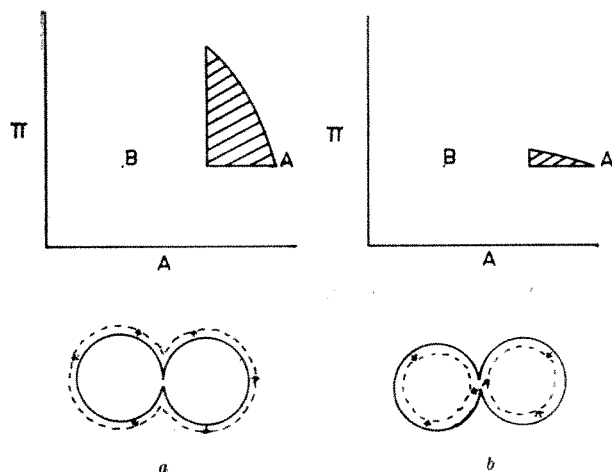


Fig. 1. Transition complex in coalescence. a, Stabilizer soluble in continuous phase; b, stabilizer soluble in dispersed phase. A, B, Initial and final states in coalescence. Hatched area, critical work of compression of monolayer. - - - - , Diffusion layer.

remains at rest in its own C.M. frame when it acquires purely thermal energy, then  $\beta$  is unchanged by the process. If the process is now examined from the C.M. frame of  $B$  one will have to conclude that the proper temperature of  $B$  should fall. It is unacceptable that one should be able to deduce that the proper temperature of  $B$  will either rise or fall according to the frame of reference in which one chooses to examine the process.

Williams's objection to Landsberg's argument fails in this case because the two systems are required to remain at rest in their respective C.M. frames. Consequently, the sign of the energy gained by either system is invariant to a Lorentz transformation and hence the direction of energy flow is invariant. This can be seen as follows. If a system suffers a change in its energy momentum four vector of  $(c \overline{dP}, dE)$ , then the value of  $(c^2 dP^2 - dE^2)$  is invariant. In the C.M. frame this value is  $-dU^2$ , where  $dU$  is the gain in the internal energy, provided the system remains at rest in this frame. Hence

$$c^2 dP^2 - dE^2 < 0$$

If this inequality is satisfied the sign of  $dE$  cannot be reversed by a Lorentz transformation.

In Williams's examples the systems do not remain at rest in their respective C.M. frames.

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## MOLECULAR STRUCTURE

### Calculation of Crystal Packing: A Novel Approach to the Phase Problem

THE structure and relative stability of helical macromolecules, consisting of repetitions of equivalent monomer units, have been analysed previously<sup>1-5</sup>. The conformational potential energy of the helices was computed as a function of the angles of rotation about the skeleton bonds, using semi-empirical potential functions to take into account intramolecular Van der Waals interactions between "non-bonded" atom pairs. The geometry of the most stable helices was deduced from the parameters corresponding to the lowest minima. It was also shown<sup>6</sup> that the crystal packing of hexamethylbenzene molecules may be predicted by locating the deepest minima of the intermolecular Van der Waals potential energy. By fixing bond lengths and angles and by knowing lattice parameters and crystal symmetries, the potential energy can be calculated for all the rotational and translational degrees of freedom which define the asymmetric unit position.

Equivalent methods, based on the analysis of intermolecular distances in molecular crystals, have been reported by Williams<sup>7,8</sup> and Rabinovich and Schmidt<sup>9</sup>. We are trying to apply this idea to the solution of the phase problem in molecular crystals when the molecular geometry is known, and have analysed the crystal structure of 5 $\alpha$ -androstane-3,17-dione (ANDR). The crystal structure of ANDR is unknown. We found the unit cell parameters of a single crystal, crystallized from normal hexane, to be in good agreement with those reported by Ohrt, Haner and Norton<sup>10</sup>, namely

$$a = 12.704 \text{ \AA}; b = 6.168 \text{ \AA}; c = 21.337 \text{ \AA}; \beta = 91^\circ 16'$$

The space group is C2, monoclinic non-centrosymmetric, with four molecules in general positions at  $x, y, z$ ;  $\bar{x}, y, \bar{z}$ ;  $\frac{1}{2} + x, \frac{1}{2} + y, z$ ;  $\frac{1}{2} - x, \frac{1}{2} + y, \bar{z}$ . Because the  $y$  co-ordinate can be fixed at zero for space group reasons, the translational

degrees of freedom are reduced to two. The experimental density,  $1.14 \text{ g cm}^{-3}$ , determined by flotation method, agrees with the calculated one,  $1.14 \text{ g cm}^{-3}$ , on the basis of four ANDR molecules per unit cell.

The conformation of ANDR was derived using bond lengths C—C =  $1.54 \text{ \AA}$ ; C—H =  $1.08 \text{ \AA}$  and C=O =  $1.23 \text{ \AA}$  and tetrahedral bond angles except for the following

$$\begin{aligned} \text{C}_2\text{C}_3\text{O}_{20} = \text{C}_4\text{C}_5\text{O}_{20} &= 125^\circ, \text{C}_{13}\text{C}_{17}\text{O}_{21} = \text{C}_{16}\text{C}_{17}\text{O}_{21} = 128^\circ, \\ \text{C}_{13}\text{C}_{17}\text{C}_{16} = \text{C}_{16}\text{C}_{15}\text{C}_{14} &= 104^\circ, \text{C}_{17}\text{C}_{16}\text{C}_{15} = 105^\circ \end{aligned}$$

and

$$\text{C}_{17}\text{C}_{13}\text{C}_{14} = \text{C}_{13}\text{C}_{14}\text{C}_{15} = 100^\circ$$

(see Fig. 1 for the atomic numbering).

The potential energy of a set of fourteen molecules in contact with the central one was calculated as a function of three rotational ( $\psi_1, \psi_2, \psi_3$ ) and two translational ( $t_x, t_z$ ) degrees of freedom, by adding the separate terms corresponding to "non bonded" pair interactions between carbon, oxygen and methyl groups. In a preliminary calculation the semi-empirical potential functions used previously<sup>2,3</sup> were adopted and the calculations were performed by means of a programme written for an IBM 7040 computer. To save computing time, angular and translational increments of  $20^\circ$  and  $1 \text{ \AA}$  respectively were used in the first run, neglecting contributions to the potential energy when the intermolecular distances were greater than  $5.5 \text{ \AA}$ . Broad regions of minima of the potential energy were analysed by means of a second run with angular and translational increments of  $10^\circ$  and  $0.5 \text{ \AA}$  respectively, including also the hydrogen atoms. All but nine minima were discarded because of some short intermolecular approaches.

All these nine minima were tested by calculating the reliability factor  $R$  for the ANDR  $h0l$  and  $0kl$  observed reflexions, which number 89 and 62 respectively, by allowing small systematic translations and rotations of ANDR molecules in the regions of the minima. Seven minima gave unsatisfactory  $R$  values, usually much greater than 0.70, and only the two deepest were considered promising. These minima have the same Eulerian angles<sup>6</sup> and translational parameters except for the  $\psi_3$  angles, which differ by  $170^\circ$  of rotation about the axis shown in Fig. 1. Moreover, most of the atoms coincide in the  $h0l$  projection, while in the  $0kl$  projection the differences are more marked.

A successive rigid body refinement by means of structure factors and Fourier synthesis calculations showed that the higher minimum should be rejected. The lower one gave the following agreement indices

$$R_{h0l} = 0.38; R_{0kl} = 0.35$$

with a mean isotropic temperature coefficient of  $3.5 \text{ \AA}$  (ref. 2). The parameters corresponding to the best  $R$  values are

$\psi_1 = -6^\circ$ ;  $\psi_2 = 343^\circ$ ;  $\psi_3 = 191^\circ$ ;  $t_x = 2.80 \text{ \AA}$ ;  $t_z = 5.21 \text{ \AA}$  and differ from those found by the potential energy analysis by

$$\begin{aligned} \Delta\psi_1 &= -4^\circ; \Delta\psi_2 = -3^\circ; \Delta\psi_3 = -1^\circ; \Delta t_x = 0.40 \text{ \AA}; \\ \Delta t_z &= 0.09 \text{ \AA} \end{aligned}$$

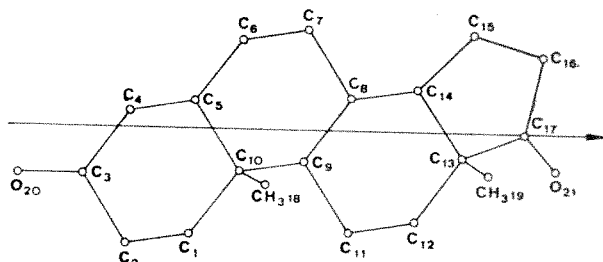


Fig. 1. ANDR molecule and atomic numbering. The axis is drawn to compare the two best models found.

Table 1. ANDR FINAL ATOMIC FRACTIONAL COORDINATES

C1	0.0631	-0.0504	0.1306
C2	0.0535	-0.1968	0.0724
C3	0.1521	-0.1700	0.0323
C4	0.2506	-0.2341	0.0714
C5	0.2602	-0.0876	0.1295
C6	0.3587	-0.1517	0.1686
C7	0.3683	-0.0052	0.2268
C8	0.2697	-0.0320	0.2668
C9	0.1712	0.0320	0.2278
C10	0.1616	-0.1144	0.1696
C11	0.0727	0.0052	0.2678
C12	0.0822	0.1517	0.3260
C13	0.1808	0.0876	0.3650
C14	0.2793	0.1144	0.3250
C15	0.3658	0.0635	0.3744
C16	0.3289	0.1769	0.4344
C17	0.2120	0.2287	0.4219
C18	0.1521	-0.3518	0.1904
C19	0.1712	-0.1497	0.3859
O20	0.1521	-0.1072	-0.0224
O21	0.1560	0.3546	0.4508

The relevant displacement along the  $x$  axis with respect to the position of the minimum brings O<sub>20</sub> into short contact with C<sub>2</sub> of the centrosymmetrical molecule and O<sub>21</sub> with C<sub>16</sub> of the molecule related by the diad axis.

The final atomic fractional co-ordinates are listed in Table 1 and the corresponding  $h0l$  and  $0kl$  electron density projections are reported in Figs. 2 and 3. Although the  $R$  values are rather high, we feel that they are adequate in view of the complexity of the structure. Fig. 4 shows the arrangement of the molecules in the crystal.

The  $h0l$  Fourier synthesis shows a low electron density of some peripheral atoms, probably due to a thermal anisotropy which is visible in the map. This effect appears to be strong and in the same direction as in the crystal structure of the parent compound 5 $\alpha$ -androstane-3 $\alpha$ -ol-17-one (ref. 11), in which the rigid body libration of the whole molecule has the largest component normal to a plane through the ring system. Moreover, the corresponding Fourier, performed with calculated diffraction amplitudes, indicates the existence of series-termination effects, which can be ascribed to the lack of  $h0l$

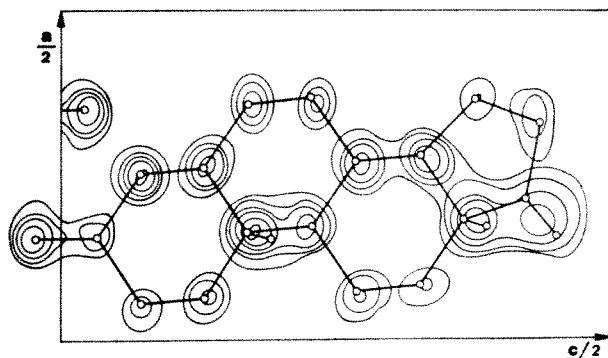


Fig. 2. (010) Fourier synthesis showing the ANDR molecular model. Contours are at arbitrary intervals.

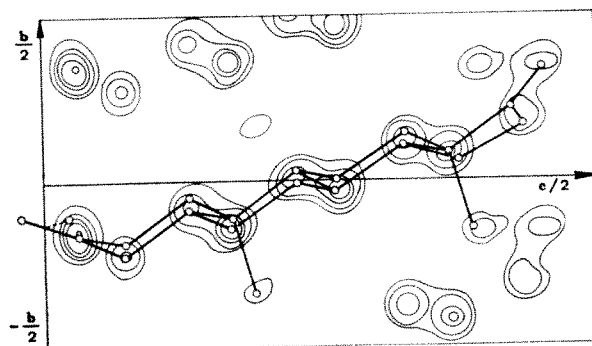


Fig. 3. (100) Fourier synthesis showing the ANDR molecular model. Contours are at arbitrary intervals.

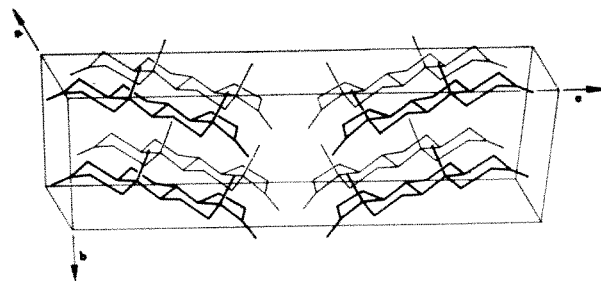


Fig. 4. View of the packing of the ANDR molecules in the crystal.

reflexions with  $2 \sin \theta > 1.5$ . On the other hand, the absence of spurious peaks in the Fourier syntheses, together with the good qualitative agreement between  $F_{\text{obs}}$  and  $F_{\text{calc}}$ , indicate that the structure is substantially correct. Better agreement could hardly be expected in view of the errors inherent in the molecular model of the pentacyclic ring structure. Three-dimensional data are being collected to refine both the molecular packing and the geometry.

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## Reconstitution of Elastin

PARTRIDGE *et al.*<sup>1</sup> observed that hydrolysis of pure insoluble elastin from bovine ligamentum nuchae by oxalic acid gave rise to soluble elastin consisting of two fractions:  $\alpha$ -elastin with a mean molecular weight of 60,000–84,000; and  $\beta$ -elastin with a mean molecular weight of 5,000. Solutions of  $\alpha$ -elastin formed a reversible coacervate phase on heating. Hall and Czerkawski<sup>2</sup> showed that coacervation could be affected by detergents. On prolonged heating of the solutions of soluble elastin, a reconstituted product was prepared with characteristics similar in many respects to those of the original elastin<sup>3</sup>.

The reconstitution of elastin involves two steps<sup>4</sup>—the unfolding of polypeptidic chains and aggregation.

We investigated the possibility of reconstituting soluble proteins prepared by alkaline and acid hydrolysis of pure elastin from bovine ligamentum nuchae. Elastin isolated by the method of Partridge *et al.*<sup>1</sup> was either dispersed in 0.5 normal sodium hydroxide, kept at room temperature and, after the dissolution was complete, the solution dialysed against water to neutrality and lyophilized; or dissolved in hot 0.25 molar oxalic acid, dialysed against water and lyophilized.

We found that hydrolysed elastin could be precipitated in an elastic gel like sediment by heparin or heparinoids under suitable conditions.

The precipitation takes place in a very narrow range of pH, ionic strength and heparin (or heparinoid) concentration as shown in Table 1. The results were obtained with a synthetic heparinoid (disulphate ester of a high molecular fraction of starch).

Precipitation could also be achieved by dialysis of solutions containing soluble elastin and heparinoid in an appropriate ratio against 0.2 molar acetic acid.

We also tried to prepare similar products using detergents in place of heparin, but elastic gels did not form under the conditions of maximum coacervation of acidic hydrolysed elastin quoted by Hall and Czerkawski<sup>2</sup>. Changing the conditions, however, resulted in precipitation with anionic detergents (dodecylsulphate) or detergents containing both cationic and anionic groups, for example, 'Empigen OB'. Precipitation did not occur when cationic detergents were used.

Elastic gel like precipitates were also prepared by the addition of hydrochloric acid to solutions containing soluble elastin and dodecylsulphate or 'Empigen OB'. The concentration of detergent was 5 mg/ml., the final concentration of the acid was 0.1 molar. Precipitates also occurred by dialysis of solutions containing soluble elastin and detergent with acetic acid as described earlier. The products obtained were similar to those prepared with heparinoid.

The amino-acid compositions of the original elastin and the precipitated products are given in Table 2. Amino-acid analysis was carried out by the method of Spackman *et al.*<sup>5</sup>.

It is obvious that only slight differences exist between elastin and the product precipitated by heparinoid. The product prepared by precipitation with the detergent shows considerable differences in the content of glycine, valine, alanine and proline.

The precipitation by heparin involves all protein fractions in the solution; consequently even  $\beta$ -elastin must undergo this type of precipitation although it is not reconstituted by the method of Wood<sup>3</sup>. On the other hand, the precipitation by detergents is incomplete.

The fraction not precipitated is rich in those amino-acids in which the precipitate is poor. This contradicts

Table 2. AMINO-ACID COMPOSITION OF ELASTIN AND OF THE PRECIPITATED PREPARATIONS

	Elastin from ligamentum nuchae	Elastin reconstituted by heparinoid	Elastin reconstituted by dodecylsulphate
Lysine	0.5	0.4	0.2
Arginine	0.9	0.8	0.7
Aspartic acid	0.7	0.9	0.9
Threonine	Traces	0.8	0.5
Serine	0.4	0.7	0.5
Glutamic acid	2.8	3.0	2.4
Proline	16.9	18.6	11.7
Glycine	24.2	22.0	29.8
Alanine	25.9	26.8	22.5
Valine	16.1	16.0	11.9
Methionine	0.4	Traces	Traces
Isoleucine	2.5	2.6	2.2
Leucine	8.0	7.3	6.2
Tyrosine	2.0	1.9	1.5
Phenylalanine	5.6	4.5	5.2
Hydroxyproline	1.86	1.69	1.78
Nitrogen	16.60	16.01	15.85

Values given as g/100g of protein.

the results given by Partridge *et al.*<sup>1</sup> and some other authors<sup>6</sup>, who found no differences in the amino-acid composition of  $\alpha$ -elastin and  $\beta$ -elastin. On the other hand, Gotte *et al.*<sup>7</sup> found that an interfibrillar protein fraction could be extracted from elastin prepared by the Partridge method, and that the composition of this fraction differed from the composition of fibrillar elastin. This might account for the different composition of the protein fractions precipitated and not precipitated by detergents.

Taking into consideration the narrow limits of pH and ionic strength in which elastic gel like sediments are formed, the results reported here support the theory that ionic interaction is involved in the reconstitution of elastin. The fact that the precipitation takes place at low values of pH suggests that an interaction occurs between strong acidic groups of the electrolyte and basic groups of polypeptides, and that the dissociation of weak acidic groups of polypeptides is suppressed.

The interaction between elastin and acidic polysaccharides may also be of importance for elastin fibrogenesis in view of the presence of acidic polysaccharides in ground substance. The fact that in identical conditions of pH and ionic strength the formation of the precipitate depends on the protein to polysaccharide ratio suggests that acidic polysaccharides contribute to the stability of elastin structure.

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Table 1a. INFLUENCE OF pH ON THE PRECIPITATION OF ELASTIN

pH	Product
	Alkaline hydrolysate      Acidic hydrolysate
1.75	Gel like elastic sediment      Gel like elastic sediment
2.00	Gel like elastic sediment      Gel like elastic sediment
2.36	No reaction      No reaction
and above	

Solutions in Britton-Robinson buffer; ionic strength 0.05 for alkaline hydrolysate and 0.2 for acidic hydrolysate, respectively. The ratio of protein to heparinoid is 10 to 1.

Table 1b. INFLUENCE OF IONIC STRENGTH ON THE PRECIPITATION OF ELASTIN

Ionic strength	Product
	Alkaline hydrolysate      Acidic hydrolysate
0.02	Non-elastic sediment      Opalescence
and under 0.05	
0.10	Elastic gel like sediment      Small amount of non-elastic sediment
0.20	Poorly elastic sediment      " " "
0.50	" " "      Elastic gel like sediment
1.00	" " "      Poorly elastic sediment

Solutions in Britton-Robinson buffer, pH = 1.80. The ratio of protein to heparinoid is 10 to 1.

Table 1c. INFLUENCE OF HEPARINOID CONCENTRATION ON THE PRECIPITATION OF ELASTIN

Concentration of heparinoid (mg/ml.)	Ratio protein: heparinoid	Product
		Alkaline hydrolysate      Acidic hydrolysate
0.0005-0.05		No reaction      No reaction
0.5	25:1	Incoherent precipitate      Incoherent precipitate
1.25	10:1	Elastic gel like sediment      Elastic gel like sediment
2.50	5:1	Poorly elastic sediment      Poorly elastic sediment
3.75	3.3:1	Opalescence      Opalescence
5.0	2.5:1	" " "
10.0	1.25:1	" " "

Solutions in Britton-Robinson buffer, pH = 1.80; ionic strength 0.05 for the alkaline hydrolysate and 0.2 for the acidic hydrolysate.

## CHEMISTRY

### Energy Transfer from Chemiluminescent Species in Polymers

PREVIOUS work<sup>1</sup> has shown that in polymers when initiators decompose into primary free radicals chemiluminescence is produced in the visible region of the spectrum. When dicyclohexylperoxy-dicarbonate (P.C.) decomposes in polycarbonate, polystyrene and polymethyl methacrylate two maxima in the chemiluminescence spectra are observed at  $\lambda = 4500 \text{ \AA}$  and  $\lambda = 5300 \text{ \AA}$ , each apparently arising from a different elementary reaction. The short-wave-



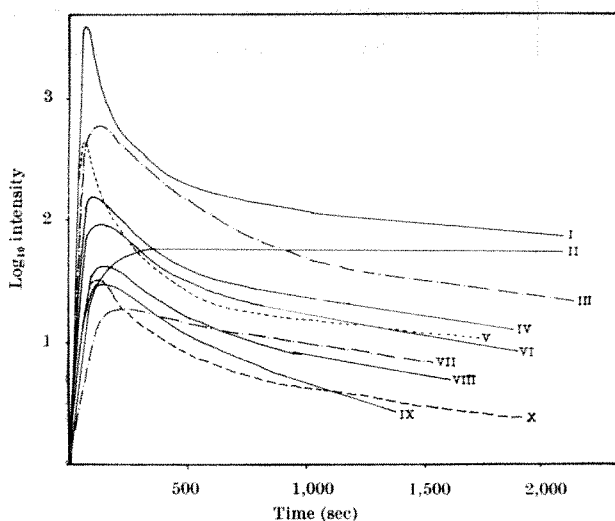


Fig. 1. Kinetics of decay of chemiluminescence. I, Rubrene (5520); II, 9,10-dibromoanthracene (4400); III, rhodamine B (5800); IV, rhodamine 6G (5800); V, 1-5 diphenyl (3 para diphenyl) pyrazoline (4580); VI, phosphine 3R (4950); VII, quinine bisulphate (4700); VIII, naphthalene (4900); IX, pyronine Y (5600); X, pyronine G (5600). Figures in brackets show wavelength in Å of emission maxima.

length chemiluminescence probably results from cage disproportionation of radicals from the initiator



giving an electronically excited cyclohexanone molecule in its triplet state. The nature of the elementary reaction giving rise to the long-wave chemiluminescence is as yet unknown.

The present work is a study of energy transfer from the chemiluminescent species to different energy acceptors introduced into the polymers. The energy transfer was studied spectroscopically, and, in general, in the presence of a large enough concentration of acceptor a new chemiluminescence spectrum is observed which coincides with the photoluminescence spectrum of the acceptor. The effectiveness of energy transfer to different acceptors is summarized in Table 1.

The first group of molecules in Table 1 accept the energy effectively from the excited cyclohexanone molecule. Thus in the presence of 9,10-dibromoanthracene the emission due to cyclohexanone disappears and the characteristic spectrum of 9,10-dibromoanthracene appears. The long-

wavelength chemiluminescence at  $\lambda = 5300 \text{ Å}$  is, however, unaffected by the presence of the 9,10-dibromoanthracene.

The acceptors in the second group in Table 1 may also accept energy effectively from the chemiluminescent species, because the characteristic spectra of these molecules are also observed. They are, however, distinguished from the first group (labelled "good" in Table 1) because the kinetics of chemiluminescence decay are different. As was shown previously<sup>1</sup>, during the decomposition of the initiator in the absence of an acceptor, the intensity of the short-wave chemiluminescence decays exponentially, and the rate constant for the decay is identical to the first order rate constant for the decomposition of the initiator. Under these conditions the decay of the long-wave chemiluminescence is much more rapid than that of the short-wavelength. With the first group of energy acceptors in Table 1, the intensity of the chemiluminescence arising by energy transfer was also observed to decay exponentially, and with a rate constant identical to that of the rate constant for the decomposition of the initiator. The emission, however, from the second group of acceptors was observed to decrease more rapidly than that of the short-wave luminescence or the "good" acceptors. This is seen in Fig. 1 where the kinetics of the decay of these acceptors are compared with those of 9,10-dibromoanthracene. The rapid decrease may result from energy transfer from species giving rise to the long-wavelength emission, or may be due to a chemical excitation of the acceptor by another reaction, for example, a direct reaction between acceptor and peroxy-initiator. Energy transfer to the third group of molecules in Table 1 could not be observed spectroscopically.

A comparison of the spectra of photo- and chemiluminescence indicates that energy transfer produces the fluorescent states of 9,10-dibromoanthracene and rhodamine 6G and the phosphorescent states of naphthalene, diacetyl and benzil.

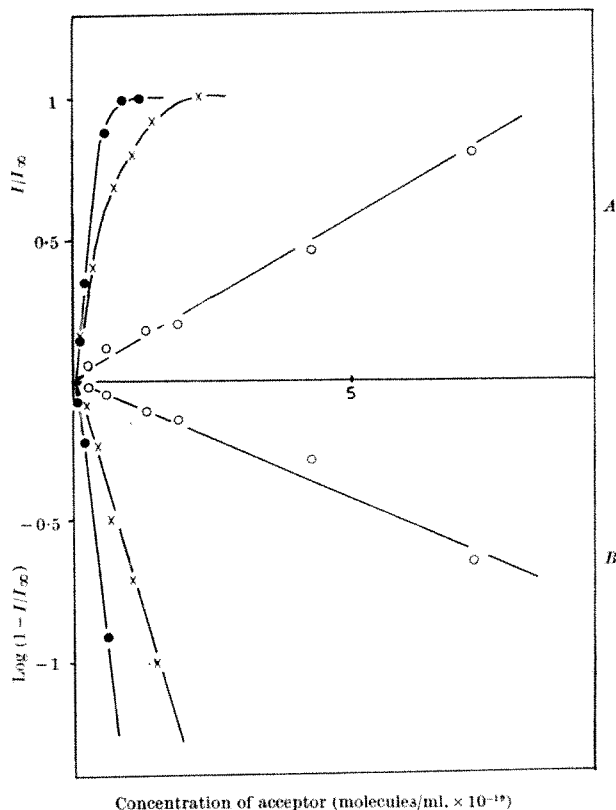


Fig. 2. A, Increase in luminescence with increase of concentration of acceptor in polycarbonate: ●, rhodamine 6G; ×, 9,10-dibromoanthracene; ○, benzil. B, Data from A fitted to equation (1).

Table 1. EFFECTIVENESS OF ENERGY TRANSFER FOR DIFFERENT ACCEPTORS

	Molecule	Concentration moles/l. $\times 10^2$	Intensity quanta/decomp. $\times 10^2$	Class
1	9,10-Dibromoanthracene	1.8	13.2	Good
2	Anthracene	3.4	5.6	
3	Diacetyl	~10	9.1	
4	Benzil	11.4	0.56	
5	Naphthalene	9.2	0.13	
6	Rubrene	0.8	610	Intensity quickly decays with time
7	1-5 Diphenyl (3 para diphenyl) pyrazoline	1.4	178	
8	Rhodamine B <sup>+</sup>	1.2	233	
9	Rhodamine 6G	1.3	58	
10	Phosphine 3R	1.7	36	
11	Pyronine G	2.0	9.9	Ineffective
12	Pyronine Y	2.0	10	
13	Naphthalene	2.2	7.6	
14	Quinine bisulphate	2.2	7.7	
15	Chlorophyll A + B	0.7	<0.1	
16	Fluorescein	1.8	0	
17	Phloxine	0.8	0	
18	Janus green B	1.1	0	
19	Benzyl orange	0.7	0	
20	Pyrene	3.0	0	
21	Bromonaphthalene	2.9	0	
22	1-4 Diphenyl benzene	2.6	0	

All at 0.175 moles/l. P.C., 343° K, except benzil, 0.35 moles/l. P.C., 353° K; naphthalene, 0.35 moles/l. P.C., 363° K; rhodamine B, 348° K.

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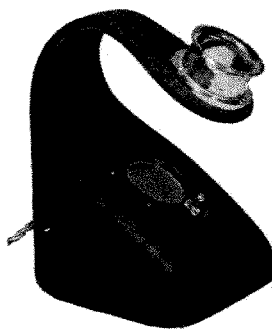
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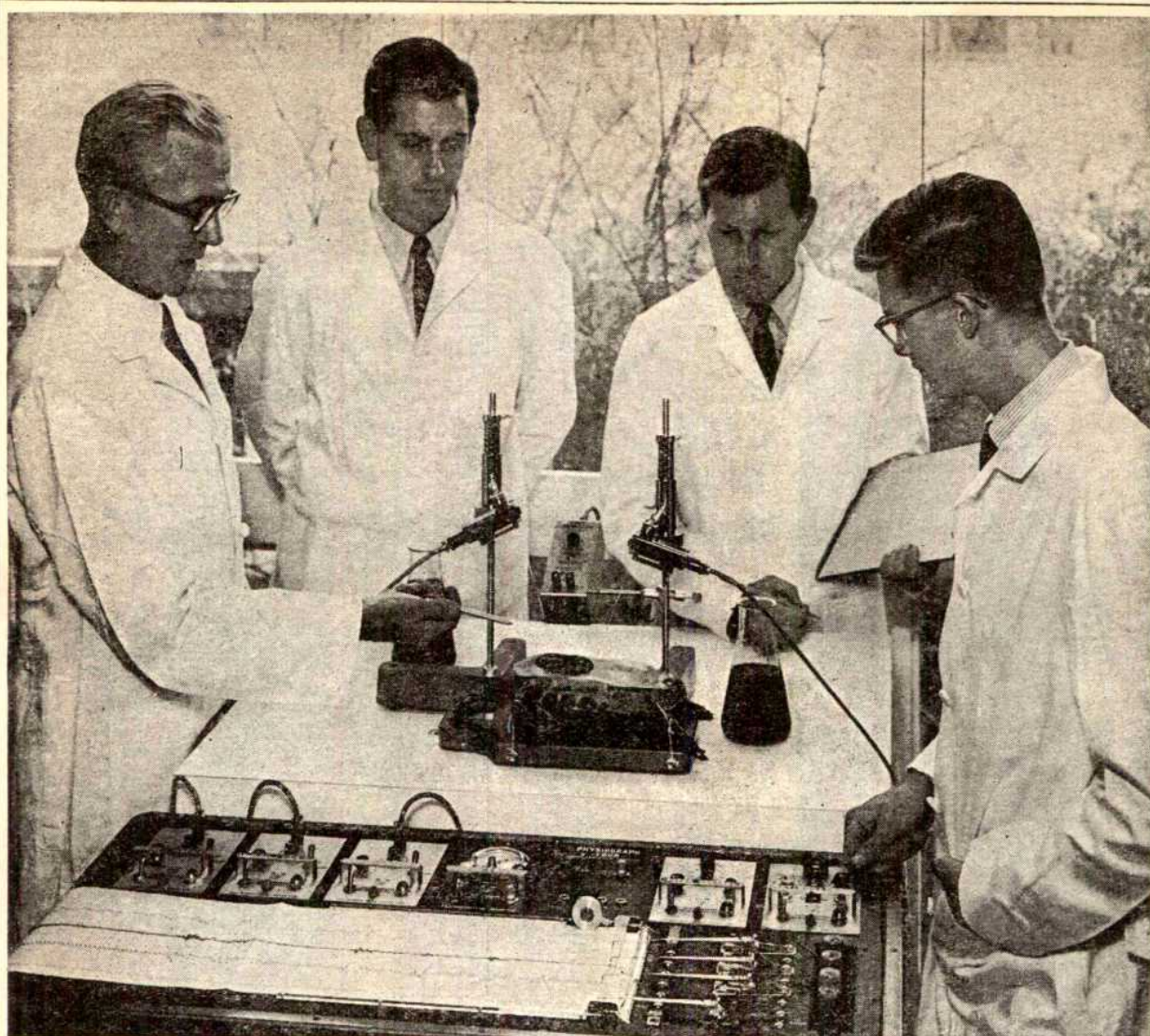
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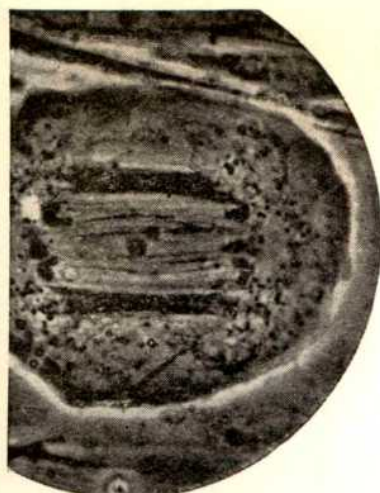
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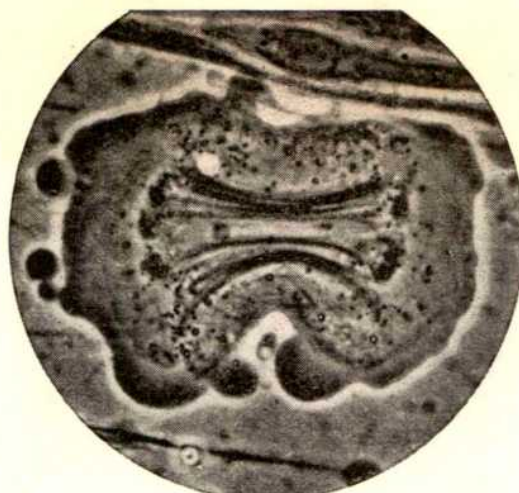
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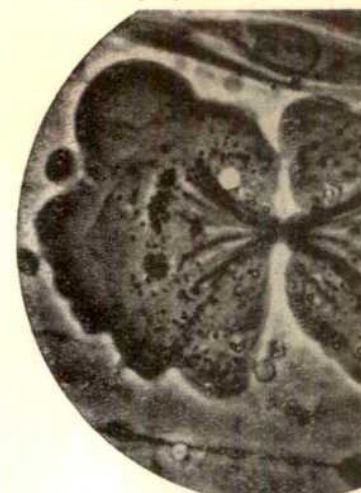




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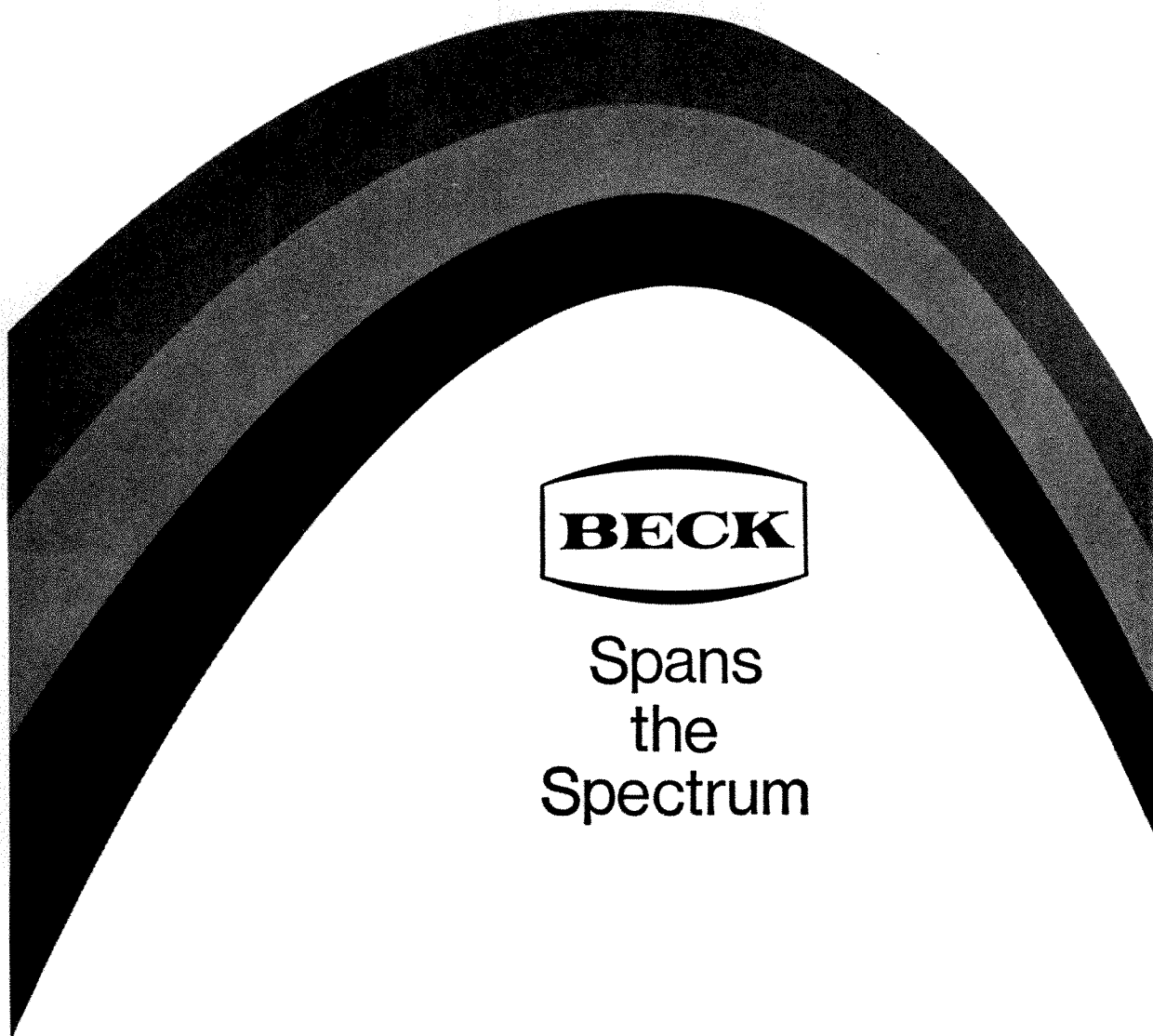
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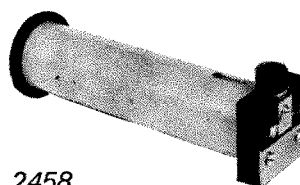
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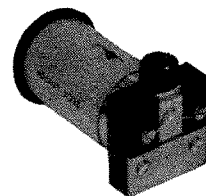
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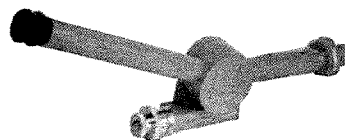
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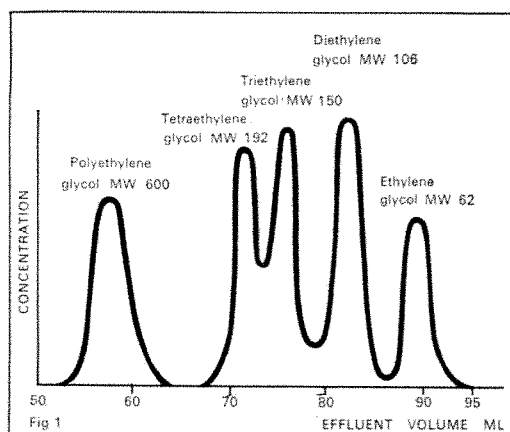


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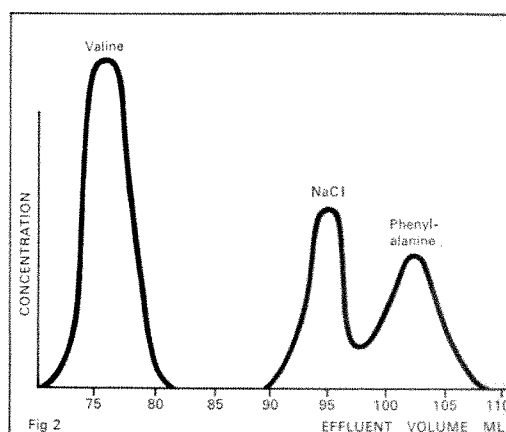


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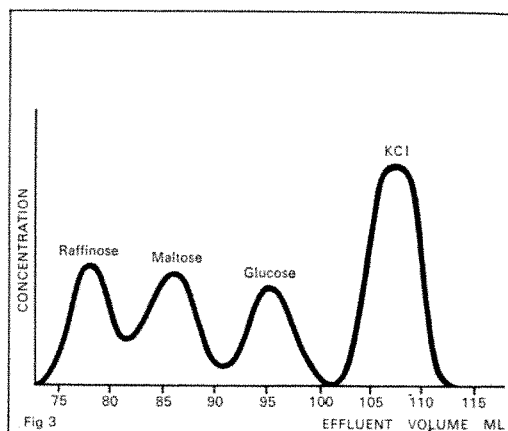


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Table 2. DISTANCE FOR EFFECTIVE ENERGY TRANSFER

Acceptor	$V \text{ \AA}^3$	$r \text{ \AA}^3$
Benzil	42,000	10
9,10-Dibromoanthracene	30,000	19
Rhodamine 6G*	75,000	26

\* Assumes that excited rhodamine 6G molecules originate only by energy transfer from cyclohexanone.

To measure the distance across which energy transfer could occur, a study of the intensity of activated chemiluminescence as a function of concentration of three acceptors, 9,10-dibromoanthracene, benzil and rhodamine 6G, was undertaken. The data fit a modification of the Perrin equation<sup>2</sup> (Fig. 2) which we use to describe the concentration dependence.

$$\frac{I_A}{I_{A\infty}} = 1 - e^{-Vn} \quad (1)$$

where  $I_A$  and  $I_{A\infty}$  are the intensity and limiting intensity of activated chemiluminescence respectively,  $n$  is the concentration of acceptor in molecules/unit volume and  $V$  is the volume of sphere inside which energy transfer occurs. The volumes of the spheres and distances  $r$  between interacting donor and acceptor molecules on this basis are shown in Table 2.

The values obtained for benzil are in good agreement with the values obtained by Ermolaev<sup>3</sup> for triplet-triplet energy transfer between different donors and acceptors. As predicted by theory<sup>4</sup>, the triplet-singlet transfer is seen to occur at greater distances.

D. PHILLIPS\*  
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Received August 1, 1967.

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<sup>1</sup> Phillips, D., Anissimov, V., Karpukhin, O., and Shliapintokh, V., *J. Amer. Chem. Soc.* (in the press).

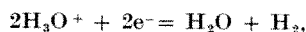
<sup>2</sup> Perrin, F., *C.R. Acad. Sci.*, **178**, 1978 (1924).

<sup>3</sup> Ermolaev, V. L., *Usp. Fiz. Nauk*, **80**, 3 (1963).

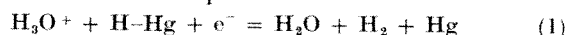
<sup>4</sup> Förster, Th., *Disc. Faraday Soc.*, **27**, 7 (1959).

### Electroactive Species produced by Electrolysis at Mercury Cathodes in Aqueous Solution

THERE is evidence that in electrolysis of aqueous solutions at mercury cathodes the accumulation of atomic hydrogen dissolved in mercury as an amalgam, as suggested by Heyrovsky<sup>1</sup> and Ilkovic<sup>2</sup>. Müller<sup>3</sup> re-investigated hydronium ion discharge at dropping mercury electrodes, and attributed long term changes in volume to a slow step in the overall reaction



either elimination of hydrogen from an amalgam, or decomposition of aqueous hydrogen molecule ions. The present work confirms that hysteresis is associated with the metal phase. We also present evidence for the occurrence of electrochemical desorption



at mercury containing dissolved hydrogen atoms.

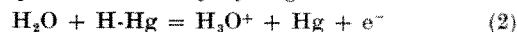
Cathodes of triply distilled mercury (Shawinigan C.P.) were polarized with respect to a 0.1 molar potassium chloride calomel reference electrode by means of a bright platinum anode, separated from the cathode by a long, narrow tube. Anaerobic conditions were maintained by use of high purity hydrogen and mercury seals. Reagent grade potassium chloride,  $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$  and  $\text{NaH}_2\text{PO}_4$ .

$\text{H}_2\text{O}$  were dissolved in distilled deionized water to make 0.1 molar potassium chloride buffered<sup>4</sup> to pH 7.5. Potentiostatic and triangular voltage measurements were made using an 'Exact 301' function generator, feeding into a 'Heath EUW 401' polarography system. Current-potential curves were recorded on a Houston Instruments 'HR98-T' X-Y recorder. A Keithley 662 differential voltmeter was used to measure potentials.

During extended cathodization at constant potentials of -1.0 to -1.5 V in hydrogen stirred solution, the cathodic current declined continuously. Subsequent open circuit decays were slow, taking several hours to reach a potential near the reference value, and current-voltage curves (Fig. 1) using a triangular sweep were considerably modified. The development of peaks in the anodic and cathodic half-cycles at -1.05 V and -1.2 V respectively was the most striking change. The effects were completely, though slowly, reversible.

The current rise at -1.3 to -1.4 V (A2, 16) is evidently caused by an essentially "irreversible" cathodic process, for the cathodic area, initially larger than the anodic, became progressively smaller (compare A15, 16 with A1, 2) because of an enhanced anodic faradaic current component. This was undoubtedly caused by the accumulation of a product of cathodization, the oxidation of which led to the balancing out of the capacitive currents at > -1.0 V, the appearance of the anodic peak at -1.05 V and the increase of the anodic area to three times the cathodic area (Fig. 1, B5), after 40 h at -1.3 V (net cathodic current falling from about 1 to 0.1  $\mu\text{amp cm}^{-2}$ ). At the same time the cathodic current at -1.4 V was suppressed, along with growth of a cathodic peak, barely noticeable in A2 and A16, at -1.2 V.

The parallel growth and decay of the anodic and cathodic peaks, at -1.05 V and at -1.2 V, respectively, suggest a common origin. The evidence suggests that the anodic peak is caused by hydrogen ionization



and the cathodic peak by electrochemical desorption (1). The more positive potential of the latter, relative to hydronium ion discharge, is consistent with the easier occurrence of (1) on metals such as mercury which adsorb hydrogen only weakly<sup>5</sup>.

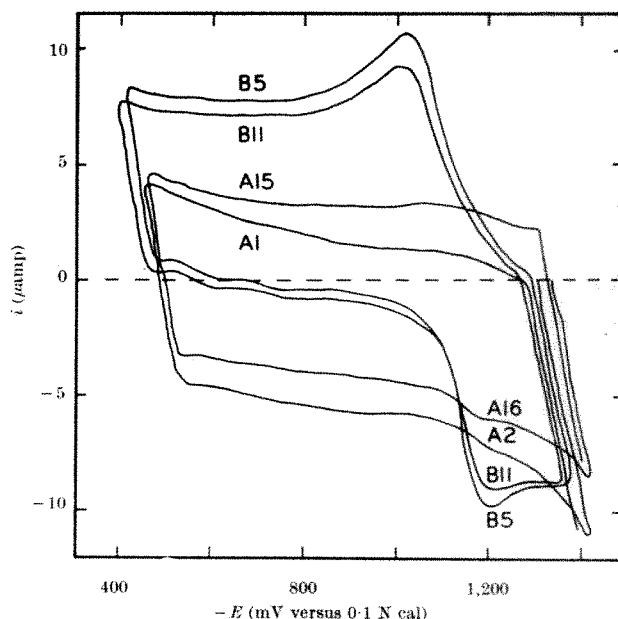


Fig. 1. Current-voltage curves in triangular voltage cycles at 0.1 c/s with continuous stirring by hydrogen before (curves A) and after (curves B) cathodization for 40 h at -1.3 V. Cycles A1 to A16 and B1 to B11, respectively, were consecutive without interruption. Extreme curves only illustrated, except B1, for which peaks were greater than B5. Electrode area about 2 cm<sup>2</sup>. Anodic currents are positive.

The rates of both (1) and (2) will increase with hydrogen atom coverage, while that of hydronium ion discharge is expected to decrease. During cathodization, in the absence of a recombination catalyst, the hydrogen atom coverage, initially very low<sup>6</sup>, will gradually rise, keeping pace with the bulk concentration of hydrogen atoms. Thus the rates of (1) and (2) will gradually rise, as observed.

The absence of a stirring effect on the curves of Fig. 1 is consistent with rate limitation by hydrogen atom diffusion in the metal, while the observation of a bubble of hydrogen around the platinum contact wire and below the mercury, when electrolysis was continued for about 1 week, is evidence that hydrogen can accumulate in mercury. No bubbles were observed in the shorter experiment of Fig. 1; in this the charge passed was equivalent to only 1.5  $\mu\text{g}$  of hydrogen, a quantity that would be difficult to see.

The possibility of an impurity effect is unlikely, for no important impurity was detected polarographically. Furthermore, the suppression of the cathodic current at  $-1.4$  V, along with the growth of a new cathodic peak, is difficult to explain on such a basis. An effect on the triangular voltage curves was apparent even after a very small number of sweeps and persisted for a great many sweeps after prolonged cathodization.

Equally, discharge of alkali ions at  $-1.3$  V can be discounted, the discharge potentials being considerably more negative. The potassium ion discharge current at  $-1.3$  V has been estimated from residual currents in deoxygenated potassium chloride at  $-1.9$  to  $-2.0$  V, assuming a Tafel

slope of  $\frac{2RT}{F}$  to be  $\sim 10^{-11}$  amp  $\text{cm}^{-2}$ , that is quite negli-

gible (unpublished work of H. U. Heintze and F. R. Smith).

Although at  $10^{-7.5}$  molar the rate of discharge of bulk hydronium ions (extrapolation of Bagotskii and Yablokova's<sup>8</sup> data) is unlikely to exceed  $10^{-9}$  amp  $\text{cm}^{-2}$ , this will be supplemented by rapid water and phosphate ion dissociations, of which the former can support a diffusion current<sup>9</sup> of 5  $\mu\text{amp cm}^{-2}$ . This is adequate to account for the observations.

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## BIOLOGY

### Role of Carbon Dioxide in Erythropoiesis

THE use of gas mixtures containing 5 per cent carbon dioxide gas has become a standard procedure for the cultivation of cells *in vitro*. In some systems, however, a higher concentration has proved essential<sup>1,2</sup>. In bone marrow cultures different concentrations of carbon dioxide have been used<sup>3,4</sup>, but the interest has centred on the role of oxygen and little attention has been paid to the function of the other gases in the mixtures. In the

present system for the cultivation of rat bone marrow *in vitro* 30 per cent carbon dioxide (which is considerably higher than any concentration previously reported) has been found to stimulate erythropoiesis<sup>5</sup>. This effect has now been investigated in detail.

Cultures were prepared from the femoral bone marrow of Sprague-Dawley rats weighing 120 g as described previously<sup>5</sup>. The cells were grown on a coverslip, in a small Petri dish using 75, 80 or 100 per cent isogenic serum as nutrient. The remainder of the medium was synthetic medium *M* 150 (ref. 6). In these conditions most of the immature cells remained on the coverslip while most of the reticulocytes and erythrocytes were released into the supernatant fluid. The fluid was therefore somewhat analogous to the peripheral blood *in vivo* and was used for reticulocyte counts in the conventional manner<sup>7</sup>.

Initially the cultures contained very few non-nucleated cells<sup>8</sup>, but when they were maintained in 3, 15 or 30 per cent carbon dioxide in air it was found that the highest percentage produced a consistently higher reticulocyte count throughout the life span of the culture (Fig. 1). When the gas mixture was composed of 30 per cent carbon dioxide, 20 per cent oxygen and 50 per cent nitrogen, the same high reticulocyte count was obtained which shows that the effect was not due to the concomitant decrease in oxygen.

When cultures were started in 3 per cent carbon dioxide and moved to 30 per cent carbon dioxide after different intervals *in vitro*, a surge of reticulocyte production occurred within 24 h after moving (Fig. 2). In the young cultures, where the initial percentage of reticulocytes was high, the increase was consistent but not very large. In the older cultures, however, where the reticulocyte percentage was negligible in 3 per cent carbon dioxide, the response to 30 per cent carbon dioxide was highly significant.

The beneficial effect of 30 per cent carbon dioxide on the production of reticulocytes was not due to low pH alone. Cultures in this concentration of carbon dioxide had a pH between 6.0 and 6.4. When low concentrations of serum (5–50 per cent) were used, the same pH was reached but very few mature cells were produced. The optimum conditions therefore required both high serum and high carbon dioxide.

Thus it appears that a high concentration of carbon dioxide in the presence of some serum component(s) stimulates the erythropoietic system of the marrow *in vitro*. Because no kidney tissue is available for the

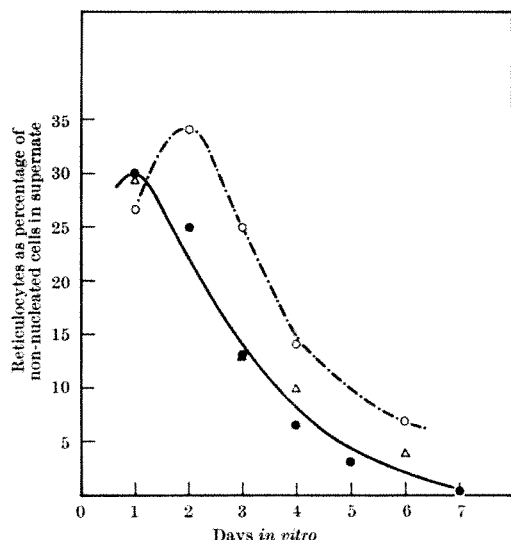


Fig. 1. The effect of continuous exposure to 3 (●—●), 15 (△—△), or 30 (○—○) per cent carbon dioxide in air on percentage of reticulocytes in the supernatant fluid.

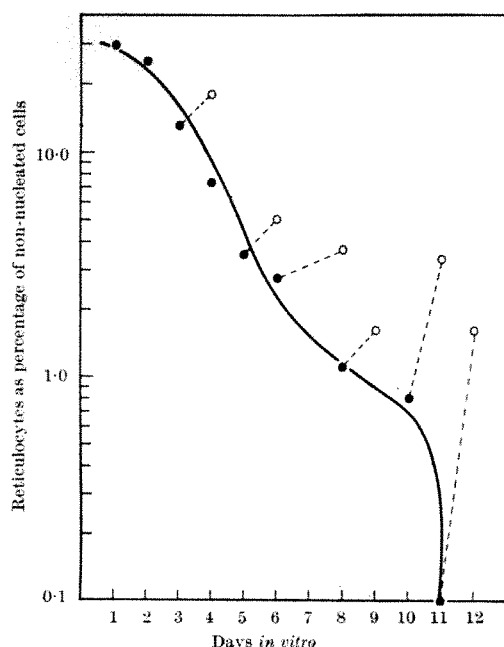


Fig. 2. Changes in percentage of reticulocytes as a result of moving cultures from 3 per cent carbon dioxide (●) to 30 per cent carbon dioxide (○) after different intervals *in vitro*.

production of erythropoietin, this response is either independent of the hormone, or involves the activation of a latent form present in the serum. An activation of erythropoietin by divalent cations has recently been suggested<sup>8</sup>. On the other hand, divalent cations are known to stimulate mitosis in bone marrow<sup>9</sup>, and prolonged exposure to high carbon dioxide stimulates the uptake of calcium by erythrocytes<sup>10</sup>. If high concentrations of carbon dioxide have the same effect on the nucleated cells of the erythron, these results could be interpreted as follows: carbon dioxide stimulates the entry of calcium into the primitive cells of the marrow, and the calcium then stimulates these cells to divide and mature. Whether or not there is any connexion between this system and activation of an inactive serum erythropoietin requires further investigation.

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## Strontium, Calcium and Magnesium in Brown Algae

THE ion exchange properties of alginates have received some attention lately, particularly because of their potential value in connexion with therapy after strontium-90 overdoses<sup>1-3</sup>. It has been shown that alginates rich in

Table 1. CONTENT OF CALCIUM, MAGNESIUM AND STRONTIUM IN DRY ALGAL SAMPLES

Algae	% Mannuronic acid	Calcium (nmole/g)	Magnesium (nmole/g)	Strontium (nmole/g)
<i>L. hyperborea</i> , stipes, Hustad, 4/5				
Outer cortex	30.5	0.373	0.190	0.0249
Inner cortex	35.4	0.355	0.195	0.0196
Peripheral tissue, upper part	41.2	0.282	0.316	0.0140
Medulla, upper part	48	0.340	0.510	0.0150
<i>L. hyperborea</i> , stipes, Munkholmen, 9/1	50	0.222	0.284	0.0107
<i>L. digitata</i> , fronds, Espevær, 8/1	54.5	0.252	0.356	0.0098
<i>Peletia canaliculata</i> , Flakk, 6/11	56.5	0.122	0.232	0.00525
<i>A. nodosum</i> , Gulosen, 6/8	58.4	0.169	0.420	0.00525
<i>L. digitata</i> , Tarva, 20/8	61.5	0.237	0.311	0.0088
<i>A. nodosum</i> , Flakk, 6/11	65	0.127	0.340	0.00536
<i>L. hyperborea</i> , new fronds, Munkholmen, 9/1	65.5	0.152	0.369	0.00605
<i>L. digitata</i> , new fronds, Være, 29/1	70.2	0.215	0.378	0.00514
Sea water <sup>7</sup> of chlorinity 19		0.0102	0.0536	0.000156

gularonic acid residues have a greater affinity for calcium in the ion exchange reaction calcium-magnesium<sup>4</sup> and for strontium in the ion exchange reaction strontium-calcium<sup>5</sup>.

The proportion of mannuronic and gularonic acid in the alginates varies from one species of brown algae to another<sup>6</sup>, and also in some cases from one type of tissue to another within a plant<sup>6</sup>. We have determined the content of calcium, magnesium and strontium in some samples of brown algae and of different parts of brown algae by extraction with 0.2 normal hydrochloric acid and analysis by means of atomic absorption. The results are given in Table 1 together with the uronic acid composition of the alginate present in the samples.

The results clearly indicate a correlation between the uronic acid composition of the alginates and the relative amounts of the three divalent metals in the plants.

If we consider the brown algae as ion exchange materials immersed in sea water, and if we assume that the different ion exchange reactions can be regarded independently of

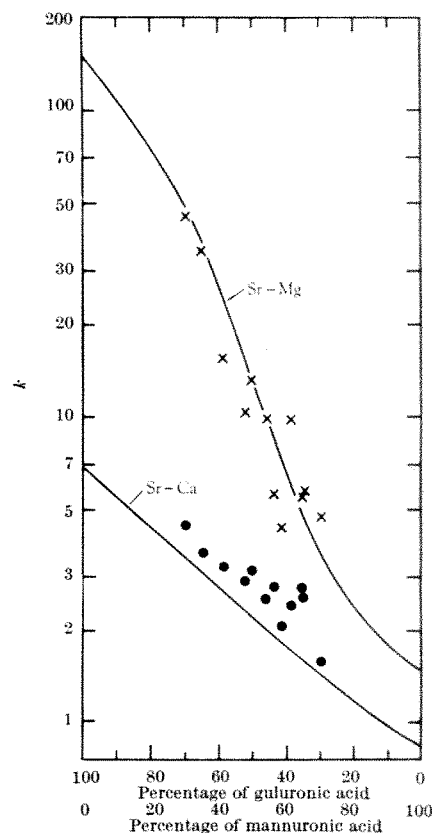


Fig. 1. Selectivity coefficients of algal samples as a function of the uronic acid composition of the alginate.  $\times$ ,  $k_{Sr-Mg}^{Sr}$ ;  $\bullet$ ,  $k_{Sr-Ca}^{Sr}$ ; —, selectivity coefficients for alginate.



each other, we can calculate the selectivity coefficients for the ion exchange reactions strontium-calcium and strontium-magnesium from the results given in Table 1.

The selectivity coefficient is defined by the equation

$$k_{\text{MeI}}^{\text{MeII}} = \frac{[\text{MeI}]_a \cdot [\text{MeII}]_w}{[\text{MeII}]_a \cdot [\text{MeI}]_w}$$

where subscripts *a* and *w* refer to algae and sea water respectively and the figures within the brackets refer to the concentration of the ions. It has been shown<sup>8,9</sup> that the activity coefficients for calcium and magnesium ions in sea water are about equal, and, assuming the same activity coefficient also for strontium ions, the ratio between the ion concentrations should be approximately equal to the ratio between the activities of the ions.

In Fig. 1 the resulting selectivity coefficients are shown as a function of the uronic acid composition of the alginates. The curves represent the results obtained from laboratory experiments with pure alginates. The agreement between the results obtained for pure alginates in salt solutions and for brown algae living in their natural environment is remarkably good. A complete agreement should not be expected, because the experiments with alginates are carried out in conditions where approximately equal amounts of the two ions are bound to the polymer; and previous results have shown that the selectivity coefficients show some dependence on the ratio of the two cations bound to the alginate<sup>10</sup>. The results therefore indicate that the content of the divalent metals calcium, magnesium and strontium in brown algae to a large extent is determined by the ion exchange taking place between the sea water and the alginate in the plants.

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### Side Effects of Dimethyl Sulphoxide

IN view of the increasing interest in the therapeutic value of dimethyl sulphoxide (DMSO) (refs. 1 and 2), we felt that the recent report by Barnett and Noel<sup>3</sup> called for some comment. The only important side effect so far reported in animal experiments has been an effect on the lens<sup>4,5</sup> and it is only as a result of these reports that widespread clinical use of DMSO has been delayed.

Both in the recent paper and the original report, the doses used were far in excess of any possible level which would be considered for clinical practice. Furthermore, the doses given to monkeys were administered by gastric intubation and no systemic use for humans is contemplated at the present time. The highest daily dermal dose so far reported in work with monkeys is 11 g/kg (ref. 6). This has been given for up to 6 months without affecting the lens. Extrapolating the reported data from monkey to man would mean that, in order to achieve even slight changes in the lens, a dose of 200 g/day would be required. At levels such as these the question of impurities becomes important and no mention of the type, source or purity of the product used in these experiments is given by the authors. This omission has been pointed out on

a previous occasion<sup>7</sup> and it is regrettable that this group should continue to overlook these basic data.

DMSO has now been used in quite extensive clinical trials for periods of up to 2 years with only one lens opacity being reported<sup>8</sup>, and in this case the author considered it to be unrelated to DMSO.

It is only because of the exceptionally low general toxicity of DMSO that such massive doses can be administered at all. Indeed, we wonder what other substance with such high physiological activity could be given in the same quantity without producing much more drastic changes in many tissues.

It is important that this ocular side effect should be seen in its proper perspective.

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### Invasion of Wheat Roots by *Pratylenchus thornei*

SEINHORST developed a model to relate the density of nematodes in a population and the damage caused to plants<sup>1</sup>. Later he developed another model to relate the increase in the population and the density of the population<sup>2</sup>. These models, based on the relationships between predator and prey of Nicholson and Bailey<sup>3</sup>, make two principal assumptions: (a) the "average" nematode is the same at all densities; and (b) the nematodes invade the host tissue randomly, that is they do not attract or repel each other.

The percentage of larvae of *Ditylenchus dipsaci* that invaded oat seedlings declined as the number of larvae in the inoculum increased<sup>4</sup>. Similar observations were made for *Heterodera rostochiensis* on potato<sup>5</sup> and for *Meloidogyne incognita* on tomato<sup>6</sup>. Larvae of *M. incognita* have been observed directly to enter a root through a single wound made by the first larva to invade. Attraction of larvae was stronger<sup>7</sup>, and invasion more rapid<sup>8</sup>, after the root had been punctured by the first larva. Wallace<sup>6</sup> suggested six reasons why invasion could be dependent on density and considered that the supporting evidence cast doubt on the validity of Seinhorst's assumption.

Seinhorst<sup>2</sup> states that the "second assumption could be tested by observing the distribution of nematodes in or on the roots". We have done this for *Pratylenchus thornei* Sher and Allen in wheat roots and the results cast doubt on the validity of the second assumption. Wheat seedlings were established in sand and, when 4 weeks old, each was inoculated with either 1,500 or 6,000 *P. thornei*. Two weeks later, the roots were removed, immersed in Fleming's fixative to stain embedded nematodes<sup>9</sup>, fixed in chrom-acetic acid overnight, passed through a graded series of alcohols and finally cleared in cedar wood oil.

Fifty pieces of root each 20 mm long were selected at random from plants inoculated with 1,500 nematodes. Forty-two pieces contained no nematodes, and the other eight contained between one and fifty-three nematodes. Long pieces of root were then selected at random from those containing nematodes, and the number of nematodes in each millimetre was counted with an ocular micrometer (Fig. 1).

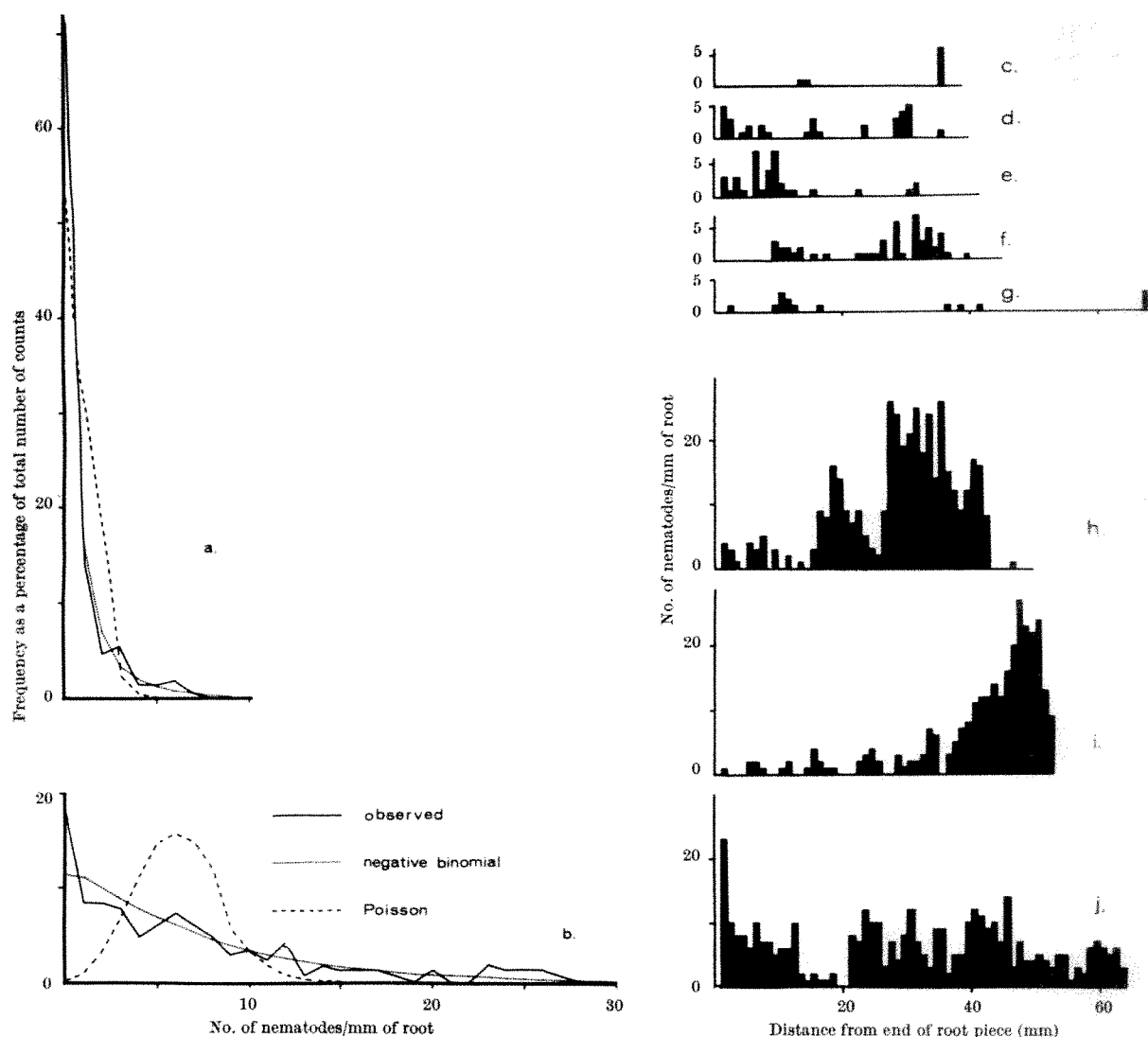


Fig. 1. The observed and calculated frequency distributions of *Pratylenchus thornei* in wheat roots of plants inoculated with (a) 1,500 and (b) 6,000 nematodes/plant. The number and distribution of *P. thornei* in lengths of wheat roots from plants inoculated with (c to g) 1,500 and (h to j) 6,000 nematodes/plant.

Because the seedlings had been inoculated for only 2 weeks the nematodes had not reproduced and, from other observations, had entered near their present site in the root. Of the roots examined, relatively few had been invaded. For the root pieces containing nematodes, if each millimetre had an equal chance of being invaded, then the observed distribution of nematodes in the root should follow a Poisson distribution. The values of  $\chi^2$  show that, for both levels of inoculum, the observed data did not fit a Poisson distribution but fitted a negative binomial distribution well (Table 1). This suggests that some parts of the root had a greater chance of being invaded than others.

Environmental differences along a root could favour invasion of some parts. The design of the experiment aimed to minimize such differences by establishing a constant temperature and moisture regime in the sand. Also, some parts of a root may have been more suitable for invasion, but no structural differences could be detected

and invasion was not constantly associated with any particular part of the root. An alternative explanation is that the initial invasion was random, but that other nematodes then entered through the wounds caused by the initial invaders. Cell contents may have escaped through such wounds and attracted other nematodes to that region of the root. These last two possibilities, both incompatible with the assumption that "nematodes act independently" during invasion, provide the most likely explanation of the localized regions of heavy invasion observed in wheat roots. These results are not unexpected because species of *Pratylenchus*<sup>9</sup> and other nematodes<sup>10</sup> orientate to chemical gradients.

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Table 1

Distribution tested	Level of inoculum	Degrees of freedom	$\chi^2$	Probability that observed data come from distribution tested
Poisson	1,500	2	97.93	< 0.005
Poisson	6,000	5	505.87	< 0.005
Negative binomial	1,500	4	6.81	0.16
Negative binomial	6,000	13	16.82	0.22

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### *Podocarpus* Root Nodules in Sterile Culture

THE morphology of the root nodules of *Podocarpus* has attracted more attention than that of any other non-leguminous plant, and the nodules have been regarded as a response to infection by bacteria<sup>1-4</sup> or by non-septate fungi<sup>5-10</sup>. The endophyte is considered not to be causal by recent workers<sup>11,12</sup>, but the nodules are thought to be arrested lateral roots with the cortex renewed annually. Attempts by Baylis *et al.*<sup>11</sup> to maintain *Podocarpus* seedlings in sterile culture long enough to permit nodule development were unsuccessful, although nodules were formed in cultures which proved to be entirely free from fungi. We have investigated the production of root nodules in sterile culture.

Shoot cuttings from *Podocarpus lawrencei* Hook., after surface sterilization, were planted in pots of autoclaved sand and peat (equal parts) in completely sterile conditions. The structures (Fig. 2b) of the nodules produced on the adventitious roots which arose from the cuttings were the same as those which have been described before, although in contrast to nodules on plants grown in the field (Fig. 2a) no endophyte could be seen. Staining of smears and sections for bacteria gave negative results. Malt-'Marmite' agar plates inoculated with crushed surface-sterilized nodules remained free of micro-organisms on incubation for 3 weeks at 20° C, and there was no evidence of any endophyte in sections examined by electron microscopy.

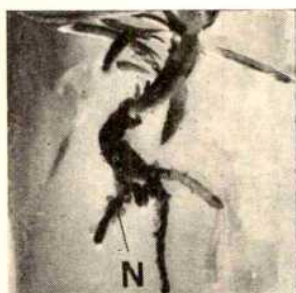


Fig. 1. Seedling (inside the culture tube) developed from excised embryo of *Podocarpus falcatus*. Note the root nodules (N). ( $\times 0.5$ .)

When the nodules were collected, however, the medium in which cuttings had been rooted was no longer sterile, a result found before<sup>11</sup>. Thus to determine whether or not nodulation is dependent on micro-organisms, excised embryos from full-size green seeds of *P. falcatus* R.Br. were grown *in vitro* on Raghavan and Torrey<sup>13</sup> tissue culture medium. When the chlorophyll had developed in the cotyledons, the embryos were transferred to a mineral agar medium. The seedlings were transferred every 4 weeks to fresh media. When 1 yr old, the roots of the seedlings nodulated (Fig. 1). The structure (Fig. 2c) of the nodules

was similar to that of field grown nodules except that no endophyte was detected in the cortical cells. Malt-'Marmite' agar plates incubated from the crushed nodules remained free of micro-organisms.

These observations suggest that unless nodulation is induced by some undetected endophyte transmitted by the embryo, nodules are a normal feature of the root system of *Podocarpus*, and that their formation is not induced by any microbiological factor. This does not exclude the possibility, suggested before<sup>11</sup>, that such nodules on which the cortex is renewed annually represent an adaptation which allows the mycorrhizal symbiont to be retained after the long roots have shed their cortex.

The idea that nodules are arrested lateral roots<sup>11,12</sup> is not supported by a comparison of the histological features of the young nodule with those of the young lateral root. Both arise endogenously but differ in their cellular configuration. The lateral root, before emergence, clearly shows a recognizable root type apical meristem with the usual open ended endodermis. The nodule, in contrast, shows, before emergence and later, a cell pattern unlike the apical meristem of any root and its new endodermis is not open-ended but over-arches the vascular system of the nodule from the beginning. Thus histologically the nodules do not seem to be modified arrested lateral roots but to be morphological features in their own right.

I thank Drs I. V. Newman and P. G. Valder for their help.

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<sup>1</sup> Bottomley, W. B., *Brit. Assoc. Advance Sci. Rep.*, **82**, 679 (1912).

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<sup>6</sup> Nobbe, F., and Hiltner, L., *Landwirtsch. Vers. Sta.*, **51**, 241 (1899).

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<sup>8</sup> Schaefer, R., *Planta*, **33**, 703 (1943).

<sup>9</sup> Shibata, K., *Jahrb. Wiss. Bot.*, **37**, 643 (1902).

<sup>10</sup> Yeates, J. S., *New Zealand J. Sci. Technol.*, **7**, 121 (1924).

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### Holographic Visualization of Plant Movement

HOLOGRAPHIC techniques have been applied to many fields with results which are adequately reported in the literature<sup>1-3</sup>. This communication describes an application of the so-called "frozen" fringe method of holography<sup>4</sup> to the visualization of plant movement or

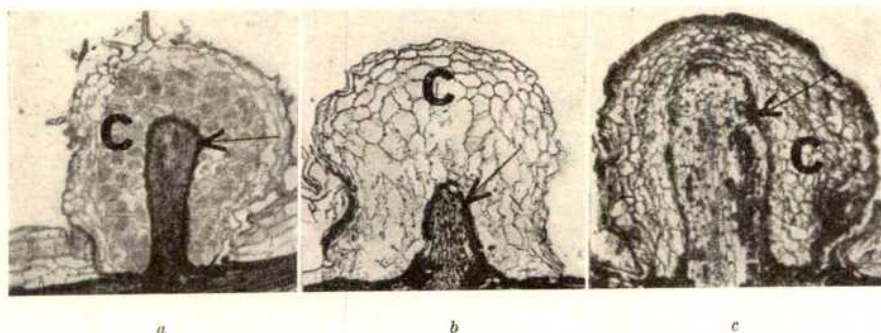


Fig. 2. Longitudinal section of root nodules of species of *Podocarpus*. Arrows indicate over-arching endodermis. C, Cortex. (a) *P. lawrencei*, field grown nodule, with fungal endophyte in the cortex. ( $\times 65$ .) (b) *P. lawrencei*, sterile nodule from adventitious roots of cutting; absence of endophyte from cortex. ( $\times 65$ .) (c) *P. falcatus*, root nodule from sterile seedling; absence of endophyte. Note the regeneration of nodule at the tip of the vascular branch underneath the endodermis. ( $\times 80$ .)



growth. No attempt has been made to analyse in detail the particular plant movement observed, partly because of the lack of control over the specimen and partly because of the limited time devoted to this experiment. Rather, I wish to show that the method described has a potential use in this field and with correct experimental procedure could yield quantitative results.

A diverging laser beam was used to illuminate the scene which in this case was a pot containing a number of wheat coleoptiles (young shoots) grown in sand. At the time of the experiment the coleoptiles were about 3 cm long and about 2 mm in diameter. The laser was a Spectra Physics, He-Ne model with an output power of about 15 mW.

The Kodak 649F plate film used for the recording received light diffusely reflected from the plants, and these waves were made to interfere with a portion of the incident beam reflected onto the plate from a plane mirror. When this latter reference beam is made to impinge on the developed plate (the hologram), a three-dimensional image of the plants is observed through the plate. This image is produced by a reconstruction of the original wavefronts reflected from the plants. The reconstruction is made possible by the controlled scattering of the reference beam by the silver grain patterns (diffraction patterns) on the developed spectroscopic plate or hologram. The diffraction patterns are created by the interference of the light reflected from the plant and the reference beam. It should be emphasized that the hologram records not only the light amplitude distribution across its plane, but also phase distributions making the reconstructed wavefronts exact replicas of the original wavefronts emanating from the original objects being studied.

With the apparatus used it was found that an exposure of 0.25 sec was suitable to produce a reasonable hologram. The important feature of the "frozen" fringe method, however, is that multiple exposures be made.

Two 0.125 sec exposures were made with an interval of about 30 sec between them. Each exposure can be considered to produce its own train of wavefronts in the reconstruction. If there was no movement of the plant during the time between exposures, then the two trains of wavefronts would be identical, and combine to form a single train which would be indistinguishable from that produced by a single exposure of 0.25 sec. If movement does occur between exposures, however, the wave trains will not coincide and the situation can be analysed by extrapolating back to the position from which they seem to emanate, that is, the virtual images of the plants. If a point on the plant surface moves towards the observer by multiples of a half wavelength of the light being used ( $\lambda = 0.6328\mu$  for the laser used in this experiment) the radiation from the first and second positions will be out of phase and cancel. This point will thus appear dark when the reconstructed wavefronts are viewed. Similarly, if points move by multiples of wavelengths, those points will appear bright. Fig. 1 is a photograph of the virtual image of a coleoptile as seen through a doubly exposed hologram, and shows dark and bright bands which map out the points of the plant surface which have moved by multiples of half and full wavelengths respectively. Measuring the separation of two dark bands on the plant, and knowing that the plant surface moved by a wavelength between them, the tilt of the plant can be calculated as being of the order of 1/2000th of a degree. The result shown in Fig. 1 is quite reproducible, and varying the time interval between exposures gives a different number of fringes.

One point which must be mentioned here is that, for the result shown, the plant surface had to be coated with a matt white paint, because a large percentage of the laser beam penetrates the outer layers of the plant tissue and is reflected from inner layers. The effect of this complicating process is to give extremely weak fringes.

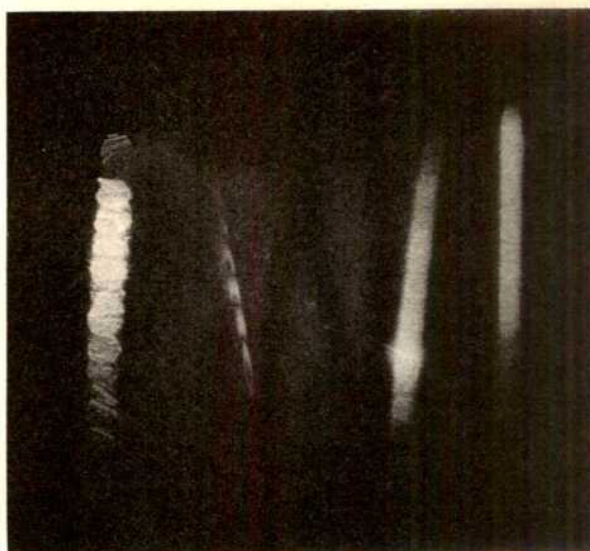


Fig. 1. Banded appearance of a coleoptile indicating movement during a 30 sec interval. (Photographed through a hologram.)

Coating the plant surface prevents this to a large extent. It is extremely debatable whether or not a plant can be coated for a controlled experiment. Researchers who might apply this technique must decide whether or not a coating of powder or paint will interfere with their experiments. Also, from some plant tissues strong reflexions might occur from the outer surfaces with little penetration. Coating in these cases would not be necessary.

A final point worth mentioning is that in some cases the "live" fringe method might be employed, that is, making a single hologram of the plant, and processing and replacing it in exactly the same position in the original apparatus so that the reconstructed image is superimposed on the plant. Any movement of the plant would give rise to fringes which in this case move as the plant moves.

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<sup>1</sup> Rogers, G. L., *J. Sci. Instrum.*, **43**, 677 (1966).

<sup>2</sup> Williams, G. T., and Owen, T., *Physics Education*, **2** (1967).

<sup>3</sup> Watrasiewicz, B. M., *Instrument Practice*, 669 (1967).

<sup>4</sup> Burch, J. M., Ennos, A. E., and Wilton, R. J., *Nature*, **209**, 1015 (1966).

### Nomenclature of the "Eastern Lowland Gorilla"

IN discussing the sub-specific taxonomy of the gorilla, Groves<sup>1</sup> has used the name *Gorilla gorilla manyema* Rothschild, 1908, for his eastern lowland sub-species. This procedure is quite contrary to both the International Rules and current practice of taxonomic nomenclature, and if not corrected will inevitably lead to further confusion in the nomenclature of this important and much-studied species. The valid name for this sub-species is *Gorilla gorilla graueri* Matschie, 1914, and the synonymy can be expressed as follows:

*Gorilla graueri* Matschie, 1914 (ref. 2).

*Gorilla gorilla rex-pygmaeorum* Schwarz, 1927 (ref. 3).

*Gorilla gorilla manyema* Groves, 1967 (ref. 1) (following Rothschild, 1908 (ref. 4)).

Groves rejected the name *graueri* because it was based on a slightly atypical montane population, although he stated unequivocally that it was indeed part of the sub-species in question. Rothschild<sup>4</sup>, from whose paper of



1908 Groves claimed to validate the name *manyema*, clearly attributed this name to Alix and Bouvier<sup>5</sup>, who in 1877 described *G. mayema* from the River Quilo, near Landana, lower Congo (that is, in the range of the western gorilla, *G. g. gorilla*). A *lapsus* (an interpretation admitted by Groves), *manyema* of Rothschild therefore has no validity and in any case clearly referred to gorillas from "South Congo Region" which from the context clearly means the lower Congo, south of Gabon, that is, the same region as referred to by Alix and Bouvier. Rothschild nowhere mentioned the upper Congo in connexion with *manyema*.

By deliberately accepting the amended spelling *manyema* with a type locality "Upper Congo", Groves has created a junior synonym of *graueri*, which is dubiously available even in the event of future sub-division of the sub-species because he gave no characters by which his sub-species could be recognized. The supposed holotype on which it is based is a skull with no locality other than "Upper Congo" (British Museum 1939.945). This skull was acquired by Rothschild from a dealer, and although the words "*Gorilla gorilla manyema* Upper Congo" are written on the skull, no details of the specimen were published by Rothschild. This specimen has no standing as the type of any name other than *manyema* Groves, 1967.

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<sup>1</sup> Groves, C. P., *Nature*, **213**, 890 (1967).

<sup>2</sup> Matschie, P., *Sber. Ges. Naturf. Freunde Berl.*, 323 (1914).

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### Nomenclature of the "Eastern Lowland Gorilla"

I GREATLY regret that I did not make myself entirely clear in my last communication<sup>1</sup>. Dr Corbet implies that I rejected the name *Gorilla graueri* because it referred to an atypical population; this is not the case. I am well aware that the name would have to stand for the sub-species were it the earliest available name. I tried to show, however, that it is not the earliest available name.

The reasons why I accepted *Gorilla gorilla manyema* Rothschild, 1908, as the valid name for the eastern lowland gorilla may be summarized as follows.

It is not clear to the casual reader of Alix and Bouvier's report<sup>2</sup> whether the locality Landana, on the banks of the Quilo (type locality of their *Gorilla mayema*), is in the (former) Belgian or French area of the Congo region. Elliot<sup>3</sup> clearly thought it was in the Belgian region, as he gives the type locality as "Upper Congo"; it is equally clear that Rothschild also did, as he<sup>4,5</sup> spells the trivial name as *manyema*—an obvious confusion with the province of that name on the Upper Congo. The specimens on which Rothschild based his description must therefore have come from the Upper Congo. Landana is in fact on the Lower Congo: so that the type localities of Alix and Bouvier's *mayema* and of Rothschild's *manyema* are not the same. This prevents the name *manyema* from coming under the heading of Emendation or of Incorrect Subsequent Spelling (International Code of Zoological Nomenclature, Art. 33, *a* and *b*), for the taxon referred to by Alix and Bouvier and by Rothschild was not the same. Also the name is not a homonym, because the difference of a single letter is sufficient to prevent homonymy (Art. 57, *d*).

In two papers, Rothschild describes his *G. g. manyema* with the characters of the Eastern lowland gorilla. In 1908 he says<sup>4</sup>, "I have recently received an adult male skull and photograph which clearly show that the Congo gorillas have . . . very sharply defined pale and dark

areas in the pelage"; while in 1923 (ref. 5) he states, "the two or three adult males examined . . . appear to have the pelage brighter in colour and more sharply contrasted". This corresponds to the difference between the eastern gorillas (both *G. g. manyema* and *beringei*) and their western relative: in the former the white "saddle-patch" of the adult male is very sharply defined from the jet black of the surrounding pelage, while in the western gorilla there is a much more gradual change. So Rothschild is clearly describing an eastern gorilla.

Rothschild never mentions more than one skull of *manyema* in his collection. He says that it is<sup>4</sup> "a still narrower and more elongated skull than those from Gabon". This is true of *B.M.* No. 1939.945 which in my communication I accepted as Rothschild's holotype: it measures 305 mm in inion-prosthion length, and 144 mm in cranial breadth, while the mean of nine Gabon skulls, all of the Rothschild collection in the British Museum, except for one which is still in Tring, is 286 mm in length and 138 mm in breadth. The Rothschild skull is certainly "narrower and more elongated". Not only is the inscription "Upper Congo" written on the skull, but application of the results of the statistical analysis to it shows that without a doubt it belongs to the Eastern lowland sub-species, most likely of the Utu deme.

This further information may make my decision to use the name *manyema* a little clearer than in my original paper; reading my paper along it is not surprising that Dr Corbet had concluded that I was at fault. It remains to be pointed out that the name *manyema* is not a *nomen oblitum* as Rothschild used it in 1923, less than fifty years ago. It is to be hoped that all is now settled satisfactorily.

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Received July 27, 1967.

<sup>1</sup> Groves, C. P., *Nature*, **213**, 890 (1967).

<sup>2</sup> Alix, E., and Bouvier, A., *Bull. Soc. Zool. France, Paris*, **2**, 488 (1877).

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### Contraceptive Action of Intrauterine Devices in the Rhesus Monkey

Kelly and Marston<sup>1</sup> have provided important information on the influence of the intrauterine device (IUD) on the distribution of ova in the spontaneously ovulating monkey.

They refer to our report<sup>2</sup> in which the influence of an intrauterine coil on ovum distribution was studied in the superovulated rhesus monkey. In this series, a 50 per cent rate of recovery of tubal ova was noted in control animals without coils. Among similarly treated animals with coils no ova were recovered from the fallopian tubes but four unfertilized ova were found in the uterine cavity. Unlike the animals used by Kelly and Marston all animals in this series were superovulated with gonadotropin, and the IUD was applied by the vaginal route. These studies have now been extended. Among eleven animals with coils which had forty-nine ovulation points, no tubal ova but seven uterine ova were recovered. The non-coil group revealed fifty-five ovulation points, sixteen tubal ova and no uterine ova. Thus, in the superovulated monkey, the coil appears to speed the rate of transfer of ova from the tube, and ova are not extruded promptly from the uterine cavity. Kelly and Marston did not find ova in the uterus in their coil group which had ovulated spontaneously.

Among spontaneously ovulating coil monkeys recently studied in our laboratory, tubal ova have been recovered. Thus our recent data<sup>3</sup> also support the conclusion that in the spontaneously ovulating animal, ova are not delivered

to the uterus immediately after arrival in the tube. The time relationship between ovulation and entry into the uterine cavity is, however, worthy of further scrutiny. A 12–24 h difference in the duration of tubal life may be crucial in terms of ability to implant.

Inasmuch as in our initial series superovulation was used, the evidence of Kelly and Marston does not “contradict” our findings. Their published studies and our studies in the spontaneously ovulating animal reveal findings which are substantially different from those obtained in the superovulated monkey. The mechanism behind the postulated enhanced speed of tubal transport in the superovulated coil animal is of great interest and worthy of further study.

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<sup>1</sup> Kelly, W. A., and Marston, J. H., *Nature*, **214**, 735 (1967).

<sup>2</sup> Mastroianni, L., and Rosseau, C. H., *Amer. J. Obstet. Gynecol.*, **93**, 416 (1965).

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## PHYSIOLOGY

### Diffusion Coefficient of Oxygen through Tissues

THE measurement of the diffusion coefficient of oxygen through actively respiring tissue is of considerable interest but presents some practical difficulty. From the work of Krogh in 1918 until the present time, a very wide range of values for oxygen diffusion coefficients has been reported<sup>1–5</sup> ranging from  $1.1 \times 10^{-4}$  to  $4 \times 10^{-8}$  cm<sup>2</sup> sec<sup>-1</sup>. Because of this very wide range of reported values we decided to measure this value again by a technique essentially similar to that described by Longmuir<sup>5</sup>, but with the difference that the measurements were made on one slice of tissue at a time without the intrusion of “edge” effects.

Tissue slices were cut under sterile conditions to 1.5 mm thickness either by hand or using a tissue slicer and the slices were then cultured in 20 per cent horse serum in ‘Medium 199’ by the organ culture method of MacDougall and Coupland<sup>6</sup>. The external oxygen tension was controlled at a steady value between 0.2 and 3.0 atmospheres pressure. The slices were left for periods varying between 4 h and 5 days at 37° C. It was found that a sharp differentiation between a superficial compact layer of approximately normal cells and a central area of obviously abnormal cells was evident as early as 8 h after slicing.

At the conclusion of an experiment, the tissues were fixed in Bouin’s solution and 8 $\mu$  paraffin sections were prepared and stained with Ehrlich’s haematoxylin and eosin. During the processing care was taken to ensure that the block of tissue was embedded so that subsequent sectioning took place at 90° to the plane of the tissue surface which had been in contact with oxygen. In some instances this surface was marked for identification with indian ink.

Measurement of the thickness of the superficial compact zone was carried out by microscopy using a calibrated eyepiece. In expressing the results allowance was made for a 20 per cent shrinkage of the tissue by the histological processing. This value was previously determined on similar tissue samples.

The results obtained using slices of rat liver are shown in Table 1. Warburg’s equation<sup>7</sup> relates the concentration of oxygen outside the tissue slices to that at any point inside the tissue in terms of the diffusion coefficient of oxygen and the respiration rate of the tissue, that is,

$$U = C - \frac{a}{2D} (Hx - x^2)$$

where  $U$  is the oxygen tension in atmospheres at a point distance  $x$  cm inside the surface,  $C$  is the external oxygen tension in atmospheres,  $a$  is the respiration rate of the tissue (in ml. of oxygen consumed/min./ml. of tissue),  $H$  is the slice thickness in cm and  $D$  is the diffusion coefficient for oxygen in Krogh’s units.

From this equation it can be shown that oxygen will diffuse into a slice of respiring tissue to a depth  $x$  given by

$$x = \left( \frac{2D}{a}, C \right)^{\frac{1}{2}}$$

Thus a plot of  $x^2$  against  $C$  should give a straight line of slope =  $\frac{2D}{a}$ , which passes through the origin. The results are shown plotted in this way in Fig. 1.

Table 1. SHOWING THE THICKNESS OF THE SURVIVING ZONE AS A FUNCTION OF THE OXYGEN PARTIAL PRESSURE DURING INCUBATION

PO <sub>2</sub> (atmospheres)	$x^1$ (cm)	$x$ (cm)	$x^2$ (cm <sup>2</sup> ) $\times 10^{-4}$
0.2	0.007	0.0087	0.76
0.6	0.018	0.023	5.3
1.0	0.020	0.025	6.25
1.5	0.025	0.031	9.6
2.0	0.030	0.0375	14.0
3.0	0.034	0.0425	18.0

$x^1$  is the measured value of the fixed and stained compact zone and  $x$  is this value corrected for shrinkage during the preparation of the slices.

In deriving his equation Warburg made the simplifying assumption that the affinity for oxygen of the terminal oxidases in the tissues is infinite. Clearly, this is not the case; while the oxygen “ $K_m$ ” needed for cytochrome oxidase is of the order of only a few millimetres of mercury<sup>8</sup>, the flavoprotein oxidases and oxygenases which contribute up to 10 per cent of the total respiration have very low oxygen affinities, that is,  $K_m$ ’s of about 150 mm of mercury of oxygen<sup>9</sup>. This makes it difficult to select a theoretically justifiable value for “ $a$ ”, the respiration rate; however, in measuring the thickness of the surviving layer it has been observed that a vacuolated zone of poorly surviving cells (see Fig. 2) lies inside the compact layer of cells of normal appearance and it is suggested that this layer represents a zone where some oxygen is supplied to the tissue but at a partial pressure insufficient for the optimal functioning of the mitochondria. Thus it seems probable that all of the zone which is measured is respiring at, or very near to, its maximum rate under the experimental conditions. This respiration rate has been measured, using very thin slices and with the same substrate, in a Cruickshank differential micro-respirometer which has the advantage that the conditions of measurement closely resemble the conditions of culture.

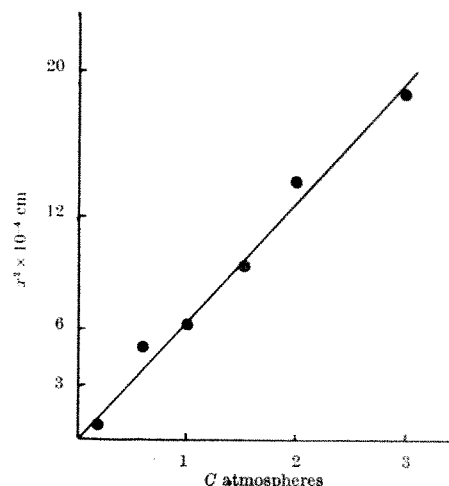


Fig. 1. Showing the relationship between the thickness (squared) of the surviving tissue and the external oxygen pressure during culture.



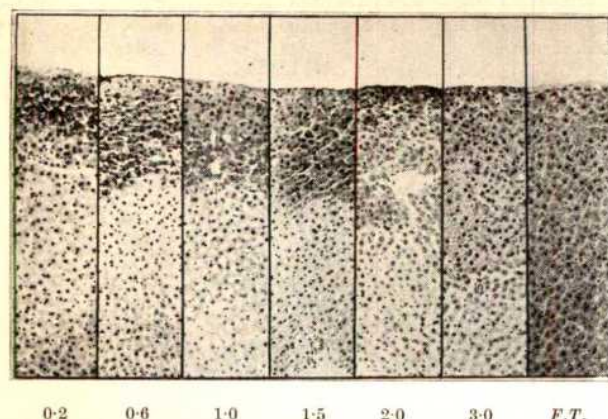


Fig. 2. Sections of liver cultured for 24 h at the oxygen pressures shown (in atmospheres absolute). Note the increasing thickness of the surviving compact zone with increasing oxygen pressure. F.T. is a section of fresh tissue for comparison. Haematoxylin and eosin,  $\times c. 68$ .

Substitution of a mean value for these results in the equation gives a value for  $D$  of  $5.2 \times 10^{-5}$  Krogh units. These units of diffusion coefficient are related to the more usual Fick units by the relation  $D_f = \frac{Dk}{60\alpha}$ , where  $\alpha$  is

the absorption coefficient of the gas in the tissue expressed in ml. gas/ml. tissue at the temperature of the experiment.

The value for  $D$  ( $O_2$ ) Fick obtained is shown in Table 2 together with several other values obtained from other sources.

Source (ref. No.)	Tissue	$D_f$ ( $O_2$ ) $cm^2 \text{ sec}^{-1}$
10	Water (25° C)	$2.12 \times 10^{-5}$
1	Rat diaphragm (37° C)	$1.4 \times 10^{-5}$
12	Bacteria	$4 \times 10^{-6}$
5	Rat liver (37°)	$1.1 \times 10^{-4}$
This work	Rat liver (37°)	$3.6 \times 10^{-5}$

It can be seen that the value is somewhat higher than that through free water<sup>10</sup> and also higher than the value obtained by Krogh<sup>1</sup>, but rather lower than the results of Longmuir<sup>5</sup>.

It is not difficult to imagine a facilitated transport of oxygen through cells as the actively respiring mitochondria have a pumping action which promotes microturbulence of the cell protoplasm. Also the presence of haem like groups may facilitate the transport of oxygen across the cells and these factors may account, as Longmuir has suggested<sup>5</sup>, for a value higher than that through free water.

On the other hand Krogh's experiments were conducted with slices of tissue exposed to haemoglobin solution which has been shown to be toxic for several tissues<sup>11</sup> while the experiments of A. V. Hill<sup>2</sup> were performed on muscle just recovering from a period of anoxia. Thus in both these experiments one might well expect a reduction of the metabolic activity of the cells including protoplasmic stirring.

The experiments of Longmuir, on the other hand, were conducted in virtually optimum conditions for respiratory activity because his slices were well oxygenated and the substrate used was carefully chosen for maximum respiration rates; and it may be that his higher values (approximately three-fold higher) are related to the higher respiration rate (approximately six times greater) of his biological system.

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### Inhibitory Action of Sodium Ions on Transmitter Release at the Motor End-plate

SEVERAL inorganic cations are important in the liberation of acetylcholine during neuromuscular transmission. Extracellular calcium ions are essential for the transmitter release during the depolarization of the nerve terminals<sup>1</sup>. The mean number of packets of transmitter liberated (quantal content  $m$ ) depends on the external concentration of calcium<sup>2</sup>. In this process magnesium ions compete with calcium ions<sup>3</sup>. Extracellular sodium ions also take part in the process of transmitter release by the nerve impulse. A reduction in the extracellular sodium concentration produces a large increase in the end-plate potential amplitude, and in  $m$ , at least under conditions of low external concentrations of calcium (refs. 4 and 5).

The effects of calcium and magnesium ions on the transmitter release by a nerve impulse have been ascribed to reactions between calcium (or magnesium) ions and a specific site  $X$  on the nerve terminal, forming  $CaX$  (or  $MgX$ ); while  $CaX$  is necessary for the release,  $MgX$  is ineffective<sup>6</sup>. It has been suggested that the effect of sodium ions can be explained in a similar way, that is, that calcium and sodium ions compete for this "strategic site" at the presynaptic membrane<sup>4,5</sup>. At low concentrations of calcium the e.p.p. amplitude (or  $m$ ) is a fourth power function of the presumptive  $[CaX]$  complex<sup>7,8</sup>. On the basis of these results it was concluded that the process of liberation of a quantum of transmitter requires a co-operative action of four calcium ions.

Thus there are at least two possible alternative explanations for the inhibitory action of sodium ions on transmitter release: they can either compete with calcium for the presumptive  $X$  sites, or they can change the number of calcium ions required for release. With normal concentrations of calcium, co-operation of four calcium ions is necessary to release a unit of acetylcholine<sup>7,8</sup>. If in low concentrations of sodium a co-operative action of a smaller number of calcium ions is needed, then the probability of release would be increased. To decide between these possibilities, the quantitative relationship between the concentration of calcium and release, at normal and low concentration of sodium, was examined.

End-plate potentials from surface fibres of frog sartorius muscle were recorded intracellularly with potassium chloride micropipettes. Conventional d.c. amplifier, cathode ray oscilloscope and photographic records were made. All experiments were performed at room temperature (19°–24° C). Solutions containing low concentrations of sodium ions were prepared by isotonic substitution of sucrose for sodium chloride. The experiments were performed in low extracellular concentrations of calcium ions; end-plate potentials were therefore fluctuating in amplitude (Fig. 1) as predicted by the quantal hypothesis<sup>9</sup>. In order to obtain a statistically reliable measure of  $m$ , a large number of end-plate potentials was averaged (256 when  $m$  was less than 3, 128 when  $m$  was between 3 and 10, and 64 when  $m$  was greater than 10). This was usually done by means of a computer for averaging transients ('Biomac 500').

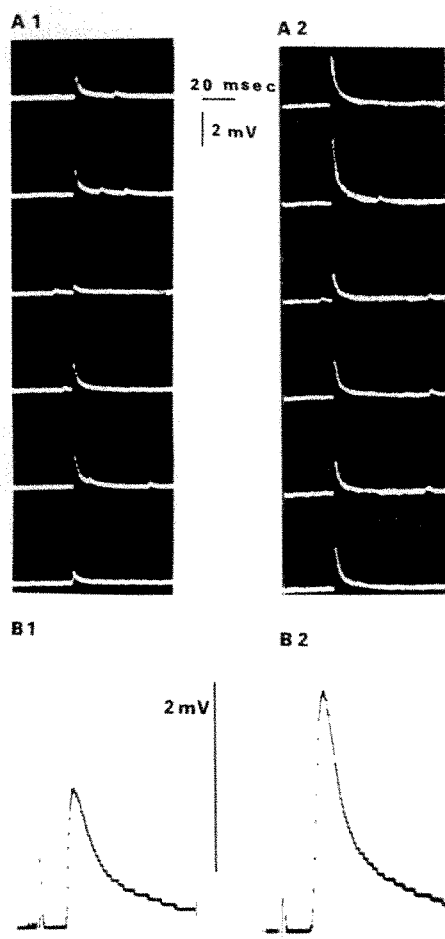


Fig. 1. End-plate potentials at normal (left) and low—60 per cent (right) concentrations of sodium.  $A_1$  and  $A_2$  are samples of individual records.  $B_1$  and  $B_2$  are automatically averaged responses to 256 stimuli. Averaging step 80  $\mu$ sec. Magnesium ions (2 mmolar) were present throughout the experiment.

When lowering the concentration of sodium, end-plate potential amplitudes are not a reliable measure of the amount of transmitter release, because there is a decrease in the postsynaptic sensitivity to acetylcholine<sup>10,11</sup>. This was also seen when measuring the mean amplitude of the miniature end-plate potential ( $\bar{a}$ ). It is interesting that in addition to the immediate reduction of  $\bar{a}$  after changing the medium to 60 per cent sodium, there was an additional slow reduction of  $\bar{a}$ . While the initial diminution in  $\bar{a}$  is probably caused by changes in the equilibrium potential<sup>11</sup>, the explanation of the second phase of decrease is still obscure. For this reason, in the subsequent analysis, only direct determinations of  $m$  were accepted as a measure of transmitter release. These were obtained from the ratio of the average end-plate potential and  $\bar{a}$  (ref. 9), which requires that all the experiments were made on uncurarized preparations.

The records in Fig. 1 illustrate the inhibitory effect of sodium ions on transmitter release. Decreasing the concentration of sodium ions from normal (116 mmolar) to 60 per cent of the normal (69.6 mmolar) causes the mean end-plate potential amplitude to increase by 87 per cent, and  $m$  to increase by 142 per cent (there was a simultaneous 23 per cent decrease in  $\bar{a}$ ). Qualitatively, these are similar to earlier results<sup>4,5</sup>.

The quantitative relation between calcium and release was examined at the same end-plate at normal and low concentrations of sodium (Fig. 2A). The non-linear relations obtained are similar to those reported before<sup>3,7,8</sup>.

It has been suggested<sup>8</sup> that the relation between calcium and release follows a co-operative equation

$$m = K \left( \frac{\text{Ca}}{1 + \frac{\text{Ca}}{K_1} + \frac{\text{Mg}}{K_2}} \right)^4 \quad (1)$$

where  $K_1$  and  $K_2$  are dissociation coefficients of  $\text{CaX}$  and  $\text{MgX}$  complexes, respectively, and  $K$  is a constant. It should be noted that in equation (1) no account is taken of possible competitive interactions between ions other than magnesium and calcium; if there is competition between sodium and calcium, this would give a spurious value of  $K_1$ .

In order to test whether sodium ions affect the power index between calcium and  $m$ , or one of the other para-

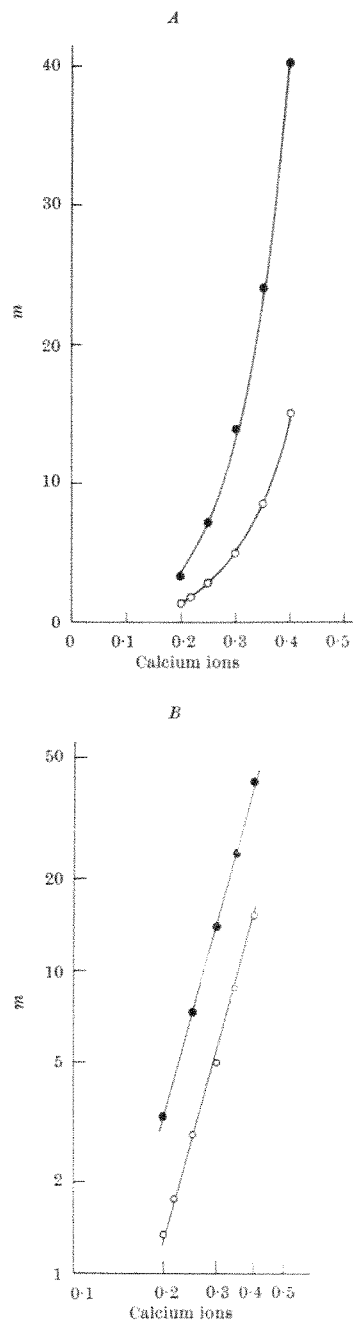


Fig. 2. Relation between concentration of calcium ion and  $m$  at 100 per cent (O) and 60 per cent (●) concentrations of sodium. All points obtained at the same end-plate. Each point represents the average response to 256, 128 or 64 stimuli. (A) Linear plot; (B) same as A on double-logarithmic co-ordinates. Magnesium (1 mmolar) was present throughout the experiment.



Table 1. EFFECT OF REDUCING THE CONCENTRATION OF SODIUM ON  $m$ 

	1 mmolar magnesium	8 mmolar magnesium
Average quantal content at normal [Na]—	11.08	0.64
$m_{1.0}$	(64)	(256)
Average quantal content at low [Na]—	27.62	0.79
$m_{0.4}$	(64)	(256)
$m_{1.0}$	2.49	1.24

The concentration of calcium was kept at 0.4 mmolar. Numbers in parentheses are averages of responses.

meters of equation (1), the experimental results were plotted on log-log scale (Fig. 2B). The resulting straight lines in low and high concentrations of sodium have almost the same slope, indicating that sodium ions do not change the power relationship between calcium and  $m$ , but only shift the relation along the abscissa. Thus it can be concluded that the requirement for a co-operative action of four calcium ions is not altered even in low external concentrations of sodium, when the release is greatly augmented. The observed shift in the log calcium-log  $m$  relation could result either from an effect of sodium on the parameter which determines the maximal release ( $K$ ), or by competition between sodium and calcium (and therefore also with magnesium) in combining with  $X$ .

In this respect some evidence was obtained by studying the interaction between sodium and magnesium ions, at a constant calcium concentration. If sodium ions compete with calcium and magnesium for  $X$ , then this would lead to equation (2)

$$m = K \left( \frac{Ca}{1 + \frac{Ca}{K^*_1} + \frac{Mg}{K_2} + f(Na)} \right)^4 \quad (2)$$

where  $f(Na)$  is a function of  $[Na]$  or  $[Na]^2$  and  $K^*_1$  is the "true" dissociation coefficient of  $CaX$  complex. From equation (2) it is expected that the increase in  $m$  caused by reduction in the concentration of sodium would be smaller at high than at low concentrations of magnesium. Table 1 shows that this is so. While reducing the concentration of sodium at 8.0 mmolar magnesium produces only a 24 per cent increase in  $m$ , at 1.0 mmolar the increase is 149 per cent. It is suggested therefore that the inhibitory effect of sodium ions on transmitter release arises from competition with calcium ions on the postulated critical sites  $X$ .

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### Unitary Excitatory Postsynaptic Potentials in Clarke's Column Neurones

It is generally believed that synaptic transmission is effected by a quantal release of transmitter substances, such as has been demonstrated for the neuromuscular junction<sup>1</sup>. The quantal nature of synaptic transmission in the central nervous system is more difficult to investi-

gate, one reason being that many presynaptic fibres usually contribute to the EPSP (excitatory postsynaptic potential), whereas at the neuromuscular junction the entire effect is evoked from a single presynaptic fibre. Thus at central synapses the total synaptic action should be described both in terms of the number of unitary EPSPs (the EPSP contributed by a single presynaptic fibre) and the quantal composition of each unitary EPSP. In motoneurones, impulses in Ia afferents (large muscle spindle afferents) usually evoke small unitary EPSPs with a low quantal content<sup>2</sup>, but larger unitary Ia EPSPs with quantal content "possibly as high as ten or fifteen" have also been found<sup>3</sup>.

The work reported here deals with transmission to Clarke's column cells giving rise to the dorsal spinocerebellar tract. These cells are effectively monosynaptically activated from primary afferents, some from Ia afferents<sup>4</sup>. Previous recordings of the EPSPs in Clarke's column cells evoked by graded stimulation of muscle afferents show a large convergence (about twenty presynaptic fibres) and suggest small unitary EPSPs (less than 1 mV)<sup>5</sup>. From an analysis of the irregularity in firing in response to muscle stretch, on the other hand, it has been suggested that large unitary EPSPs may exist in Clarke's column cells<sup>6</sup>.

The experiments were performed on spinal cats, anaesthetized with chloralose and immobilized with 'Flaxedil'. One hindlimb was denervated except for the nerves of the ankle muscles. The tendons of these muscles were cut near their insertions and by means of threads and pulleys the individual tendons could be loaded with weights. Intracellular records of Clarke's column cell were obtained in spinal segments LIV to LII by micropipettes filled with potassium citrate or potassium chloride. All neurones were identified by antidromic activation from the dissected dorsolateral funicle of the cord and by group I monosynaptic activation from one of the muscle nerves.

The impaled cells analysed had a resting membrane potential of 35–60 mV. At the lower levels the spike mechanism was inactivated, but local responses occurred which could be confused with "synaptic noise". When the cells were hyperpolarized this "noise" disappeared and a resting discharge occurred. For the analysis of unitary EPSPs the cells were hyperpolarized to a level at which no firing was produced by a moderate muscle stretch. Stronger hyperpolarization was used to prevent the firing evoked by a synchronous group I volley in muscle afferents. A maximal volley may produce an EPSP of 25–30 mV. Graded stimulation reveals steps of widely different sizes. We found it difficult to determine the exact degree of convergence with this technique but have confirmed that there is a considerable degree of convergence of primary afferents onto each Clarke's column cell. A more convenient technique in the study of unitary EPSPs is to investigate the asynchronous effect of muscle stretch<sup>7</sup>. The records in Fig. 1 are from a cell that was monosynaptically activated by group I afferents from the soleus muscle. With the tendon slack there were only very small fluctuations in membrane potential. A load of 20 g on the tendon produced rhythmic short-lasting depolarization consisting of a rapid rising phase followed by a slower approximately exponential return to the resting level (Fig. 1A). They have all the characteristics of unitary EPSPs produced by afferent impulses from muscle stretch receptors. Their time course resembles that of the electrically evoked EPSP and they occur at very regular intervals as long as the muscle stretch is constant (compare interval distribution in Fig. 1C). From its amplitude and time course the unit of Fig. 1A could be recognized at a higher degree of muscle stretch (Fig. 1B). Its rate of appearance is higher and now it appears alternately with other units of smaller amplitude. When coinciding in time the EPSPs summate. The mean amplitude of the large unitary EPSP in Fig. 1A is 4.3 mV.

Three other unitary EPSPs of the same cell identifiable by the rhythmicity and time course had mean amplitude of 2.4, 1.8 and 1.4 mV. Unitary EPSPs of smaller size, which were difficult to identify individually, were frequently evoked by stretch.

Other group I activated Clarke's column cells showed an appreciable unitary synaptic activity even with the activating muscle slack. Such resting activity may originate in muscle stretch receptors because primary endings of the muscle spindles commonly have a resting discharge in the slack muscle. We could usually demonstrate that a greater part of the resting synaptic activity did originate in the muscle stretch receptors by its disappearance in the period immediately following release of the muscle from a strong pull. In the cells with a resting synaptic activity it was difficult to recognize the individual unitary EPSPs evoked by muscle stretch.

We conclude that many primary afferents contribute excitatory action to each Clarke's column cell. The unitary EPSPs range from a maximal size of about 5 mV down to a fraction of 1 mV.

The amplitude of a unitary EPSP shows appreciable fluctuations (Fig. 1A). Assuming that the release of the quanta of transmitter occurs with a certain probability the fluctuation in amplitude of an individual unitary EPSP is a function of the number of quanta released by each presynaptic action potential. If the average probability of release of an individual quantum is small the number of quanta released and accordingly the distribution of amplitudes of an individual unitary EPSP are described by Poisson's distribution. Without any information on the size of the individual quanta in the unitary EPSP of the Clarke's column cells the estimation of their quantal content could be based on the frequency of occurrence of complete failures in a rhythmic series like that of Fig. 1. We found no failures in our observations of unitary EPSPs. The unit of Fig. 1A was followed for more than 450 occurrences, and the absence of failures gives only an estimate of the minimal quantal content of more than seven.

In the absence of failures the quantal content ( $m$ ) can be estimated from the coefficient of variation

$$CV = \frac{S.D.}{\text{Mean}}$$

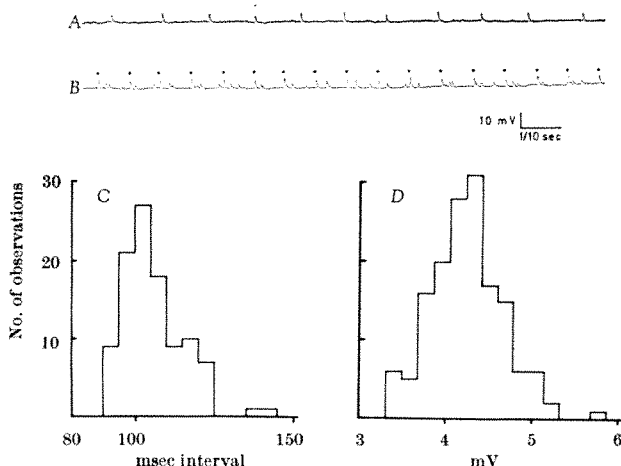


Fig. 1. Intracellular record from Clarke's column cell. The resting membrane potential was 60 mV and the cell hyperpolarized by passage of 10 nAmp through the recording microelectrode. A, Rhythmic unitary EPSP evoked by a load of 20 g on soleus tendon. B, Same as A with a load of 50 g on soleus tendon. The largest unitary EPSP is marked with dots, and is the same as that in A. C, Distribution of intervals between consecutive unitary EPSPs, measured from record like A with a load of 20 g on tendon. Ordinate, No. of observations in each class. Abscissa, duration of intervals. Class size 5 msec. D, Distribution of amplitudes of unitary EPSPs of A. Total No. of observations 151. Mean amplitude 4.3 mV. S.D. 0.42 mV. Ordinate as in C. Abscissa, amplitude of EPSP.

of the distribution of amplitudes. In the simplest case<sup>7</sup> the relationship is the following

$$m = \frac{1}{(CV)^2}$$

Fig. 1D illustrates the distribution of amplitudes of the unitary EPSP of Fig. 1A. The distribution is fairly symmetrical and approximately Gaussian. Because the Poisson distribution approximates the normal distribution for large average numbers of events, the form of the amplitude distribution indicates that many transmitter quanta are liberated by each presynaptic impulse. The mean amplitude of the EPSP of Fig. 1D was 4.3 mV and the S.D. 0.42 mV. This gives quantal content of approximately 100. For five other large unitary EPSPs the CV varied between 16 and 11 per cent, which corresponds to a mean quantal content of 39 to 83. The estimates are subject to the assumption of a Poisson law governing quantal transmitter release and certain additional approximations<sup>7</sup>. It seems reasonable to accept the Poisson model because it has proved adequate at the neuromuscular junction where its implications have been tested directly. The accuracy of the present estimate of  $m$  is reduced by a number of factors<sup>7</sup>. A rough appreciation of the importance of these suggests that the true number of quanta released is not less than half of the estimate. Accordingly, the number of quanta released by an impulse in a group I afferent fibre producing a large unitary EPSP in a Clarke's column cell would be at least between 20 and 50. Such a large quantal content, perhaps half of that released by an impulse at the mammalian neuromuscular end-plate, might be correlated with the extensive synaptic contacts between primary afferent fibres and Clarke's column cells<sup>8</sup>.

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### Influence of Denervation on Localization of Neurotoxins from Clapid Venoms in Rat Diaphragm

$\alpha$ -BUNGAROTOXIN<sup>1,2</sup> isolated from the venom of *Bungarus multicinctus*, as well as cobra neurotoxin<sup>3,4</sup> from the venom of *Naja naja atra*, has been found to block neuromuscular transmission by an antidepolarizing action similar to that of *d*-tubocurarine. These conclusions have been further supported by the autoradiographic findings that  $\alpha$ -bungarotoxin labelled with iodine-131 (ref. 5), as well as cobra neurotoxin<sup>6</sup>, accumulates on the motor end-plate zone of the mouse diaphragm in the same way as *d*-tubocurarine<sup>7</sup>.

In the rat diaphragm preparation treated with either labelled  $\alpha$ -bungarotoxin ( $5 \times 10^{-6}$  g/ml.) or cobra neurotoxin ( $10^{-6}$  g/ml.) *in vitro*, the radioactivity on the motor



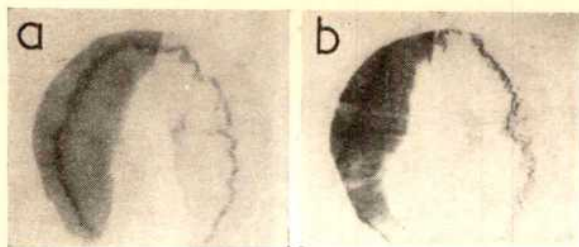


Fig. 1. Effect of phrenicotomy on fixation by  $\alpha$ -bungarotoxin labelled with iodine-131 after 14 days (a) and 60 days (b). Right side: innervated hemidiaphragm; left side: phrenicotomized hemidiaphragm. Each rat was injected subcutaneously with 0.3  $\mu$ g/g of  $\alpha$ -bungarotoxin and died at 75 min (a) and 110 min (b) respectively after the injection.

end-plate zone remained unchanged even after more than 4 h of washing with Tyrode solution, whereas the portion of toxins taken up non-specifically by the muscle tissue other than the motor end-plate zone disappeared almost completely after washing. This finding is consistent with the irreversible nature of the neuromuscular block by these toxins.

By cutting the left phrenic nerve we examined the influence of denervation on the localization of these toxins in the rat diaphragm. At 14 and 60 days respectively after phrenicotomy, the labelled toxins were injected subcutaneously in a dose of 0.3  $\mu$ g/g body weight and the autoradiograph of the denervated side was compared with that of the intact right side of the diaphragm. In the hemidiaphragms denervated for 14 days, the radioactivity spread over the whole area of the muscle tissue, although a well defined motor end-plate zone could still be distinguished (Fig. 1a). This finding, together with that of Waser<sup>8</sup> using C14-curarine, gives support to the contention that supersensitivity of denervated muscles to acetylcholine results from an increase in the receptor area rather than from a marked change in the receptors themselves<sup>9,10</sup>. In contrast to the finding of Waser<sup>8</sup> that in the denervated diaphragms the radioactivity disappeared completely within 60–120 days, we found almost the same pattern of localization of radioactivity in the hemidiaphragm denervated for 60 days (Fig. 1b).

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### Progesterone in Cerebrospinal Fluid during Human Pregnancy

PROGESTERONE has been found to be an effective anaesthetic agent when given intraperitoneally to animals<sup>1,2</sup> and 21 hydroxy pregnandione succinate has been used clinically for general anaesthesia<sup>3</sup>. Of practical importance in clinical anaesthesia is the fact that in animals progesterone potentiated the effect of ether and chloroform<sup>2</sup>.

No clinical confirmation of this effect in humans is, however, available. Paradoxically, it is believed that progesterone renders the respiratory centre more sensitive to carbon dioxide and that this may account for the lowered  $p\text{CO}_2$  in blood during pregnancy.

In view of the higher concentration of progesterone in the peripheral circulation during pregnancy and the apparent lack of clinical effects on the central nervous system, we were interested to obtain some information concerning the concentration of progesterone in the cerebrospinal fluid especially in pregnancies approaching term. A search of the literature revealed that this information had not previously been reported.

The method which we used to assay the progesterone in cerebrospinal fluid was identical to that used for plasma<sup>4</sup>. The highest reliable sensitivity obtained with the method was 0.004  $\mu$ g/ml. Using this method we confirmed that the concentration of progesterone in the peripheral blood rises progressively during pregnancy and that there is no reduction in concentration until separation of the placenta<sup>5</sup>. It is now generally believed that there is no reduction of progesterone in the peripheral blood before the onset of labour at term.

Peripheral blood and cerebrospinal fluid were obtained at the same time from each of four patients at term. In each case the cerebrospinal fluid was obtained before the administration of spinal anaesthesia. The results of the assays are shown in Table 1.

Table 1		
Patient	Progesterone in plasma ( $\mu$ g/100 ml.)	Progesterone in cerebrospinal fluid ( $\mu$ g/100 ml.)
A	10.00	Undetectable
B	8.74	0.30
C	9.00	0.55
D	11.10	Undetectable

The results strongly suggest that there is an effective blood-brain barrier for progesterone and this may explain the lack of apparent effect on the central nervous system as pregnancy advances. The marked difference in concentration of progesterone has also been shown to exist for cortisol<sup>6</sup>, and it is interesting that the concentration of cortisol found by Baron and Abelson in cerebrospinal fluid is similar to that reported here for progesterone.

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### Effects of Norepinephrine on Tissues of the Frog Heart Atrium poisoned by Tetrodotoxin

THE acceleration of the sinus venosus of the frog's heart induced by epinephrine may be the result of an increase in the conductance of sodium<sup>1</sup>. We also know that the action potential of the frog atrium is abolished in sodium

free solutions<sup>2</sup>. We have tested the effects of tetrodotoxin on these responses because this poison selectively blocks the increase in the conductance of sodium which is responsible for the generation of action potentials in many tissues<sup>3</sup>.

The excised frog atrium and sinus venosus were immersed in oxygenated Ringer's solution (115 mmolar sodium chloride; 3 mmolar potassium chloride; 1.0 mmolar calcium chloride; 2.0 mmolar sodium bicarbonate; and 0.05 mmolar sodium phosphate, pH 7.2). We used pairs of small platinum wires for stimulation and extracellular recording. Intracellular recordings were made by means of conventional microelectrode techniques.

When  $10^{-8}$  g/ml. of tetrodotoxin was added to the bath the electrical activity of atrial muscle was abolished without eliminating the initiation of impulses at the sinus venosus (Fig. 1A and B). In the experiment illustrated here, the rate of activity in the sinus venosus decreased from 44 to 28 beats/min after the addition of tetrodotoxin. When norepinephrine ( $5 \times 10^{-6}$  g/ml.) was added, the sinus rate increased to 47 beats/min. More striking than the increase in rate was the recovery of excitability of atrial muscle. It now propagated action potentials in response to impulses from the sinus venosus (Fig. 1C) or to electrical stimuli.

The propagation in the atria treated with tetrodotoxin plus norepinephrine was sluggish. In the experiment illustrated in Fig. 1, the conduction time before treatment was 80 msec. After treating the preparation with tetrodotoxin plus norepinephrine the time was 400 msec. In two other preparations a similar prolongation of conduction time was observed.

The most striking effect observed in intracellular records of atrial muscle treated with tetrodotoxin plus norepinephrine was a slowing of the rate of rise of the action potential (Fig. 2A and B). In nineteen cells in three atria penetrated before treatment the rate of rise was  $37.7 \pm 11.4$  V/sec (mean  $\pm$  S.E.). In eighteen cells of the same atria impaled after adding  $10^{-8}$  g/ml. of tetrodotoxin plus  $5 \times 10^{-6}$  g/ml. of norepinephrine the rate of rise was  $8.1 \pm 3.5$  V/sec.

The minimal dose of norepinephrine sufficient to recover atrial excitability varied between 2 and  $8 \times 10^{-6}$

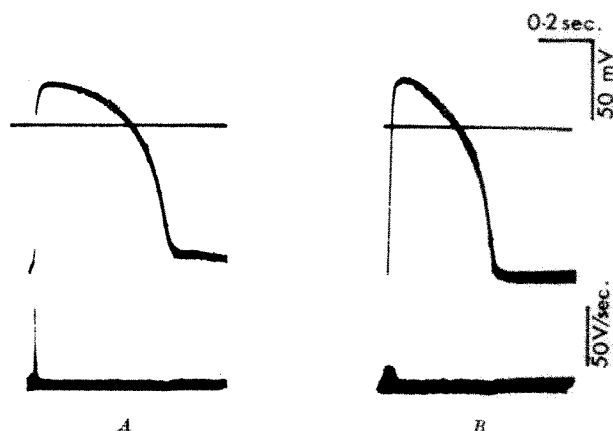


Fig. 2. Intracellular records of two cells of the same atrium. The upper tracing is the action potential. A horizontal line was drawn through the zero potential level. The lower tracing is the electrically differentiated record of the action potential. (Time constant of the differentiating network: 100  $\mu$ sec.) (A) Control; (B) after treatment with tetrodotoxin plus norepinephrine.

g/ml. In several experiments, we tried to find whether this minimal dose of norepinephrine necessary for recovery was modified by varying the concentration of tetrodotoxin. We found that the minimal dose of norepinephrine that caused a given atrium to recover was not altered by increasing the tetrodotoxin from  $10^{-8}$  to  $10^{-6}$  g/ml.

Of the most likely explanations for these results are: (a) much of the acceleration of sinusal rate caused by norepinephrine results either from an increase in the conductance of sodium through a site insensitive to tetrodotoxin or from a change in conductance to an ion other than sodium; (b) in atrial muscle norepinephrine increases the voltage dependent changes in conductance through sites different from those carrying the currents responsible for the normal action potential. An alternative explanation would be that norepinephrine restores in a non-competitive manner the ability of the site poisoned with tetrodotoxin to increase the conductance of sodium ions in response to depolarization of the membrane.

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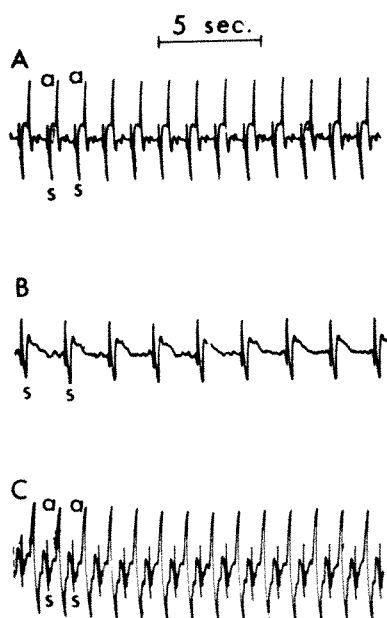


Fig. 1. Tracings of the extracellular activity of the excised atrium. (A) Control record: the sinusal action potential (s) and atrial muscle action potential (a) can be clearly distinguished. (B) After the addition of  $10^{-8}$  g/ml. of tetrodotoxin, the atrial muscle action potential is abolished while spontaneous sinusal activity continues, although at a lower rate. (C) After treatment with norepinephrine ( $5 \times 10^{-6}$  g/ml.) the sinusal rate increased and the atrial muscle propagated impulses again.

## Interaction of "Stress" and the Response to Mescaline

THE clinical effect of mescaline is very variable both between different subjects and in the same subject at various times. One factor that has been noted to play a part here is the anxiety proneness of the subject. Klerman<sup>1</sup> has shown in a double blind study that passive, anxious, intellectual introverts react in a far more psychotic manner to mescaline than do non-anxious extroverts. Our clinical experience confirms this.

The formula of mescaline suggests that it might act on some biochemical mechanism concerned with the catecholamines—in particular catechol-O-methyl transferase. There might therefore be an exacerbation in its effects



if we increase the central adrenergic activity associated with anxiety. In order to gain further data on this point we have investigated in the rat the effect of mescaline on a stable behavioural baseline before and after the instigation of the conditioned emotional response (CER). Previous to this, experiments in our laboratory had shown that on positive reinforcement schedules, animals with prior negative reinforcement history (Sidman avoidance) showed a much heightened susceptibility to the effects of mescaline.

In the experiments described a rat deprived of water is first trained to press a lever for water reward on a differential reinforcement of a low rate, limited hold (DRL-LH) schedule. Fig. 1A shows the response obtained after more than 100 daily training sessions. The rat is now producing a characteristic DRL type inter-response time distribution with a mode around the lower bound of the reinforced interval. Fig. 1B shows that a dose of 8.5 mg/kg mescaline intraperitoneally has no effect on this lever-pressing response. A higher dose of mescaline (at least 12.5 mg/kg) will induce a dose dependent period of inhibition with time of onset between 5 and 10 min after injection. The CER was then established by repeated presentation of a 2 min buzzer conditioned stimulus (CS) terminated by brief electric shock (0.5 m.amp, 0.5 sec). Response shock pairings were preceded by habituation to the conditioned stimulus and CER procedure was maintained for forty daily sessions. The rat quickly adapts by complete suppression of lever pressing during the CS periods but with no apparent disruption of the baseline behaviour (Fig. 1C).

Fig. 1D shows that mescaline (at the previously inactive dose level) now has a profound effect on behaviour, suppressing all responding for about 30 min. The figures show the response of one animal from a group of three,

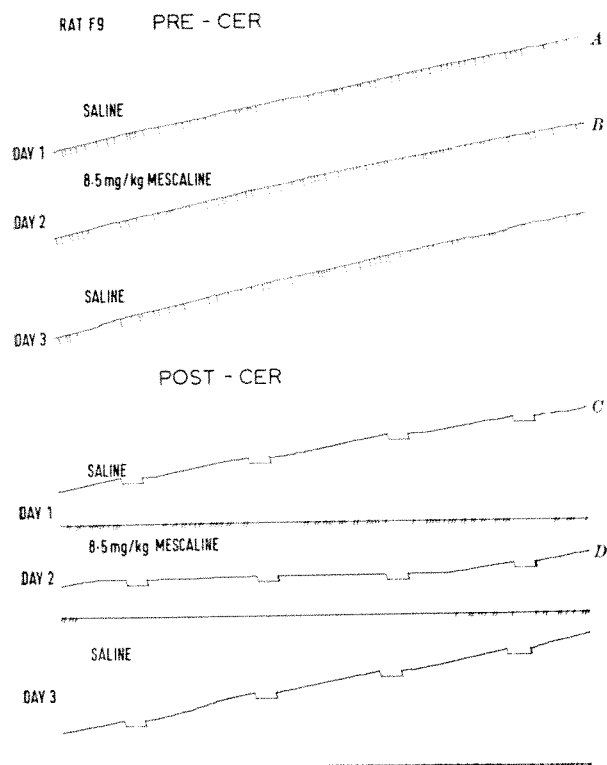


Fig. 1. The effects of 8.5 mg/kg mescaline before and after the establishment of the conditioned emotional response. Pre-CER: Each response step the record in the vertical direction. A downward slash indicates reinforcement. A, Control saline injection; B, 8.5 mg/kg mescaline intraperitoneally. Post-CER: In the top record periods of deflection represent the 2 min CS presentations. Reinforcements are shown on the bottom horizontal record. C, Control saline injection; D, 8.5 mg/kg mescaline intraperitoneally.

all of which showed this interaction. Thus the behaviour disrupting effect of mescaline is markedly potentiated following the establishment and maintenance of the CER procedure. This is compatible with the hypothesis that the mescaline acts on the central mechanisms mediating "anxiety" or "stress".

An alternative hypothesis would be that the effect is caused by summation of disruption of behaviour by two independent processes because both mescaline (at higher doses) and the CER schedule (at higher shock intensities) may by themselves disrupt behaviour. It is difficult, however, to distinguish between two hypotheses using any behavioural studies where the behavioural dependent variable is influenced in the same direction by both manipulations. Nevertheless the experimental design used here, and these results obtained, offer a way of determining the interaction between mescaline and central adrenergic activity if additional biochemical data are obtained.

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### Cell Proliferation during the Development of Stress Erosions in Mouse Stomach

THE surface of the gastric mucosa, which is continually replaced by rapidly proliferating epithelial cells<sup>1</sup>, is prone to erosion under stress. These lesions occur in man<sup>2,3</sup> and are induced in animals by various experimental procedures<sup>4,5</sup>. Stress of short duration decreases the frequency of mitosis in the stomach of the rodent<sup>6</sup>.

We have measured the number of cells incorporating tritiated thymidine and entering into mitosis in the stomach of the mouse during prolonged stress, before and during the development of erosions. Experimental and control groups, each of thirteen male CFW mice weighing 25–30 g, were housed in individual cages for one week. After this period, each mouse in the experimental group was restrained at midnight in a wire mesh apparatus<sup>7</sup>, and mice were deprived of food and water. Beginning 12 h later, at intervals of 1–3 h two mice, one under stress and one control, were injected with tritiated thymidine, (25  $\mu$ Ci/mouse, specific activity 6.7 Ci/mmole). All animals were killed 1 h after injection, so the first mouse killed in the experimental group had been under stress for 13 h and the last mouse for 42 h.

Stomachs were removed from all animals, opened along the greater curvature, placed on a cork board, and sectioned through the mid-portion of the gastric body. The specimens were fixed in neutral formalin, embedded in paraffin and sectioned at 3  $\mu$  with tissues oriented to pass longitudinally through the columns of the gastric pit. Microautoradiographs were prepared by dipping the sections in 'NTB' liquid emulsion (Kodak). Slides were then exposed for two months in darkness, in a sealed dry box, developed with Kodak 'D'19, and stained with haematoxylin and eosin. Additional slides were prepared with periodic acid-Schiff (PAS) stain.

Histological examination of gastric mucosa under light microscopy after stress caused by restraint revealed scattered focal areas containing moderate vacuolization of cytoplasm in epithelial cells and slight to moderate decrease in PAS-positive mucopolysaccharide. Desquamation of surface cells was increased, and in a few areas early collapse of the mucosa was seen, the cells having an

abnormal eosinophilic cytoplasm with pyknotic nuclei; there was no oedema, inflammatory exudate or necrosis.

Counts of metaphase figures, cells labelled with tritiated thymidine and unlabelled cells were made. Background levels averaged one grain in an area occupied by twenty nuclei in these sections. In each specimen 4,000 epithelial cells were counted randomly. Eleven of the thirteen animals which had been under stress had reduced mitoses compared with the controls, and twelve of the thirteen had reductions in labelled cells. Table 1 compares the group averages of controls and all animals under stress from 13–42 h, and gives the counts made of cells in metaphase and cells labelled with tritiated thymidine, together with standard errors of the group averages. The level of  $P$  for differences between control and experimental groups indicates that the frequencies of mitoses and labelled cells were significantly decreased in the stress group. These decreases were in areas of mucosa containing no histological changes, and were pronounced in areas showing the histological alterations mentioned earlier. Despite cell damage and loss therefore, during the development of the erosions the rate of replacement of epithelial cells was significantly lower than that of the non-stressed controls.

Table 1. COUNTS OF MITOSES AND LABELLED CELLS

Group averages and standard errors of counts made on cells in metaphase and cells labelled with tritiated thymidine					
No.	Average count/ 1,000 ( $\pm$ S.E.)	Stress	$t$ ratio and associated $P$ level for difference between control and stress groups		
Stomach					
Mitosis	13	4.1 $\pm$ 0.39	2.7 $\pm$ 0.39	$t_{24} = 2.536$	0.02 $> P > 0.01$
Labelled cells	13	96.0 $\pm$ 7.74	53.9 $\pm$ 14.9	$t_{24} = 2.506$	0.02 $> P > 0.01$

Four thousand cells counted in each stomach specimen of the thirteen animals in each group. The counts for each group were examined for distribution properties and these were found to be consistent with the normal distribution.

Tritiated L-leucine-4,5 (specific activity 5 c./mmole, 100  $\mu$ C./mouse) was also injected intraperitoneally into two additional groups of stressed and control animals maintained under the same conditions; animals were again killed 1 h after injection. Using microautoradiography, the comparative rates of protein synthesis in intestinal epithelial cells were estimated\*. The results showed active incorporation of leucine into all cell types. Grain counts over cytoplasm indicated equivalent amounts of leucine incorporated in both groups (epithelial cells 12.9 and 11.7 grains/cell, respectively, in control and experimental mucosa; parietal cells 44.2 and 40.9 grains; zymogen cells 44.4 and 45.8 grains, always with  $P > 0.5$ ). Throughout the mucosa, epithelial cells showing histological abnormalities were well labelled with leucine, as seen after early radiation damage\*.

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## Effect of Calcium and Phosphates on Gastrointestinal Absorption of Strontium and Calcium in Newborn Rats

An increase in the calcium and phosphate in the diet has been shown to cause decreased absorption of strontium from the gastrointestinal tract in growing rats<sup>1-4</sup>. The same effect of phosphates on strontium absorption was also observed in pregnant female rats and in their litters, suggesting that an increased phosphate intake into the nursing mother provides an effective means of decreasing the uptake of radioactive strontium in her litter<sup>5</sup>. This effect could be caused by either the action of the higher phosphate content in mothers' milk on calcium and strontium absorption from the gastrointestinal tract in newborn rats, or by a decreased ratio of strontium to calcium in the milk, caused by the influence of phosphates on calcium and strontium metabolism in the nursing mother. We have tried to clarify this point.

Our experiments were carried out on rats 5 days old which were artificially fed on cows' milk and on cows' milk to which  $\text{KH}_2\text{PO}_4$  or  $\text{CaCl}_2$  and  $\text{KH}_2\text{PO}_4$  had been added to increase dietary phosphorus only (Table 1) or

Table 1. INFLUENCE OF PHOSPHATE ADDITIVES TO MILK IN ARTIFICIALLY FED RATS ON CALCIUM AND STRONTIUM ABSORPTION FROM THE GASTROINTESTINAL TRACT IN 5-DAY-OLD ANIMALS

Milk (mg/100 ml.)		No. of rats	Retention in carcass % oral dose		
P	Ca		Strontium-85	Calcium-45	Strontium-85/ Calcium-45
45	140	52	82.32 $\pm$ 1.47	81.93 $\pm$ 1.46	1.01 $\pm$ 0.005
230	140	68	82.35 $\pm$ 1.27	82.81 $\pm$ 1.55	1.00 $\pm$ 0.072
500	140	59	82.84 $\pm$ 1.46	84.52 $\pm$ 1.67	0.97 $\pm$ 0.014

Strontium-85, calcium-45 and  $\text{KH}_2\text{PO}_4$  were added to cows' milk, which was fed to rats by means of a dropper; each rat received an average of twenty-five drops (0.5 ml.) during 12 h.

Table 2. INFLUENCE OF CALCIUM AND PHOSPHATE ADDITIVES TO MILK IN ARTIFICIALLY FED RATS ON CALCIUM-45 AND STRONTIUM-85 ABSORPTION FROM THE GASTROINTESTINAL TRACT IN 5-DAY-OLD ANIMALS

Milk (mg/100 ml.)		No. of rats	Retention in carcass % oral dose		
P	Ca		Strontium-85	Calcium-45	Strontium-85/ Calcium-45
95	140	30	86.55 $\pm$ 1.16	85.87 $\pm$ 1.18	1.00 $\pm$ 0.006
230	400	30	76.05 $\pm$ 2.29	74.49 $\pm$ 2.13	1.02 $\pm$ 0.010
500	1,000	37	65.85 $\pm$ 2.36	67.00 $\pm$ 2.61	0.98 $\pm$ 0.009

Strontium-45, calcium-45,  $\text{CaCl}_2$  and  $\text{KH}_2\text{PO}_4$  were added to cows' milk, which was fed to rats by means of a dropper during 12 h; each rat received an average of twenty-five drops (0.5 ml.).

to increase both the calcium and phosphate intake (Table 2). Tracer amounts of carrier free calcium-45 and strontium-85 were added to the milk.

The average concentration of calcium and phosphate in cows' milk used in these experiments was 140 and 95 mg/100 ml., respectively, as analysed by standard methods<sup>6,7</sup>. By adjusting the concentration of calcium and phosphate in the milk to 400 mg and 230 mg/100 ml., respectively, the normal concentration of calcium and phosphorus in rats' milk was found<sup>8</sup>. The milk with the highest content of calcium and phosphate (1,000 mg of calcium and 500 mg of phosphate/100 ml.) corresponds to the calcium and phosphate concentration in the diet of a normal adult rat. The baby rats were fed by means of a dropper for a period of 12 h, each rat receiving 25 "standard" drops equivalent to 0.5 ml. They were returned to their nursing mothers overnight and killed 12 h later. The content of strontium-85 and calcium-45 in the carcass was determined after removal of the gastrointestinal tract. The results are presented as percentage of the oral dose retained in the carcass.

Increase in the phosphate content of the milk had no effect on the retention of either strontium or calcium in 5-day-old rats as shown in Table 1. A simultaneous increase of both calcium and phosphate in the diet caused a small but statistically significant reduction in the absorption of radioactive strontium and calcium from the gastrointestinal tract (Table 2). The absorption of calcium-45 was only some 20 per cent less in rats fed on milk with a seven times greater calcium content (1,000 mg/100 ml.) and so the total absorption of calcium was some

five to six times greater in this group of animals. The ratio of strontium-85:calcium-45 in the carcass, if corrected for the calcium content of the diet, would be seven times lower in the same group of animals.

These results confirm the earlier findings of a relatively high absorption of strontium and calcium from the gastrointestinal tract in young babies<sup>9</sup> and very young animals of other species<sup>10</sup>.

Despite this high absorption efficiency and almost complete lack of discrimination against strontium in animals of this age, an increased content of calcium and phosphate in the milk seems to influence strontium and calcium absorption in the gut.

These experiments, however, indicate that the much greater reduction of the retention of radioactive strontium in baby rats observed in our previous experiment<sup>9</sup> was caused by the decreased ratio of strontium to calcium in mothers' milk caused by the increased content of phosphate in the diet of the mother.

The possibility of influencing the absorption of strontium from the gastrointestinal tract of the newborn rat is still being investigated.

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### Correlation between Kinetically Defined Calcium Compartments and Contractile Response in Rabbit Atrium

THE importance of calcium ions in the contraction mechanism of skeletal and cardiac muscle has been established on the basis of previous work<sup>1-15</sup>. Calcium seems to be stored in the sarcoplasmic reticulum and plays a critical part in coupling excitation with contraction when released into the sarcoplasm of these tissues after depolarization of the membrane. The exact way in which calcium performs this function is not clear, and, furthermore, calcium in cardiac tissue is divided into at least two components by kinetic studies with calcium-45 (refs. 16-19). This communication presents evidence for the applicability of kinetic analysis to the contraction process in cardiac tissue.

Left atria removed from freshly dissected rabbit hearts were inflated with a gas mixture containing 95 per cent oxygen and 5 per cent carbon dioxide. They were depleted of calcium by soaking in a Krebs-Henseleit solution containing 0.2 mmolar calcium for 2 h and then treated in one of two ways; either soaked for 3 min (group 1a) or 2 h (group 2a) in a Krebs-Henseleit solution containing 5 mmolar calcium chloride. When the atria are returned to a low calcium solution (0.2 mmolar) and stimulated

electrically at a frequency of once a min, an initial rapid decline in tension is noted in both groups. But only the atria of group 2a show a slow linear decline in tension, with a half-time approximating 60 min (Fig. 1). Apparently, initial decline in tension in this experiment results from release of calcium from superficial binding sites, whereas the continuous linear response is caused by a release of a calcium moiety more firmly bound and stored within cellular compartments.

To corroborate kinetic analysis with results of the twitch-tension study, a time course of calcium loss from the tissue after soaking for short and long periods in isotopic calcium was determined. The atria so employed were also depleted of calcium and then soaked for either 3 min (group 1b) or 2 h (group 2b) in a Krebs-Henseleit solution containing calcium-45. Washout in successive changes of non-isotopic Krebs-Henseleit solution followed.

The efflux of calcium-45 occurs rapidly in group 1b while the half-time of desaturation of group 2b is about 90 min (Fig. 2). Thus the time course of loss of tension in the twitch-tension studies can be explained on the basis of a two compartment (superficial and intracellular) concept of calcium storage. Furthermore, Fig. 2 shows that, when the linear portion of curve 2b is extrapolated to zero time and subtracted from the original curve, a second curve which corresponds to the slope of curve 1b is obtained. The calcium-45 washout curve for group 2b is therefore approximated as the sum of two separate functions: (a) a fast component similar to group 1b representing superficially bound calcium, and (b) a slower one with first order kinetics, representing calcium stored within cell compartments.

There is a correlation between kinetically defined calcium compartments and twitch-tension response in cardiac muscle. Initial rapid loss of contraction in these experiments is caused probably by release of calcium from superficial binding sites, whereas slow linear decrease in tension is an effect of slow release of calcium from the intracellular compartments.

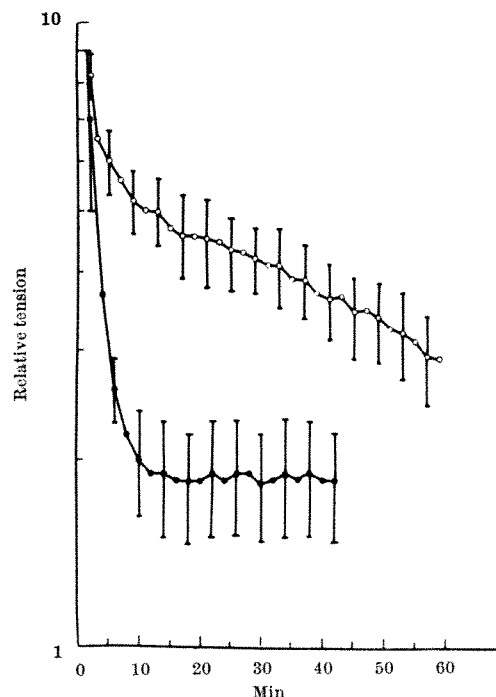


Fig. 1. Comparison of the decline of twitch-tension of rabbit atria previously depleted of calcium and then soaked for either 3 min (●, group 1a) or 2 h (○, group 2a) in a Krebs solution containing 5 mmolar calcium chloride. Electric stimulation was applied at a frequency of once each min in a Krebs solution containing low calcium (0.2 mmolar). Each point is the mean of four experiments. Vertical bars represent standard error of the mean.

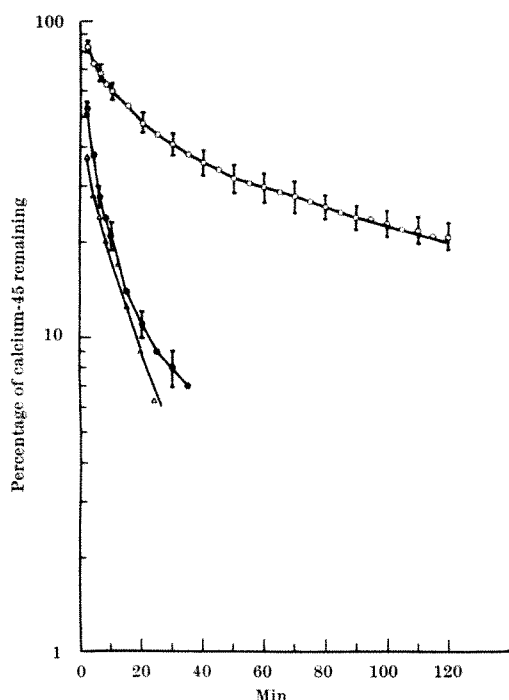


Fig. 2. Desaturation curve of calcium-45 for rabbit atria washed out in non-isotopic Krebs solution after soaking in Krebs solution containing tracer amounts of calcium-45 for 3 min (●, group 1b) and for 2 h (○, group 2b). Each point is the mean of five experiments. Vertical bars represent standard error of the mean. △, Curve obtained after linear portion of group 2b curve is extrapolated to zero time and then subtracted from original curve.

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## PATHOLOGY

### Effect of Chlordiazepoxide on Stomach Ulcers in Rabbit Induced by Stress

In an earlier communication<sup>1</sup> we reported that premedication with chlordiazepoxide (CDP), a 1,4-benzodiazepine derivative, prevented the eosinopenia caused by emotional stress in rabbits, acting in a manner antagonistic to adrenocorticotrophic hormone (ACTH). Furthermore,

CDP behaves antagonistically towards another hormone involved in stress, namely, antidiuretic hormone<sup>2</sup>. The effect of premedication with CDP on stomach ulcers induced by stress has now been investigated.

Male rabbits weighing 1.5–2.0 kg were used. Emotional stress was applied daily for 21 days as previously described<sup>1</sup> using the method of Colfer *et al.*<sup>3</sup>. Three rabbits received electroshock only and acted as controls. Three rabbits received daily intraperitoneal injections of CDP (50 mg/kg) 30 min before electroshock. Daily blood counts showed that eosinopenia occurred in the control group only. After 21 days the animals were killed and the stomachs examined with a hand lens. Stomachs from the control group all showed extensive congestion, numerous haemorrhages, shedding of the epithelium over large areas and distinct ulceration. Among the experimental group there was slight shedding of epithelium in one rabbit and some congestion in all three, but no haemorrhage and no ulceration.

It thus appears that premedication with CDP affords protection against the effects on the stomach of emotional stress. It is unlikely that gastric acidity played any part in these experiments, because the pH of the stomach contents was the same in both groups. In a separate investigation<sup>4</sup>, it has been shown that premedication with CDP prevents the stomach damage in rats usually caused by the stress of restraint. In other experiments, we have found that stomach ulcers produced by chronic administration of cortisone to rats were not prevented by similar premedication with CDP.

It may be assumed that stomach lesions following emotional stress are caused by adrenocortical hormone released in response to increased concentrations of ACTH in the blood. It seems reasonable to infer from the present investigations that the antagonism between CDP and ACTH prevents the release of adrenocortical hormones in amounts sufficient to damage the stomach.

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### Pyrogen in the Urine of Febrile Patients with Hodgkin's Disease

THE mechanism whereby bacterial infections induce fever has been elucidated in some detail<sup>1,2</sup>. Bacteria and bacterial pyrogens (exogenous pyrogen) interact with polymorphonuclear granulocytes of the host, resulting in release into the circulation of a soluble product of the granulocyte (endogenous pyrogen) which is the proximate cause of the fever through its action on the temperature control centre of the host. Exogenous pyrogen and endogenous pyrogen may be distinguished in several ways, but the most striking difference between their actions is that tolerance to the first develops rapidly, whereas endogenous pyrogen continues to cause fever after a long sequence of daily injections.

Less is known about the mechanisms responsible for fever not associated with bacterial infection. Such fever is frequently seen in patients with Hodgkin's disease. This study was undertaken to determine whether an analogous mechanism (that is a circulating pyrogen of the "endo-



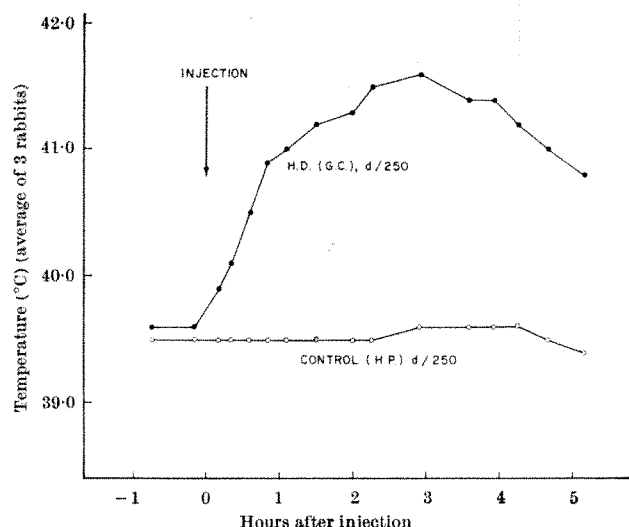


Fig. 1. Rectal temperature of rabbits injected with urine concentrates representing 0.4 per cent of 24 h collections from a febrile patient with Hodgkin's disease and a healthy control.

genous" type) could be demonstrated during febrile episodes in uncontrolled Hodgkin's disease. Initial studies were similar to those described by Snell<sup>3</sup>. Plasma was obtained from febrile patients, stored, and re-infused into the same individuals after remission of Hodgkin's disease had been induced. Although the results of a few experiments of this type were more encouraging than those described by Snell, the same conclusion was reached, namely, that this was not a feasible technique for the study of circulating pyrogens in man. We then investigated the possibility that the presumed pyrogen in Hodgkin's disease might be excreted in urine and might induce fever in a foreign species.

Urine was collected in glassware free of pyrogen in the presence of antibiotics, dialysed and concentrated by ultrafiltration to 10–15 per cent of its original volume. Urine processing was monitored bacteriologically and sterility of the final product was demonstrated before use. Urine concentrates were injected intravenously in volumes of 2–3 ml. (representing 0.03–1.0 per cent of 24 h urine collections) into trained rabbits, known to maintain stable rectal temperatures during 8 h of restraint in the pyrogen laboratory. Temperatures were determined by an electronic thermometer, coupled to sensors in rectal probes. Usually, groups of three animals were used for assay of individual doses of urine concentrate. Controls were injected with concentrates from urine of healthy individuals, collected and processed under similar conditions. These doses of urine concentrate were well tolerated by the rabbits.

Fig. 1 presents data from a representative experiment. Doses of concentrate representing 0.4 per cent of the respective 24 h urine collections from a febrile patient with Hodgkin's disease and a healthy physician were administered to groups of three rabbits. No rise in temperature followed injection of the control material. A prompt monophasic fever with a peak temperature elevation of 2.1° C, however, was induced by the Hodgkin's urine concentrate. This fever persisted throughout the experiment.

Urine concentrates from six of seven patients with uncontrolled Hodgkin's disease and temperatures exceeding 102° F daily regularly induced fever in rabbits. Unequivocal fever followed injection of concentrate representing as little as 0.06 per cent of some 24 h urine collections. Material from two different collections from the seventh patient, in amounts representing up to 1 per cent of the 24 h urine volumes, did not induce fever. Concentrates of urine from four different healthy individuals, injected in amounts representing 0.4–1 per cent of the 24 h

urine volumes, failed to induce fever. Both monophasic and biphasic fevers were induced by pyrogenic urine fractions. The degree of temperature elevation and the shape of the fever curve appeared to reflect, at least in part, individual differences among rabbits as well as characteristics of the material injected; thus the same urine concentrate injected in the same dose might induce monophasic fever in some rabbits and biphasic fever in others.

These urinary pyrogens were compared with two types of exogenous pyrogen in the following experiment. Four groups of three rabbits received daily injections for one month as follows: group 1, typhoid-paratyphoid vaccine, USP, 0.01 ml. daily; group 2, partially purified lipopolysaccharide from *Escherichia coli* 026-B6, 0.3 µg daily; group 3, pyrogenic urine concentrate from a febrile patient with Hodgkin's disease in a daily dose representing 0.063 per cent of a 24 h urine collection; group 4, pyrogenic urine concentrate from another febrile patient in a daily dose representing 0.25 per cent of a 24 h urine collection. These doses were chosen after preliminary testing in other rabbits had shown that they produced comparable febrile responses. Fig. 2 presents temperature curves obtained on different days of the experiment, in groups 2 and 3. The upper panel shows the febrile responses to *E. coli* endotoxin on the first, third and eighth days. A biphasic fever which lasted almost 5 h was observed on the first day. On the third day, the fever was monophasic and lasted a little less than 3 h. By the eighth day, all 3 rabbits were tolerant to this dose of endotoxin; this tolerance was maintained during the remainder of the experiment. The lower panel presents temperature curves for group 3. On the first day, a biphasic fever, almost identical to that obtained in group 2, was observed. On the third day, the fever was monophasic and lasted about 3 h. On the eighth day, the febrile peak was slightly lower and somewhat delayed. The response on the twenty-eighth day of consecutive injections was indistinguishable from that on the eighth day. Fever curves for group 1 were very similar to those shown for the *E. coli* endotoxin; all rabbits became tolerant to the

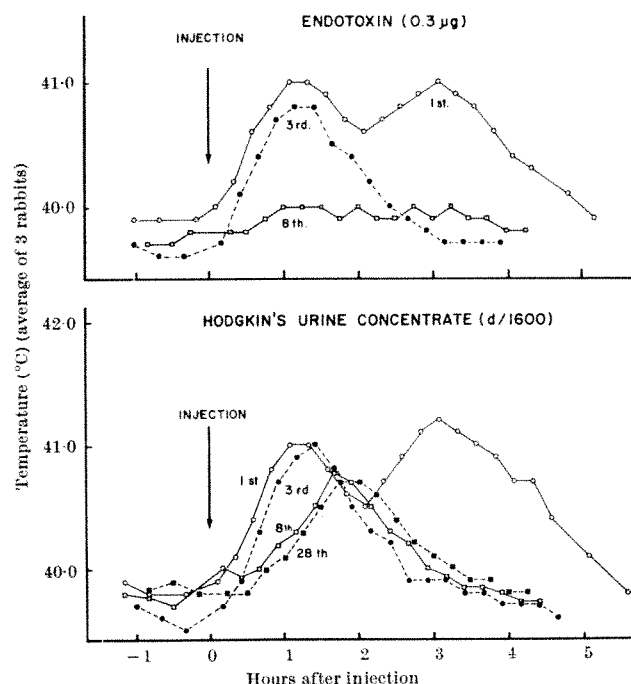


Fig. 2. Rectal temperature on various days of rabbits receiving daily injections of *E. coli* endotoxin (above) and urine concentrate from a febrile patient with Hodgkin's disease (below).

pyrogenic effects of typhoid-paratyphoid vaccine within 8 days. The curves for group 4 resembled those obtained with the more potent pyrogenic concentrate used for group 3; although a decrease in the duration of the febrile response occurred during the first week of daily injections this material continued to induce fever throughout the month long experiment.

Pyrogenic urine concentrates were then administered to rabbits which had been proved to be completely tolerant to the pyrogenic effects of *E. coli* endotoxin, typhoid-paratyphoid vaccine, or both. Febrile responses were observed in five of six experiments. The maximal temperature elevations were very similar to those obtained in non-tolerant rabbits given the same doses, but the duration of clear cut fever was distinctly shorter. Fig. 3 presents data from a typical experiment. Urine concentrate from patient S.S., in a dose representing 0.13 per cent of a 24 h collection, induced peak temperature elevations of 1.2° C both in non-tolerant rabbits and in animals tolerant to both *E. coli* endotoxin and typhoid-paratyphoid vaccine. A biphasic fever lasting 5 h was observed in the former, however, whereas the latter manifested a shorter, monophasic response. These results are similar to those obtained by Atkins and Huang<sup>4</sup> with administration of endogenous (serum) pyrogen to rabbits tolerant to an exogenous pyrogen.

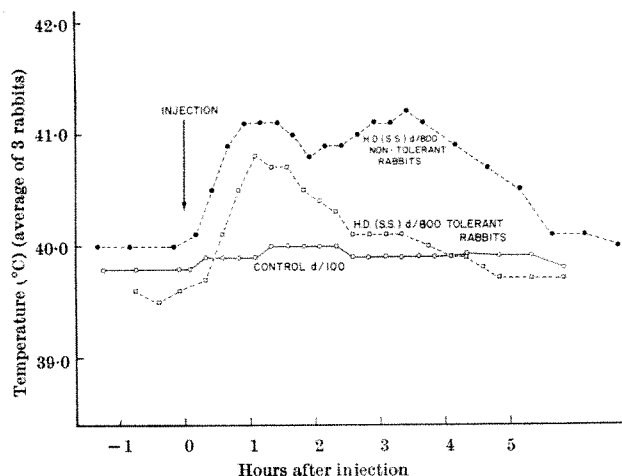


Fig. 3. Effect of the same dose (0.13 per cent of a 24 h collection) of a pyrogenic urine concentrate from a patient with Hodgkin's disease on temperature of normal and bacterial pyrogen tolerant rabbits. Controls received a urine concentrate representing 1 per cent of a 24 h collection from a healthy individual.

Our inability to induce tolerance to the pyrogenic effect of urine from patients with Hodgkin's disease, and the production of fever by these urine concentrates in rabbits completely tolerant to two bacterial pyrogens, indicate that the material excreted by these patients resembles "endogenous" rather than "exogenous" pyrogen. These results would appear to exclude bacterial pyrogen, whether derived from unrecognized infection in the patients or from contamination during urine processing, as the cause of the febrile responses observed in test animals. We conclude therefore that many febrile patients with uncontrolled Hodgkin's disease excrete in their urine a material which has some of the characteristics of endogenous pyrogen. Such material was not found in urine collected from healthy individuals and tested in equal or greater doses. Work is currently in progress to identify some of the biochemical characteristics of this pyrogen and to determine whether it is specific for Hodgkin's disease or whether it may reflect the presence in the circulation of a final common mediator for the production of various types of fever in man.

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## MICROBIOLOGY

### Isolation of Half Molecules of DNA from Gram-negative Bacteria

THE isolation of intact DNA of the same size and form as that present in the organism is possible in the case of viruses<sup>1</sup> but presents problems in bacteria and higher organisms, principally as a result of the action of degrading enzymes and mechanical shear. Isolated bacterial DNA usually has a relatively low molecular weight ( $S^{20,w}$  between 11–33)<sup>2,3</sup> except in the cases of *E. coli*<sup>4</sup>, *B. subtilis*<sup>4</sup> ( $S^{20,w}$  of 81) and *H. influenzae*<sup>5</sup> ( $S^{20,w}$  of 94).

We report the results of attempts to minimize the degrading factors in preparations of DNA from Gram-negative bacteria. The organisms used were strains of *Salmonella typhimurium*, *Proteus mirabilis*, *Alcaligenes faecalis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Shigella flexneri*. Organisms were grown overnight in 4 l. Difco nutrient broth at 37° C and cells were collected by centrifugation in the cold. The yield of about 10 g packed cells was suspended at room temperature in 50 ml. of 0.1 molar *tris*-0.015 molar NaCl buffer, pH 9.0. To this sodium dodecyl-sulphate was added at a final concentration of 1 per cent. With gentle agitation the mixture assumed a jelly like viscous consistency as a result of massive cell lysis. Fifty ml. of a 90 per cent freshly distilled phenol solution<sup>4</sup>, at 55° C, was added to deproteinize the mixture which was shaken at 5 r.p.m. for 20 min and then centrifuged at 3,000*g* for 60 min. The mixture separated into three layers. Between the upper phase and the lower brown phenol fraction a white gel like material consisting chiefly of denatured protein was present. The upper, very viscous, layer was carefully removed by means of a special pipette with an orifice of 5 mm to minimize shearing. The phenol and other interfering substances of low molecular weight were removed by dialysis against a 0.15 molar NaCl-0.015 molar citrate buffer, pH 7.0. Four changes of the buffer at intervals of 4 h were sufficient. The crude DNA was incubated for 1 h at 37° C with 50  $\mu$ g RNase/ml. solution. The mixture was cautiously agitated for 20 min at room temperature after the addition of 0.79 g activated charcoal<sup>6</sup>/ml. of solution. The charcoal was removed by spinning at 10,000*g* for 10 min. The absence of RNA and protein was established by the Smellie and Krotkov method<sup>7</sup> and the Folin-Ciocalteu reaction<sup>8</sup>. The concentration of DNA was determined according to the modified Burton method<sup>9</sup>. Sedimentation velocity centrifugation in the Beckman model *E* analytical centrifuge, using ultra-violet optics, indicated a homogeneous preparation. Sedimentation coefficients were determined in the same apparatus. To minimize shear forces, DNA solutions were placed in the centrifuge cell with a wide bore pipette while one of the quartz windows was removed. All runs were conducted at 9,945 r.p.m. to avoid the anomalous effects observed with large DNA molecules<sup>10</sup>. Films were traced on a Beckman analytical Type R6 microdensitometer. The results are shown in Fig. 1.

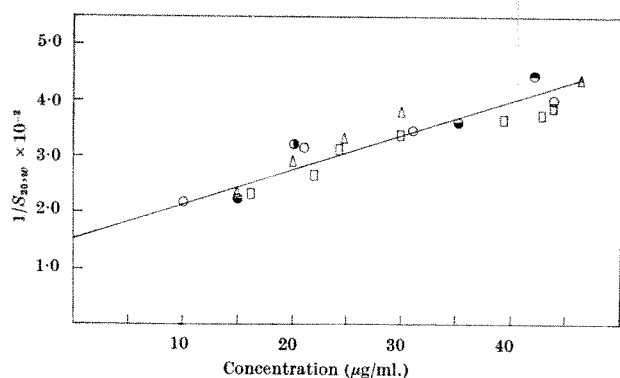


Fig. 1. Reciprocal of the sedimentation coefficient as a function of concentration. *S. typhimurium*, *P. mirabilis*, *A. faecalis*, *P. aeruginosa*, *E. coli* and *S. flexneri* are presented by  $\circ$ ,  $\square$ ,  $\triangle$ ,  $\bullet$ ,  $\blacksquare$  and  $\bullet$  respectively.

As can be seen, all results fall almost on the same line which extrapolates to give  $S_{20,w}^0$  equal to 62.5. For the sake of interest, this value is in good agreement with the  $S_{20,w}^0$  of whole DNA molecules from bacteriophages  $T_2$  and  $T_4$  (refs. 2,11,12), which does not necessarily imply that phage DNA occurs in units half the size of bacterial DNA. When the value of 62.5 is compared with that of Massie and Zimm<sup>4</sup> and Berns and Thomas<sup>5</sup>, it is obvious that degradation due to shearing has taken place. The most probable step at which shear degradation could have occurred is the removal of RNA by charcoal.

If the value of 62.5 is assumed to be the  $S_{20,w}^0$  for half molecules, a value of about 83 should be obtained for whole molecules. The value of 83 is in good agreement with the result of Massie and Zimm<sup>4</sup> but not with the value according to Berns and Thomas<sup>5</sup>. When the DNAs for *P. mirabilis* and *E. coli* were subjected to increasing shear forces, the molecules were shear degraded to molecules with  $S_{20,w}^0$  of 45, 31 and 22, respectively. These values correspond then to quarter, eighth and sixteenth molecules.

Taking all these results into consideration it seems that half molecules were isolated with a  $S_{20,w}^0$  of 62.5 and a molecular weight of about 120 million as calculated according to the equation of Crothers and Zimm<sup>11</sup>. Furthermore, it appears that the so-called whole or intact DNA molecules of bacteria have a  $S_{20,w}^0$  of approximately 81 and a molecular weight in the region of 250 million. It is thus hard to avoid concluding that in bacteria the DNA occurs in the organism in an assembly of sub-units of about  $250 \times 10^6$  molecular weight probably held together by protein.

We thank Professor J. N. Coetzee for supplying the strains *S. typhimurium*, *P. mirabilis*, *P. aeruginosa*, *E. coli* and *S. flexneri*.

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## Association of AAV-1 with Simian Adenoviruses

THE discovery of small virus particles resembling AAV<sup>1</sup> in preparations of the simian adenoviruses SV<sub>11</sub>, SV<sub>15</sub> and SV<sub>34</sub> (refs. 1-4) suggested that these agents might be widely distributed among the simian adenovirus group. We therefore examined electron microscopic preparations<sup>1</sup> of several recognized simian adenoviruses for the presence of these agents and tested hyperimmune simian adenovirus antisera for the presence of complement-fixing (CF) and precipitating antibodies to AAV-1 (ref. 5), a serotype first isolated from a simian adenovirus stock<sup>1</sup>. In addition, it was of interest to determine whether normal monkey sera contained antibodies to AAV-1 and whether the presence of these antibodies would correlate with the presence of adenovirus antibodies.

A compilation of our results on the correlation between the demonstration of AAV-like particles on one hand and the demonstration of antibodies to AAV-1 in hyperimmune rabbit sera on the other is presented in Table 1. Small particles resembling AAV were found in seven out of sixteen adenovirus preparations examined by electron microscopy. All these adenoviruses had been purified by terminal dilution in rhesus kidney cells in the presence of SV<sub>5</sub> and SV<sub>40</sub> antisera. Thus it does not seem that this method of purification eliminated the small viral particles from these adenovirus stocks. This is interesting in view of the communication of Hoggan *et al.*<sup>6</sup>, who reported that AAV could be eliminated from human adenovirus stocks by limiting dilutions as well as by plating.

Table 1. CORRELATION OF AAV-1 ANTIBODIES IN HYPERIMMUNE RABBIT SERA WITH THE DEMONSTRATION OF AAV-LIKE PARTICLES BY ELECTRON MICROSCOPY

Simian adenovirus	Direct examination by electron microscopy	Demonstration of AAV-1 antibodies in simian adenovirus hyperimmune rabbit sera		
		CF titre		Ouchterlony (immune sera, 1:2)
		Pre-immune	Immune	
SV1	—	—*	AC†	+
SV11	—	—	AC	—
SV15 (3464)	+	—	256	+
SV17 (Hull)	—	—	1,024	+
SV17 (NIH1-2)	+	AC	AC	—
SV20	+	—	8	—
SV23	+	4	32	—
SV25	—	NT‡	NT	NT
SV27	—	—	32	—
SV30	—	—	64	—
SV31	+	—	AC	—
SV32	+	AC	AC	—
SV33	—	—	512	—
SV34	—	AC	AC	—
SV36	+	8	256	+
SV37	—	AC	AC	—
Total positive/ total examined	7/16	8/15	4/15	

\* Less than 2.

† Anti-complementary.

‡ Not tested.

Antibodies to AAV-1 were detected in eight out of fifteen hyperimmune sera examined by the CF test and in four out of fifteen sera examined by the Ouchterlony method (Table 1). Of the seven simian adenoviruses found by electron microscopy to contain AAV-like particles, CF antibodies were demonstrated in four out of seven hyperimmune rabbit sera prepared against these agents (three out of seven were anti-complementary) while only two out of seven of these sera were positive by the Ouchterlony test when AAV-1 was used as antigen. It is interesting that four additional simian adenovirus sera were found to contain CF antibodies to AAV-1, although no AAV-like particles were seen in the simian adenovirus preparations examined by electron microscopy. One of these four sera was also positive by the Ouchterlony test. Finally, one serum, too anti-complementary for significant CF testing, was found to contain precipitating

antibodies for AAV-1. Because the successful demonstration of the small viral particles by electron microscopy would require the presence of approximately  $10^6$  particles/ml. in the original material, it was not surprising to find that although several of the sera reacted by complement fixation and/or Ouchterlony tests with AAV-1, no AAV-like particles were discerned by electron microscopy. Apparently, in these instances there was, none the less, sufficient AAV-1 to act as antigen in stimulating the production of specific antibodies in the inoculated animals.

Several AAV serotypes have been found in human adenovirus stocks<sup>6-8</sup>. Passage of the SV<sub>15</sub> stock reported here (3464) three times with AAV-1 antiserum in African green of LLC-MK<sub>2</sub> cells has failed to eliminate all of the small viral particles, in spite of the demonstration of AAV-1 antibodies in SV<sub>15</sub> (3464) hyperimmune sera. This suggests that this stock was contaminated by at least two AAV serotypes, one of which was type 1. Contamination of human adenovirus stocks by two or more AAV serotypes has been observed<sup>6</sup>, and a type 4 serotype has been isolated from a strain of SV<sub>15</sub> (ref. 4). Attempts are now being made to isolate and identify the second AAV contaminant in SV<sub>15</sub> (3464).

Table 2. DEMONSTRATION OF COMPLEMENT FIXING ANTIBODY TO AAV-1 AND ADENOVIRUS GROUP-SPECIFIC CF ANTIGEN IN NORMAL MONKEY SERA

Serum	No. positive*/No. examined AAV-1	Adenovirus group-specific
Rhesus	7/14	9/14
Grivet	0/12	0/12
Vervet	0/5	2/5
Patas	0/3	0/3

\* Titre equal to or greater than 1:4.

The results of complement fixation tests on a small number of monkey sera collected from our animal quarters are presented in Table 2. Of fourteen rhesus sera examined, nine contained adenovirus group-specific CF antibodies and, of these, seven also had CF antibodies to AAV-1. Complement fixing titres for AAV-1 and adenovirus varied in these animals from 1:4 to 1:32. Although adenovirus CF antibodies were detected in certain grivet and vervet sera (titres varying from 1:4 to 1:128), no AAV-1 antibodies were demonstrated. The three patas sera examined did not contain CF antibodies for either of the two antigens.

While the origin of AAV contaminants in simian and human adenovirus preparations is uncertain, these findings suggest that AAV-1 (and perhaps other serotypes as well) may be a defective, covert agent of rhesus kidney cells the presence of which cannot be detected without the aid of an adenovirus "helper". Thus tests of uninoculated control cultures for the presence of adventitious agents may have to include examination of these cultures after inoculation of a test adenovirus "helper", previously shown to be free of AAV. It is interesting that field isolates of human adenovirus are apparently free of AAV, but many AAV-1 contaminated stocks have been passaged in monkey kidney cells<sup>9</sup>. Failure to find AAV in adenovirus preparations examined by the electron microscope is, obviously, no guarantee of the purity of the stock. Other criteria should also be satisfied such as failure to elicit antibodies to any known AAV serotype on hyperimmunization of a competent animal (for example, rabbit, guinea-pig), or failure to demonstrate any immunofluorescence with known AAV antisera in tissue culture cells infected with the stock adenovirus. Finally, the demonstration of AAV-1 antibodies in rhesus sera and the finding of AAV-1 virus in human adenovirus stocks suggested that it might be fruitful to conduct a survey of human as well as rhesus sera for AAV-1 antibodies. Such a survey has been completed and in a preliminary account it was reported that AAV-1 antibodies were found in nineteen out of ninety-two normal human sera<sup>5</sup>.

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### Immunogenicity of an Attenuated Strain of Vaccinia Virus on Rabbits and Monkeys

WE have made a study of a virus preparation which shows some promise as a potentially safer live smallpox vaccine. The virus, called the DI<sub>1</sub> strain, is a highly attenuated mutant of dermovaccinia virus which has been isolated<sup>1</sup> by successive 1 day egg passages of the DIE virus—a strain at present authorized for smallpox vaccination in Japan. The DI<sub>1</sub> strain is characterized by the production of tiny pocks on chicken chorioallantoic membrane and is distinguished from DIE by its reaction to fluorocarbon (unpublished results). DI<sub>1</sub> is not pathogenic for mice, guinea-pigs or rabbits, and produces more interferon when irradiated with ultra-violet light and inoculated onto chick embryo fibroblast cultures<sup>2</sup>. We have studied the pathogenicity of DI<sub>1</sub> in monkeys and the immunogenicity of the strain in rabbits and monkeys.

The immunogenicity of the DI<sub>1</sub> strain in rabbits seems to be poor. In our study, pairs of albino rabbits (2.0–2.5 kg) free from pox virus antibody were inoculated either with DI<sub>1</sub> or with DIE subcutaneously and intradermally, with and without Freund's adjuvant. In all cases the inoculum was obtained from infected chorioallantoic membrane, equal parts inoculated at four consecutive weekly intervals and antibody formation was determined<sup>3,4</sup> 6 weeks after the last injections. Antibody formation was insignificant except with subcutaneous inoculation associated with adjuvant. Pathogenic changes were not observed, and the transient local reddening within 30 h of inoculation was presumably a consequence of a non-specific reaction of the treated site.

Inoculation of monkeys with DI<sub>1</sub> preparations gives markedly different results. In our study, two female cynomolgus monkeys (about 3.5 kg) were inoculated with DI<sub>1</sub> virus and one, a control, with DIE (Table 1). Reactions were observed for 7 days and serum antibody was extracted for 6 weeks after inoculation. It is clear from the results in Table 1 that DI<sub>1</sub> strain is pathogenic to cynomolgus monkeys by dermal inoculation. The reaction was characteristic of dermovaccinia virus but less severe than that due to the original strain DIE, whether the inoculation was carried out by multiple pressure, intradermal injections or scarification. Dermal pulp taken from the scarified lesion of monkey No. 2 7 days after inoculation contained  $3.4 \times 10^6$  pock-forming



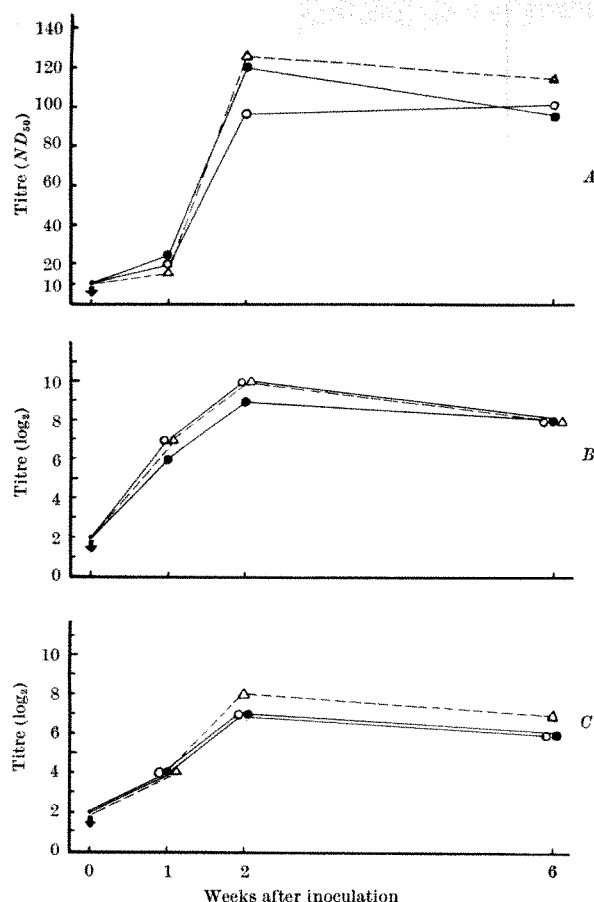


Fig. 1. Antibody response of cynomolgus monkeys to the primary inoculation of vaccinia virus, strain DIs or DIE. ○—○, Monkey 1 (DIs); ●—●, monkey 2 (DIs); △—△, monkey 3 (DIE). A, Neutralizing antibody; B, complement-fixing antibody; C, haemagglutination-inhibiting antibody.

units/g, which suggests that the virus had replicated there. The antibody response of the monkeys inoculated with DIs was similar to that inoculated with DIE (Fig. 1) and roughly proportional to the dose of virus administered. Neutralizing antibody was more or less constant after the second week, but complement-fixing and haemagglutination-inhibiting antibodies decreased after that time.

These results demonstrate that even though the DIs strain is remarkably non-pathogenic in certain experimental animals and cell cultures, the strain is pathogenic in cynomolgus monkeys and gives an immunity which is comparable with that provided by the original strain DIE. Our data suggest but do not substantiate the possibility

that DIs is less pathogenic in monkeys than DIE. The possibility that DIs may be a strain of variola virus such as alastrim, suggested by the characteristic host range which is at present confined to chick embryo and monkey, can be excluded because DIs does not form hyperplastic foci in the HeLa monolayers<sup>1,5</sup>.

Because of the serious complications of smallpox vaccination with live vaccinia virus, which is pathogenic for several species of animals, several workers are seeking a different procedure using either inactivated or attenuated virus. The DIs strain, which has not yet been tested in humans, is a promising candidate for a safer live smallpox vaccine, for our experiments with laboratory animals show that the virus is highly attenuated but that it is potentially immunogenic in at least one species of primates.

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## BIOCHEMISTRY

### Inhibition of RNA Polymerase by Histones

*In vitro* nucleohistones are known to be less efficient templates for RNA polymerase than free DNA<sup>1-3</sup>, but there has been some disagreement as to whether this is due to a real inhibition of RNA synthesis by histones or to their aggregation into precipitates which the reagents cannot penetrate<sup>4</sup>. Sonnenberg and Zubay<sup>5</sup> supported the second suggestion by showing that ultrasonication of nucleohistone which was initially inactive as a template for RNA synthesis caused an increase in activity to about one-third that of free DNA, and Roy and Zubay<sup>6</sup> have recently made similar observations using a mammalian polymerase. Bonner and Huang<sup>7</sup>, however, have repeated their claim that it is possible to prepare soluble nucleohistones which show the inhibition by histones.

In an attempt to resolve this, we have compared the decrease in template activity caused by histones and the precipitation of DNA under similar conditions.

*Escherichia coli* RNA polymerase (fraction 4) was prepared by the method of Chamberlin and Berg<sup>8</sup> and DNA and whole histone were prepared from calf thymus<sup>9,10</sup>. The template activity was measured at two concentrations of DNA. At the higher concentration, which was close to the quantity required to saturate the enzyme, the ratio of histone to DNA was varied between 0.04 and 0.4, and at the lower concentration between 0.2 and 2.0. The reaction mixtures, which contained 10 µg or 50 µg DNA, histone as indicated, 1 mg bovine plasma albumin, 200 µmoles each of ATP, CTP, GTP and UTP, 0.2 µc. tritiated UTP (Schwarz) and 40 µg RNA polymerase in 0.5 ml. 0.01 molar magnesium chloride, 0.01 molar *tris*-HCl (pH 7.9), 0.002 molar β-mercaptoethanol and 0.0001 molar EDTA, were incubated for 10 min at 37° C and assayed essentially as described by Barr and Butler<sup>11</sup>: incorporation in the absence of primer was subtracted. The same solvent was used for the precipitation experi-

Table 1. REACTION OF CYNOMOLGUS MONKEYS TO THE DERMAL INOCULATIONS OF VACCINIA VIRUS, STRAINS DIs AND DIE

Monkey No.	Inoculum	Inoculation route	Days p.i.	Maximum reaction	Notes
1	DIs	M.p.* 5 spots (1.5 × 10 <sup>5</sup> P.F.U./spot)	7	Reddening, 10 × 10 mm, with pustule	Slightly febrile on day 5-6 p.i.
2	DIs	Left: serf. † 1 spot (10 × 10 mm) (1.5 × 10 <sup>5</sup> P.F.U.) Right: i.d. ‡ 2 spots (1.5 × 10 <sup>5</sup> P.F.U./spot) 2 spots (1.5 × 10 <sup>5</sup> P.F.U./spot)	6 7	Purulent, scar formed on seventh day Reddening: 13 × 13 mm, with pustule 8 × 9 mm, with no pustule or vesicle	Slightly febrile on day 5-6 p.i.
3	DIE	M.p.* 5 spots (1.8 × 10 <sup>5</sup> P.F.U./spot)	6	Reddening, 15 × 15 mm, with pustule	Febrile on day 4-6 p.i.

\* Multiple pressure inoculation, 0.05 ml. of the inoculum was extended on a circle of 5 mm diameter and inoculated with 20 pressures by the routine vaccination technique.

† Scarification, a square of 10 × 10 mm is scarified and inoculated with 0.05 ml. of the inoculum.

‡ Intradermal inoculation.

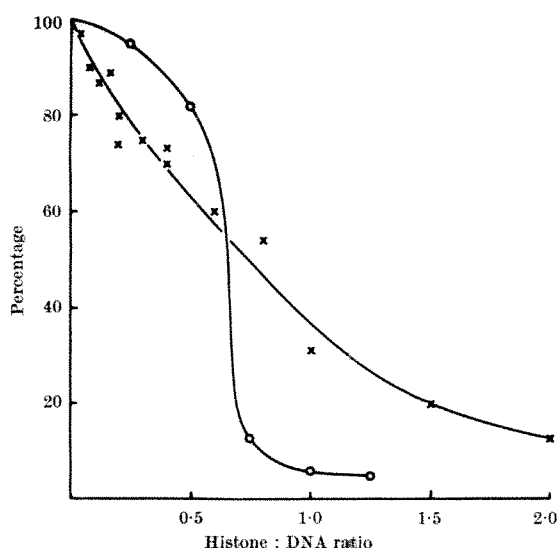


Fig. 1. Solubility and template activity of DNA in the presence of histone. Results are expressed as percentages of controls to which no histone was added. ○—○, Solubility; ×—×, template activity.

ments except for the omission of  $\beta$ -mercaptoethanol and the four nucleotide triphosphates, all of which would interfere with the estimation of DNA by its optical density at 260 m $\mu$ . The precipitation of DNA (40  $\mu$ g/ml.) was measured after centrifugation at 2,000g for 30 min.

The reduction in template activity is by no means parallel with the amount of DNA precipitated, as Fig. 1 clearly shows. We conclude that histone causes a real decrease in the template activity.

Table 1. EFFECT OF ULTRASONICATION ON THE TEMPLATE ACTIVITY AND SOLUBILITY OF CALF THYMUS NUCLEOHISTONE

Primer	Sonication time (min)	Incorporation of UMP ( $\mu$ moles)	Solubility of nucleohistone (percentage of whole)
DNA	—	272	—
None	—	0	—
Nucleohistone	0	12.5	0.04
"	0-25	—	0.04
"	0.5	25.2	0.05
"	1	23.8	0.04
"	2	20.2	0.06
"	3	15.6	—
"	4	17.5	0.08

Calf thymus nucleohistone prepared by the method of Zubay and Doty<sup>17</sup> was treated with ultrasonication for the periods shown, using an MSE-Mullard 60 W ultrasonic drill: template activity and solubility were measured as described in the text.

Experiments were also carried out in which native nucleohistone was ultrasonicated for varying times. After a short period of sonication there was an increase in template activity but no corresponding increase in solubility (Table 1). The template activity decreased somewhat with the longer periods of sonication and the solubility increased only slightly. The results suggest that once the larger particles are broken further sonication in the conditions used has little effect. We did not use longer periods or more intense sonication, because it is well known that DNA may be degraded by such treatment<sup>12</sup>. It is possible that the increases of activity observed in these experiments and those of Sonnenberg and Zubay<sup>5</sup> are due to the loss of small amounts of histone, especially near the ends of the DNA fibres.

The solubility of nucleohistones in our conditions is very low. Like Sonnenberg and Zubay<sup>5</sup> we have been unable to prepare native "soluble" nucleohistones from calf thymus by the methods described by Bonner and Huang<sup>7</sup>.

The conclusion to be drawn is that the inhibition of RNA polymerase by histones is not related to the solubility as estimated by the criterion which we have chosen. This result, like those obtained with rather different experi-

mental approaches<sup>13-15</sup>, is in accord with the hypothesis that histones are true gene-repressors<sup>16</sup>.

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### Incorporation of <sup>14</sup>C-Choline and Tritiated Inositol into Rat Adipose Tissue Phospholipid *in vitro*

PHOSPHATIDIC acid is known to be the only labelled phospholipid which is produced in significant amounts in rat white adipose tissue *in vitro*<sup>1</sup> from 1-<sup>14</sup>C-palmitate, 1-<sup>14</sup>C- or <sup>32</sup>P-glycerophosphate. Steinberg *et al.*<sup>2</sup>, on the other hand, reported that a certain amount of the radioactivity (9-25 per cent) of the total phospholipid, after incubation of adipose tissue homogenates with 1-<sup>14</sup>C-palmitate, was not in the phosphatidic acid fraction. We have investigated the problem of phospholipid biosynthesis in rat adipose tissue and have shown that, together with phosphatidic acid, phosphatidylcholine and monophosphoinositide are probably formed<sup>3</sup>.

In this communication, we present evidence to show that phosphatidylcholine and monophosphoinositide are synthesized *in vitro* in the white adipose tissue of rats from labelled 1,2-<sup>14</sup>C-choline and tritiated inositol, respectively, as precursors.

As shown in Table 1, phosphatidylcholine is formed in white adipose tissue from radioactive choline, but at a very low rate. Weak incorporation into the same lipid has also been obtained from labelled phosphorylcholine incubated under similar experimental conditions.

Inositol is also utilized by adipose tissue for phospholipid biosynthesis (see Table 2). Uptake of inositol into monophosphoinositide is even lower than that of choline into phosphatidylcholine and has been related in these experiments to the phosphorus content of the total phospholipid extract because the quantity of monophosphoinositide detected in the adipose tissue was too low to be reliable. Synthesis of diphosphoinositide or triphosphoinositide from labelled inositol has never been observed when these two phosphatides were properly extracted,

neither has phospholipid formation been detected *in vitro* from either ethanolamine, phosphorylethanolamine or serine under various experimental conditions.

Phosphatidylcholine and monophosphoinositide have been identified by comparison of the radioactive material with authentic reference samples on thin-layer chromatograms and by analyses of the water-soluble phosphorylated products obtained after mild hydrolysis<sup>8</sup> of pooled spots of the phospholipid synthesized from several chromatograms. Glycerolphosphorylethanolamine and glycerolphosphorylinositol were the only labelled products which could be detected and which retained all of the original radioactivity of the parent phospholipid.

Thus our results and those of previous workers<sup>1-3</sup> indicate that phosphatidic acid takes up 10-12 per cent of the <sup>32</sup>P-glycerophosphate radioactivity after 30 min of incubation with rat adipose tissue, while phosphatidylcholine and monophosphoinositide take up at the most 0.5-1 per cent of the radioactivity from labelled choline and inositol, respectively. These findings must be interpreted with some caution, however, because for each phospholipid the nature of the precursor and the composition of the incubation system are different. Low activities of the enzyme systems concerned with the synthesis of phosphorylethanolamine, cytidine-diphosphatidylglyceride and cytidine-diphosphate-choline, which are biological intermediates of the corresponding phospholipid moieties, may explain the weak incorporation of the label into phosphatidylcholine and monophosphoinositide.

Experimental evidence has been reported recently<sup>9,10</sup> which shows that rat adipose tissue phospholipid may be formed *in vitro* from <sup>32</sup>P-orthophosphate as a precursor. Detectable synthesis has been observed for phosphatidyl-inositol, phosphatidylcholine, phosphatidic acid, lyso-phosphatidylcholine and possibly phosphatidylglycerol. Because of the different nature of the precursors the results cannot, however, be strictly compared with those reported here.

Table 1. 1,2-<sup>14</sup>C-CHOLINE INCORPORATION INTO PHOSPHATIDYLCHOLINE OF RAT EPIDIDYMAL FAT PADS *in vitro*

Experiment	Time (min)	nc. 1,2- <sup>14</sup> C-choline incorporated/ $\mu$ g of P
1	30	3.7
2	30	2.7
3	90	4.3
4	90	4.5

The homogenates (400 mg of wet weight of tissue), prepared as "defatted homogenates"<sup>11</sup>, were incubated in a final volume of 3 ml. in the following incubation mixture: Krebs-Ringer bicarbonate (equilibrated with 95 per cent O<sub>2</sub> and 5 per cent CO<sub>2</sub>) buffer, pH 7.4; glucose, 10 mmolar; MgCl<sub>2</sub>, 12 mmolar; CTP, 2 mmolar; ATP, 4 mmolar; Na<sub>2</sub>HPO<sub>4</sub>-NaH<sub>2</sub>PO<sub>4</sub>, pH 7.4, 10 mmolar; cysteine, 12 mmolar; Na malate, 2.5 mmolar; 1,2-<sup>14</sup>C-choline chloride, 1 mmolar (6  $\mu$ Ci). Temperature, 37° C. Incubation in air.

Phospholipid was extracted and washed after incubation according to standard procedures<sup>12</sup>. Phosphatidylcholine was revealed by autoradiography of silica gel G thin-layer chromatograms developed in chloroform, methanol, 35 per cent aqueous ammonia (14:6:1 vol.), and identified by comparison with an authentic reference sample. The radioactivity was determined by liquid scintillation of the phosphatidylcholine scraped from the plate<sup>13</sup>, and accordingly corrected for quenching. The phosphorus of the phosphatidylcholine spot was estimated by a micro-scale modification of the method of Strickland *et al.*<sup>14</sup>. The results are expressed as nc. labelled choline/ $\mu$ g of phosphatidylcholine P.

Table 2. PHOSPHATIDYLINOSITOL BIOSYNTHESIS FROM TRITIATED-INOSITOL BY RAT EPIDIDYMAL FAT PADS *in vitro*

Experiment	Time (min)	C.p.m. tritiated-inositol incorporated/ $\mu$ g of P
1	30	505
2	30	461
3	90	601

The homogenates (400 mg of fresh weight of tissue) prepared as described in Table 1, were incubated in a final volume of 3 ml. in the following incubation mixture: *tris* buffer, pH 7.4, 50 mmolar; potassium phosphate, pH 7.4, 10 mmolar; MgCl<sub>2</sub>, 14 mmolar; CTP, 1 mmolar; ATP, 1 mmolar; glucose, 10 mmolar; NAD, 1 mmolar; <sup>3</sup>H-inositol, 0.5 mmolar (3  $\times$  10<sup>6</sup> c.p.m.). Incubation in air at 37° C.

The phospholipid material was extracted with chloroform, methanol (1:1 vol.), and the phospholipids then separated on 'Whatman No. 1' formaldehyde treated paper<sup>15</sup> using, as developing solvent, the upper phase of a mixture of butanol, acetic acid, water and diethyl ether (20:5:25:6, v/v/v/v). The radioactivity of the separated and identified monophosphoinositide (*R<sub>F</sub>* = 0.47) was determined as reported in Table 1. Phosphorus of the total phospholipid extract estimated as described in Table 1. The results are expressed as c.p.m. labelled inositol incorporated/ $\mu$ g of total phospholipid P.

Our results on the *in vitro* biosynthesis of phospholipid in the adipose tissue show that these compounds are formed *in situ* from the appropriate precursors, although at a slow rate. These findings imply that phosphatides in the adipose tissue have an important function. The recent work of Morril and Rapport<sup>11</sup> on the determination of the phospholipid content of newborn rat adipose tissue has shown that both the phospholipid concentration and the DNA content rise at the beginning of the lipid deposition, thus indicating that a net synthesis of phospholipid per cell unit takes place during the time of the lipid deposition, at least in the neonatal period.

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### Selective Purification of Phosphoserine Peptides by Diagonal Electrophoresis

WHEN a mixture of amino-acids and/or peptides is separated on paper by ionophoresis and a strip containing all of them is sewn to a new sheet of paper and run again by ionophoresis, under the same conditions, in a direction at right angles to the first, the amino-acids or peptides will finally lie in a diagonal line because the mobility in both dimensions is the same. If the compounds are subjected to some chemical reaction before being run for the second time, however, all those with an altered ratio of charge to mass will have a different electrophoretic mobility and so should not appear on the diagonal line. This technique has been used to identify different kinds of peptides, namely, histidine peptides of the active site of phosphoglucomutase<sup>1</sup>, C-terminal peptides of the  $\gamma$  chain of human foetal haemoglobin<sup>2</sup>, and disulphide bridges of chymotrypsinogen A (ref. 3). In these cases the treatments before the second ionophoretic run were, respectively, photo-oxidation, digestion with carboxypeptidase B *in situ* and performic acid oxidation.

I have applied this method to the identification of phosphoserine peptides, which have in the past been studied either by radioactive techniques, in which the phosphate can be labelled, or by running the partial acid hydrolysate of a protein through a 'Dowex 50' column in the acid form<sup>4</sup>. The second method has the disadvantage that it is not specific and that it is not suitable for peptides which include a basic residue, as could be the case for tryptic peptides (which usually have a basic residue as the C-terminal amino-acid). In the case of phosphoglucomutase, for example, when studying the amino-acid sequence around the active site, the peptide obtained by tryptic hydrolysis contains basic residues and is not retained by a 'Dowex 50' column. Furthermore, Flavin<sup>4</sup>, applying his method of separation in acid hydrolysates of ovalbumin, could not find the dipeptide SerP.Ala; he found Glu.SerP.Ala in one experiment and in very low yields, in spite of the fact that his hydrolysis conditions



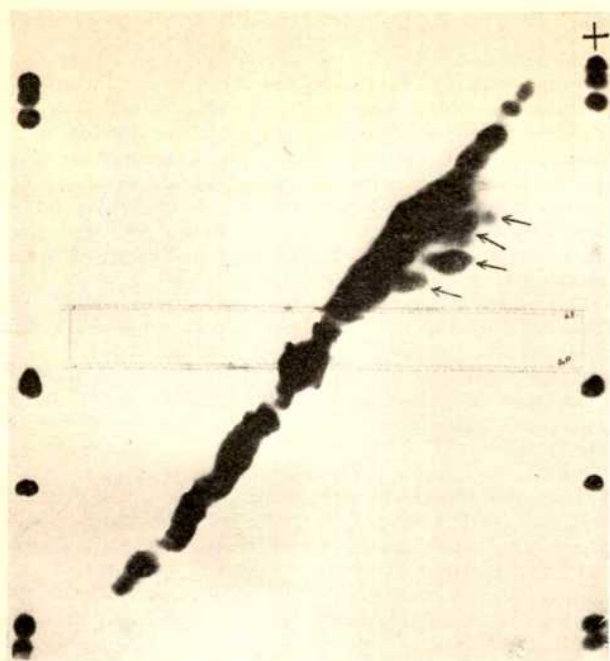


Fig. 1. Ovalbumin oxidized with performic acid and digested with pepsin in 0.01 normal hydrochloric acid for 17 h at 37° C. Protein concentration 2.5 mg/ml., enzyme-substrate ratio 1 : 100 by weight. Hydrolysate was run at pH 6.5, 53 V/cm for 1 h, the strip was treated with alkaline phosphatase, as described in the text, and run in the second dimension at right angles, under the same conditions. Arrows show peptides which lost the phosphate group and became more basic.

(11 normal hydrochloric acid, 37° C for 3–4 days) would cause minimum inversion of dipeptide sequences. This type of difficulty is overcome by the technique described here which, besides being selective for phosphoserine peptides, possesses all the advantages of sensitivity and speed offered by paper ionophoresis.

The procedure as applied to a protein hydrolysate is as follows. The hydrolysate is first run in one direction at a given pH and then a marker strip is digested with alkaline phosphatase. This treatment releases the phosphate from the serine phosphate peptides and the negative charges of the phosphate are therefore lost, so that when the strip is run in the second dimension those peptides which are affected by the enzyme undergo a change in mobility and do not appear on the diagonal line.

The method has been tested with serine phosphate which is hydrolysed by alkaline phosphatase from *Escherichia coli* into serine and inorganic phosphate. Several samples, each 10 µl. of a 5 mmolar solution of serine phosphate, were applied in bands of 1 cm on strips (3 × 2 cm) of chromatography paper (Whatman No. 1) and carefully wetted with a solution of the enzyme in 0.2 molar ammonium carbonate, pH 8.8. The strips were kept in a damp chamber at room temperature, dried and sewn to a new sheet of chromatography paper. Serine and serine phosphate were identified by their different electrophoretic mobilities after running the samples by high voltage electrophoresis at pH 3.5, 3 kV for 20 min, and staining with ninhydrin. Different concentrations of enzyme and different times of incubation were tried; 0.3 mg/ml.—the concentration which gave total hydrolysis of serine phosphate in 1 h—was selected for further experiments.

The method has been successfully applied to ovalbumin. The protein was first oxidized with performic acid and then digested with different proteolytic enzymes. One sample was digested with trypsin, one with chymotrypsin and one with pepsin. Each digest was treated as described earlier and when the papers were developed with cadmium-ninhydrin reagent<sup>5</sup> the number of peptides which did not

appear on the diagonal was different in each case. Two principal peptides were obtained in the tryptic digest. The chymotryptic digest gave two principal peptides and the peptic (Fig. 1) four. The peptides were purified and their amino-acid analysis showed that they were fairly large (10 to 20 residues). Two of the peptic peptides had a closely related amino-acid composition and seem to be related to the more acidic of the two principal tryptic peptides. They were found to contain the sequence SerP.Ala reported by Flavin<sup>4</sup> and Shaw<sup>6</sup>. This is the sequence which according to Flavin<sup>4</sup> is susceptible to the action of prostatic alkaline phosphatase.

The other principal tryptic peptide obtained seems to be related to a third peptic peptide and to one of the chymotryptic peptides. They probably include the sequence Asp.SerP. Work on the elucidation of the two sequences is now in progress.

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### Solubilization of Insoluble Collagens by Rat Liver Lysosomes

AN enzyme or enzyme system which can degrade calf skin collagen soluble in acid at an acid pH is present in the lysosomes of rat liver cells<sup>1–4</sup>. It has also been shown<sup>5–8</sup> that there is a collagenolytic enzyme system in homogenates of rat bone, and that this system, which can convert rat bone collagen to a soluble form, is localized in sub-cellular particles with some of the properties of lysosomes<sup>6,7</sup>. Experiments with radioactively labelled rat bone collagen<sup>8</sup> suggested that this material is not made appreciably soluble by treatment with large granule fractions from homogenates of kidney, brain, leucocytes and liver at pH 7.4. In this report, the effect of rat liver lysosomes on various insoluble collagens at a variety of pH values is described.

Enzyme activity was released from rat liver lysosomes<sup>9</sup> by five cycles of freezing at –60° C and thawing at 37° C, and the mixture was clarified by centrifuging at 100,000g for 30 min. Collagen fibrils isolated by the Nishihara procedure<sup>10</sup> from the Achilles tendons of ox and aged human and from aged human thigh skin were used as substrate. In addition, rat tail tendons which had been exhaustively extracted with cold 0.5 molar acetic acid were used. The course of the reaction was followed by determination of hydroxyproline in the soluble fraction. In practice, 10 mg of insoluble collagen was incubated with a quantity of lysosomal extract containing 0.25 mg protein for 18 h in 0.2 molar acetate buffer. At each pH collagen was also incubated without enzyme. Hydroxyproline was determined<sup>11</sup> after the removal of undissolved collagen by centrifugation at 0° C for 30 min at 120,000g and hydrolysis with 6 normal hydrochloric acid at 103° C for 20 h. For comparison, collagen was also incubated with 1 mg of crystalline pepsin.

The degradation caused by lysosomes varies from one kind of collagen to another (Table 1). Insoluble rat tail tendon collagen, in many ways an atypical collagen, is most easily solubilized. At the other extreme, collagen from aged human tendon is not affected. It may be significant that the least affected collagens are those which are reported to embody the greatest degree of cross-linkage (ref. 12 and F. S. Steven, personal communication).



Table 1. EFFECT OF LYSOSOMES AND PEPSIN ON VARIOUS COLLAGEN PREPARATIONS AT DIFFERING TEMPERATURES AND pH

Collagen source	Enzyme	Temp. (° C)	pH	Per cent collagen solubilized as measured by hydroxyproline release	
				Test	Control
1. Rat tail tendon	Lysosomes	35	4.0	18.6	0
" " "	"	35	5.0	0	0
" " "	"	35	6.0	0	0
2. Ox tendon Nishihara (A)	"	18	4.0	3.9	1.7
" " "	"	35	4.0	8.0	2.9
" " "	"	35	5.0	2.9	2.1
" " "	"	35	6.0	1.1	1.0
" " "	Pepsin	35	4.0	4.2	2.8
" " "	"	18	4.0	2.8	1.9
3. Human tendon Nishihara	Lysosomes	35	4.0	0	0
4. Ox tendon Nishihara (B)	"	35	4.0	2.2	0.25
5. Human thigh skin Nishihara	"	35	4.0	0.5	0.2

Pepsin conc., 2 mg/ml. Lysosome protein conc., 0.5 mg/ml. Collagen: pepsin, 10:1. Collagen: lysosomal protein, 40:1.

The collagenolytic system is active at pH 4.0. This result contrasts with those of Woods and Nichols<sup>7</sup>, who showed optimum solubilization at pH 6.0 with rat bone homogenates. In their study of rat liver lysosomes these authors determined the solubilization at pH 7.4 (ref. 8). Pepsin has considerably less effect on ox tendon Nishihara collagen than lysosomal extract. At 35° C only 4.2 per cent of the collagen is solubilized by pepsin, whereas 8.0 per cent is solubilized by the lysosomes, in spite of the fact that the total concentration of protein in the crude lysosomal extract is only one quarter of the concentration of crystalline pepsin. To minimize the risk of collagen denaturation, some experiments were performed at both 18° and 35° C. Significant solubilization by lysosomal extracts was observed at 18° C.

These experiments demonstrate conclusively that there is present in rat liver lysosomes an enzyme or enzyme system which degrades undenatured insoluble collagen. The small amount of collagen released may be due to the heterogeneity of the incubation mixture where the available surface area of the substrate is small. A preliminary examination of the solubilized products of ox tendon collagen on polyacrylamide gel electrophoresis<sup>13</sup> suggests that the molecular weight of the larger portion is considerably less than that of the  $\alpha$ -chains of collagen.

Two types of collagenase activity have now been reported in mammals, one having an acid pH optimum<sup>3</sup>, the other showing maximum activity at a pH value which is nearer neutrality<sup>7</sup>. The second enzyme, found chiefly in bone in rats, appears to be similar in its properties to that found in tadpoles<sup>14</sup>. The tadpole enzyme is not stored in the cell but released continuously in tissue culture into the culture medium. It is interesting to suggest that the collagenase acting at neutral pH is essentially extracellular in its action whereas the acidic collagenase can find suitable conditions for action in the phagosome within the cell.

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## Sequential Appearance of Phosphoglycerides and Protein in Developing Myelin

MYELINATION is not a sudden event but rather the gradual assembly of various chemical components which appear at the same or different times to form mature myelin. Electron microscopy has shown the gradual morphological development which occurs during myelination<sup>1,2</sup>. The purpose of this study was to inquire into some aspects of the chemical development of myelination of the corpus callosum of the rat, with the use of reliable histochemical techniques.

The brains of sixty rats, 1–16 days after birth, were fixed in various fixatives and embedded in paraffin or cut as frozen sections, as the methods required.

Three methods were used for this study; (a) the gold hydroxamate method for phosphoglycerides; (b) 'Luxol'-fast blue for protein; and (c) osmium tetroxide for unsaturated lipids.

Phosphoglycerides were demonstrated by the gold-hydroxamate method devised by Adams and Davison<sup>3</sup>, modified<sup>4</sup> and remodified<sup>5</sup>; proteins were stained with 'Luxol'-fast blue<sup>6</sup> and the unsaturated lipids were demonstrated with osmium tetroxide<sup>7</sup>. Control sections were also stained by these methods after extraction of lipid with chloroform-methanol (1:3). The phosphoglycerides and unsaturated lipids were thus eliminated whereas the proteinaceous material, stained with 'Luxol'-fast blue (frozen and paraffin section), was not.

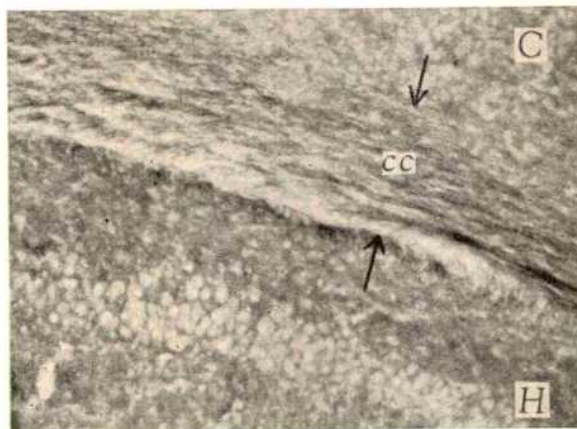


Fig. 1. Phosphoglycerides in the "pre-myelin" fibres of the corpus callosum of the developing rat on the first day. C, Cortex; H, hippocampus; cc, corpus callosum. The gold hydroxamate method was used. ( $\times$  c. 100.)

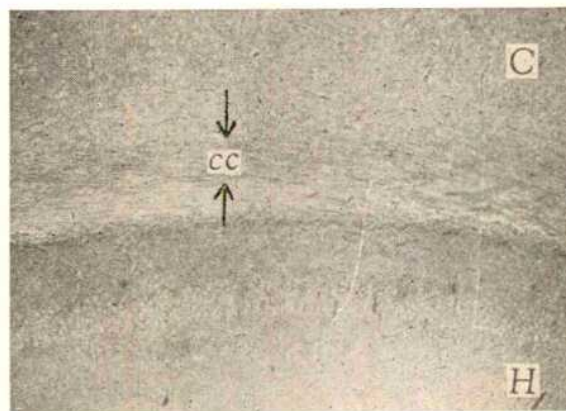


Fig. 2. A protein component of "pre-myelin" fibres in the corpus callosum of a young rat on the eighth day, demonstrated by the 'Luxol'-fast blue technique. ( $\times$  c. 50.)



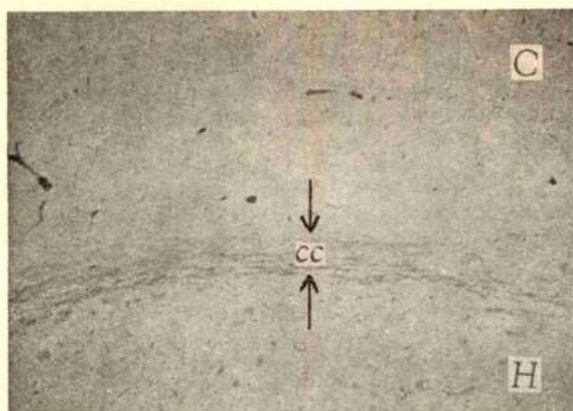


Fig. 3. Osmophilia of myelin in the fibres of the corpus callosum of a young rat on the twelfth day. ( $\times c. 50$ .)

Sections were also stained with haematoxylin and eosin, silver-iodate, periodic acid-Schiff, myelin and sudan black B.

Phosphoglycerides are present in the "pre-myelin" fibres of the corpus callosum on the first day. 'Luxol'-fast blue demonstrated "pre-myelin" fibres on the eighth day and not before. Koenig<sup>8</sup> and Clasen *et al.*<sup>9</sup> have shown that after extraction of lipid 'Luxol'-fast blue stains a protein related to neurokeratin in myelin and not phospholipids as was previously supposed. Osmophilia demonstrates the presence of unsaturated lipids for the first time on the twelfth day and suggests that the myelin is mature<sup>10</sup>.

This work supports the suggestion that the chemical correlate of myelination is a sequential rather than a simultaneous process.

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### Degradation of Human Serum Proteins in the Digestive Tract of Mosquitoes

In the course of investigations aimed at finding the origin of blood ingested by mosquitoes, we took the opportunity of observing the sequence of breakdown of serum proteins in mosquito stomachs. This breakdown may be watched by means of conventional immunological techniques using specific precipitating antisera. We used immunoelectrophoresis and double diffusion in agar gel. The digestive tracts of freshly killed mosquitoes provided the antigen; the tracts were teased apart and placed directly into wells (1.5 mm in diameter) containing agar gel. Antisera prepared against whole human serum and against the following single human proteins, albumin,  $\alpha 2$ -macroglobulin, transferrin, IgM and IgG, have been

used. The results reported here concern only albumin and IgG.

When mosquitoes are killed shortly after ingestion of blood, serum proteins can easily be demonstrated in the stomach by the occurrence of specific precipitation in agar gel. Precipitin reactions, however, become progressively less obvious the longer the time-lapse between feeding and death, and ultimately the reactions become negative. This sequence is likely to indicate digestion of the blood meal. Using *Culex fatigans*, the following observations were made. (a) The rate of disappearance of the different serum proteins varies with the nature of the protein and with the experimental conditions. Sixteen mosquitoes reared in the laboratory were allowed to gorge on several human volunteers and were then kept in a cage. They were killed at various intervals after feeding. Between 24 and 48 h after feeding the albumin could not be detected but IgG continued to react with the specific antiserum for 4 days and sometimes 5 days after feeding. Mosquitoes caught in the wild were similarly allowed to gorge on volunteers but were then left free in a room for 48 h. Eight of these mosquitoes were subsequently captured, killed and dissected. These gave different results: the reactions for albumin were strongly positive but reactions for IgG were negative. The stomach contents were tested from two mosquitoes which had been caught in the wild and fed on human blood in the same house, then kept in a tube in a humid atmosphere for 4 days. The results were similar to those obtained with mosquitoes reared in the laboratory.

(b) When stomach contents were tested with a specific human IgG antiserum, it was found that for all mosquitoes, including those reared in the laboratory, digestion of IgG seems to begin about 5 h after engorgement of blood. At this time a single precipitin line occurring in double diffusion systems is replaced by two lines (Figs. 1 and 2). Because the change from single to multiple lines is reminiscent of the change which occurs when human IgG is exposed to proteolytic enzymes such as papain or trypsin, further double diffusion studies were made to compare the immunological identity of fragments obtained from mosquitoes' stomachs with those produced by enzyme digestion. Pure human IgG was digested with papain after the method of Porter<sup>1</sup> and with trypsin according to Burtin<sup>2</sup>, and the sequence of enzyme digestion followed immunoelectrophoretically. The two fractions obtained by enzyme digestion were then compared in double diffusion systems with the two fractions obtained from the

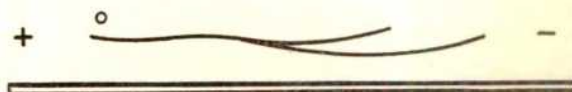


Fig. 1. Immunoelectrophoretic analysis of a mosquito blood meal developed by a rabbit anti-human IgG serum.

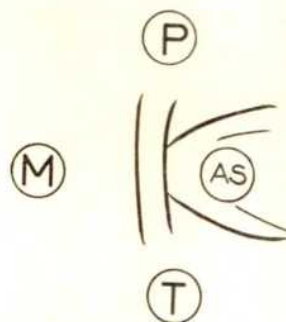


Fig. 2. Agar double diffusion system. M, Mosquito digestive tract; P, human IgG treated with papain; T, human IgG treated with trypsin; AS, anti-human IgG serum (from rabbit).

stomach contents of mosquitoes. The fragments formed during the different digestion process were not identical. Fig. 2 illustrates the broad similarity of the fractions obtained by digestion with papain and trypsin and the obvious dissimilarity of the fractions resulting from digestion of IgG by mosquitoes. This finding suggests that protein cleavage occurring in the stomach of mosquitoes is quite different from that produced by papain or trypsin.

Immunological techniques have been widely used to identify the host on whom mosquitoes feed<sup>3</sup>. Our present findings indicate that the interpretation of the results obtained by such techniques must be made carefully. Environmental conditions could materially influence the rate of protein digestion in mosquitoes and possibly even its sequence. The value of immunological techniques depends largely on the nature of the antiserum used. Our findings indicate that the best results are likely to follow the use of a combination of highly specific antisera rather than the commonly used single polyvalent antiserum.

In our studies the digestion of IgG by the alimentary enzymes of mosquitoes yielded protein fragments which differed from those formed during digestion with papain and trypsin. Further investigation of proteolytic enzymes existing in mosquitoes seems to be merited.

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## Deuterium Exchange in Wool

GRAVIMETRIC hydrogen exchange measurements with purified wool confirm an estimate<sup>1</sup> of the amount of crystalline  $\alpha$ -helical material in keratin fibres made from measurements of the intensities of the wide angle X-ray diffraction pattern. Between 90 and 95 per cent of the cross sectional area of wool is occupied by cortical cells and these cells are largely filled with the characteristic keratin matrix-microfibril structure<sup>2</sup>. The deuterium exchange technique may therefore give an approximate estimate of the proportion of crystalline  $\alpha$ -helical material in the matrix-microfibril composite structure.

We believe we can make a clear distinction between two classes of labile hydrogen atoms using the gravimetric technique. The sample of wool contained 0.75 equiv. of labile hydrogen atoms in the side chains and 0.86 equiv. of main chain imide hydrogen atoms, a total of 1.62 equiv. of labile hydrogen atoms/100 g of wool (Table 1). The clear plateaux in Fig. 1 show that in our experiments 1.40 equiv. of labile hydrogen atoms/100 g of wool exchanged with deuterium oxide either instantaneously or at a measurable rate. The remaining 0.22 equiv. exchanged much more slowly. A reasonable interpretation is that all the labile hydrogen atoms in the side chains exchanged instantaneously or at measurable rates, but that parts of the main protein chains were held strongly in stable configurations and so exchanged only very slowly. Thus, in our sample, a fraction 0.22/0.86, or 26 per cent, of the protein chains were in a very stable configuration. We have made experiments with other types of wool and obtained values between 21 and 52

per cent for the fraction of main chain imide hydrogen atoms which exchange only very slowly.

Turner and Woods<sup>1</sup> examined the equatorial X-ray diffraction pattern from Lincoln wool at various extensions up to 100 per cent. Making certain assumptions, they estimated the ratio at each extension of the total amount of crystalline material,  $\alpha$ -helix plus  $\beta$  extended chain, to the original amount of  $\alpha$ -helical material in the unextended fibre. This ratio fell to a minimum, 0.75, at 45 per cent extension. They estimated the amount of non-crystalline material in the unextended fibre and at various extensions from the scattered intensity at 6 Å. During extension this increased to a maximum of 11 per cent above the value at zero extension. From these two results they calculated the fraction of crystalline  $\alpha$ -helical material in unextended Lincoln wool to be about 0.3, and in a merino wool sample 0.24.

We conclude from our deuterium exchange experiments that 26 per cent of the main chain imide hydrogen atoms in merino wool are in very stable regions of the wool protein structure and not freely exposed to solvent. We suggest therefore that these hydrogen atoms are taking part in intramolecular hydrogen bonds between the turns of the helix in the crystalline  $\alpha$ -helical regions of wool<sup>3</sup>. Crystalline  $\alpha$ -helical regions may be so stable that small changes in conformation are almost impossible, exposure of main chain imide hydrogen atoms to the solvent extremely rare, and replacement of imide hydrogen atoms by deuterium therefore very slow<sup>4</sup>.

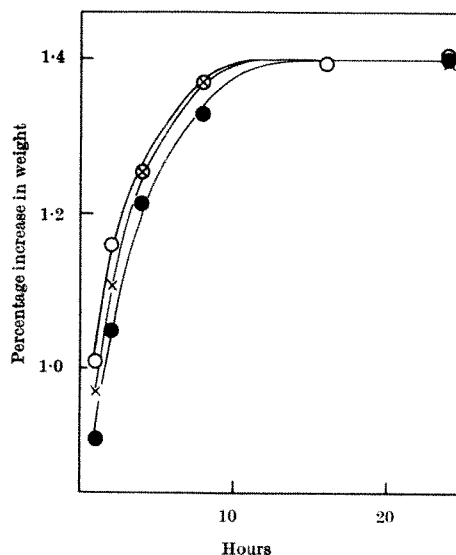


Fig. 1. The rate of increase in weight of a merino wool sample exposed to deuterium oxide at 0°C (—●—), at 20°C (—×—), and at 37°C (—○—). The rate of deuterium exchange with purified wool was measured with a quartz spring microbalance, as described by Burley *et al.*<sup>5</sup>, modified by surrounding the spring (sensitivity 1,000 cm/g) with a water jacket held at a controlled temperature. The spring chamber can be evacuated by means of a mercury diffusion pump protected by liquid nitrogen traps and backed by a rotary pump, which gave a vacuum better than 10<sup>-4</sup> mm mercury. In the exchange experiments at 37°C a sample of purified wool weighing 20–30 mg was hung on the quartz spring. The spring tube was evacuated for 24 h. Because there was no measurable change in weight after 12 h the sample was assumed to be completely dry. The spring tube was isolated from the pumps, and deuterium oxide (99.97 per cent, Norsk-Hydro) was carefully distilled into the tube. It condensed on the wool sample. Exchange was allowed to occur for 1 h and then the tube was evacuated for 24 h. The new dry weight of the sample was noted, and then it was exposed again for 1 h to deuterium oxide, and dried as before. The procedure was repeated in six stages until the wool had been exposed to deuterium oxide for a cumulative time of 24 h. In the experiments at 20°C a new sample of wool was first dried for 24 h at 37°C, then the water jacket was brought to 20°C before the deuterium oxide was admitted to the spring tube. After each period of exchange the tube was evacuated at 20°C for 1 h and then at 37°C for 23 h. In the exchange experiments at 0°C, exchange and the first hour of drying were carried out at 0°C. Drying was completed at 37°C for 23 h. In one experiment a sample was exposed to deuterium oxide for 108 h at 20°C. There was no detectable difference between the dry weight after exposure for 24 h and 108 h.



Table 1. AMINO-ACID ANALYSIS OF SOUTH AFRICAN MERINO WOOL

	mmoles/g	Hydrogen atoms attached to nitrogen or oxygen atoms equiv./100 g	
		Imide	Side chain
Alanine	0.462	0.0462	—
Arginine	0.608	0.0608	0.2432
Aspartic acid	0.515	0.0515	0.0644*
Cysteine	0.560	0.1120	—
Glutamic acid	1.031	0.1031	—
Glycine	0.801	0.0801	—
Histidine	0.078	0.0078	0.0078
Isoleucine	0.260	0.0260	—
Leucine	0.630	0.0630	—
Lysine	0.236	0.0236	0.0472
Methionine	0.038	0.0038	—
Phenylalanine	0.235	0.0235	—
Proline	0.708	—	—
Serine	1.120	0.1120	0.1120
Threonine	0.620	0.0620	0.0620
Tryptophan	0.046	0.0046	0.0046
Tyrosine	0.309	0.0309	0.0309
Valine	0.518	0.0518	—
Amide nitrogen	0.902	—	0.1804
Totals:	—	0.863	0.753

\* 0.0644 is the sum of the values for aspartic and glutamic acids minus half the value for amide groups.

The wool was purified as follows. The tips of the fibres were removed, and the wool was extracted with ether in a Soxhlet apparatus. Burrs and vegetable contaminants were removed by hand, and the wool was rinsed several times in cold ethanol and several times in distilled water. It was carefully dried between filter papers and then in air at room temperature. The dry weight of about 25 mg of purified wool was accurately measured, before deuterium exchange, with the quartz spring microbalance. After the exchange experiments it was placed in a phial and 1 ml. 5.7 normal hydrochloric acid/10 mg of wool was added. Dissolved air was removed from the hydrochloric acid by repeated freezing and thawing. Then the phial was sealed under vacuum and the wool hydrolysed for 24 h at 105° C. In parallel experiments samples were hydrolysed for 48 and 72 h. Amino-acid analyses were carried out with a Technicon analyser. Peak areas were calibrated with Technicon standard amino-acids and standard norleucine. Hydrolysis of wool for three different times allowed us to correct for slow decomposition of serine, threonine and cystine, and the corrected analysis is given in the first column. Amide groups were estimated by the method of Corfield and Robson<sup>6</sup> and tryptophan by the method of Graham and Statham<sup>7</sup>. Fletcher, Robson and Todd<sup>8</sup> showed that the cysteine content of wool is about 0.003 equiv./100 g, which is negligible in our experiments. In 12 analyses of different wool samples, the sum of the weights of the component amino-acid residues, after correction for decomposition of serine, threonine and cystine during hydrolysis, was always within 1.5 per cent of the weight of wool contained in the volume of hydrolysate applied to the analyser column. This indicates the accuracy of the analyses and shows that not more than 1.5 per cent of wool is non-protein material, for example lipid or nucleic acid.

This suggestion is supported by an experiment in which a sample of merino wool was reduced overnight at 4° C in 0.2 molar thioglycolic acid, 0.1 molar disodium hydrogen phosphate adjusted to pH 10.5. About half the protein dissolved from the fibres in these conditions. The fibrous residue was re-oxidized by immersing it in 0.45 normal magnesium chloride, 0.15 normal potassium phosphate adjusted to pH 7.0, and bubbling air through the buffer for 24 h. The re-oxidized fibres were rinsed in distilled water. They did not show any sharp wide angle X-ray diffraction pattern: the protein chains appeared to be completely disordered by this treatment. An amino-acid analysis showed 1.57 equiv. of labile hydrogen atoms/100 g of disordered fibres. In an exchange experiment, all the labile hydrogen atoms were replaced by deuterium after 24 h at 37° C.

Bendit<sup>5</sup> has recorded the changes which take place in the infra-red spectrum of a longitudinal section of horse-hair exposed to deuterium oxide, when the radiation is polarized parallel or perpendicular to the fibre axis. There is evidence that in 16 h at 35° C some exchange of imide hydrogen by deuterium occurs in  $\alpha$ -helices. It is possible that these may be single helices approximately aligned with the fibre axis, and not part of the crystallites. There is also evidence that exchange of the labile hydrogen atoms in arginine side chains may not be instantaneous at 35° C. If the proportion of imide groups in crystalline  $\alpha$ -helices which exchange in several hours at 35° C, and the proportion of arginine side chains which do not exchange, are not negligible, then the agreement we found between X-ray and gravimetric deuterium exchange measurements of the proportion of crystalline  $\alpha$ -helix in wool may be fortuitous. Bendit points out, however, that it is difficult to make the infra-red method quantitative and to be certain of the assignment of wavelengths.

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### Concentrations of RNA in the Brain during Oestrus in the Deermouse

THERE has recently been extensive research into the role of nucleic acids in biological systems, and the part played by RNA in protein synthesis has now been established as has that by oestrogen in protein synthesis of certain tissues.

With this in mind, we have analysed the hypothalamus, amygdala and frontal cortex of adult female deermice (*Peromyscus maniculatus bairdii*) for total RNA content during dioestrous, pro-oestrous and oestrous stages of the oestrous cycle. Vaginal smears were made daily to determine the stage of oestrus. As soon as the stage of the oestrous cycle was established, the mice were killed by decapitation and their brains quickly removed; the hypothalamus, amygdala and frontal cortex were dissected out, frozen in acetone-dry ice, and weighed to the nearest 0.1 mg. About eight identical brain areas from deermice in identical stages of the oestrous cycle were pooled to obtain sufficient tissue for each analysis. At each stage of the oestrous cycle, twelve such pools of tissue were made for a total of 288 deermice in the three oestrous stages. The total content of RNA of the different brain areas was determined by the specific extraction procedure for brain tissue<sup>1</sup> and a quantitative estimation of RNA was made<sup>2</sup> using yeast RNA, Type XI (Sigma), as the standard. Data were analysed statistically by means of a standard *t* test and standard deviations were also determined.

The results indicate that total concentrations of RNA in all three areas studied were the same during the dioestrous stage (Table 1). With the onset of pro-oestrus, there was a significant increase ( $P < 0.01$ ) in total RNA in these three areas, ranging from a minimum of 19 per cent in the amygdala to a maximum of 28 per cent in the frontal cortex. With the onset of oestrus no further changes occurred in the RNA content of the hypothalamus and frontal cortex. There was a significant decline ( $P < 0.001$ ) in RNA content, however, in the amygdala.

This is the first attempt to correlate the concentrations of RNA in the brain with different stages of the oestrous cycle, so any attempt to explain the significance of the changes found would be mere conjecture. They may, however, reflect increased RNA turnover and stimulation of protein synthesis as a result of increased production of ovarian oestrogen with the onset of pro-oestrus and oestrus<sup>3-5</sup>. Another possibility is that changes in con-

Table 1. TOTAL CONCENTRATION OF RNA (IN  $\mu$ g/100 MG) IN THE HYPOTHALAMUS, AMYGDALA AND FRONTAL CORTEX DURING THE OESTROUS CYCLE IN THE FEMALE DEERMOUSE (*Peromyscus maniculatus bairdii*) ( $\pm$  S.D.)

Oestrous condition	Hypothalamus	Amygdala	Frontal cortex
Dioestrus	178.1 $\pm$ 9.4	187.4 $\pm$ 10.2	178.5 $\pm$ 5.3
Pro-oestrus	224.2 $\pm$ 4.2	223.2 $\pm$ 3.4	229.5 $\pm$ 8.3
Oestrus	236.3 $\pm$ 6.8	166.7 $\pm$ 7.4	227.0 $\pm$ 5.9



centrations of RNA reflect a differential increase in neural activity associated with the onset of oestrus.

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### Mechanism of the Toxic Effects of Hyperbaric Oxygen

THE mechanism of the toxic effects of hyperbaric oxygen has long been a subject of discussion and Haugaard<sup>1</sup> has summarized the evidence in favour of the view that the toxicity is due to oxidation of sulphhydryl groups. More recently, however, Chance *et al.*<sup>2</sup> observed that 11–17 atm. of oxygen selectively inhibit the energy-linked reduction by succinate of nicotinamide-adenine dinucleotide (NAD). Because this inhibition occurs much more rapidly than oxidation of sulphhydryl groups, Chance *et al.* suggested the energy-linked reduction of NAD by succinate as the site of the toxic effects of hyperbaric oxygen. The oxygen pressures used by Chance, however, were much greater than those customarily used therapeutically<sup>3</sup>—that is 3 atm. Because toxicity is also observed at these pressures, it seemed desirable to examine the effects of oxygen at 3 atm. on the energy-linked reduction of NAD by succinate.

In the experiments described here, the reduction of NAD was studied by coupling it to a system which utilizes NADH, namely the synthesis of glutamate from  $\alpha$ -oxoglutarate and ammonia<sup>4</sup>. Table 1 shows that oxygen at 3 atm. has no effect on the synthesis of glutamate, even after pre-incubation for 60 min at 25° C. The reaction at 3 atm. of oxygen was carried out in the high-pressure chamber described by Boerema<sup>5</sup>.

A sensitive method of following the state of oxidation-reduction of the nicotinamide nucleotides in rat liver mitochondria is to measure the deamination of glutamate. Papa *et al.*<sup>6</sup> have shown that deamination is slight when the mitochondrial NADP is kept low by the combined action of the energy-linked reduction of NAD by succinate and the nicotinamide nucleotide transhydrogenase. Inhibition of the former by malonate, for example, causes extensive deamination. The amount of ammonia formed after oxidation of glutamate for 60 min was found to be the same with 3 atm. of oxygen as with 0.2 atm.

Thus according to both tests used, oxygen at 3 atm. was found to have no effect on the energy-linked reduction of NAD by succinate in rat liver mitochondria, and so this system does not appear to be the site of the toxic action of oxygen at this pressure.

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### Preparation Parameter for Macromolecules

SOME measurable parameter which could characterize the methods of isolation of a natural macromolecule from its source would be useful. Such a parameter, it seems, could be provided by a measure of the polydispersity of the buoyant density of the molecules when in solution and subjected to ultracentrifugation. The parameter of interest is the standard deviation  $\gamma$  of the Gaussian distribution of buoyant densities and can be obtained from centrifugation experiments alone if an empirical relationship between sedimentation coefficient  $s$  and molecular weight  $M$  is known for the material being investigated. In this communication, the theoretical basis for the use of  $\gamma$  is suggested and some preliminary experimental results for various samples of DNA are given.

If a steady density gradient is created within a liquid held in a cell which is itself subject to centrifugal forces and a macromolecular solute be present, then, provided the solute is either homogeneous or has a Gaussian distribution in buoyant density, the variation of concentration of the solute particles with distance down the cell will follow a Gaussian distribution. According to Sueoka<sup>1</sup>, the density  $\rho$  varies with the distance  $r$  from the axis of rotation according to the expression

$$\rho = \rho_0 + A(r^2 - r_0^2) \quad (1)$$

where  $\rho_0$  is the mean density at distance  $r_0$  and  $A$  is a constant for the particular gradient.

According to Meselson *et al.*<sup>2</sup>, if  $\sigma$  is the standard deviation of the concentration versus distance curve, the spread of which results from thermal agitation alone, then for a sample homogeneous in both molecular weight and density,

$$M = RT \{ \bar{v} \omega^2 r_0 \sigma^2 (d\rho/dr)_{r=r_0} \}^{-1} \quad (2)$$

in which  $\bar{v}$  is the apparent partial specific volume of the macromolecules,  $\omega$  is the angular velocity and the other symbols have their usual meanings. If, however, the isolation procedure is such as to produce material which

Table 1. EFFECT OF 3 ATM. OF OXYGEN ON THE SYNTHESIS OF GLUTAMATE BY RAT LIVER MITOCHONDRIA COUPLED WITH THE AEROBIC OXIDATION OF SUCCINATE

Series: No. of experiments:	1 11		2 6		3 5	
	0.2 atm. O <sub>2</sub>	3 atm. O <sub>2</sub>	0.2 atm. O <sub>2</sub>	3 atm. O <sub>2</sub>	0.2 atm. O <sub>2</sub>	3 atm. O <sub>2</sub>
$\Delta O$ ( $\mu$ atoms/mg)	3.08 $\pm$ 0.20	3.77 $\pm$ 0.28	4.46 $\pm$ 0.25	5.56 $\pm$ 0.30	3.54 $\pm$ 0.76	5.00 $\pm$ 1.25
$\Delta$ Glutamate ( $\mu$ moles/mg)	1.17 $\pm$ 0.10	1.17 $\pm$ 0.09	2.57 $\pm$ 0.45	2.50 $\pm$ 0.45	0.95 $\pm$ 0.23	0.97 $\pm$ 0.20

Rat liver mitochondria, isolated by the method of Myers and Slater<sup>4</sup>, were suspended in a medium containing 15 mmolar KCl, 2 mmolar EDTA, 25 mmolar *tris*-HCl buffer (pH 7.4), 10–15 mmolar potassium phosphate buffer (pH 7.1), 0.1 mmolar ADP, 25 mmolar sucrose (derived from the mitochondrial suspension), 24 mmolar  $\alpha$ -oxoglutarate, 24 mmolar NH<sub>4</sub>Cl, 1 mmolar arsenite, 25 mmolar succinate, and, in series 2, 10 mmolar ATP. The concentration of mitochondria varied between 3 and 6 mg protein/ml. In series 3, the suspension was pre-incubated for 1 h at 25° C before addition of succinate. Reaction at 25° C for 60 min. Uptake of oxygen was measured in Warburg flasks with 0.1 ml. 30 per cent (w/v) potassium hydroxide in the centre well. The reaction was stopped with 0.3 ml. 35 per cent (w/v) perchloric acid, and glutamate determined according to Bernt and Bergmeyer<sup>7</sup>. The values given are means  $\pm$  standard error.

is heterogeneous in density, then the observed standard deviation becomes  $\varphi$  where<sup>3</sup>

$$\varphi^2 = \sigma^2 + \delta^2 \quad (3)$$

and

$$\delta = \gamma / (d\rho/dr) \quad (4)$$

Here  $\gamma$  is the standard deviation of the distribution in buoyant densities.  $\varphi^2$  can be found directly from the concentration distribution curves;  $\sigma^2$  can be determined using equation (2) if  $M$  and  $\bar{v}$  are known. Then from equations (3) and (4) a value for  $\gamma$  can be found.  $M$  can be determined using an independent technique or more readily from measurements of the sedimentation coefficient, using an equation of the form

$$s = \alpha M^\beta \quad (5)$$

in which  $\alpha$  and  $\beta$  are predetermined constants.

Studies have been made using a Spinco model *E* ultracentrifuge on samples of DNA from *Micrococcus lysodeikticus* and *Escherichia coli* prepared by the method of Marmur<sup>4</sup> and on four strains from *Protomyces inundatus* and one from *E. coli* which were isolated by the procedure of Ahmad (in preparation). These groups are designated 1 and 2, respectively. The group 1 *E. coli* DNA was used to calibrate the density gradient by taking its buoyant density as 1.710 g cm<sup>-3</sup> as recommended by Schildkraut *et al.*<sup>5</sup> For the gradient experiments, buffered aqueous solutions of optical grade caesium chloride (Harshaw Chemical Co., Cleveland, Ohio) were used as the density gradient liquid. The procedure outlined by Schildkraut *et al.*<sup>5</sup> was followed. The centrifuge was run at 44,770 r.p.m., which corresponded to a value of 0.0092 for  $A$  (equation (1)). The values of  $\alpha$  and  $\beta$  (equation (5)) were taken as 0.063 and 0.37, respectively<sup>6</sup>, but here it is strictly the weight average molecular weight  $\bar{M}_w$  which is obtained. For samples which are monodisperse in  $M$ ,  $\bar{M}_w \equiv M$ .

Table 1. DENSITY GRADIENT AND VELOCITY SEDIMENTATION RESULTS FOR DNA

Source	Isolation procedure	Buoyant density (g cm <sup>-3</sup> )	$S_{20,w}^0$	$\bar{M}_w(10^{-5})$
<i>M. lysodeikticus</i>	1	1.731	24.1	94
<i>E. coli</i>	1	1.710*	27.0	130
<i>E. coli</i>	2	1.710	10.3	9.9
<i>Protomyces inundatus</i>				
(1) Haploid +	2	1.717	9.7	9.5
(2) Haploid -	2	1.717	8.1	8.4
(3) Natural diploid	2	1.732	9.4	9.4
(4) Artificial diploid	2	1.711	10.2	9.1

Haploids are designated +, -, for convenience. Buoyant densities are to  $\pm 0.001$  except for haploid - which is to  $\pm 0.002$ .

\* Assumed value used to calibrate the density gradient.

All results are shown in Table 1. The buoyant density of *M. lysodeikticus* DNA agrees with the value obtained by Schildkraut *et al.*, confirming the gradient calibration. All group samples gave a value of  $5.1 \times 10^{-4}$  cm<sup>2</sup> for  $\delta^2$  from which  $\gamma = 0.0028$  g cm<sup>-3</sup>, while for group 2 samples  $\gamma = 0$ . The latter value implies samples which have constant buoyant density although they may be polydisperse in molecular weight. For group 2 *E. coli* DNA, however,  $\bar{M}_w$  was also obtained from the density gradient data by performing the vigorous integration over the whole band<sup>2</sup>. The value obtained was, within the experimental uncertainty, that obtained from the sedimentation velocity experiments (equation (5)).

In equation (2) the apparent partial specific volume is used, and its average value may vary with the spread in buoyant density of the macromolecules. For different samples of a single material, each with the same density distribution, the average value of  $\bar{v}$  is not likely to alter significantly. It may, however, vary from distribution to distribution. The values of  $\gamma$  reported here may therefore need slight correction. Nevertheless,  $\gamma$  will still characterize the distribution of buoyant densities within each group.

The equality of  $\gamma$  for the two group 1 samples isolated by the same technique thus gives a measure of support to the use of  $\gamma$  as a preparation parameter; further support comes from  $\gamma = 0$  for all group 2 preparations. Further study is, however, necessary on samples isolated by more techniques and on material from a better identified source.

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## Circular Dichroism of Rhodopsin and Isorhodopsin

A COMPARISON between the optical rotatory dispersion curves of rhodopsin before and after irradiation has been previously reported<sup>1</sup>. Unbleached rhodopsin solutions show a weak Cotton effect in the visible range, and another distinguishable Cotton effect in the ultra-violet range, attributable to the polypeptide conformation (probably  $\alpha$ -helix) of the protein moiety, opsin. After bleaching, however, the samples have a simple dispersion curve showing lower values of optical rotation in the visible range. The Cotton effect in the visible range is thus presumably caused by the optical activity of the prosthetic group conformation (11-*cis*), although this is not certain. We have made measurements of the circular dichroism of rhodopsin and isorhodopsin (the artificial pigment with a prosthetic group in the 9-*cis* configuration) in order to elucidate the weak Cotton effect demonstrated by optical rotatory dispersion.

A digitonin extract of cattle rhodopsin was prepared by the usual procedures<sup>2</sup> (outer segments of rod were hardened with potassium alum, freeze dried, washed with light petroleum ether and extracted with 2 per cent aqueous digitonin). The  $E_{400}/E_{500}$  and  $E_{278}/E_{500}$  of the extracts were 0.22 and 2.5. The concentration of digitonin in the extract was about 1.2 per cent. Isorhodopsin was prepared by irradiating rhodopsin solution at the temperature of liquid nitrogen through a filter transmitting more than 590 m $\mu$  (ref. 3) until the  $\lambda_{max}$  of the sample shifted from the initial 498 m $\mu$  (rhodopsin) to 487 m $\mu$  (isorhodopsin<sup>4</sup>). Assuming the molar extinction coefficient of isorhodopsin to be 43,000 and that of rhodopsin to be 40,600 (ref. 4), the irradiated sample was estimated to contain more than 95 per cent of isorhodopsin and less than 5 per cent of rhodopsin, and no other photoproducts (Fig. 1).

Measurements of the circular dichroism between 600 and 200 m $\mu$  were performed at 20° C with the Roussel-Jouan Dichrograph, Type B. The pH of the sample was adjusted to 6.8 with M/15 phosphate buffer. The circular dichroic absorption was expressed as  $\Delta\epsilon_{ir} = \Delta A_{ir}/c.l$ , where  $\Delta A_{ir}$  is the observed difference in absorbance between the left and right circular-polarized components,  $c$  is the molar concentration of rhodopsin or isorhodopsin, and  $l$  is the light path in cm. In the visible range the light path was 1 or 2 cm, but in the ultra-violet range it was only 0.5, 0.2 or 0.1 cm (because of the strong absorbance in this region). Absorption spectra were recorded with the Hitachi spectrophotometer (EPU-2A).

Fig. 1 shows the absorption spectra and circular dichroic absorption spectra of rhodopsin and isorhodopsin in the visible range. Both rhodopsin and isorhodopsin have positive circular dichroic absorptions, each possessing two optically active absorption bands corresponding to the main peaks at 500 and 487 m $\mu$  and to the respective *cis* peaks. It is noteworthy that the optical activity in the *cis* peak region is remarkably high in both cases (for rhodopsin it is rather greater than the optical activity of the main peak).

After irradiation of rhodopsin with  $10^4$  lux for 3 min, the circular dichroic curve in the visible "collapsed" completely to the base line, a result which was also achieved by heating for 5 min at  $90^\circ$  C.

Fig. 2 shows the circular dichroic spectra of rhodopsin in the range 200–250 m $\mu$ . The two lower curves are those before ("dark") and after ("irradiated") bleaching. The upper curve is that of the "dark" sample after four-fold dilution. This curve indicates that rhodopsin contains a considerable amount of polypeptide in the  $\alpha$ -helix conformation in comparison with other helical (synthesized) polypeptides and natural proteins<sup>5</sup>. The decrease in circular dichroic absorbance after irradiation suggests that the change of conformation in the protein moiety occurs when retinal is released from opsin. The circular dichroic spectra in the ultra-violet range are consistent with the earlier data on optical rotatory dispersion<sup>1,14</sup>. A clear circular dichroic spectrum near 278 m $\mu$  corresponding to the absorption of aromatic residues could not be observed, indicating little or no activity of the band in spite of its high absorption.

In the previous report<sup>1</sup>, we ascribed the decrease in  $[\alpha]_D$  on irradiation to be caused chiefly by a conformational change in the protein (because the Cotton effect in the visible was much weaker than in the ultra-violet). The present results, however, suggest that conformational change in the chromophore contributes in a considerable degree to this decrease.

What has induced the optical activity of 11-*cis* retinal? The 11-*cis* retinal molecule is bent in the middle and twisted because of steric hindrance<sup>6</sup>. When bound to opsin, this twisting could be enhanced in one direction—right or left handed—and consequently an optically active (asymmetric) structure might be induced, as is observed in many optically active biaryl compounds<sup>7</sup>. Against this, however, is the fact that although 9-*cis* retinal (in isorhodopsin) has a relatively plain structure<sup>8</sup>, it

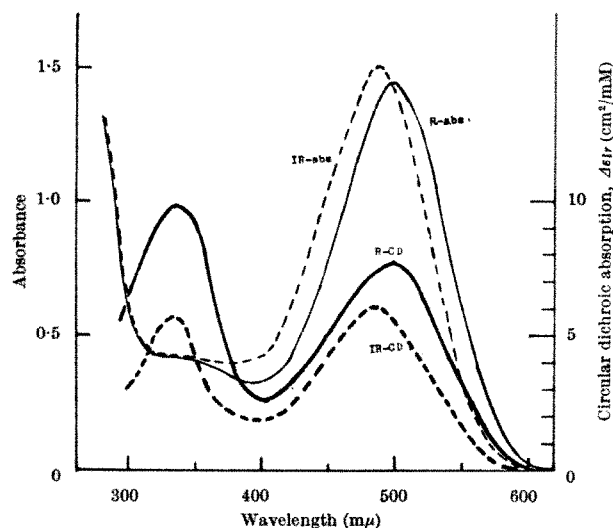


Fig. 1. Absorption spectra and circular dichroic absorption spectra of rhodopsin and isorhodopsin in the visible range. *R-abs* and *IR-abs*: absorption spectra of rhodopsin and isorhodopsin. *R-CD* and *IR-CD*: circular dichroic absorption spectra of rhodopsin and isorhodopsin. Measurements at  $20^\circ$  C for 1 cm of light path.

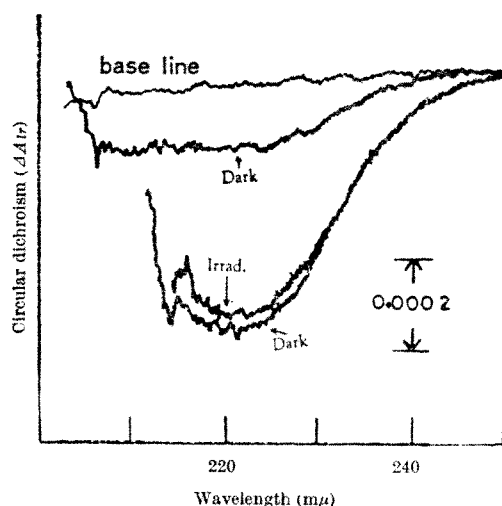


Fig. 2. Circular dichroic absorption spectra of rhodopsin in the ultra-violet range. Bottom curve: rhodopsin solution (at five-fold dilution of Fig. 1. sample) in the visible range. Middle curve: irradiated solution at the same dilution. Top curve: rhodopsin solution at twenty-fold dilution. Measurements at  $20^\circ$  C for a 0.1 cm light path.

likewise becomes optically active, though less so than the 11-*cis* isomer.

In any case, it is probable that the bathochromic shift in the absorption spectrum of retinal-opsin could arise from any strong interaction between retinal and opsin—that would lead to an unsymmetrical conformation for the retinal<sup>14</sup>. The circular dichroic absorption and optical rotatory dispersion in the ultra-violet suggest that an asymmetric conformation of retinal could be imposed by attachment to a rigid conformation (probably  $\alpha$ -helix) of the protein moiety. The fact that the circular dichroic absorption of rhodopsin is greater than that of isorhodopsin suggests that 11-*cis* retinal can be more rigidly fixed to opsin than can 9-*cis* retinal. If this is so, we can understand why the  $\lambda_{\max}$  of rhodopsin is longer than that of isorhodopsin.

The reason why the circular dichroic absorption corresponding to the *cis* peak is so high cannot yet be explained. The electronic transition corresponding to the *cis* peak was tentatively explained by Wald<sup>8</sup> as being perpendicular to the transition corresponding to main peak. The fact that  $E_{cis\text{ peak}}/E_{main\text{ peak}}$  of free 11-*cis* retinal is about 0.5 (ref. 6), while that of bound retinal in rhodopsin is about 0.2, indicates that the electronic transition corresponding to the *cis* peak is much more strongly affected than the main peak by interaction with opsin. We cannot decide, however, whether asymmetric conformation of retinal has been caused by a change in the steric orientation of a local group in the polyene chain, or by a conformational change in the whole polyene chain.

In a late stage of the preparation of this communication we read the article by F. Crescitelli *et al.*<sup>9</sup> dealing with the circular dichroism of rhodopsin and porphyropsin. These authors discussed the induced circular dichroism of visual pigments in the light of the fact that optically inactive dye molecules, when asymmetrically arranged along the ordered conformation of synthesized polypeptides, become optically active<sup>10,11</sup>. They tentatively suggested that the induced circular dichroism of 11-*cis* retinal arose from its steric fitness with opsin, as proposed by Wald *et al.*<sup>12</sup>. We agree with this idea. Takagi *et al.*<sup>13</sup> reported that when flavin adenine dinucleotide (FAD) was bound with D-amino-acid oxidase, the optical activity of FAD was inverted and enhanced. Similar phenomena were found in other natural chromoproteins with a single prosthetic group that was originally optically active. We can find, however, no example other than rhodopsin

in which optical activity has been induced by interaction of a single optically inactive prosthetic group with the ordered structure of a protein moiety. In order to elucidate the retinal-opsin linkage in rhodopsin, and the functional mechanism of rhodopsin in vision, further studies on circular dichroism are required.

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### Some Physical Properties of Bimolecular Lipid Membranes produced from New Lipid Solutions

ALL bimolecular lipid membranes (BLM) (which are also known as bilayer or black lipid membranes) so far investigated have been formed from solutions containing phospholipids, with perhaps one exception<sup>1,2</sup>. In an attempt to understand more fully the structure of BLM, and in particular the factors responsible for BLM formation and stability, we tried to generate BLM from a variety of substances and to measure their physical properties. Previous measurements of properties have been restricted chiefly to electrical characteristics, thickness and water permeability<sup>3</sup>, but a technique has recently been developed which permits direct quantitative measurements of interfacial tension.

The composition of the lipid solutions which produced stable BLM is given in Table 1. The compounds are all readily available. Cholesterol obtained from various commercial sources was recrystallized twice from absolute ethanol before use, and oxidized cholesterol was prepared by a procedure previously described<sup>2</sup>.

The electrical properties of BLM were measured in a chamber basically similar to the one used by Mueller *et al.*<sup>3</sup>. We measured various electrical properties (Table 1) by inserting two saturated calomel electrodes into the bathing solution across the BLM. The polarizing voltages were applied to the BLM using an instrument designed by Rudin, Mueller and Chock (Electronics for Life Sciences, Rockville, Md.), with which the input resistance can be varied between 0.1 and 1,000 megohms and the polarizing potential between 0 and 5.5 V. The potential across the BLM was monitored with a Keithley electrometer. For quantitative measurements of interfacial tension, the BLM was interposed between two identical aqueous solutions. An infusion-withdrawal pump was used to change the hydrostatic head in one chamber. The resulting pressure difference across the membrane was detected by a sensitive pressure transducer, and was continuously recorded. The pressure reached a maximum when the membrane was hemispherical. The interfacial tension ( $\gamma_i$ ) was calculated from this point using the equation

$$P = \frac{8\gamma_i}{d} \quad (1)$$

where  $P$  is the pressure difference across the BLM and  $d$  is the diameter of the hemisphere. In some experiments, the inner chamber for membrane support was replaced by a length of 'Teflon' tubing inserted vertically in the outer chamber. The experimental apparatus is illustrated in Fig. 1 and the results of interfacial tension measurements are also given in Table 1.

Attempts to form BLM with purified cholesterol, dioctadecyl phosphite (DODP), dodecyl acid phosphate (DAP) or hexadecyltrimethylammonium bromide (HDTAB) alone in *n*-dodecane were not successful. Stable black membranes were obtained, however, when cholesterol was present in combination with a surface active agent (Table 1, lipid solutions A, B, and C). On the other hand, lecithin, glycerol distearate and oxidized cholesterol could all form BLM alone. Low interfacial tension at the oil/aqueous solution interface, although important for the formation of BLM, is not a sufficient criterion to predict the formation of a stable membrane. From our measurements on a number of systems, we conclude that the interfacial tension must be in a given range between slightly higher than 0 and about 6 dynes/cm and that the packing of molecules in the membrane must satisfy some sort of space-charge requirements.

The results given in Table 1 are of particular interest because the Davson-Danielli model of the cell membrane<sup>4</sup> was based on an expected value of  $\gamma_i$  of some 20 dynes/cm for the lipid layer. Because the interfacial tensions measured for cell membranes were of the order of 0.1 dynes/cm, Davson and Danielli concluded that an adsorbed protein layer was responsible for the reduction of the interfacial tension of the lipid leaflet. Although the data presented in this report do not rule out the presence of an adsorbed layer of protein being responsible for low  $\gamma_i$  in the cell membrane, they do indicate that protein adsorption might not be the only cause of the low  $\gamma_i$  found for cell membranes.

Table 1. COMPOSITION OF LIPID SOLUTIONS FOR THE FORMATION OF BIMOLECULAR LIPID MEMBRANES AND SOME PHYSICAL PROPERTIES AT 25° C

Lipid solution (w/v)	Aqueous phase	Interfacial tension (dynes/cm)	Conductance $\times 10^4$ (mhos/cm <sup>2</sup> )	Capacitance ( $\mu F/cm^2$ )	Breakdown voltage (mV)
A, Dioctadecyl phosphite* (0.08 per cent) + cholesterol (0.8 per cent) in <i>n</i> -dodecane	0.1 normal NaCl	3.9 $\pm$ 0.5	1-10	0.74 $\pm$ 0.01	350
B, Dodecyl acid phosphate* (0.35 per cent) + cholesterol (0.93 per cent) in <i>n</i> -dodecane	0.01 normal NaCl	1.1 $\pm$ 0.1†	1	0.69 $\pm$ 0.03	800
C, Cholesterol (~1 per cent) in <i>n</i> -dodecane	0.001 normal NaCl + 0.008 per cent HDTAB	0.65 $\pm$ 0.1	0.5	0.79 $\pm$ 0.03	300
D, Egg lecithin (1 per cent) in <i>n</i> -dodecane	0.1 normal NaCl	0.9 $\pm$ 0.1	0.5	0.45 $\pm$ 0.04	120
E, Glycerol distearate (sat.) in <i>n</i> -octane	0.1 normal NaCl	1.5 $\pm$ 0.1	0.5	0.39 $\pm$ 0.01	120
F, Oxidized cholesterol (4 per cent) in <i>n</i> -octane	0.1 normal NaCl	1.9 $\pm$ 0.5	0.5	0.57 $\pm$ 0.01	310

\* Supplied by Hooker Chemical Co., NY

† In 0.1 normal NaCl

Conductance measurements can be duplicated within an order of magnitude or better, and breakdown voltage usually within 15 per cent. Capacitance values are given with standard error for 3-9 experiments.



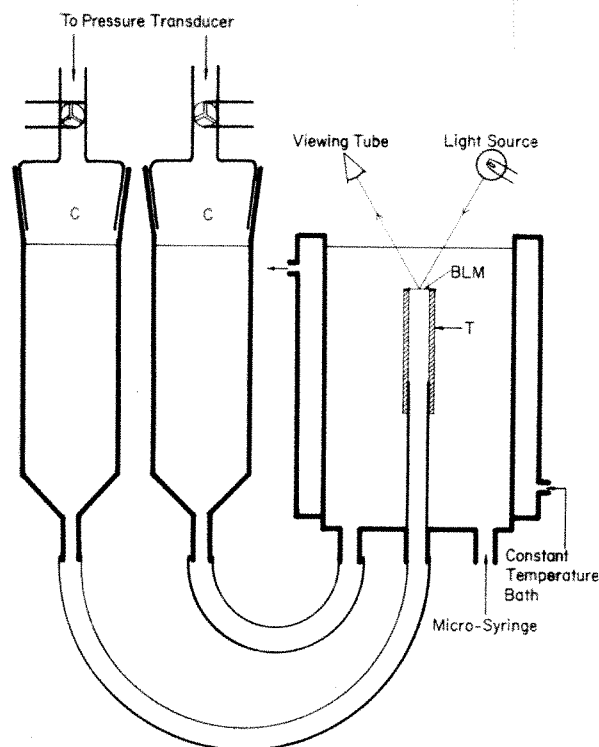


Fig. 1. Apparatus for measuring interfacial tension of bimolecular lipid membranes. C, Ballast chamber; T, 'Teflon' tubing; BLM, membrane or thin lipid film.

Finally, the apparatus described can be used to study the effects of adsorption of electrolytes, drugs and other substances on bimolecular lipid membranes. These membranes have already been shown<sup>5</sup> to be a promising tool in the understanding of biological phenomena in relation to their reactions and behaviour. The apparatus should also be useful for the study of other types of interfacial films and associated adsorption phenomena at oil/water interfaces<sup>6,7</sup>.

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### Substrates of Kinin-releasing Enzymes isolated from Horse Plasma

KININOGEN has been isolated from horse plasma<sup>1</sup>, bovine plasma<sup>2-4</sup>, human plasma<sup>5</sup> and rabbit plasma<sup>6</sup>. Several authors, however, have shown that plasma contains at least two kininogens with affinities for different releasing enzymes<sup>7-10</sup>. Recently, Jacobsen<sup>11</sup> detected the presence of two kininogens in human, dog and rabbit plasma—one which forms kinin with plasma kallikrein and another which forms kinin only when incubated with glandular kallikrein.

We have previously shown<sup>1,10</sup> that although a kininogen isolated from horse plasma released kinin when incubated with trypsin and snake venom enzyme, it did not do so when incubated with plasma kallikrein. The observation that fresh or inactivated plasma was rich in substrate for plasma kallikrein<sup>10</sup> induced us to try to separate these two substrates in the course of the same chromatographic procedure. The first kininogen which emerged from the DEAE-cellulose column was the substrate specific for plasma kallikrein and will be referred to here as kininogen I; the second kininogen was the same as that isolated previously<sup>1</sup> as "bradykininogen", and will here be called kininogen II.

Fresh oxalated horse plasma was heated to 61° C for 20 min to destroy the endogenous formation of kinin<sup>12</sup>. Ammonium sulphate was added to 20 per cent saturation, and after separation of the inactive precipitate the concentration of ammonium sulphate was increased to 35 per cent. The precipitate was separated by centrifugation, dissolved in a small volume of water and dialysed in the cold (4° C) in 'Cellophane' tubing, first against water and then against 5 mmolar sodium phosphate buffer, pH 7.0, until the diffusate was free from  $\text{NH}_4^{++}$  ions. The residue was then passed through a DEAE-cellulose column equilibrated with the same buffer. A gradient was established by introducing 0.02 molar phosphate buffer, pH 6.5; 0.05 molar phosphate buffer, pH 6.0; 0.05 molar  $\text{Na}_2\text{HPO}_4$  in 0.1 molar NaCl; and 0.1 molar  $\text{Na}_2\text{HPO}_4$  in 0.2 molar NaCl, from a separating funnel into a chamber containing 5 mmolar sodium phosphate buffer, pH 7.0. The ultra-violet absorption of the fractions was recorded at 254 m $\mu$ . The presence of the substrates in the various fractions was determined by assay on the guinea-pig ileum of the bradykinin formed by incubation of the fractions with purified plasma kallikrein<sup>10</sup> and the kinin-releasing enzyme from the venom from *Bothrops jararaca*<sup>12</sup>. Kininogen I was eluted from the anion exchange column at a sodium phosphate concentration of approximately 0.017 molar. Kininogen II was eluted at a concentration of 0.09 molar in sodium phosphate and 0.16 molar in sodium chloride and was further purified as previously described<sup>1</sup>. The active fractions containing kininogen I were further purified by gel filtration on a column of 'Sephadex G-100' in 5 mmolar phosphate buffer, pH 7.0 (Fig. 1). Fractions 30-40, which contained the peak of

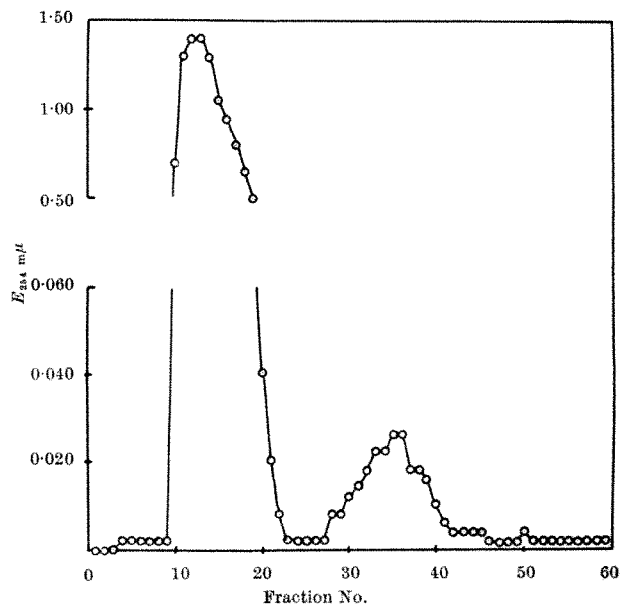


Fig. 1. Fractionation of kininogen I on 'Sephadex G-100'. Elution pattern of DEAE-cellulose purified material (12.5 ml.; 437 mg protein). Column dimensions: 2.2 cm × 35 cm. Fraction volume was 7 ml.

activity, were combined and the protein content was determined by the method of Lowry *et al.*<sup>14</sup>. An overall purification factor of about 200 was obtained in terms of capacity to produce kinin when incubated with plasma kallikrein, as compared with the globulin fraction precipitated at 35 per cent saturation with ammonium sulphate. The chromatographic and gel filtration experiments were performed at 4° C.

Kininogen I releases kinin when incubated with plasma kallikrein, snake venom enzyme and trypsin, while kininogen II releases kinin when incubated with trypsin, venom enzyme, urinary kallikrein and plasmin<sup>10</sup> but not with plasma kallikrein.

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## HAEMATOLOGY

### Reaction Sequence of Blood Coagulation

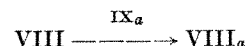
IN 1964 Macfarlane<sup>1</sup> proposed a reaction mechanism for the interaction of blood coagulation factors, known as the enzyme cascade. In this hypothesis the blood coagulation factors as they occur in plasma are considered to be zymogens (except factor I; namely, fibrinogen). Coagulation is initiated by the conversion of one of these zymogens into an enzyme as a result of contact with anything other than intact vascular endothelium. This enzyme then acts on another clotting factor to form another active enzyme. All clotting factors thus interact in a given order until prothrombin (factor II) is converted into thrombin. Thrombin then brings about the actual coagulation by converting fibrinogen into fibrin.

The prothrombin converting activity (prothrombinase activity) was first thought to be exerted by activated factor V (ref. 1). As the result of further work it now seems that the prothrombinase activity is displayed by a complex of activated factor X (*f.X<sub>a</sub>*), calcium ions (II) and factor V (*f.V*) which are adsorbed together on a phospholipid surface.

The evidence for this concept is of two kinds. (a) It can be shown that *f.X<sub>a</sub>* and *f.V* are bound by phospholipid micelles, and that calcium ions favour the binding of *f.X<sub>a</sub>*. High concentrations of calcium, however, inhibit the binding of *f.V* (refs. 2-5). (b) The kinetics of the generation of prothrombinase activity in mixtures of *f.X<sub>a</sub>*, *f.V*, phospholipid and calcium ions can be explained by assuming that a product of all four reactants is the

*f.II* converting substance. It cannot be explained by a reaction of the cascade type<sup>6</sup>.

This communication shows that evidence can also be provided for the existence of a complex consisting of the clotting factors IX (in activated form) and VIII, and phospholipid, similar to that described in section (a). The interaction between *f.IX<sub>a</sub>* and *f.VIII* previously has been considered to be of the cascade type, that is



The experiments described in Table 1, however, show a strong similarity between the characteristics of adsorption

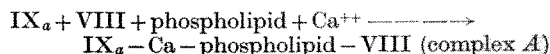
Table 1. ADSORPTION OF COAGULATION FACTORS BY PHOSPHOLIPID

Calcium ion conc. (mmolar)	Percentage of clotting factor adsorbed			
	V	VIII	IX	X
1	60	58	27	38
50	<1	41	45	60
100	<1	3	68	76

The figures give the means of three different experiments. Each coagulation factor determination was carried out eight times. The material used as a source of *f.V* and *f.VIII* was barium sulphate adsorbed bovine oxalated plasma. For *f.IX<sub>a</sub>* and *f.X<sub>a</sub>* oxalated bovine serum was used as a source. To prevent active thrombin being formed from residual amounts of prothrombin, 5 mcg/ml. hirudin was added. The phospholipid used was a suspension of inosithin prepared by homogenizing the crude material in *tris-HCl* buffer 0.02 molar, pH 7.5 containing 0.14 molar NaCl. The reaction mixture consisted of 0.9 ml. plasma or serum, 0.4 ml. CaCl<sub>2</sub> solution in *tris-HCl* 20 mmolar pH 7.5; 0.4 ml. of a suspension of inosithin 5 mg/ml. Half the mixture was centrifuged for 30 min at 100,000g at 4° C; in the supernatant the original concentration of phospholipid was restored. The other half of the mixture was stored at 4° C and served as a control. The concentration of the clotting factors in the centrifuged sample was determined and expressed as a percentage of that in the control. The percentage adsorbed was calculated as the amount not recovered in the supernatant. Factors VIII and IX were estimated according to Veltkamp<sup>7</sup>, factors V and X were estimated as described in ref. 10. The concentration of hirudin used did not interfere with the clotting factor determinations.

onto phospholipid of *f.X<sub>a</sub>* and *f.IX<sub>a</sub>*, on the one hand, and of *f.V* and *f.VIII*, on the other. Calcium ions favour the adsorption of *f.X<sub>a</sub>* and *f.IX<sub>a</sub>*; excess calcium inhibits the adsorption of *f.V* and *f.VIII*. Control experiments show that it is the phospholipid that adsorbs the clotting factors. The activity that disappeared from the supernatant could be recovered from the resuspended phospholipid sediment. No activity was recorded from the phospholipid in experiments in which no activity was adsorbed from the sample.

The role of phospholipid in the interaction of *f.IX<sub>a</sub>* and *f.VIII* has so far remained obscure, although it has been proved that this role is a mandatory one<sup>7</sup>. Our experiments strongly suggest that phospholipid interacts with calcium ions, *f.IX<sub>a</sub>* and *f.VIII* in a manner similar to the interaction of phospholipid, calcium, *f.X<sub>a</sub>* and *f.V*. If this is true, the reaction equation of the interaction would be



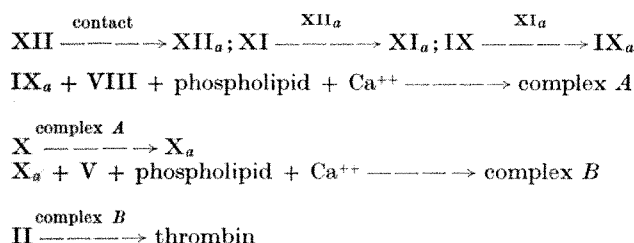
Theoretically, it is possible to obtain more evidence for the existence of complex A by investigating the kinetics of the generation of *f.X* converting activity from the purified factors IX<sub>a</sub> and VIII, that is, by a procedure analogous to the experiments cited in section (b). Technical difficulties in obtaining the pure factors IX<sub>a</sub> and VIII as well as in the measuring of the conversion *f.X*  $\longrightarrow$  *f.X<sub>a</sub>* seem to prevent this approach in the near future.

The fact that kinetic evidence obtained with impure systems has so far suggested that *f.VIII* is converted enzymatically by *f.IX<sub>a</sub>* (ref. 8) remains disturbing. From the results of experiments on the kinetics of the interaction of factors X<sub>a</sub> and V, it was evident that if only a limited range of concentrations was investigated, the results obtained simulated that of an enzyme inter-

action. If the experiments were carried over a wide range of concentrations then the stoichiometric interaction is suggested<sup>6</sup>.

We have paid no attention to the form in which f.V and f.VIII participate in the reactions. By this we do not mean to say that we suppose them to be in the unmodified form in which they occur in intact plasma.

The considerations presented here suggest the following scheme of the intrinsic reaction mechanism of blood coagulation



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## IMMUNOLOGY

### Tolerance in Adult Rats to a Purified Protein, Flagellin, from *Salmonella adelaide*

THE capacity of flagellar antigens from *Salmonella adelaide* to induce immunity and tolerance in rats has been described in detail before<sup>1</sup>. The monomeric protein, flagellin, prepared from the flagella of this organism has been purified. It has a molecular weight of about 38,000 and contains in particular three methionine residues, but no cysteine, histidine or tryptophan. Flagellin was readily polymerized by treatment with ammonium sulphate to form linear polymers which under the electron microscope looked the same as the original flagella particles<sup>1,2</sup>. These proteins were highly immunogenic in the adult rat; amounts of less than 1 µg, when injected in saline, gave rise to readily detectable amounts of antibody<sup>3</sup>. Tolerance to flagellin has been previously described in newborn<sup>4</sup> but not in adult rats. Tolerance has now been achieved in adult rats by the injection of flagellin degraded by cyanogen bromide, a reagent which cleaved the polypeptide chain at methionine residues.

The polymer (20 mg) in aqueous solution was deposited by centrifugation (30,000g for 45 min) and the residue was

dissolved in formic acid (1 ml., 100 per cent). Cyanogen bromide (40–60 mg) was added and the mixture was incubated at 20° C for 16 h. The reaction products were examined by polyacrylamide gel electrophoresis. At all pH values used, flagellin showed a single band of protein, and so did flagellin treated with formic acid alone. The best resolution of the reaction products resulting from cyanogen bromide treatment was achieved in an acetate buffer of pH 2.7 containing 8 molar urea<sup>5</sup>. The separation achieved is shown in Fig. 1. There were four chief protein bands called fragments A, B, C and D which were well separated from the position where intact flagellin would band. There were in addition three or four very narrow, minor bands immediately above fragment A but below the expected position of intact flagellin. It was initially thought that these minor bands might represent incomplete cleavage of the flagellin molecule. Amino-acid analysis of the reacted protein showed, however, that the destruction of methionine seemed to be complete so that the three or four minor bands in the polymer digest might represent recombined fragments of the flagellin molecule<sup>6</sup>.

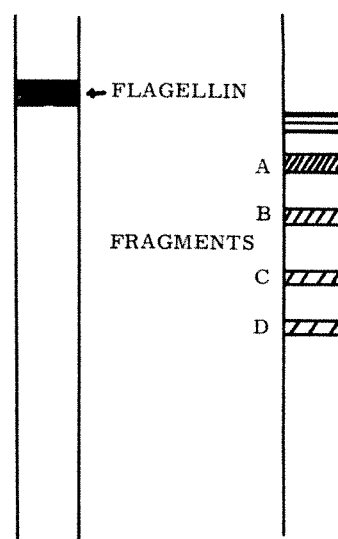


Fig. 1. Polyacrylamide gel electrophoresis in an acetate buffer, pH 2.7, containing 8 molar urea of flagellin and of a cyanogen bromide digest of the polymer. Protein bands were stained with amido black (0.25 per cent, w/w) in 7 per cent (v/v) acetic acid containing mercuric chloride (0.5 per cent, w/w). Flagellin gave a single band (left) whereas the digest gave four main bands, designated fragments A, B, C and D. The degree of cross hatching approximates the intensity of staining observed.

The antigenic properties of the protein digest (hereafter called the digest) were examined by studying the reaction with anti-flagellin rabbit serum. Immunodiffusion analysis showed a complete line of identity between flagellin, the digest and fragment A, suggesting no destruction of antigenic determinants during the cyanogen bromide treatment. When tested by a micro precipitin reaction (carried out by Dr R. Wistar) the digest was found to retain completely the ability to react with anti-flagellin serum. The digest was also compared with flagellin for its ability to neutralize the immobilizing activity of a given amount of anti-flagellin serum when mixed with a motile suspension of organisms (*S. derby*, which shares the same H antigens as *S. adelaide*). The digest was found to be about 50 per cent as efficient as flagellin in this regard.

The *in vivo* behaviour of the digest was compared with that of the intact antigen. When a single dose of different preparations of the digest in aqueous solution was injected into the footpads of adult rats (10 µg each rat—randomly bred Wistar rats were used), either no antibody or very low titres of antibody was detected (antibody titres were

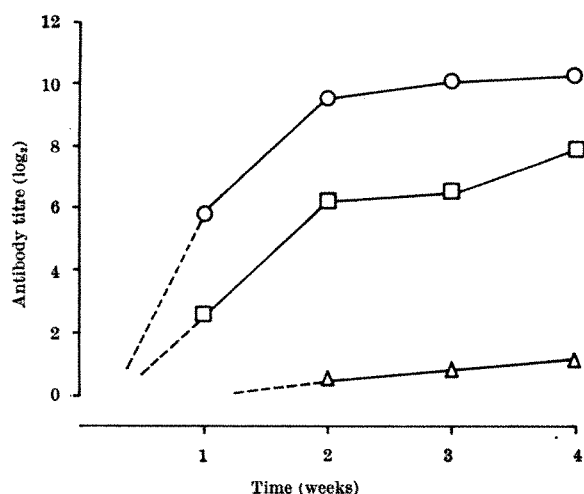


Fig. 2. Antibody titres in rats which had been subjected during 4 weeks to daily injections of either polymer (O), flagellin (□) or a cyanogen bromide digest of flagellin (Δ).

measured by the bacterial immobilization technique which has a sensitivity of about 15 ng/ml. of antibody—unpublished results of Lang and Ada). In contrast, the digest was as efficient as flagellin in (a) inducing antibody formation when injected with complete Freund's adjuvant and (b) initiating a secondary response in rats previously injected with flagellin. The possibility that the digest injected in saline was effective in inducing tolerance in adult rats was studied by injecting groups of 6–7 week old rats intraperitoneally on day 1 with 500  $\mu$ g, and on each successive day for 4 weeks with 100  $\mu$ g of either polymer, flagellin or the complete digest. The antibody titres which resulted are shown in Fig. 2. The repeated injection of either polymer or flagellin resulted in high titres of antibody whereas very low titres of antibody were observed after injection of the digest. Thirteen out of twenty rats in the latter group showed no detectable antibody production. These thirteen rats were challenged immediately at the end of the 4 week period by injections into the footpad as follows: five with 100  $\mu$ g of flagellin in saline; five with 100  $\mu$ g of flagellin in complete Freund's

adjuvant; and three with 100  $\mu$ g of polymer in saline. At the same time other rats (not previously injected) were injected with 100  $\mu$ g of flagellin in saline or in complete Freund's adjuvant. In addition, those rats which had received the course of injections of 100  $\mu$ g of flagellin for four weeks were challenged with a further dose of 100  $\mu$ g of flagellin (injected into the hind footpads). The results in Fig. 3 clearly show that rats receiving the complete course of flagellin injections maintained high antibody titres. In contrast, those rats which had received the full course of injections of digest were almost completely tolerant, not only to flagellin in saline but also to flagellin in complete Freund's adjuvant. Each of the three rats which had been challenged with polymer gave low but definite antibody titres, the average value at 3 weeks being less than 10 per cent of control values. At present experiments are in progress to see whether only fragment A of the digest is necessary to confer tolerance in adult rats. It seems likely that a decrease in the size of the antigen molecule reduces the immunogenic potential but allows adult tolerance to be induced.

It seems important to extend this work to other systems (unpublished results of Bonavida, Miller and Sercarz). It is possible, for example, that if a histo-compatibility antigen could be partially degraded without loss of antigenic determinants injection of such a preparation could bring about tolerance in adult animals not only to the intact antigen but possibly also to the cells from which the antigen was derived.

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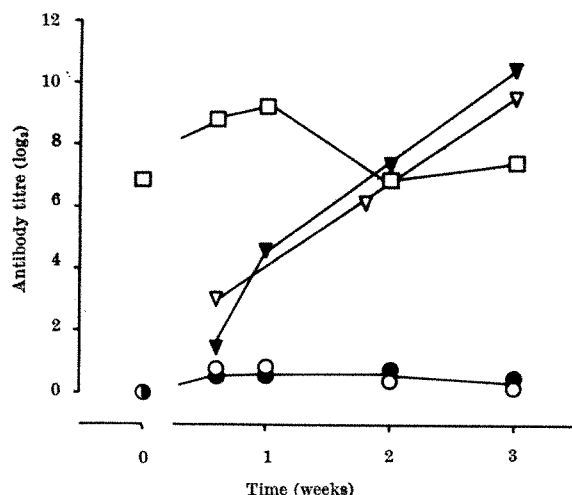


Fig. 3. Antibody titres in rats which had been given for 4 weeks a course of injections of (a) flagellin and challenged with flagellin in saline (□), and (b) cyanogen bromide digest of polymer and challenged with a single dose of flagellin in saline (○), or flagellin in complete Freund's adjuvant (CFA) (●). Control (previously uninjected) rats were injected with flagellin in saline (▽) or flagellin in CFA (▼).

## PSYCHOLOGY

### Timing of Cognitive Responses in Naming Tasks

THE processes of perception are currently envisaged as organized into hierarchies of tests for criterial attributes of stimuli<sup>1-3</sup>. Analysis of stimulus information may be total or partial, and the tests arranged in series or in parallel. This general conceptual approach derives primarily from studies of intelligibility in dichotic listening, but finds support from studies of word recognition, and from visual search studies in which time is the principal<sup>4</sup> measure of performance.

Theories of perceptual organization have been based primarily on error data, but it seems important to take latency data into account also in any attempt to develop current models. This view is encouraged by the fact that in a short term memory task, error and latency data may behave quite differently in response to experimental treatments<sup>5</sup>. There is less direct evidence from perceptual studies, but Pollack's demonstration<sup>6</sup> of differences in reading speed according to whether subjects named individual items or their class membership may be com-



pared with Moray's<sup>7</sup> failure to find differences in recognition thresholds assessed on the basis of item or superordinate responses.

Oldfield and Wingfield<sup>8</sup> recently showed that latencies of discrete naming responses to drawings of objects depend on the frequency of the object name in the language. They interpret the latency differences as reflecting relative access times to the requisite parts of a "neural dictionary", and hence as throwing light on underlying systems of classification in the brain. The general method seems versatile and is suitable for the investigation of other wider aspects of cognitive organization often subsumed under "thinking" and "sensorimotor skills"<sup>9,10</sup>.

The experiments reported here were planned to illustrate some advantages of the approach, rather than to produce evidence at this stage favouring any specific theory. They concern principally the effect on response latencies of the nature of the coding operations carried out. In both experiments, the responses were single common words relating to stimulus dimensions. The tasks could be regarded, in part at least, as search tasks without a spatial component.

Each stimulus complex comprised a superimposed letter and digit stencilled on white card, in contour to avoid mutual occlusion. One member of each digit-letter pair was red, the other green. Twenty-four letters, I and O excluded, occurred twice, once in each colour. The digits 2-9 inclusive occurred in random combination with the letters, with frequencies balanced as far as possible. The cards were exposed in a tachistoscope for 0.5 sec in experiment 1, and 1.0 sec in experiment 2.

Verbal response latencies were measured with a 'Digitron' timer which was started at the onset of presentation and stopped by the subject's voice through a throat microphone and voice key relay. Presentations were manually controlled by the experimenter in the first experiment. In experiment 2, the subject was given control of presentation in order to try to increase the sensitivity of the method.

Twelve male undergraduates in each experiment met all conditions (see Table 1) in balanced Latin square designs. They were told to respond as rapidly as possible and to avoid errors. Each condition occurred in a block of eight consecutive trials in experiment 1 and of twelve consecutive trials in experiment 2. The conditions were described to the subject before the experiment began, and again before each block of trials, and specific instructions were given before each single trial. In conditions 5 and 6, the specified class and colour respectively alternated on successive trials. In experiment 1, there was no training beforehand. In experiment 2, one practice series was given for each condition in counterbalanced order.

Table 1. CONDITIONS AND MEAN MEDIAN LATENCIES

Condition	Task requirement	Reaction time (msec)			
		Exp. 1	$P < 0.05$	$P < 0.01$	Exp. 2
1	Name number, given its colour	739	}	}	594
2	Name number	778			627
3	Name colour, given the number	884			
4	Name item, given a colour	955	}	}	
5	Name class, given a colour	1,109			
6	Name colour, given a class	1,163			

Brackets enclose means which do not differ significantly at the specified levels.

The overall error rate was very low (around 1-2 per cent). In experiment 1, the significances of differences in response speeds between conditions were assessed by a Keuls extension to a parametric analysis of variance of individual median latencies for correct responses<sup>11</sup>. The results are summarized in Table 1. A similar analysis of variance in experiment 2 showed that the difference between conditions 1 and 2 was significant ( $P < 0.05$ ) in this case. Effects of order (practice effects) and subjects were significant in both cases.

The results seem to warrant the following conclusions.

**Item Responses.** A small advantage can be gained if the subject knows the colour of the target item. This

finding extends the generality of results of visual search tasks<sup>12,13</sup> to a situation in which relevant and non-relevant shape information have a common spatial source (conditions 1 and 2 in experiment 2).

Item naming is slowed by enlarging the stimulus ensemble, or more probably by mixing response classes (compare Pollack<sup>4</sup>) (conditions 2 and 4).

**Colour Responses.** Cognitive operations take longer on the basis of prior class information than if the "target" item is specified (conditions 3 and 6).

**Class Responses.** Superordinate responses are considerably slower than item responses (conditions 1 and 4 versus condition 5). Handling of superordinate information in general (conditions 5 and 6) seems rather inefficient in terms of speed, although there was little or no evidence of lower accuracy of performance.

**Other Comparisons.** Advance colour information seems more advantageous than advance shape information (conditions 1 and 3). This may be because colour analysis is usually carried out at earlier stages in the central nervous system than shape analysis, and may be completed sooner.

When the colour is known, the naming of digits is fastest, the naming of items (either class) is next, and naming superordinate classes is slowest.

Insofar as digits are named more quickly when some sensory attribute (colour) of the target item is known, the results appear consistent with a hierarchical classificatory system like that of Treisman<sup>3</sup>. The markedly longer latencies for specifying the broader, superordinate class, however, would not be predicted on her model. They suggest that the identification of individual items may represent the end of a hierarchical chain, and are more consistent with the slightly different cognitive grouping of Forgas<sup>2</sup>. It is probably relevant that class identifications concern the utilization of disjunctive stimulus attributes.

The data thus suggest inferences about the organization of classificatory processes in cognitive tasks. These depend on more systematic study of the effects of extended practice and on more detailed analysis of the data than has been attempted here. It seems probable, however, that the results as they stand could be accommodated by three principles. (a) Information handling is speeded if the subject is able to focus on a relevant item immediately; (b) an increase in the amount of information to be handled leads to slowing of response; (c) Response latency increases with the number of processing stages through which information passes (compare Fitts<sup>10</sup>). Furthermore, a verbal latency method may yield reliable differences between conditions with a surprisingly small number of readings.

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## BOOK REVIEWS

### AN INSTRUMENT DEVELOPS

#### Historical Aspects of Microscopy

(Papers read at a one-day conference held by the Royal Microscopical Society at Oxford, 18 March, 1966.) Edited by S. Bradbury and G. L'E. Turner. Pp. 227. (Cambridge: W. Heffer and Sons, Ltd., 1967.) 42s. net.

#### The Evolution of the Microscope

By S. Bradbury. Pp. x+357. (Oxford, London and New York: Pergamon Press, Ltd., 1967.) 80s. net.

THE papers from a meeting of the Royal Microscopical Society at Oxford are now published in the form of a book of essays in which experts deal with six subjects connected more or less directly with the history of the microscope. Some of these topics are of a general philosophical kind, some deal particularly with the instruments collected in the Oxford Museum of the History of Science and elsewhere. The contribution by Dr A. C. Crombie deals with the emergence of the physiology, psychology and philosophy of vision, the most fascinating of the special senses.

The establishment of communication between the mechanisms of nature and the spirit of man clearly involved difficulties of conception which did not disappear when the optical system of the eye had been recognized as analogous to that of artificial instruments. Kepler at the outset of the seventeenth century, and Descartes a few decades later, pursued the problem, and physiological optics remained attractive for the remainder of the century to Christopher Wren and to Isaac Newton himself.

A paper by Joseph Needham and Lu Gwei-Djen reveals from a study of local historical sources a group of men of the seventeenth century who worked in south-east China on making spectacles and other instruments. There is some difficulty about translating and interpreting the names of these instruments; they seem to include both telescopes and microscopes as well as spectacles, magic lanterns, and searchlight projectors.

A paper by Dr J. R. Levene is concerned with the accommodation mechanism of the eye as it appeared before, and early in, the nineteenth century. Young presented a paper on the subject in 1793, and Sir Everard Home devoted his Royal Society Croonian lecture in 1794 to experiments to find whether the crystalline lens and its elastic capsule were responsible for accommodation. He came to the conclusion that accommodation was possible when the lens had been surgically removed, and with the collaboration of the celebrated instrument maker Ramsden, who devised an instrument for measuring changes in the curvature of the cornea, he concluded that curvature changes in this could produce some of the accommodation and that changes in the length of the eyeball could produce the rest.

An interesting paper by Dr S. Bradbury specifically devoted to the instrumental history of the microscope gives the results of applying modern test objects and comparison with modern optical systems to early microscopes in museum collections, particularly English instruments of the era 1700-1840. The old instruments were corrected individually and empirically, and not by the application of systematic optical design methods, and so it is not surprising that they are inconsistent in quality one with another. They are usually heavily afflicted with chromatic and spherical aberration, even after the aperture has been stopped down, so that the resolution is much reduced compared with that usually attained by

modern optical systems of the same magnifying power. The theoretical work of J. J. Lister about 1830 on the removal of aberration was followed by the improvements in technology which allowed the production of satisfactory achromatic objectives.

A paper by Mr G. L'E. Turner is concerned with the quantitative aspect of the increase in microscope resolution over the years. This can be followed largely because of the contemporary use of test objects in the form of gratings ruled on glass by the German Nobert. The production of these plates had a definite effect in encouraging the improvement of objectives, because they were made with progressively finer rulings and the last of them went beyond the theoretical limit of the optical microscope. Mr Turner and Dr Bradley examined one of Nobert's test plates owned by the Oxford Museum, using an electron microscope and a replica technique.

The paper by T. Mulvey deals with the early history of the electron microscope which lies quite near to our own day, but which is very interesting to read in summary form. When Abbe had shown that the wavelength of light set an inescapable limit to the resolution of the optical microscope, he realized that a new sort of radiation of short wavelength was needed, but it was not then apparent that electrons filled this need. In the 1920s electron beams had an important application in oscillography and were being studied, particularly in Germany, on that account. The science and technology of electron optics arose in the hands of Gabor, Busch and others. Gabor, working then in Berlin, was responsible for the short ironclad coil as an electron lens, and from this device arose the two-lens microscope of Ruska and Knoll. In 1934 it was found possible to use an electron microscope to photograph a botanical specimen stained with a heavy metal, and to surpass the resolution of the optical microscope. Development and commercial construction of electron microscopes took place in Germany, Britain and the United States, France, Holland and Japan. The instrument entered the decade of 1940 in a well-engineered state capable of continued development, which, of course, goes on.

During the past four hundred years the microscope has kept its place as the most characteristic instrument of biological science, and it is most interesting to have its history laid out in the book by Dr Bradbury. For a physicist it is chastening to find how little has arisen from the systematic use of optical theory—the first man to do so with effect was probably J. J. Lister, the father of the surgeon, who in 1830 showed that a doublet lens could be freed simultaneously of chromatic and spherical aberrations for a particular position of object and image. More recently, about 1880, Abbe applied wave optics to microscope resolution, revealing that the smallest distance which could be resolved was limited by the wavelength of light and the angle of the light which could be accepted by the object glass. With Abbe the microscope entered the phase of fully scientific design and advanced manufacture, for he not only recognized the natural limit of resolution, but he designed apochromatic objectives with unexampled sophistication of correction. This enterprise required the foundation of a new optical glass industry, and Abbe was mainly responsible for the administrative and industrial innovations of the Zeiss-Schott organization which established for the time the overwhelming superiority of the German optical industry. But until these comparatively recent times the microscope developed by the efforts of isolated scientific enthusiasts and craftsmen-artists. In early days the "single microscope", a powerful magnifying glass, competed successfully with the two-lens "compound microscope", and one of Bradbury's chapters is devoted to single microscopes, particularly between 1650 and 1820. Their most conspicuous disadvantage was the very short working distance, which made them uncomfortable to use. One of their most famous users was Leeuwenhoek,

who worked at Delft about 1700, and who communicated regularly with the Royal Society. He has some claim to be considered the founder of the sciences of bacteriology and protozoology. Some of the simple microscopes which he constructed for himself survive and show a resolution of three or four microns. Compound microscopes were regularly constructed in the eighteenth century, and achromatic objectives began to appear about 1800

H. J. J. BRADDICK

## MATHEMATICS AND PROBABILITY

### Green's Function Methods in Probability Theory

By Julian Keilson. (Griffin's Statistical Monographs and Courses, No. 17.) Pp. viii+220. (London: Charles Griffin and Co., Ltd., 1965.) 40s. net.

THE formal identity between mass distribution and probability distribution is so exact that it is always surprising to see how independently the mathematical methodologies of the two fields have developed. The statistician who reflects on the stimulus the problem of moments gave to Karl Pearson's foundation of the modern theory of statistics is apt to wonder whether a second such stimulus is not imminent. This book is not it. Yet this project has been so thoroughly and effectively carried out that one is tempted to infer that the two fields have evolved beyond the point where hybridization remains fertile.

Dr Keilson gave the material of the book as lectures when he was visiting the University of Birmingham and some of us in other universities were able to read the cyclostyled course-notes. The published version has been heavily revised with the help of Dr D. M. G. Wishart so that not only is it extremely clear and easy to read but one may be sure that no possible application to probability of the Green's function methods discussed here will have been overlooked. The facts that (as earlier reviewers have noted) substantially no result has been obtained that is not already known to probabilists in the chosen field of application (Markov processes with associated boundary problems), and that on the whole the probabilists' methods are simpler, if more *ad hoc*, suggest that Green's function methods serve no function in probability theory.

D. E. BARTON

## HARDWARE AND SOFTWARE

### Computer Programming and Computer Systems

By Anthony Hassitt. (Academic Press Textbooks in the Computer Sciences edited by Anthony Ralston.) Pp. x+374. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1967.) 86s.

Now that we have sophisticated problem-oriented languages, writing a computer programme is easy. It is still difficult, however, to write a good programme because to do this we need to understand how the software interacts with the hardware, that is, how the systems programmes that convert the higher-level language into the machine-level language and control the job-sequence interact with each other and with the processor, the store and the peripheral devices. This book is a praiseworthy attempt to introduce these concepts to scientific programmers with a basic knowledge of Fortran or another compiler language, and to impart to them a "reading knowledge" of the language produced by the compiler. It is not intended to teach the student, still less to encourage him, to write programmes in machine-level languages; as the author states, the days are past when machine time could be taken as the sole index of efficiency in programming.

That this laudable object of getting the programmer to understand the backstage workings of the compiler is not likely to be wholly attained is not so much the fault of the author, who obviously has considerable relevant experience and expounds his material clearly, as of the

time when the book was written—a time of great change in computers and the ways of using them. Much of the discussion is based on the 7090 and its monitor system and Fortran compiler, and so has to describe the surmounting of many difficulties that would not arise with a more modern computer system and a language better adapted for character and file handling. Although the current practice is described also, it would be much less confusing for the student if the historical tribulations were confined to the excellent introductory chapter.

The dual intention, of providing a textbook for the student of computer science and a reference book for the scientific research worker using the computer as a tool for solving his own problems, has caused the treatment of machine-level language to fall between two stools: it is rather too shallow for the former and too detailed for the latter, who might benefit more from a macroscopic flowchart approach to compilers than from the microscopic code approach. The book is arranged sensibly for use as a student text, with problems at the end of each chapter, but the organization of the material is not ideal for its potentially more valuable use as a reference book. The index in particular is not satisfactory, and one has to refer frequently to the contents list as a supplement: for example, although system 360 is discussed in detail in several chapters, the only index reference to it is to the explanation of virtual memory and time-sharing principles as exemplified in the 360/67. One of the most useful features is the excellent critical comparison of different implementations of Fortran, but the index entry "Fortran" gives ten references without stating which are general, which are to specific dialects and which are to the comparative discussions.

The book should be very useful to conscientious programmers using batch-processing techniques, despite the shortcomings mentioned. A second edition could be considerably improved by the inclusion of more information on disk-oriented systems, an introduction to the functioning of command-language systems for conversational working, and some drastic pruning of the discussion of obsolescent systems. An aesthetic improvement would be the upgrading of the printing of some tables which have been reproduced direct from line-printer output, an irritating design fault in an otherwise well-produced book.

JOHN HAWGOOD

## ADVANCES IN BASIC SCIENCE

### Some Recent Advances in the Basic Sciences

Edited by A. Gelbart. Vol. 1: 1962, 1963, 1964. (Belfer Graduate School of Science—Annual Science Conference Proceedings.) Pp. xii+228. (New York, N.Y.: Belfer Graduate School of Science, Yeshiva University, 1966. Distributed by Academic Press, New York and London.) 68s.

THE Belfer Graduate School of Science at Yeshiva University has sponsored annual conferences on "Some Recent Advances in the Basic Sciences" since 1962. This beautifully produced volume contains the proceedings of three such conferences.

Basic science here means mathematics and physics, with equal emphasis on both subjects. We are thus presented with fourteen discourses which cover the entire field of the exact sciences. The authors are, as one would expect, of impeccable standing in their subjects and they have interpreted their task in very varying ways. He would be a bold reviewer who attempted to do justice to this fare.

Starting with physics, two contributions, one by C. H. Townes, R. Y. Chiao and E. Garmire on "The Interaction of Intense Light Waves and Mechanical Motions in Extended Media", the other from W. A. Fowler, "A Quasar Model based on Relaxation Oscillations in Supermassive Stars"

are models of scientific writing, and can be read with enjoyment by any scientist. C. N. Yang's brief note, "The Mass Formula of  $SU_3$ ", deals with a problem which has since found a reasonably satisfactory solution, whereas Professor Dirac's lecture on the "Foundation of Quantum Mechanics" is concerned with showing that the Heisenberg and Schrödinger pictures do not necessarily provide the same answers in quantum field theory. Both these contributions are really highly technical, although written in a way which obscures this fact. R. Serber's talk on "High Energy Scattering" deals very nicely with the application of optical and diffraction theoretical techniques in this area, and an amusing discourse on "Weather Modification: Prospects and Problems", by G. J. F. McDonald, makes for light reading. Finally, P. Bergmann's talk on "General Relativity in Contemporary Physics" is a non-technical exposition in a somewhat conservative hue.

Turning to the mathematics, things get very tough. Only Chern on "Geometric Structures on Manifolds and Submanifolds" makes any concessions to the non-initiated. Harish-Chandra on "Harmonic Analysis on Semisimple Lie Groups", N. Jacobson on "Forms of Algebras", A. Beurling on "Local Harmonic Analysis", D. C. Spencer on "The Theory of Harmonic Intervals", O. Zariski on "Equisingular Points on Algebraic Varieties", L. V. Ahlfors on "Kleinian Groups", and S. Bochner on "Analytic Measures on Compact Bohr Groups" are contributions moving near the very frontier of mathematical research, and all demand a highly sophisticated level of mathematical education. The tempestuous development of modern pure mathematics towards ever greater generalization and abstraction coupled with extreme precision is here displayed once more, and much hard work will be required before the gap separating these truly marvellous achievements from all the natural sciences is reduced to manageable proportions.

S. ZIENAU

## METALLURGICAL THERMOCHEMISTRY

### Metallurgical Thermochemistry

By O. Kubaschewski, E. L. Evans and C. B. Alcock. (International Series of Monographs in Metal Physics and Physical Metallurgy, Vol. 1.) Pp. xix+495. (Oxford, London and New York: Pergamon Press, Ltd., 1967.) 75s. net.

REVISED and enlarged, the new edition of this well known book retains the general form and content familiar from the editions of 1955 and 1958. A direct interchange in the previous order of the fourth and fifth chapters, however, now emphasizes the book's dual character. For it essentially comprises two separate but related monographs: the first provides an introduction to the principles and practice of applied thermochemistry, and the second is a convenient collection of thermochemical data relevant to metallurgical problems.

The concise survey of important basic principles and relationships presented in the first chapter has been slightly extended. With the aim of providing a clearer and more logical presentation, occasional modifications have been made in the division and order of the material and in the treatment of some concepts. Brief presentations of some interpolation formulae and atomistic solution models are now included. The latter provide an improved introduction of the concepts of Raoultian, Henrian and regular solution, but the treatments of spinodal decomposition and order-disorder appear somewhat out of context and are insufficiently developed to be of great value.

Although slightly shortened, the chapter surveying experimental methods still provides a comprehensive and once more up to date survey of techniques. Again minor

rearrangements have been made, as, for example, in adopting the contemporary classification of calorimeters as isoperibol, isothermal or adiabatic in character; the newer applications of high temperature adiabatic calorimetry, microcalorimetry and fluorine bomb calorimetry are noted. Developments in the techniques for the measurement of vapour pressures and the extensive application of solid oxide electrolytes for measurements of e.m.f. are also reflected in the review.

Least changed is the chapter dealing with the estimation of thermochemical data, but brief mention is now made of more recent attempts at the empirical estimation of entropies and of the relation of electronegativity to heats of formation.

The pedagogically invaluable fourth chapter, which provides worked examples of the thermochemical treatment of metallurgical problems, has been expanded from eight to thirteen examples. Among the new examples are calculations pertaining to the extraction of nuclear and other metals, the evaporation of ceramic materials, and metal-refractory equilibria at very high temperatures. These and the modified examples of equilibrium diagram calculation appropriately reflect the current and developing interests of metallurgists and demonstrate the importance of thermochemical studies in these fields.

As would be expected after an interval of nine years, perhaps the greatest changes are to be found in the collection of thermochemical data. This monograph within a monograph has been brought up to date (1965) and now runs to 183 pages and lists 661 references to original publications or other reviews of data. The intense experimental activity of past years has resulted in the revision of many of the previous values; a considerable amount of additional information, some from unpublished work, is also incorporated.

The publication of this fourth edition will be appreciated by workers in the field who have come to regard "Kubaschewski" as their normal first source of information on experimental techniques and available thermochemical data. By its clear demonstration that metallurgical thermodynamics is a challenging experimental science, providing a powerful tool for the treatment of real problems, it will also continue to assist in convincing further generations of students that the fundamentals and applications of thermodynamics are subjects meriting their earnest attention.

JOHN N. PRATT

## FUROPYRANS AND FUOPYRONES

### Fuopyrans and Fuopyrones

By Ahmed Mustafa. (The Chemistry of Heterocyclic Compounds: a Series of Monographs.) Pp. xii+376. (London and New York: Interscience Publishers, a Division of John Wiley and Sons, 1967.) 135s.

THE publication of a book concerned with the chemistry of oxygen heterocyclic compounds is always very welcome, especially when it fills an interesting and well defined gap in the chemical literature.

The plant phenols are a group of compounds of great interest to certain organic chemists, some of whom give a prominent position to naturally occurring products which have the fuopyrans and fuopyrones as parent compounds. Knowledge of these and related compounds has developed through the structural determinations of products obtained from plants and plant extracts with certain intriguing properties such as their use as an aid in catching fish or as medicinals and insecticides.

The chapters of the book all follow the same general pattern, starting with an introduction to a specific topic followed by a mention of the chemistry of a number of naturally occurring compounds. Accounts dealing with the determination of structure of such compounds are very concise, but include all the relevant degradation



steps and chemical properties of the original compounds and their degradation products. In fact, in a number of cases, the expressed evidence for the structure of a particular naturally occurring compound is a précis of the original paper. This presentation of the information gathered from an investigation is very useful to a researcher working in related fields and may save time when he wishes to consult the literature. Quite frequently the physiological activity of compounds is emphasized, and a brief reference is made to the biosynthesis of furocoumarins. Adequate coverage is given to the physical properties of compounds. Chapters deal with the chemistry of the furocoumarins, furochromones, furoxanthones, furoflavones, furoisoflavanoids and chromanochromanones (the Rotenoids) in some detail, giving an extensive background knowledge to anybody interested in these topics. In reviewing a book of this type it is very difficult to single out any particular item for special reference, because the number of compounds mentioned is so very large and nearly all of them are treated with the same degree of importance.

It is easy to see that a great deal of work and endeavour has gone into producing this excellent book and it will without doubt find a place in all chemical libraries. This book provides a useful summary of information which will be of great value to research workers undertaking investigations in the oxygen heterocyclic field. The layout of the book is very good and it is easy to praise the excellent diagrams and tables, which are a great help to the reader. In mentioning the inclusion of the more than adequate number of references, it is worth drawing attention to the fact that every effort has been made by the author to include papers indexed by *American Chemical Abstracts*, up to and including 1964, and subsequent papers in the more important journals up to December 1965.

R. LIVINGSTONE

## SORTING STEROIDS

### The Gas Liquid Chromatography of Steroids

Edited by J. K. Grant. (Memoirs of the Society for Endocrinology No. 16.) (Proceedings of a Symposium held at the University of Glasgow on April 4-6, 1966.) Pp. viii + 294. (London and New York: Cambridge University Press, 1967.) 70s. net; \$13.50.

THIS further edition in the series of Memoirs of the Society for Endocrinology contains the sixteen papers (one as an abstract) presented at the symposium on the "Gas Liquid Chromatography of Steroids" and also the verbatim report of the subsequent discussions. The stated purpose of the symposium was to bring together the European workers in the steroid field to discuss and assess the present position of gas liquid chromatography and to consider new techniques to be used in this field and also in conjunction with it. Some of the speakers were not working on steroids and so it was hoped that their contribution would prove both critical and provocative. As the editor states in the preface, however, more was gained in informal rather than formal discussion and, as a result, such benefit is not recorded. As was to be expected, the standard of papers is, on the whole, high.

The first three papers deal mainly with the basic technical problems of gas liquid chromatography and include an excellent discussion of the characteristics of capillary columns, as well as a useful and informative description of the handling of micro-litre quantities. Solutions to some of these basic problems are proposed and, while most are acceptable, perhaps it is a little unreasonable to hope that "instrument manufacturers will improve the design of all their Chromatographers" (page 6—an unfortunate transposition).

There follows an excellent paper on the conditions required for the separation of steroids by this technique.

This is a very condensed and precise contribution which deals with such aspects as the effects of column and column packing, alterations in the stationary phase, the preparation of and the effects of derivative formation and the relationship between the structure of steroids and their behaviour when subjected to gas liquid chromatography.

The principles of the systematic analysis of steroids are then considered briefly, with references to the practical details already published. The results of the application of such a scheme to the study of free and conjugated steroids in human placenta are of considerable interest and the scheme itself indicates the amount of chemical manipulation required before such extracts can be applied to gas liquid chromatography columns. The need for this preparation is borne out very well in subsequent papers on determination of oestrogens, the use of electron capture in the ultramicro detection of steroids and the fractionation of steroids before gas chromatography. The combined use of an electron capture detector with heptafluorobutyrate derivatives of steroids appears to offer greatly increased sensitivity and may well lead to the greater use of these chromatographic techniques in steroid analysis.

The many aspects of the quantitative determination of steroids are dealt with very adequately and this paper underlines the great care that is required before useful results can be obtained.

The estimation of cortisol and prednisolone and the use of haloalkylsilyl ether steroid derivatives are discussed in subsequent papers, and also the problems of investigating such complex mixtures as the metabolites of plant sterols in faeces.

Techniques, such as mass spectrometry and those involving radiochemistry, which are less commonly used in steroid gas liquid chromatography are the subjects of the following three papers. It is perhaps unfortunate that only an abstract is given of one of these papers, for its inclusion would have afforded an opportunity to evaluate the relative merits of the discontinuous and the continuous isotope counting techniques.

The organizers of the symposium are to be congratulated on the success of their meeting and this book is a useful addition to the literature.

D. B. HORN

## CELLULAR ORGANIZATION

### Molecular Insights into the Living Process

By David E. Green and Robert F. Goldberger. Pp. xii + 420. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1967.) 78s.

THE authors describe this popular account of molecular biochemistry and cellular organization as being written in a narrative style. It soon transpires, however, that this amounts to serving the molecular "meat" in an anthropomorphic "gravy". Thus the reader is asked to admire "Nature as a brilliant chemist" or "Nature's exploitation of transition state chemistry"—banalities which neither improve the literary flow nor aid the understanding. The authors' own uneasiness about this approach has unfortunately only prompted them to emphasize a Darwinian view of the evolution of molecules which is surely misapplied and will undoubtedly add to the general confusion about this subject.

The central theme of this book, as might be expected, is contained in a ninety page account of the mitochondrion and electron transport system (energy transduction) and the structure and properties of cell membranes. The descriptions are clear and the accompanying diagrams and electron micrographs are good. It does not matter that the ultimate location of enzymes within the mitochondrion is still a subject of some controversy and may require some reappraisal in the future.

Unfortunately, the remainder of the book is very patchy in quality. The opening chapters augur well with an

account of the fitness of elements and molecules for their roles in living systems, although the functional roles of nitrogen, oxygen and sulphur might have been considered as a contrast to the structural role of carbon. Perhaps more disturbing are the decisions to commit a description of the hydrogen bond to a footnote and omit any account of pH and ionization phenomena, which result in much of the subsequent account of proteins and enzymes, in particular, seeming rather unrealistic.

As the authors state, enzymes play a central part in all biological processes. The clarity of exposition of what enzymes are and how we think they work is a needle test of any elementary treatment. *Molecular Insights* . . . does not come out well. A confusion between the structures of fumaric and maleic acid unhappily opens a general account of the properties of enzymes, which neglects to explain the concept of the active site and provides an inadequate background for the discussion of enzyme control (illustrated by the over-complex phosphorylase system) which follows. An attempt to explain the mechanism of catalysis by chymotrypsin fails largely because of small, confusing diagrams (how beautifully this can be presented is shown by the large, clear diagrams by Hartley in *Structure and Activity of Enzymes*). Understanding is not helped by a text statement that two histidines are involved while only one is shown in the diagrams. A later account of the acetokinase reaction is easier to follow but again the accompanying diagrams are much too small.

Coenzymes and prosthetic groups are treated in a fairly conventional way, but emphasizing the functional atoms of each molecule, and the section concludes with an interesting discussion of the chemistry of reduction of DPN<sup>+</sup> (the recommendations of the International Commission on Enzymes are not followed) although not everybody would agree that the carboxamide group plays no part in the process.

Consideration of cell energy processes begins with a chapter on bioenergetics which leans heavily on the "high energy bond"—a concept which even if ultimately explained is better avoided. Surely it is no more difficult to explain at the outset that forming the structures of some molecules requires considerable energy, most of which can be released again when the molecule is broken down, rather than baldly stating that the energy is held in the bond and leaving the reader to puzzle over why some molecules can have such bonds and others cannot. The more rational nature of this approach would have benefited the subsequent discussion in which various reactions of ATP are considered.

The nucleic acids and protein synthesis are not well handled. A diagram of the cytosine-guanine base pair shows only two hydrogen bonds and, in contrast to the mitochondrion, the structures of tRNA and the ribosome are completely ignored while the function of the latter in simultaneously binding the messenger and tRNA only emerges in a later discussion of methods for determining the genetic code. The diagrams at this point are poor and confusing. In contrast, a consideration of the extent to which the amino-acid sequence of a protein determines its three-dimensional structure is followed by a stimulating discussion of the problems involved in the biosynthesis of organelles which loses none of its impact for being, in part, outdated by the march of recent scientific events.

The twelfth chapter, a good general account of control mechanisms, is significant for the return to sanity in presenting biological ideas in a scientific manner and underlines the difficulties in understanding how such complex cellular processes evolved. This is followed by a particularly lucid and commendable account of the relationship between biochemistry and disease. Beginning with a simple genetic lesion, haemoglobin S, examples of increasing complexity are considered ending up with myocardial infarction. Each is analysed to show how complex physiological processes may be described in terms of their contributing biochemical components, an

understanding of which may ultimately lead to successful combat of the disease. The theme is continued by a chapter on the use and design of drugs for therapeutic purposes.

The book concludes by enumerating the biochemical features common to all forms of life and discussing the problems involved in the evolution of even the simplest functional cell and how future research might fruitfully be directed towards improving our understanding of organization at the molecular level.

This book is intended as a unified work to be read and appreciated as a whole. Undoubtedly, most students and many teachers would benefit from the diversity of modern ideas presented, for the most part, in an attractive and palatable way. It is a pity that the inadequate diagrams and poor content of some sections preclude its unqualified recommendation.

D. C. WATTS

## ALL ABOUT THE BROWN TROUT

### The Trout

By W. E. Frost and M. E. Brown. (The New Naturalist.) Pp. 286 + 46 plates. (London: William Collins, Sons and Co., Ltd., 1967.) 25s. net.

In the authors' preface it is stated that "We have tried to avoid the anglers' approach and equally that of the experimentalist and to present a true picture of the brown trout and how it lives in its complex environment". The book is certainly a most readable account, and will be of interest and use to anyone who wishes to know about the brown trout. It must be emphasized that the book deals with the brown trout, and the sea trout is given a very cursory treatment. Perhaps "The Brown Trout" would have been a more appropriate title.

The first chapter deals with the anatomy and physiology of the trout, and works through the various aspects of normal body function. Reproduction is discussed fully in the fourth chapter and behaviour in the eighth chapter. In some ways this first part of the book is a disappointment, because much of the information is so general that it applies to almost any species of bony fish. The treatment is superficial for the expert, and relatively unstimulating for the lay reader.

The other nine chapters are much more stimulating, indeed in some ways it might have been better if the book had started here. The second chapter discusses the taxonomy, and emphasizes the fact that the "many species" of trout described from continental and British waters belong to the one polytypic species *Salmo trutta* L. Closely related species are briefly described. In the third chapter the distribution of the trout is indicated. On page 55 six sub-species recognized by L. S. Berg are listed, but unfortunately the reader is not informed of the characters used to separate these. In view of the emphasis placed on the one polytypic species in the previous chapter it would have been interesting to know this. In addition, it is not stated when Berg arrived at these conclusions and the English translation of the fourth edition of his book<sup>1</sup> gives a rather more complex account.

The fourth chapter describes in detail the life history, gonad development, spawning migrations, spawning and development of the egg, alevin and fry stages. It is concluded with a consideration of the length of life and ultimate fate of the trout. The authors note that the trout has a natural term of life, and individuals which exceed this are rare, although the effective causes of death have not been investigated.

Age and growth form the subjects of the next chapter, describing the Petersen method for ageing fish, use of tagging and the reading of bones and scales. The structure and use of the scales for age determination are dealt with in detail and are well illustrated by photographs. The back calculation of lengths from scales is explained fully, and

the various means of expressing growth patterns are discussed. Specific growth rate is considered the most useful form of expression of these data and Appendix V gives the procedure for the calculation of the specific growth rate. Appendix VI lists specific growth rates of brown trout from various waters in the British Isles and some of these data are duplicated in Table 7 (page 101).

The heredity of the trout is considered in the sixth chapter. The trout is not a convenient subject for genetical experiments because it takes two years or longer to mature and then they breed only once a year. The studies of Alm<sup>2</sup> and Donaldson and Olson<sup>3</sup> are correctly stressed. The chapter concludes with a short section on hybrids.

The longest chapter in the book considers the physical environment. The topographic features are described and the vegetation and fauna associated with each type of environment listed. The reasons for the occurrence of trout in particular types of water are discussed. Climate is related to the annual cycle of events in the life of the trout, and the presence of an internal physiological rhythm in trout kept in a constant temperature and light intensity for a constant number of hours each day is noted. The effect of the introduction of trout to the Antipodes is also discussed in this connexion. The effects of light, temperature and water chemistry on growth of the trout are given and the chapter concludes with a consideration of the results of chemical fertilization of acid waters.

The authors emphasize in the eighth chapter that despite many records there are great gaps in our knowledge of the food of the trout. Figure 22, Tables 22-27, 29, 31 and 32 and Appendix VII compare the food of trout with the bottom fauna, show seasonal feeding, bottom fauna, vegetation and associated fauna, fauna at different depths in lakes and in rivers of increasing hardness. Unfortunately, the species of animals involved are expressed in the form "Caddis l., Mayfly n., Molluscs, snails," and so on. I am inclined to wonder if data shown in this fashion have serious scientific value. It is convenient for the lay reader, but surely the generic if not specific names of the animals could have been included? In addition, there is no discussion of the methods used for the analysis of the stomach contents of fish. Some reference to a critical review, for example that of Hynes<sup>4</sup>, would have been appropriate.

The biological environment is considered in the ninth chapter, and topics such as competition, territorial behaviour and population density are discussed. Inter-specific competition between trout and other salmonids is considered, but the authors state that almost nothing is known from British waters with the exception of the relationship between trout and char in Windermere. It is noted that such studies have been carried out in Norway and Sweden, but these receive no further comment. It would have been appropriate that at least some of these works were discussed, for example those of Nilsson<sup>5</sup>.

A rather inadequate section on parasites concludes the chapter on the biological environment. The authors comment that rather little is known about the effect of parasites on wild trout. This is essentially correct, but no reference is made to any of the work which has been carried out on the parasites of natural populations of brown trout in Continental Europe, the Soviet Union, or even in the British Isles (see, for example, Thomas<sup>6,7</sup>). There is a further adverse criticism about this section, and that is that only generic names of the various parasites are given, and only some members of these genera infect trout.

The final chapter is entitled "Trout and Man" and gives a short account of trout fishing, pollution, management of trout fisheries and improvement of the environment.

There are eight appendices dealing with the routine examination, age and growth determination from scales, data on brown trout from rivers, lakes, the calculation of specific growth rates, specific growth rates from various waters in the British Isles, examination of stomach con-

tents and a list of waters for which data on total hardness are available.

There is a list of 170 references, and a further ten authorities were quoted in the book, but have been omitted from the list. Nine authorities quoted in the text with dates are given different dates in the list of references. Frequently in the text an authority was quoted without indicating the date, a procedure which renders it difficult to refer to the original paper if more than one is listed for that person. On page 196 Jones is referred to, without indication whether J. W. Jones or J. R. E. Jones is meant. The index, however, confirms (page 280) that the work of J. R. E. Jones is being quoted. A similar comment applies to the mention of Berg on page 197, although here the context makes it clear which authority is being quoted.

The plates and figures which illustrate the book are of good quality. The index is useful and is mainly accurate. *The Trout* is, despite a few adverse comments, a very useful book. The authors are to be congratulated on writing it in such a manner that it will be of interest to layman and scientist alike. I must emphasize that in reality the book only summarizes our knowledge of the brown trout, because the sea trout receives a very cursory treatment. It is also relevant to note that the book does not always include or do justice to foreign work on the biology of the trout. Nevertheless, it is excellent value and can be recommended.

JAMES C. CHUBB

<sup>1</sup> Berg, L. S., *Freshwater Fishes of the USSR and Adjacent Countries*, fourth ed., 1 (Israel Program for Scientific Translations, 1962).

<sup>2</sup> Alm, G., *Rept. Inst. Freshw. Res. Drottningholm*, 29, 29 (1949).

<sup>3</sup> Donaldson, L. R., and Olson, P. R., *Trans. Amer. Fish. Soc.*, 85, 93 (1957).

<sup>4</sup> Hynes, H. B. N., *J. Anim. Ecol.*, 19, 36 (1950).

<sup>5</sup> Nilsson, Nils-Arvid, *Rept. Inst. Freshw. Res. Drottningholm*, 46, 58 (1965).

<sup>6</sup> Thomas, J. D., *Proc. Zool. Soc.*, 142, 459 (1964).

<sup>7</sup> Thomas, J. D., *Parasitology*, 54, 263 (1964).

## MAN AND PLANT

### Plants and Archaeology

By Geoffrey W. Dimbleby. Pp. 187 + 23 plates. (London: John Baker, 1967.) 50s. net.

PROFESSOR DIMBLEBY's book pleads a cause. He first asks those who excavate to ensure that plant remains do not escape notice, and then asks those who interpret the discoveries to do so positively and imaginatively. The book has three parts—a survey of the many diverse and ingenious uses to which man has put plants and their products, an account of the ways in which different parts of plants may be preserved and discovered, and an indication of the information that may be derived from the discoveries. One hundred and sixty books and papers are referred to, but the text is aimed at the general reader. A similar concession is not made by the illustrations, which are mainly microphotographs of very high standard of wood, fibres, seeds and pollen, more appropriate for study at the laboratory bench than in the domestic armchair.

In addition to calling for more information, Professor Dimbleby drives home in a very valuable way the profound need to separate those vegetational changes which are caused by climatic change alone, from those in which the hand of man may have been concerned. Gone are the days when we could happily rattle off as a climatic sequence Boreal, Atlantic, Sub-boreal, Sub-atlantic. Iversen and Troels-Smith have demonstrated the profound effect of the Neolithic farmer. The book cites ample evidence for Mesolithic influence on vegetation, and refers to West's tantalizing suggestion of Palaeolithic forest clearance at Hoxne.

The author pays well-merited tribute to Helbaek's work, both in northern Europe and more recently in the near East, on cereal plants and the weeds which accompany them in cultivation. Professor Dimbleby notes that the carrying of crop seed by migrating groups has been an important factor in weed distribution. Detailed

work on the first appearance of specific weeds in different areas might well throw light on the migration of different culture groups. Like King Alfred and the cakes, those who in prehistoric time were given the task of heating grain to prevent its germination during storage sometimes neglected their task, so that the mass of grain became charred and had to be jettisoned. Study of samples of such charred grain often reveals surprising differences. Sometimes the samples are full of many different weed seeds, sometimes they are remarkably clean. Different agricultural practices must lie behind such differences.

Professor Dimbleby deserves our thanks for a stimulating book.

G. F. MITCHELL

## PSYCHIATRIC SUMMING UP

### The State of Psychiatry

Essays and Addresses. By Aubrey Lewis. Pp. ix + 310. (London: Routledge and Kegan Paul, Ltd., 1967.) 63s. net.

### Inquiries in Psychiatry

Clinical and Social Investigations. By Aubrey Lewis. Pp. vii + 335. (London: Routledge and Kegan Paul, Ltd., 1967.) 63s. net.

IN these two volumes, published at the insistence of his present and former pupils, Sir Aubrey Lewis has collated the pick of his contributions to the literature of psychiatry during the past forty years. For once, the term "literature" is not inappropriate: in many of these papers, the writer shows a turn of phrase and a relish for the unfamiliar yet precise word calculated to excite the envy of a Vladimir Nabokov. This aesthetic element is apparent not only in the first volume, sub-titled "Essays and Addresses", but also in the second where it informs the writer's imaginative insight into the phenomena of mental illness—as in the earliest paper, which deals with "The experience of time in mental disorder". This penetrating clinical report has an added significance today because distortion of the sense of time is one of the striking characteristics of intoxication with cannabis or LSD.

The major work in the second volume is undoubtedly Sir Aubrey's massive clinical and prognostic study of depressive states, a study which led him to conclude that the customary practice of categorizing depression as either "endogenous" or "reactive" was an erroneous oversimplification. Curiously enough, more than thirty years after the publication of these papers, the illusory dichotomy still holds the field, although empirical studies designed to test its validity have shown conflicting results. When one re-reads Sir Aubrey's classic paper, one finds that although he suggests that the term "reactive depression" should be done away with (volume 2, page 112) he follows this almost immediately by the suggestion that in each case an assessment should be made of the degree to which reactive processes or endogenous factors enter into the illness. This suggestion, in turn, is not advanced directly but only by endorsing the words of a German neuro-psychiatrist. Here, and again in the two papers on obsessional illness, we encounter the paradox of Sir Aubrey's clinical teaching: he shows a keen perception of the subtleties of the phenomena, but is so conscious of the limitations of present knowledge that he finds it almost impossible to commit himself to a firm theoretical formulation.

This acute awareness of the complexity of psycho-physiological phenomena and of the paucity of definite knowledge about their underlying processes was what characterized his teaching. One might say that he specialized in making trainee psychiatrists realize how little they knew: but he also demonstrated, in his own work and in his discussions of current research, how this great continent of ignorance could be explored, surveyed, measured and conquered piecemeal.

His first volume contains three concise and concen-

trated articles summing up his views on the teaching of psychiatry, and also a number of papers addressed to a wider audience. Here, his two major interests are apparent: first, the history of ideas about mental disorder and the relationship of these ideas to the contemporary social and intellectual climate in earlier periods: and second, his tireless concern to promote a spirit of true scientific empirical verification in contemporary psychiatric research. If such research has a keener cutting edge today than forty years ago—as few would question—this is because its tools have been honed by the whetstone of Sir Aubrey's intellect.

G. M. CARSTAIRS

## OBITUARIES

### Dr Ann Horton

ANN CATHERINE HORTON (née Davies), who died in Cambridge on July 15, was the first woman to be appointed to the lecturing staff of the Cavendish Laboratory. She was born in London in April 1894, the daughter of Robert Davies, merchant tailor, and studied physics at Royal Holloway College where she took her B.Sc. in 1915 and her D.Sc. in 1922. Most of her published work, comprising about twenty-five papers in the *Proceedings of the Royal Society* and elsewhere appearing between 1919 and 1936, were carried out at the College in co-operation with Professor F. Horton, whom she married in 1939. The most important of these papers were concerned with radiation from and ionization potentials of the rare gases; the work emphasized the importance of a high degree of purity in gases under study, and in the years following Bohr's theory of stationary states contributed to its full verification. Her last paper "The Production of Radiation and Ionization from Helium Atoms by Potassium Positive Ions" reports work which she carried out in the Cavendish Laboratory, published by the Royal Society in 1936.

After holding a position as staff lecturer at Holloway College, Miss Davies was appointed fellow and lecturer in physics at Newnham College, Cambridge, a position she held until 1957; she was vice-principal of the college from 1936 to 1946. From 1935 until 1961 she was a university lecturer in physics and continued demonstrating to Part I classes until a few months before her death. She had many outside activities. For instance, she was a member of the Trustees of Homerton College, Cambridge, and in this capacity was particularly interested in the problems a training college has in producing good science teachers with an experimental approach to their subject. She was a member of the Council of New Hall and at a time when several groups in Cambridge had been working to secure more places for women students in the university she played a great part in formulating the practical policy, and her moderation, good sense and willingness to listen played an important part in the foundation of the college in 1954.

When her main interests had moved from original research to teaching and planning, she never lost her independent approach to the sort of problems which always arise in an active laboratory. When an experiment in the practical class gave unexpected results, or when students wanted to know something more than the working manuscript had told them, she was always ready to explore it with them, whether it concerned an unevenly loaded strip, a zone plate with badly drawn zones or a radioactive source with unexpected lifetimes. Her wide experience, her readiness to tackle difficulties and her patience in giving help where help was needed were of value not only to successive generations of students but also to her academic colleagues and the assistant staff. It would be fair to say that the unassuming way in which she took her place and her full part in a laboratory which had not previously had women lecturers made it easier for those who came after her.



## University News:

DR C. KAPLAN, at present head of the Virus Vaccine Department in the Lister Institute of Preventive Medicine, has been appointed professor of microbiology and will succeed Professor B. C. J. G. Knight as head of the Department on his retirement in September 1969. Professor V. H. Heywood, at present professor of botany in the University of Liverpool, has been appointed to the chair of botany in succession to Professor T. M. Harris.

## Reading

## Appointments

DR E. V. D. GLAZIER has been appointed director of the Royal Radar Establishment at Malvern. Dr Glazier took his BSc in 1935 and his PhD, also in engineering, in 1942. He has worked in the GPO and the Signals Research and Development Establishment. Since 1959 Dr Glazier has been at RRE and his appointment as director took effect from September 1. His predecessor Dr Macfarlane now holds the post of controller of research in the Ministry of Technology.

DR BORIS GRINBERG has been appointed director of the International Atomic Energy Agency's Division of Research and Laboratories, in succession to Dr Garman Harbottle, who is returning to Brookhaven National Laboratory.

DR JAMES R. HEIRTZLER, at present senior research associate at Columbia University's Lamont Geological Observatory, has been appointed director of the university's Hudson Laboratories in succession to Dr Alan Berman, who has been appointed director of research at the US Navy's Naval Research Laboratory.

## Announcements

PROFESSOR J. BLAMONT, scientific and technical director of the Centre National d'Etudes Spatiales and director of the Laboratoire d'Aéronomie du Centre National de la Recherche Scientifique, has been awarded the Guggenheim international astronomical prize.

THE second Jansky Lectureship has been awarded to Professor Jan H. Oort, director of the Leiden Observatory, The Netherlands, and he will deliver a lecture entitled "Large-scale Distribution and Motion of Hydrogen in the Galaxy" on November 29 at Gilmer Hall Auditorium, Charlottesville, Virginia.

PROFESSOR A. CORRADETTI, director of the Department of Parasitology and Entomology, Istituto Superiore di Sanità, Rome, and Dr Clay G. Huff, chief parasitologist at the Naval Research Institute in Washington, have been elected honorary fellows of the Royal Society of Tropical Medicine and Hygiene.

APPLICATIONS for a Meres senior studentship for medical research are invited by the Council of St. John's College, Cambridge. The candidate must be a university graduate, and will be elected for a period of not less than one year or more than three years. Further information can be obtained from the Master, St. John's College, Cambridge.

A MEDAL and award of one hundred guineas is being offered by the Royal Agricultural Society of England for research work carried out in the United Kingdom which has proved of benefit to agriculture. Recommendations for this award can be made by heads of university departments, research stations and institutes and other research organizations, and further information can be obtained from the Secretary, Royal Agricultural Society of England, 35 Belgrave Square, London, SW1.

A NUCLEOTIDE Chemistry Group of the Chemical Society is being formed in order to encourage the discussion of the chemistry of nucleosides, nucleotides and nucleic acids. Membership of the group is not confined to fellows of the society and further information can be obtained from

Dr A. S. Jones, The Chemistry Department, The University of Birmingham, P.O. Box 363, Birmingham, 15.

THE inaugural meeting of the Fisheries Society of the British Isles is to be held at the Meeting Rooms of the Zoological Society of London on Saturday, October 21, at 2.30 p.m. Further information can be obtained from Mr. Lionel E. Mawdesley-Thomas, Fisheries Society of the British Isles, Huntingdon Research Centre, Huntingdon.

## Meetings

HIGH Pressure Engineering, September 11-15, Imperial College of Science and Technology (Mr R. Glynn, Head of the Conference Department, The Institution of Mechanical Engineers, 1 Birdcage Walk, Westminster, London, SW1).

MOLECULAR Model Building, September 18-29, State University of New York at Buffalo (Mrs W. J. Browne, Laboratory of Molecular Biophysics, Department of Zoology, University of Oxford, Oxford).

CONCRETE Roads for the Future, September 20, London (The Operations Department, The Concrete Society, Terminal House, Grosvenor Gardens, London, SW1).

AMORPHOUS and Liquid Semiconductors, September 28-October 3, Bucharest (Professor R. Grigorovici, Institute of Physics of the Academy, Calea Victoriei 114, Bucharest).

ENERGY Sources for Space Power, October 2-6, Brussels (Office of the Director Plans and Programmes, AGARD, 64 Rue de Varenne, Paris).

CROP LOSSES: Evaluation of Field Losses caused by Pests and Diseases and of Factors affecting Epidemics and Outbreaks, October 2-6, Food and Agriculture Organization, Rome (Dr L. Chiarappa, Crop Protection Branch, FAO, Viale delle Terme di Caracalla, Rome).

DIELECTRIC Properties of Biological Macromolecules, October 3, Queen Elizabeth College (Dr E. H. Grant, Queen Elizabeth College, Campden Hill Road, London).

# CORRESPONDENCE

## Unfair to Aristotle

SIR,—If a dead man's reputation is wrongly and needlessly attacked, it is up to the man's friends to defend him, and if the man has been dead for more than two thousand years, the friends must be posthumous ones. In view of this, may I comment on the statement in the review of a translation of Albertus Magnus's *Book of Minerals* (*Nature*, 215, 443; 1967), that Aristotle's doctrine prevented true scientific advance based on observation for a thousand years?

In the first place, surely the writer does not really believe that Albertus was influenced by Aristotle; if his book was no advance—of no value—why translate it, or review it once translated?

In the second place, Aristotle's doctrines were not well known through most of the Christian era until the tenth or twelfth century. Until then, Greek influences were more neo-Platonic than Aristotelian; indeed, only the logical treatises were known until about the tenth century. People could not be under the influence of doctrines which they did not know.

Yours faithfully,

H. L. ARMSTRONG

Department of Physics,  
Queen's University,  
Ontario.

## FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Sunday, September 10—Tuesday, September 12

BOTANICAL SOCIETY OF THE BRITISH ISLES, in association with the LINNEAN SOCIETY OF LONDON (at the Hartley Botanical Laboratories, the University of Liverpool)—Conference on "Modern Methods in Plant Taxonomy".

Monday, September 11

INSTITUTION OF MECHANICAL ENGINEERS, AUTOMOBILE DIVISION (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Mr R. C. Teasel and Mr R. D. Miller: "Characteristics of New Ignition Systems to Improve Engine Performance".

Monday, September 11—Friday, September 15

INSTITUTION OF MECHANICAL ENGINEERS, APPLIED MECHANICS GROUP (at Imperial College of Science and Technology, London, S.W.7)—Conference on "High Pressure Engineering".

Tuesday, September 12—Wednesday, September 13

BRITISH OCCUPATIONAL HYGIENE SOCIETY (in the Clement Stephenson Theatre, School of Agriculture, University of Newcastle upon Tyne), at 9.30 a.m. daily—Conference on "Hazards in Docks and Shipyards".

Friday, September 15—Saturday, September 16

SYSTEMATICS ASSOCIATION (in the Department of Botany, The University of Birmingham, Birmingham)—Symposium on "Chemotaxonomy and Serotaxonomy".

## APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

DEMONSTRATOR or SENIOR DEMONSTRATOR in the DEPARTMENT OF PURE MATHEMATICS—The Registrar, The University, Liverpool, 3, quoting Ref. RV/158/N (September 15).

LECTURER (with qualifications in physiology or applied physiology) in PHYSIOLOGY in the Department of CHEMISTRY AND BIOLOGY—The Deputy Registrar (Personnel), University of Wales Institute of Science and Technology (Designate), King Edward VII Avenue, Cathays Park, Cardiff, quoting Ref. NAT88 (September 16).

SCIENTIFIC ASSISTANT in the DEPARTMENT OF MEDICINE for experimental work of a physiological character on the lung—The Registrar, The University, Sheffield, 10 (September 16).

TUTORIAL RESEARCH STUDENT (graduate) in PHYSIOLOGY for duties which include the supervision of laboratory classes—The Secretary, Bedford College (University of London), Regent's Park, London, N.W.1 (September 16).

SCIENTIFIC OFFICER/SENIOR SCIENTIFIC OFFICER, ZOOLOGIST (with a good honours degree combined with approved postgraduate experience) at the Nature Conservancy Scottish Headquarters, Edinburgh, to study deer behaviour and movements—The Natural Environment Research Council (E), State House, High Holborn, London, W.C.1 (September 22).

CHEMIST (with a degree or equivalent with interest in soil, applied or analytical chemistry) to work in the MICROBIOLOGY SECTION on the evaluation of effects of herbicides on chemical activities of the soil affecting soil fertility—The Secretary, A.R.C. Weed Research Organization, Kidlington, Oxford, quoting Ref. 15/67 (September 23).

JUNIOR RESEARCH ASSOCIATE (graduate with an honours degree in zoology) in the DEPARTMENT OF ZOOLOGY to work on the taxonomy of trichopteran larvae—Dr G. N. Philipson, Department of Zoology, University of Newcastle upon Tyne (September 27).

LECTURER in BIOCHEMISTRY in relation to veterinary studies—The Secretary of the University Court, The University, Glasgow (September 28).

CHAIR OF PHYSIOLOGY in the Faculty of Medicine, University College of Rhodesia, Salisbury—The Registrar (RS), University of Birmingham, P.O. Box 363, Birmingham, 15 (September 30).

EXPERIMENTAL OFFICER (with H.N.C. or equivalent qualification, and a good knowledge and practical experience of basic electronic circuit techniques using both vacuum-tube and solid-state devices) in the DEPARTMENT OF ELECTRICAL AND ELECTRONIC ENGINEERING—The Registrar, The University, Nottingham (September 30).

LECTURER (veterinary graduate) in VETERINARY STATE MEDICINE and PUBLIC HEALTH in the DEPARTMENT OF ANIMAL HEALTH, Royal (Dick) School of Veterinary Studies—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh (September 30).

RESEARCH FELLOW/ASSISTANT to work on a study of the response to vibrations and ride motions in transport vehicles—The Registrar, University College of Swansea, Singleton Park, Swansea, South Wales (September 30).

SENIOR LECTURER or LECTURER in ZOOLOGY at the University of Natal, Pietermaritzburg—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, September 30).

CHAIR OF ANTHROPOLOGY at the London School of Economics—The Academic Registrar, University of London, Senate House, London, W.C.1 (October 3).

LECTURER in ANATOMY at the University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 13).

SENIOR LECTURER in CHEMICAL ENGINEERING at the University of Cape Town—The Secretary-General, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1; and The Registrar, University of Cape Town, Private Bag, Rondebosch, Cape Town, South Africa (October 15).

CHAIR OF VETERINARY PHYSIOLOGY at the Royal Veterinary College—The Academic Registrar, University of London, Senate House, London, W.C.1 (October 25).

LECTURER in the DEPARTMENT OF NUCLEAR AND RADIATION CHEMISTRY at the University of New South Wales, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 30).

GEORGE HOIT CHAIR OF PATHOLOGY—The Registrar, The University, Liverpool (November 7).

SECOND CHAIR OF OBSTETRICS AND GYNAECOLOGY at the University of Melbourne, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, November 30).

BIOCHEMIST (Senior or Principal grade) (with an honours degree in science or its equivalent and considerable experience in hospital biochemistry) in the DEPARTMENT OF PATHOLOGY—The Secretary, Darlington Memorial Hospital, Darlington.

JUNIOR TECHNICIAN (with previous experience in histology and histochemical techniques) to join a small group working on problems of intersexuality in domestic mammals—The Secretary, Paediatric Research Unit, Guy's Hospital Medical School, London Bridge, S.E.1.

PHYSICAL CHEMIST (preferably with research experience in the field of polymer characterization, optical studies or electrical behaviour) to join a group concerned with long-range research into the study of new materials—The Personnel Officer, Arthur D. Little Research Institute, Inveresk Gate, Musselburgh, Midlothian, Scotland.

POSTDOCTORAL CHEMICAL ENGINEER, CHEMIST or PHYSICIST to investigate reactions of hydrocarbon dispersions heated in shock tubes—Professor A. G. Gaydon, F.R.S., Department of Chemical Engineering and Chemical Technology, Imperial College of Science and Technology, London, S.W.7.

POSTDOCTORAL RESEARCH ASSISTANT in the DEPARTMENT OF ZOOLOGY for the study of certain aspects of the biochemistry of oogenesis in Amphibia—Dr H. C. Macgregor, Zoology Department, University of St. Andrews, Fife, Scotland.

RENTOKIL RESEARCH ASSISTANT (graduate or prospective graduate in biochemistry, with a strong interest in animal behaviour) in the DEPARTMENT OF BIOLOGY to undertake a study of the olfactory response of rodents—The Academic Registrar (EMS), Brunel University, Kingston Lane, Hillingdon, Uxbridge, Middlesex.

RESEARCH ASSISTANT in the DEPARTMENT OF ZOOLOGY to work under Dr A. F. G. Dixon on the effects of aphids on tree growth—The Secretary, Department of Zoology, University of Glasgow, Glasgow, W.2.

RESEARCH ASSISTANTS (good honours graduates) in the PHYSICS, CHEMISTRY or ENGINEERING DEPARTMENTS—The Registrar, West Ham College of Technology, Romford Road, London, E.15.

S.R.C. POSTDOCTORAL RESEARCH FELLOW in the DEPARTMENT OF INORGANIC, PHYSICAL AND INDUSTRIAL CHEMISTRY for work on reactions of methylene (CH<sub>2</sub>) and imidogen (NH) radicals, with particular reference to their photofragmentation and reactions in condensed and gaseous phases—The Registrar, The University, Liverpool, 3, quoting Ref. RV/153/N.

SCIENCE GRADUATE to work in a metabolic unit under the Professor of Psychiatry doing full-time research on biochemical aspects of mental disorder—The Secretary, Institute of Psychiatry, De Crespigny Park, Denmark Hill, London, S.E.5.

## REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

## Great Britain and Ireland

Office of Health Economics. Pharmaceutical Research: The Case for Growth in Britain. Pp. 32. (London: Office of Health Economics, 1967.) 2s. 6d. [177]

University of Oxford. Annual Report of the Curators of the Bodleian Library for 1965-6. (Supplement No. 7 to the *University Gazette*, Vol. 97, June 1967.) Pp. 48. (Oxford: The University, 1967.) 7s. 6d. [177]

Meteorological Office. Scientific Paper No. 26: A Study of Vertical Air Motion and Particle Size in Showers Using a Doppler Radar. By Dr. P. G. F. Canon. (Met. O. 788.) Pp. 33. (London: H.M. Stationery Office, 1967.) 5s. net. [177]

Tate and Lyle, Ltd. This is Ravensbourne. Pp. 57. (Keston: Tate and Lyle, Ltd., 1967.) [187]

University of Oxford. Lord Nuffield's Benefaction for the Advancement of Medicine—Report for 1965-6. Pp. 30. (Supplement No. 8 to the *University Gazette*, Vol. 97, June 1967.) (Oxford: The University, 1967.) 2s. 6d. [197]

London and Home Counties Regional Advisory Council for Technological Education. Bulletin of Special Courses in Higher Technology, Management Studies and Commerce, 1967-68. Part 1: Autumn Term. Pp. 127. (London: London and Home Counties Regional Advisory Council for Technological Education, 1967.) 8s. 6d. [197]

## Other Countries

Canada: Department of Energy, Mines and Resources. Memoir 341: Whitbourne Map-Area, Newfoundland. By W. D. McCartney. Pp. 135 (12 plates). \$3.50. Memoir 348: Willbobe Lake and Thompson Map-Areas, Quebec and Newfoundland. By M. J. Frarey. Pp. 73 (17 plates). \$2.25. Paper 66-58: Progress Report on Low-Level Aeromagnetic Profiles over the Labrador Sea, Baffin Bay, and Across the North Atlantic Ocean. By Peter J. Hood, P. Sawatzky and Margaret E. Bower. Pp. iii+11. \$1. Paper 67-12: New Occurrences of Jurassic Rocks and Fossils in Central and Northern Yukon Territory. By Hans Frebold, Eric W. Mountjoy and D. J. Tempelman-Kluit. Pp. vii+35 (3 plates). \$1. Paper 67-23, Part 1: Progress Report on Biogeochemical Research at the Geological Survey of Canada, 1963-1966. By J. A. C. Fortescue and E. H. W. Hornbrook. Pp. xii+143 (9 plates). \$2. Paper 67-26: Further Considerations on Certain Statistical Methods in Palaeomagnetism. By A. Laroche. Pp. 10. \$0.75. (Ottawa: Queen's Printer, 1967.) [37]

Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft, Berlin-Dahlem. Heft 122 (Mai 1967): Elektronenmikroskopie von Pflanzenviren—Bibliographie 1939-1965/Electron Microscopy of Plant Viruses—Bibliography 1939-1965. Zusammengefasst von Dr. J. Brandes. Pp. 91. (Berlin-Dahlem: Biologischen Bundesanstalt für Land- und Forstwirtschaft, 1967.) 20 D.M. [57]

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## HEAD ON THE BLOCK

THE report of the Science Research Council for 1966-67 (HMSO, 6s. 9d.) is a curious mixture of resignation and cheerfulness. Like the best martyrs, the council seems determined to keep its head high and its lip stiff even in adversity. In particular, to judge from the evidence in the report, it has resolved not to cry out with anguish even when outsiders can tell that it is being starved of funds to the point where jobs which need doing cannot be done properly. This, of course, will suit the administrators and, if it comes to that, there is always the possibility that the council has been dealt with fairly in the sharing out of what is called the national cake in the past few months. But that, fortunately for the council, is somebody else's responsibility. Within its own terms of reference, it could usefully have been less diplomatic and more outspoken.

What is the outlook? In strictly financial terms, the immediate future for the Science Research Council and its dependants is not entirely black. For one thing, there is now a measure of financial stability, and the council is right to boast a little about this. After spending several years with the uncertainty of never quite knowing whether forward commitments on research grants would be honoured by the government of the day, the council (backed up by the Council for Scientific Policy) has been able to establish the view that budgets shall be agreed with the Treasury three years or so in advance. It is probably just bad luck that the system should have started operating at a time of financial pressure on public funds, but the result is to make the agreement on forward planning seem, this year, more like an unpleasant boundary condition than a guarantee of peace of mind. In the event, spending is to increase from £34.3 million in 1966-67 to £43 million in 1969-70, which represents an annual increase of just over eight per cent. Compared with the rate of growth elsewhere in the British economy, this may seem more like an explosion than mere growth. This, however, is not the point. The tests which should be applied to the question of whether the council has enough money to spend are much simpler and more specific than that. What needs to be decided is whether it can do what needs doing properly. There are unwelcome signs that it cannot.

In the council's view, the allocations for the years ahead should permit "modest progress". Plainly it has been under some pressure from the three boards which help to make decisions on the allocation of funds and which are said to have "reduced their demands below what they felt was desirable in the interests of science". Sir Bernard Lovell, the chairman of the Astronomy, Space and Radio Board, has been much more outspoken than that. After the publication of

the report he took care to emphasize that, although his own subject of radio astronomy seems to have come off quite well in the share-out, elsewhere people were being forced to make economies within forward plans which they had considered to be inadequate two years ago, when the Science Research Council was first established. And the truth is, of course, that what seems to be the ending of a period of rapid growth has come at a time when all kinds of new demands on the council's finances should have been openly acknowledged. This, after all, is a time when a great many new universities are coming into service, with fresh needs of funds for carrying out research. The time is ripe for a number of new ventures in the physical sciences. Sophistication—which is the euphemism for the increasing cost of doing good work—abounds. And there is a continuing but clamant need for effective steps somehow to realize the pious hopes there have been in recent years that postgraduate teaching would soon come of age in Britain as it has elsewhere. In the long run, it would have done more good if the council had drawn attention more sharply to these and other problems. To promise implicitly, as has been done, not to be a thorn in the flesh of the Treasury in the years ahead is both unnecessary and unwise.

The danger of a stagnation in postgraduate teaching is particularly serious. According to the figures quoted in the report, there was actually a decline in the numbers of new postgraduate studentships awarded by the council, from 1,947 in 1965 to 1,831 in 1966. Given that the council is the chief source of financial support for postgraduate students at British universities, this is an unhappy tendency. It is true that 1965 seems to have been a particularly good year, but this is a field in which it is reasonable to ask that every year should be a good year. The fact that the council has done something to soften the effects of this tendency by introducing a scheme by means of which students can sign on for postgraduate courses and then spend several years in industry before completing a higher degree is a useful innovation, but not strictly relevant to the problem of finding ways of creating a sufficient stock of highly trained young people. This is why it is, if anything, a step backwards that the council has now taken to the dubious expedient of asking that ten per cent of all training awards in physics and chemistry (and half that proportion in other subjects) should be awarded only to people working on problems which are of direct interest to some industrial company. This rule has already led to some unseemly scrambling by academics for somewhat artificial links with industry. Everybody will appreciate the council's wish to find some way of inducing more young people into industrial occupations. At this stage there is no



reason to think that the expedient which the council has hit on is more than an irritating gesture in that direction.

On research grants, penny wise may also mean pound foolish. The council says that lack of funds has forced it to turn away projects which would otherwise have qualified for grants, but, in spite of this, spending on grants has gone up from £7.8 million to £10.2 million. And it will probably do very little harm if the high-flux magnet laboratory and the high-flux beam reactor—both of them potential candidates for houseroom at Harwell—are postponed for a year or so. Those who back the reactor (at a capital cost of £7 million) will be lucky if it is provided with funds by the time the council's next report is out. It is also entirely proper that the proposal to increase the intensity of the proton beam in the NIMROD accelerator has been postponed—indeed it would have been unseemly if the nuclear physicists had pressed hard both for the NIMROD project and for a British contribution to the new CERN machine. Large items of capital equipment seem also to have done well in astronomy and in other related fields. It is particularly pleasing that the Isaac Newton telescope will soon be working, and that there will be some access to the Australian instrument in the seventies. But is it also wise to cut down on grants for high energy physics when there is evidence accumulating of how scientists from Britain make much less use than they might do of central installations such as the CERN machine at Geneva, sometimes for lack of funds? The council is right to boast of what it has done to support biology in the past few years (although its boast in the report is somewhat too reverential), but it would have been splendid if it could have given some real evidence that it is alive to the needs and interests of all the newer universities—even the smaller ones. And what, in any case, about the polytechnics?

All this implies that the council is if anything too modestly unwilling to acknowledge the central position which it occupies in the planning of academic research in Britain. Unlike the other research councils, it is deeply involved in higher education, partly through the research grants and partly through its support of postgraduate students. It has stronger industrial links than the other councils (and there are some passages in the new report which suggest that the council is not entirely sure where it stops and the Ministry of Technology begins). But this, of course, implies that the council could with advantage occupy some valuable ground which has so far tended to stay untenanted—it could play a much more vigorous part in planning the development of science in Britain than has been its inclination in the past two years. This by itself, of course, is no complaint. The council is still only two years old, and it is only now becoming clear what kinds of tasks the Council for Scientific Policy can undertake and what it must leave to others. Yet it is becoming plain that academic science is going quite soon to be as much in need of a strong and independent buffer between itself and the Department

of Education and Science as the universities were in need of the University Grants Committee several years ago. The council could make a good beginning by seeing that it represents in public, not just at committee meetings, any discontents which it may have about the financing of research.

## HOVERCRAFT AHEAD

THESE are happy days for hovercraft enthusiasts. Next month the largest hovercraft yet built will emerge from the works of the British Hovercraft Corporation at Cowes. Within the next few months, work will also begin on a flat strip of fenland north of Cambridge, where the first tracked hovercraft will be built and tested. If appearances are to be believed, the development of hovercraft in Britain is being pursued with vigour. The National Research Development Corporation will no doubt be grateful if the bustle of activity drowns for a time the criticism of the way in which development has been handled in the past.

The NRDC has been responsible for hovercraft since it first backed them in 1957. It set up a subsidiary, Hovercraft Development Limited, to handle research and the issuing of licences to companies wanting to build hovercraft. Research was carried out by a technical group at Hythe, but this was transferred earlier this year to the control of the National Physical Laboratory. Development work was carried out by the companies licensed by HDL. There seems from the start to have been a determination to concentrate the industry in a small number of powerful companies—as recently as 1966, for instance, NRDC was congratulating itself in its annual report on the merger of the two largest licensees, Westland Aircraft and Vickers, into the British Hovercraft Corporation, now the most powerful company making hovercraft in Britain. Most of its £5 million equity (65 per cent) is owned by Westland. The NRDC itself keeps 10 per cent. At the time only one other company (Cushioncraft) had been licensed, but more recently there has been a gradual change of policy on licensing agreements. In February this year, NRDC awarded a licence to Hovermarine, a Southampton company formed to specialize in submerged wall hovercraft, and work started on the first two versions of a sixty-seater hovercraft. The licence was the result of long negotiation, for the company had been formed nearly eighteen months before. This week, NRDC has announced that Vosper Thornycroft is also to be allowed into the market. The basis of the industry is thus becoming broader, although there is no evidence that NRDC has abandoned its scorn of duplication. Elsewhere, both Bell Aerosystems in the United States and Mitsubishi in Japan have negotiated licensing agreements with BHC; although this has been the occasion for some criticism, it was really no more than bowing to the inevitable. Few of the patents on hovercraft exclude other manufacturers, and sales from Britain to the United States would be

particularly difficult without a licensee. The argument is that for each hovercraft Bell and Mitsubishi build, BHC will receive royalty payments and that, if hovercraft establish themselves, there will be enough business for all three.

The British Hovercraft Corporation is hoping that the breakthrough to profitability will be soon. Next month, its first serious attempt to capture a share of the transport market, the *SRN 4*, will be rolled out from the factory at Cowes. (BHC firmly uses aircraft terminology, so that the *SRN 4* will not be "launched"; the Board of Trade, not yet sure how to define hovercraft, is to invent a special category for them.) Although the nine-ton *SRN 6*, which can carry 38 passengers, has been used on regular scheduled services within the British Isles and for pleasure trips from seaside beaches, it is a calm-water hovercraft. *SRN 4*, as well as being nearly twenty times as big, will have to operate in far worse conditions. It will weigh 160 tons, and be capable of carrying 800 commuter passengers, or 256 people and 30 cars at a speed of 70 knots. This is clearly a great advance, but potential buyers are behaving warily. So far, only two have appeared. Hoverlloyd, a Swedish company, has ordered two for a service between Ramsgate and Calais, and British Rail has ordered one. If these two operators are successful, other buyers will undoubtedly come forward.

It is hard to feel as optimistic about another hovercraft project announced last week. Several months after NRDC proposed the idea, the Ministry of Technology approved the construction and operation of a tracked hovercraft in East Anglia. The tersely worded announcement from the ministry gave few details of the project, the broad outlines of which have been established after computer studies and model testing at Hythe. The design provides for hovercars 50 feet long by 10 feet wide, weighing 10 tons and running on a T-shaped reinforced concrete beam 4 feet from the ground. The cars would be propelled by the linear induction motor developed by Professor E. R. Laithwaite at Imperial College, London, and would reach a speed of 300 m.p.h. In order to test these ideas at full scale, twenty miles of fenland north of Cambridge have been obtained on lease from the Great Ouse River Authority, and a track will be built there. The ministry says that the cost will be £2 million over the first two to three years, but nobody seriously believes that will be the final cost. The track alone will cost more—probably £0.25 million a mile for a device of 70 tons. The experiment may well be cheaper, but if 300 m.p.h. is to be reached at least 20 miles of track will be needed. It is hard to see how this could be done for less than £3 million, and then it will be necessary to build a vehicle filled with electronic equipment. By publishing a figure of £2 million, even with the proviso that it will cover only the first few years, the ministry has laid itself open to criticism when, in five or ten years time, the cost turns out to be £5 million or even £10 million.

Certainly it will be possible for tracked hovercraft

to provide a more rapid service than conventional trains or the aircraft now in service. Even allowing for improvements in transport between airports and city centres, a hovercraft travelling at 300 m.p.h. would still be quicker on journeys of up to 600 miles. This assumes that hovercraft would be allowed to operate from city centres, but that is plausible. Hovercraft may be able to compete with aircraft, but this calculation takes no account of the improvements which are possible in conventional train services.

The estimates produced by HDL make tracked hovercraft seem a dubious proposition. Double track would cost about £0.5 million a mile, and one car, seating 200, would cost £420,000. For routes handling fewer than 2 million passengers each year, air travel would be cheaper. At higher densities, the figures begin to look more attractive, but only on routes handling more than 6 million passengers a year would hovercraft be able to offer cheaper transport than trains. The greatest density on any British Railway route in 1965 was 5 million passengers a year, so that there seem at present to be no routes in Britain on which the hovercraft would be cheaper than the trains. The ideal hovercraft route must be one on which the density of traffic makes the idea economically attractive but long enough to make use of the greater speed the hovercraft offers. Nothing in Britain can compete effectively with the Northeast Corridor in the United States as a route for introducing hovercraft. The hovercraft men in Britain may have to turn their sights to Stansted if the Government really decides to make that London's third airport, but that is another matter—political as well as economic.

All this is not to say that hovercraft will never supply an acceptable alternative to conventional systems of transport, but they are obviously more attractive where alternative systems are slow and inconvenient, as with the cross-Channel ferries. Even there, the Channel Tunnel may reduce the amount of traffic carried above the surface. Where existing systems operate at higher speeds and are capable of considerable development, the case for hovercraft looks slim. British Rail, losing £130 million a year on rail services, has been castigated for its reluctance to embrace the hovercraft. In retrospect, it may turn out to have been wiser to concentrate on the improvement of existing services.

## ASK FOR MR JONES

THE British Government is well on the way to making a great muddle of its policies towards the nationalized industries, particularly in fuel and power. The immediate cause of trouble is the announcement two weeks ago of higher prices for the electricity sold to domestic consumers. It has been known since May this year that electricity prices would have to go up, but domestic consumers (who are also usually voters) have not taken kindly to the suggestion that they will have to pay an extra 16 per cent. Mr Richard Marsh, the Minister of Power and the custodian of electricity

prices, only has himself to blame that his holiday in Yugoslavia was cut short by the excitement, and that the Prime Minister has agreed that all future charges levied for products or services by the nationalized industries shall first of all be examined by the Prices and Incomes Board. It does not take a particularly long memory to appreciate that this will bring pleasure to Mr Aubrey Jones, the chairman of the Prices and Incomes Board, for his complaint that the Government should have given him a chance to examine this latest increase of price seems to have been fully vindicated. If things go on like this, people will be wondering who is the Prime Minister—Mr Wilson or Mr Jones.

Although the problem of what prices the nationalized industries should charge may on the face of it seem to be an exceedingly academic issue, the fact that these industries between them are responsible for more than a sixth of the industrial activity in Britain is a sufficient proof that the London School of Economics cannot be left entirely in charge. By what may seem to some to be a pleasing poetic justice, the rules by which the nationalized industries are at present regulated have their roots in the late fifties, when the electricity industry invited the enmity of the Treasury by the size of its capital investment each year in new power stations and transmission plant. In the competition for capital, private industry was starved of funds, and it became apparent that nothing in the original acts of nationalization laid down how much the nationalized industries should seek to earn by way of surplus on their trading. From there it was a short step to the White Paper of April 1961 by means of which each nationalized industry was told to earn a surplus each year defined as a certain percentage of the net capital assets employed. Electricity was asked to shoulder the heaviest burden, presumably but illogically because it was the only one of the nationalized industries to have consistently made a profit in the fifties. Ironically, the chief reason why electricity prices must now be increased is that the industry is hard pressed to raise the whole of the 12·4 per cent of the surplus which is specified.

So should the charges be abolished? This is what a great many people will say when bigger bills come in for payment in the months ahead. The simple answer is that they are indispensable. Just as other kinds of industries must make provision out of their earnings for the depreciation of plant and for the raising of capital, so nationalized industries must keep their books in proper order. In 1965–66, for example, the electricity industry put aside £330 million to cover these charges, and also earned a surplus of £85 million. Taken altogether, these sums of money were 12·6 per cent of the net capital employed, or more than enough to satisfy the industry's statutory obligation. Yet in the same year investment cost £595 million, so that the electricity industry consumed more public money than it paid to the Exchequer. Obviously the only sensible question to ask about the charges is whether they should be varied by some small amount, one way or the other.

To begin with, there is a strong case for asking that nationalized industries in competition with each other—coal, gas and electricity, for example, or the two nationalized airlines—should have similar charges laid against them. One of the anomalies of the past few years has been that electricity has been more highly taxed than gas—a sign of how the gas industry seemed to be languishing when the charges were first specified in the early sixties. There is now the most urgent case for equalizing these two and also for making the coal industry pay a fair return on the capital it employs.

The charges should also be administered more flexibly. The sharp increase of electricity prices last week has come about because the industry has had to meet a number of unexpected costs in the course of a single year's operation. The demand has fallen behind the forward estimates on which the industry has based its calculations, principally because the Government's own economic plan has fallen into disarray. Some power station equipment has been slow in coming into service, which has also increased the industry's costs without adding to its productive capacity. In circumstances like these, it is essential that the nationalized industries should be able to spread hardship over more than the year in which it occurs. It would even make sense if the financial objectives set for the industries could be defined not by a single number but by some variable relationship with the parameters which affect the industry's operations. It would make sense, for example, to have a lower financial objective when the economy as a whole is lagging.

It is not at this stage clear whether the Prices and Incomes Board will have a chance to examine issues like these. According to the Prime Minister, the financial objectives are to remain the Government's prerogative, and a new version of the White Paper of 1961 is due to appear soon. But Mr Jones is a resourceful man, and it will be surprising if he does not find some way of pointing out that the present system is so cut and dried that it is an impediment to efficiency, not a help. But, even if the Prices and Incomes Board has for a time to examine the side issues, there are plenty of important questions to be asked. It would, for example, be good to know whether the electricity industry is entirely justified in its devotion to such a closely integrated transmission network that all the generating stations in the country can operate effectively as a single unit. A more general version of the same question is whether the public interest would be served if the industry deliberately offered a poorer service for a lower price. Certainly it is pertinent to ask why the industry should have accumulated an extra 17 per cent of generating capacity to guard against the chance that the coming winter will be exceptionally severe. In other words, by asking the right questions the Prices and Incomes Board can do a great deal not merely to rationalize the way in which the nationalized industries make use of scarce resources but also to ensure that they conduct themselves efficiently.

## POLYPEPTIDES OR AMINO-ACIDS

THE origin of biological macromolecules and life on Earth is, like the possibility of extra-terrestrial life, a subject for speculation and experiment. There is no likelihood of producing protein and nucleic acid self-replicating systems from simple inorganic molecules in the foreseeable future. This, quite rightly, does not deter everyone from seeing what can be obtained from simple mixtures of the gases that were probably present in the primeval atmosphere.

The primeval atmosphere was almost certainly a reducing environment containing hydrogen, methane, ammonia and hydrogen cyanide, so it seems reasonable to believe that proteins somehow arose from condensation products of these gases and water. When Miller (1955) in his pioneering experiments—repeated many times since—found that mixtures of ammonia, methane and water yield  $\alpha$  amino-acids when subjected to high energy radiation or electrical discharge, it was speculated that proteins arose in the primeval seas by polycondensation of  $\alpha$  amino-acids. Matthews and Moser, on page 1230 of this issue, question this idea. They suggest that polypeptides and proteins in fact arose directly from the condensation products of hydrogen cyanide, ammonia and other gases and not by the condensation of pre-existent  $\alpha$  amino-acids. They have shown that the products from mixtures of ammonia and hydrogen cyanide when dissolved in water yield polypeptides but no free amino-acids. On hydrolysis, this peptide material yields no fewer than fourteen  $\alpha$  amino-acids, assuming that the amide precursors of aspartic and glutamic acids were formed. As expected, because the gas mixture used contained no acetylene or hydrogen sulphide, aromatic and sulphur containing amino-acids were not formed.

Matthews and Moser propose that dimerization and subsequent polymerization of the hydrogen cyanide forms polyaminonitriles which then react with water to give the peptide material. They also believe that the free  $\alpha$  amino-acids found by other workers were in fact produced by the hydrolysis of peptides. If, as is likely, the primeval atmosphere contained acetylene and hydrogen sulphide, it is perfectly possible that polypeptides containing all twenty  $\alpha$  amino-acids were produced in this way and subsequently gave rise to today's proteins. It will be a long time before anybody can hope to know for certain whether polypeptides gave rise to  $\alpha$  amino-acids or whether things happened the other way about, but the idea is stimulating.

## NEWS GATHERING

LATER in the year, *Nature* will begin a new venture in gathering and presenting news about science to the public and the scientists. Under an agreement with the *Times* (of London), this journal will provide the *Times* with regular news of important developments in

science. This will take the form of a daily column written by the staff of *Nature*. At the same time, it will be possible for this journal to improve enormously on the news of developments within the profession of science which it at present offers to its readers. Not only will there be more people to do the work, but *Nature* will have access to the network of international communications at present used to provide the *Times* with a foreign news service.

Readers of both journals will benefit. *Nature* is particularly glad of an opportunity to help with the dissemination of news about science to the general public, for the excitement and significance of modern science are inadequately appreciated. It is important, however, that an arrangement like this should not compromise the independence of the two journals, which is why it has been agreed that *Nature* should be in sole charge of the contributions offered to the *Times* each day and that the service thus provided will supplement and not replace the coverage of science now provided by the *Times*. It goes without saying that contributors to *Nature* can be sure that their communications will in future, as in the past, be dealt with in confidence and objectively. If there is any interaction at all between the news service and the function of *Nature* as a vehicle for original communication, it will probably take the form of a further increase in the speed with which manuscripts are turned into print.

## NO LINGUA FRANCA

THE resignation last week of the Indian Foreign Minister, Mr M. C. Chagla, has again drawn attention to language problems in India and to the Indian Government's determination to implement immediately the decision to replace English by regional languages as the medium of teaching in Indian universities. It is easy enough to understand the Indian desire to remove what may seem to them to be vestiges of a colonial past, and there is plenty of evidence from elsewhere—Wales, for example—that language ranks high in national pride. But in India, with no universally spoken or accepted language, the change is impractical and unrealistic. It will inevitably lead to a lowering of the standards of education and to a further isolation of Indian universities which India can ill afford. Until Hindi, now spoken by only about 40 per cent of the population of India, has become a universal language, there are to be no fewer than fifteen accepted regional languages. Will India face the chaos of translating textbooks and journals into all of these? And will teachers be obliged to lecture in a language in which they lack proficiency? Many hoped that the English language might remain as the most useful legacy of imperial India, and it is ironic that the Indian Government has felt obliged to replace English by obscure regional languages. Will their decision help to ruin Indian unity as well as Indian universities?



## NEWS AND VIEWS

### Only Specialists Need Apply

THE first physical evidence of the drive to get British scientists back from the USA was unveiled this week in New York. Management Selection Ltd, which the Ministry of Technology recruited earlier this year to lead the drive, opened an information and recruitment centre in Park Lane. The centre is intended to provide help and information for engineers, scientists and managers who wish to return to Britain. Other centres are planned for San Francisco and Toronto.

Simply writing to MSL or the ministry is absolutely no guarantee that a job will be found. Since the ministry started its campaign, 400 British scientists in the USA have written asking for jobs in Britain, but very few have yet been placed. No doubt part of the reason for this is the difficulty of arranging interviews (which should be simplified now the MSL office is open), but the real reason is simply that industry in Britain has few vacancies to fill. Employers are not interested in flying to New York to recruit chemists if they can find men as good in Manchester. In some areas—data processing, electronics, management services, and some branches of engineering—there are genuine shortages, but in others employers are easily able to fill their needs within Britain. In the first quarter of 1967, only half as many jobs were advertised as in the first quarter of 1966. In these circumstances, British scientists wanting to come back to Britain are deluding themselves if they expect to be offered jobs on the spot.

MSL is tackling the problem by first finding out from British companies what their needs are. It may then be able to find among those who go to the office in New York candidates who have the necessary qualifications and experience. So far several hundred companies have been contacted, and asked what they will be needing over the next twelve months. Their needs are not always obvious; chemical companies may well be more interested in statisticians than in chemists, and engineering firms may be looking for systems analysts rather than engineers. At the moment, apart from the special fields, it looks as if there are more applicants than there are jobs. With the number of annual vacancies running at about 25,000, against 40,000 a year ago, this is not surprising. Like everything else, recruitment on a large scale from the USA is likely to have to wait for the end of the squeeze.

### How Many Social Scientists?

THE Social Science Research Council, set up in 1965 as a result of the Heyworth Report, is the body which makes the awards for postgraduate research in social science in British universities. Estimates as to the likely number of eligible graduates are given each year to the Treasury, which then decides the proportion which will be given awards. The money required for these grants (about £630 each) is, surprisingly, not the only criterion; the number of people tied up

in academic research rather than let loose on the world is also taken into account. At present, the Treasury and the SSRC have agreed that about 50 per cent of those eligible should be given awards. Although the proportion is rising every year it is taking longer than the SSRC would like to reach the 60 per cent recommended by the Heyworth Report.

Several universities have recently complained that large numbers of their eligible students (first or upper second class degrees) are being refused grants. In a letter to the *Times* on September 9, Professor Meek of the University of Leicester gave figures for his department—only four of the thirteen applicants received awards.

The SSRC allocates awards to each university on the basis of previous figures. A reserve of about 10 per cent is held back until the summer, and further grants are given on the results of final examinations. Here again there seems to be a shortage of awards. The London School of Economics, for example, filled its original quota and applied for fifty-seven further awards, only seventeen of which were granted. It has been found in the past, however, that some awards that were granted were not taken up, when universities have over-estimated their own appeal. The SSRC takes this into account when the allocations are made.

### Pugwash Meets

THE latest Pugwash conference has ended after a week of discussion at Ronneby, Sweden. Members of the conference met in a mood of self-examination: indeed, the organizers took the occasion of the tenth anniversary of the Pugwash movement to call a more comprehensive and larger conference (of about 200 members) than ever before, and to declare that one of its main purposes would be to review the aims and organization of the movement—to discuss its past achievement and to plan its future.

The history of the Pugwash conferences is remarkable. On an absurdly small budget—of about £4,000 a year—at its central office, under the dedicated and dynamic supervision of its originator and secretary-general, Professor J. Rotblat, the movement now has 22 national groups—some of them extremely active—and a continuing programme of international and national study groups and conferences.

What have been its achievements? At this conference one heard on all sides the conviction that they were considerable even though immeasurable and that the movement must continue. Although the conferences are private, reports of their proceedings are given to the press, to governments and to other interested institutions. Observers attend from many international organizations, such as the organs of the UN. But the main achievement is thought to be, in the now accepted jargon, its "fall-out" or "spin-off". Perhaps the most obvious fall-out is on the participants themselves, many of whom hold important advisory or even executive positions in their own countries. The unique feature of these conferences seems to be that the participants, bound by common commitment to science and scholarship—and by common habits of thought—find themselves discussing political issues with the familiar friendliness of ordinary scientific conferences. Regular Pugwashites will assert that they can detect changes in attitude between one

conference and the next in those with whom they have argued most keenly.

But some of the fall-out has been detectable and concrete. An example is the new Stockholm International Institute for Peace Research. This is an independent organization financed by the Swedish Government but, as the Prime Minister of Sweden told the conference at its opening session, it owes its existence to Pugwash.

The delegates came from nearly 40 countries, broadly representative as between East and West, and between advanced and developing nations. Indeed, one of the trends in recent years has been the increasing attendance from countries other than the UK, USA and USSR—though not at present including China. Representation from the USSR has always been high (it included Kapitza on this occasion); here Pugwash has probably been ahead even of conventional scientific conferences.

The work of the conference was done at many levels—in plenary sessions, in working groups, and in *ad hoc* committees. More than 70 invited or contributed papers were read, covering all aspects of science and world affairs, such as arms control and arms regulation; the resolution of current conflicts; the role of UNO international scientific programmes; assistance to developing nations; social responsibilities of scientists; the underlying reasons for conflict. Of course, no problems were solved; no doubt the final statements varied in real content in roughly inverse proportion to their political sensitivity. But there was a notable absence of complacency about the international situation or about the contribution of Pugwash. On the international scene an apparent détente between the “super-powers” comes at a time when “local” wars are perhaps more numerous than ever before. The conference was well aware of the instability of this situation, as the statement by the Continuing Committee shows.

The contribution of Pugwash in the future under its new President, Cockcroft, and chairman, Powell, will be by intensification of its own work. Conferences and study groups will be smaller but much more numerous, much more closely focused on particular problems, and much more professional in character. Perhaps even more important, the Pugwash movement must attract many more scientists—especially younger scientists—and must increase its budget. It is the only movement of its kind: the only independent international forum for scientists concerned about world affairs.

G. O. JONES

## Pugwash Statement

The safeguards system of the International Atomic Energy Agency got a nod of approval at the 17th Pugwash conference which has just taken place in Rönneby, Sweden (see preceding story). In the report of the continuing committee, which describes what went on, some details are given of the discussion on the problems of disarmament. While welcoming the tabling of the draft non-proliferation treaty, the conference regretted the omission of Article 3, which covers safeguards. Fears about inspection should be allayed, as far as possible, by minimizing the intrusiveness of the inspection, and ultimately it would be better if the nuclear states were subject to the same inspection as

the non-nuclear states. “The control system of the IAEA,” the statement says, “appears to be entirely adequate for the required inspection.” It goes on to say that because of the great importance of the non-proliferation treaty, countries should not impose conditions—such as other disarmament measures—on its acceptance. These are more likely to be successfully negotiated after the treaty is signed. One suggestion is that nuclear powers should undertake not to use nuclear weapons against states which have signed the treaty, which do not possess nuclear weapons and which are prepared to promise that there are no nuclear weapons on their territory.

The conference agreed that it is now possible to detect nuclear tests underground, so that there is no objection on technical grounds to extending the test ban treaty. The repeated outbreak of local wars not involving nuclear weapons alarmed the conference, and this statement warns of the dangers of the militarization of the oceans and of outer space, and of chemical and biological warfare. All nations were urged to adhere to the Geneva Protocol of 1925, and a formal treaty prohibiting the use and transport of chemical and biological weapons should be negotiated.

The conference agreed that all states should recognize the German Democratic Republic, and that both West and East Germany should be admitted to the United Nations without prejudicing the eventual reunification. The bombing of North Vietnam should be stopped immediately and unconditionally, peace negotiations should begin and a conference should be convened to establish a stable peace in South East Asia.

The conference returned to a discussion of a proposal previously made at Pugwash—the formation of an International Science Foundation within the UN to permit young scientists in developing countries to undertake research they would not otherwise be able to afford. The International Biological Programme seems to be going well in the developed countries but has so far failed in the underdeveloped countries for lack of funds. Too little is known, says the statement, about the production of essential foodstuffs in the tropics; the technical problems are linked with economic, religious and social ones. On a more optimistic note, the conference had hopes of the creation of what the statement calls “agro-industrial power complexes” in coastal deserts or in partly fertile areas. In essence, these would be large nuclear reactors producing electricity for desalination and fertilizer production.

## Task Force for COMSAT

THE restrictions governing communications in the United States have now become so archaic and contradictory that the corporations in command of satellites, computers and cables must hesitate before taking logical steps ahead, lest they run foul of the law. The fairly young Communications Satellite Corporation (COMSAT) is probably the most hamstrung of all. At the moment it is waiting to hear from the Federal Communications Commission whether it can spend some of its capital of \$200 million to provide an experimental domestic satellite service for the United States. Worse still, the corporation will have to hang on until 1969 to know what part it is ultimately to play in the International Telecommunications

Satellite Consortium (INTELSAT) of which it is now temporary manager. When COMSAT will know whether it can be permanent owner of an American domestic network is anybody's guess.

President Johnson has agreed at last that the Federal Communications Commission has neither the will nor the ability to deal with these questions, so that there is to be a task force under Mr Eugene Rostow, Under Secretary of State for Political Affairs, to analyse current regulations and the agencies which administer them. It would not be surprising if the group recommended elevating communications to the status of a full department. It may also suggest that anti-trust laws should be changed to allow COMSAT and the big communication companies to split. Hitherto COMSAT has felt at a disadvantage at the international bargaining table. There is an impression, which would not be confirmed by a study of British policy on satellites, that men from the British General Post Office can make clear what they want as prices, for example, while COMSAT's negotiators must keep in mind not only the national interest but that of the Federal Communications Commission, the small shareholders and the giant communications carriers—particularly the American Telephone and Telegraph Company, which owns half its stock.

The establishment of the task force may mean that the Federal Communications Commission will feel free to let COMSAT go ahead with a domestic satellite system on an experimental basis. The commission is well known to hate making up its mind; now the difficult decision about permanent ownership can be postponed until the task force reports. COMSAT has kept its critics in mind in drawing up its plan. It has offered—quite needlessly—to relay telephone calls by sending one voice by satellite and the answering party by ground line in the hope that this might quieten the fears of AT & T, which is worried about its investment in ground lines. COMSAT also hopes to crush the Ford Foundation's proposal to have a non-profit corporation set up to do the same thing by offering free channels for educational television.

One of the principal motives in the appointment of a communications task force was undoubtedly the imminent expiration, in 1969, of the interim agreement on INTELSAT. A permanent agreement on the ownership and operation of a global commercial satellite network must then be thrashed out. President Johnson has pledged the United States to work for a continuation of the consortium, in which the representatives of 58 countries are now members. He has asked the Soviet Union to think again about joining (instead of forming a rival network of its own). And, as bait, he suggested that COMSAT might be allowed to drop down from its present 54 per cent share of the votes in the consortium. 40 per cent is being mentioned as a possible ceiling, but it is unlikely that this will satisfy foreign critics of COMSAT's dominance.

## Japanese Space

THIS autumn may see the launching of the first Japanese preliminary satellite, which would be a small one. The launch vehicle Lambda 4S will have its fourth and fifth firings in the next two months, in the hope of overcoming difficulties which led to the failure of the first three launches. Since the failures, a violent

press attack has been directed at the Institute of Space and Aeronautical Science at Tokyo University, whose Professor of Engineering, Professor Itokawa, has been the inspiration behind the Japanese space effort. When a government inquiry into the affairs of the institute was begun in the spring, Professor Itokawa resigned, nominally to make way for a younger man, and, despite the support of his colleagues, has refused to be reinstated.

If all goes well, the first launching of a full scale satellite by the larger Mu rocket should take place in March 1968. The Science and Technology Agency recently admitted, however, that the programme has fallen behind schedule. One of the reasons for this seems to be the opposition of local fishermen, both to the erection of shore buildings and to the proposed orbital launchings at Uchinoura near Kagoshima. Already local opposition has caused the cancellation of a programme in which ten meteorological rockets were to have been launched. As a first step in resolving the muddle, greater integration of Japan's main space agencies was begun this summer. As a result, the Space Development Long Range Planning Office was set up, with representatives from the Science and Technology Agency, the Meteorological Agency, and the Ministries of Transport, Posts, Construction and Trade. The Institute of Space and Aeronautical Science at Tokyo is not represented, however, as "this might violate the autonomy of the university".

## Canals for Pleasure

THE Ministry of Transport is to spend £340,000 to improve and maintain 1,400 miles of canals so that they can be used for fishing and boating. The canals, most of them in the Midlands, are of no commercial value and their upkeep would in any case cost £600,000, so that the comparatively small expense of keeping them open for pleasure craft is fully justified. A mere twenty-eight miles of the canal system is to close, either because the canal passes through unattractive countryside, as in the case of the final section of the Leeds-Liverpool Canal, or because there are too many locks over a section serving too little traffic, as with part of the Sheffield-South Yorkshire Canal. By encouraging the development of facilities for pleasure craft, there is hope that the Canal system will come to cost the taxpayer less. There is even a possibility that some of the 600 miles of unnavigable waterways owned by the British Waterways Board may be reopened to traffic. A new Inland Waterways Advisory Council is to be established to deal with suggestions from those interested in the development of the canals, though the Waterways Board itself has co-operated admirably in the past with voluntary organizations, as, for instance, in the recent development of the Stafford and Worcester Canal.

## Profitable Airports

THE British Airports Authority was set up eighteen months ago to run the principal international airports in Britain on a profitable commercial basis. The first annual report of the authority shows that, within these terms of reference, the first year was successful. Finance is not the only consideration, however, as the authority also aims to provide good services, facilities

and public relations for both travellers and local residents.

Heathrow, Gatwick, Stansted and Prestwick—the four international airports formerly owned by the Ministry of Aviation—were handed over to the authority in April 1966. They represent a small proportion of the eighteen airports in Britain with scheduled services, but between them they handle 60 per cent of the total passenger traffic. Gatwick, struggling with seasonal traffic problems, could not balance its winter deficits by its summer profits. Stansted, on the brink of development, also failed to produce an operating surplus. Prestwick succeeded, however, and Heathrow, handling more international passengers than any other airport in the world, made enough for the authority to record a net profit before tax of £2.7 million.

Prestwick, owned by the authority, is as yet less busy than Abbotsinch, the other airport in Glasgow which is owned by the city. The two are being developed side by side, however; Prestwick, with tartan trimmings and welcoming early morning music, is to concentrate on long distance intercontinental flights, leaving short hauls and domestic routes to Abbotsinch.

Although the authority seems to have started well, there is as yet little talk of its taking on any other airports. The Board of Trade runs several airports and the rest, apart from a few private ones, are municipally owned. Not all of them are making a profit and, unless there is a chance of financial viability, it is unlikely that the authority will be able to take them on. Talks are at present going on, however, between the authority and the Board of Trade about the future of Edinburgh airport which the city council has declined to look after. The authority is building up a specialized staff of airport developers, and is prepared to act on a consultancy basis if its advice is sought.

## Death of an Elephant

THE post-mortem on Diksie, the London Zoo's twenty-seven year old African elephant which died last week after falling into the dry moat around her enclosure, has revealed that she had serious injuries from which she could not have recovered. The main nerve of the left front leg was severed—a result of the way in which she fell and landed off balance. Her right tusk was broken, and she was concussed—she hit her head on the front wall of the moat. With injuries such as these nothing could have been done to save the four ton elephant. The zoo's lifting equipment could have lifted her into a standing position had she not been injured. With her damaged leg it would have been necessary to destroy the elephant even if the rescue operation had been successful.

Elephants have fallen before during the past twenty years. In her previous enclosure Diksie fell into the moat, which was deeper than the present one, and was removed safely. An Indian elephant has also fallen into the same moat in which Diksie died, but was unhurt and was brought out with little difficulty. Last week's was the first fatality, the result of an awkward fall.

The zoo says that it has no reason to doubt the efficiency of the moat. It is, however, considering banning all feeding of elephants by the public, because the animals would not then be encouraged to stand at

the edge of the moat and stretch out their trunks to catch items of food. It was this that was partly responsible for Diksie's fall. A ban on feeding, with warning notices and constant supervision by staff, works effectively in continental zoos, and its introduction in London would not be untimely.

## Software for Teaching

EDUCATIONAL technology sounds like a grand name for teaching machines, but in fact it includes a great deal more than hardware. Closed circuit television and language laboratories cannot work by themselves, and the provision of the right programmes for such machines is a task which is receiving increasing attention. On a more fundamental level, research is now being done to find the best use for each type of machine and the most effective means of using the machines in teaching actual subjects. The creation of the National Council on Educational Technology is a sign of the times.

The latest group to take up this type of work in Britain is the newly established Institute for Educational Technology at the University of Surrey. Professor L. R. B. Elton, head of the physics department, will be taking charge of the institute, which he believes to be the first of its kind in Britain concentrating on teaching aids in universities. The Massachusetts Institute of Technology has such a department, but most of the work on audio-visual aids in Britain, at Leeds and Sussex, for example, has been for the secondary school level.

The new institute at Surrey will be working in close touch with the several departments in the university, and Professor Elton hopes that this unusual relationship, which will link the educational research directly with the teaching methods in use in each subject, will prove fruitful. The teaching of first year students is one of the first important areas for investigation, and this will be studied in connexion with sixth form teaching, as the two are so closely related.

In the present buildings in Battersea there is little room for the institute to take on students, but when the university moves to Guildford in 1968 it is hoped that students will be able to take higher degrees in this expanding field. Students who can take postgraduate courses in educational technology will be well equipped to raise standards when they begin to teach. Studies of this nature may also encourage more science students to apply themselves to teaching. The institute hopes also to encourage school teachers in the area to join in this work so that the results of research can be put into practice.

A grant of £1,350 has been awarded to the Institute by the Social Science Research Council for a particular project concerned with the transition between school and university. Philips Industries has offered support, particularly in the form of machinery, and other finances are being provided through the usual university channels.

## Brakes without Skids

A RECENT study by Mr J. K. Meades of the Road Research Laboratory shows that different tread patterns have only a small effect on braking efficiency on wet roads. Mr Meades tested five different cross ply



tyres and five radial tyres on four different wet road surfaces. Of the two groups, radials were slightly the better, but within each group the differences attributable to tyre tread and material are less than the scatter of the experiment. Only one type, an Italian tyre not recommended for British roads, was significantly worse than the rest. The generally better performance of the radials, Mr Meades concludes, is consistent with the view that they are better at removing water from the contact region; at 80 m.p.h. on polished concrete the peak braking coefficient for radials was 0.6, while that for cross ply tyres was 0.5.

The experiments also showed that when the wheels lock, braking coefficients are frequently reduced by a factor of two or three, and occasionally by as much as eight. Drivers are therefore in danger of over-estimating the braking power available to them. Automatic braking devices which prevent the wheels from locking offer a chance of making the best use of the friction available. Perhaps more important, though, is the need to retain directional control, which is almost completely lost when the wheels lock. Skidding, the laboratory estimates, is involved in about 70,000 accidents each year; 33 per cent of all accidents in wet conditions involve skidding.

Several systems of automatic brake control have been announced. The Dunlop Maxaret system uses a rubber tyred wheel driven by the road wheel which it controls. Within the driven wheel is a flywheel kept in driving contact by a spring; normally the flywheel rotates at the same speed as the driven wheel, but when brakes are applied and the driven wheel decelerates, the flywheel continues to rotate against the resistance of the spring and operates valves which release the brakes. The driven wheel then accelerates again, catches up with the flywheel, and the brakes are re-applied. Experiments almost ten years ago at the RRL by R. D. Lister and R. D. Kemp showed that units of this type can reduce braking distances and give much better control during braking.

Lockheed have also produced a system of control, called the Lockheed Antilock. This operates on the rear wheels only, and uses an inertial system driven from the propeller shaft to prevent the rear wheels locking. The system has demonstrated that braking distances can be reduced and directional stability maintained. Another system, the Kerr Antilocking device, makes use of the forces produced in the suspension of the vehicle to release the brakes when the wheels begin to slip. Yet another, the Autostable braking system, makes use of torque reactions on the front brake shoes to regulate the pressure to the rear shoes.

Although several of these systems have been convincingly demonstrated, their use is still very restricted. The Dunlop Maxaret system, used on aircraft, has been fitted to some commercial articulated vehicles, at a cost of £150 per axle converted. Cost may well be more significant than technical factors, and it is probable that antilock devices will not achieve widespread acceptance until their use is compelled by legislation.

## Keeping Armies in Touch

BRITAIN has joined the United States, Australia and Canada in a collaborative project to develop a tactical trunk communications system for their armed forces.

The United States, Canada and Australia ratified an agreement to proceed with the project, called Mallard, in April of this year, but Britain deferred a decision because the problem of the sharing of costs and work had not been resolved. The project will cost about £45 million over 8 years in research and development, of which the United States will pay about 60 per cent, Britain about 30 per cent and Australia and Canada about 10 per cent. When the scheme goes into operation towards the end of the seventies, it is expected to cost between £200 million and £350 million. Each country will have the right to place contracts for up to one-half of its production requirements where it wishes, but the balance will be open to competitive tendering.

The Mallard system is a development of the British "Hobart Plan" for a digital tactical communications system. The United States authorities considered that this sort of system could not be developed until the early eighties instead of the middle seventies, and so had been developing a less sophisticated analogue system. The Mallard system will provide secure, fully automatic, switched communications in the battlefield area from army headquarters down to battalion level and will link up with the "Clansman" system down to forward battle positions. It will also link up with other strategic communications systems including "Skynet", the satellite system. It will have facilities for transmitting and receiving speech, telegraph, data and facsimile, and will replace the "Bruin" system, a microwave trunk telephone communications system due to come into service next year.

In the initial development phase of the programme, the competitive system design studies will be carried out by United States and British electronics companies. A consortium of GEC, Marconi, Plessey and STC, co-ordinated by the Signal Research and Development Establishment, has been formed to do the design studies in Britain. Other companies will be called into the consortium as required, and when the system is developed it is hoped that other countries in NATO will buy it.

## EMBO Fellows

SIXTEEN long-term fellowship awards for 1967/68 have been announced by the European Molecular Biology Organization. These awards, tenable for a year, allow research workers in molecular biology to attend centres to continue their research. Although the organization is European, it does award fellowships to non-Europeans who wish to work in Europe and to Europeans who wish to work outside Europe.

Among the fellowships awarded, Dr Ch. Phelps, of Bristol, has received one to work on conformational changes and enzyme kinetics at the Istituto di Chimica Biologica, Centro di Biologia Molecolare, Rome; Dr L. Cordone, of Palermo, has received one to work on messenger-ribosome relations at the Laboratory of Genetics of Brussels University; and Dr J. P. Richardson, of Cambridge, Massachusetts, has received one to work on the structure of RNA polymerase at the Institut de Biologie Moleculaire, Geneva University. EMBO has also renewed the fellowship awards of Dr C. Vesco, at the Department of Biology, MIT; Dr J. Witz, at the Laboratory of Molecular Biology, Cambridge University; Dr E. Pojnar, at the Department

of Botany, Nottingham University; and Miss S. A. Bonanou, at the National Institute for Medical Research, London.

During the year EMBO has sponsored five summer courses in molecular biology. These covered separation methods, immunology, steroid biochemistry, ribonucleic acid and electron microscopy.

## Depth Perception

from a Neurophysiology Correspondent

FOR several years Julesz has studied binocular interactions in human vision using pairs of patterns which give a depth percept, when fused binocularly, exactly as in a normal stereoscope, but in which no structure is apparent monocularly. The stimuli are patterns of equal numbers of black or white square picture elements generated by computer and arranged at random in square arrays 100 elements square. The two members of a pair are identical except for the central array ( $40 \times 40$  squares) of which one is shifted horizontally by an integral number of elements with respect to the centre of the other. When the two patterns are fused binocularly in a stereoscope, this disparity leads to the percept of a central square. At first, subjective development of the square may be slow; when it is seen it appears in a plane in front of or behind the surround, depending on the direction of the disparity. This demonstrates that depth perception and the cross-correlation necessary to establish the disparity of two images can be an entirely central process, independent of monocularly visible structure and complex pattern recognition and of eye movements and vergence, but a function of time. The experiment therefore provides an ingenious technique for investigating purely central mechanisms, as any structure apparent in the fused image is a central percept (*Science*, **145**, 356-362; 1964).

In recent publications, Julesz and his colleagues describe extensions to their first experiment. When there is a monocularly apparent structure in one of a pair of random dot patterns which also contains a shape only visible when binocularly fused, the binocular "central" percept entirely predominates. This is true for patterns containing strong bilateral or higher order symmetries seen monocularly, as well as for patterns containing familiar words (Julesz, *Bell Syst. Tech. Journal*, **46**, 1203; 1967). Thus, when binocular interactions occur, they dominate the perception of monocularly perceived symmetries.

Julesz and Spivack (*Science*, **157**, 563; 1967) describe experiments with patterns made up of thin straight line segments. These, too, are drawn by computer, and consist of grids of thin vertical or horizontal lines. The lines are black, one dot thick and ten apart. For a vertical grid each line segment is in the vertical meridian of a 10 dot square element, or is displaced horizontally by two dots. The two types of segment are arranged at random, so that the monocular percept is of a grid of narrow lines with random breaks at intervals of ten dots, or an integral multiple of ten. As before, the two images of a pair are identical except for the central array of  $40 \times 40$  picture elements which in one is shifted horizontally by a single (10-dot) element. Even when the targets are viewed at a distance at which the breaks subtend only 16 seconds

of arc at the retina, perception of a square in depth occurs on fusion.

This is below the limits of normal visual acuity, but within those for vernier acuity. The authors suggest that the process used in vernier acuity is used in the cross-correlation on which global stereopsis depends. They also demonstrate an anisotropy for global stereopsis with these targets: stereopsis with horizontal line grids was stronger than with vertical grids, withstanding a disparity of the central square up to three times larger. This suggests that global stereopsis does not depend on local fusion, which is roughly isotropic (disparities in any direction between two retinal images less than about 6 seconds of arc are fused easily), but on local stereopsis, which is anisotropic, occurring for disparities only in the horizontal plane shared by the two eyes. Another interesting feature of these results is that even though horizontal displacement of the central square in one image will produce, by chance, 50 per cent of lines with zero disparity and 25 per cent each of lines with two dot disparity in either direction, a locally acceptable depth percept in terms of these disparities is rejected in favour of a more global percept, which must require more central processing for its detection.

Fender and Julesz (*J. Opt. Soc. Amer.*, **57**, 819-830; 1967) have further investigated the limits of fusion, using pairs of images stabilized on the retinae. With their stabilization technique, the two images of a stereoscopic pair can be moved apart without the possibility of the movement being counteracted by vergence movements of the eyes. Under these conditions, once fusion is established within Panum's fusional area (that is, with a binocular disparity of less than 6 seconds of arc for foveal vision), the two targets can be separated—literally pulled apart on the retinae—without loss of fusion for a distance of up to  $2^\circ$  provided that the pulling is not too fast and that the stimuli are never occluded. If the pulling velocity is above a certain limit, or the stimulus is occluded, fusion breaks down and can only be re-established within Panum's fusional area. With random dot patterns fusion is maintained for a greater distance of movement than with a pair of single lines. The authors interpret their results as follows: a labelling process, operating within Panum's fusional area, establishes correlations between corresponding points of the images on the two retinae. This process takes time, but once labels are established they can be preserved by a "cortical registration process", allowing the images to be pulled apart, within limits, without loss of fusion. Finally, there is a process preserving labelling against saccadic eye-movements, which do not destroy fusion in normal vision.

Julesz' work emphasizes the relation between the spatial and temporal aspects of fusion, while showing that in some ways it is a simpler process than had been thought. In normal vision, eye movements and vergence as well as object movements might all upset fusion without the hysteresis phenomena characteristic of binocular interaction.

## Cold Star

from an Astronomy Correspondent

RECENT interest in quasars has somewhat obscured the extraordinary development that has been occurring in

infra-red astronomy in the past few years. The latest surprise, announced in the most recent number of *Astrophysical Journal Letters* (149, L1; 1967), is the discovery by D. E. Kleinmann and F. J. Low of a new source of infra-red radiation in the Orion nebula. This has been detected at a wavelength of 22 microns and covers an area of sky 30 secs of arc in diameter. Using the observed flux and angular diameter, the brightness temperature of the source is only 70° K (about the temperature at which nitrogen liquefies). But at all wavelengths, it probably emits at least one hundred thousand times as much radiation as the Sun. Looking at 5 microns in another "window" where the Earth's atmosphere is sufficiently transparent to observe the sky, Kleinmann and Low failed to detect the source, thus confirming its low temperature. Nearby, however, is another source of infra-red radiation known as Decklin's star after its discoverer. The latter, with a higher temperature (about 600° K), emits powerfully at 5 microns but is not detected at 22 microns by Kleinmann and Low. The relation, if any, between these sources is an exciting puzzle for the theorists.

If the 22 micron source were in front of the Orion nebula, it would be seen in optical wavelengths as a black cloud superimposed on the bright background. Since no such black region exists, it is behind or inside the nebula. So far, two theories have been advanced to account for this peculiar object. One, championed by A. G. W. Cameron and W. K. Hartmann, is that the radiation comes from the gravitational contraction of a proto-cluster of stars which are still forming from dust and gas. On this view, it is far behind the Orion nebula, and the fact that it is in the same direction as the nebula, another important star formation region, is a coincidence. Another point of view put forward by M. Harwit and K. Davidson is that it is a single very luminous star in the midst of the Orion nebula which is cocooned in the dust cloud from which it was formed. The "cocoon-star" would be surrounded by a sphere of hydrogen ionized by the star's radiation and contained within the cocoon. This gives hope of distinguishing between the two theories by radio observations of the bremsstrahlung emission from the ionized hydrogen region.

## Transport of Secretory Protein

from our Cell Biology Correspondent

OVER the past decade the beautiful work of Palade and his collaborators has revealed the essential features of the secretory cycle in pancreatic exocrine cells. The cycle can be divided into four steps: (1) the synthesis of enzymes and zymogens on ribosomes attached to the endoplasmic reticulum; (2) transfer of the protein to the cisternae of this reticulum; (3) intracellular transport of the protein to the Golgi complex where it is packed in zymogen granules; and (4) discharge of the zymogen granules into the glandular lumina. The third of these steps is least understood. Caro and Palade (1964) showed with autoradiography that, within 10 min of synthesis, the protein reaches the condensing vacuoles of the Golgi complex where it is concentrated, but because of the low resolution of autoradiography they were unable to answer the vital

question: does the protein migrate to the Golgi complex through the cytoplasmic matrix or is it transported there in membrane bound vesicles? Data obtained in the early days of cell fractionation suggested the former route, but, as Jamieson and Palade (*J. Cell Biol.*, **34**, 577 and 597; 1967) have now shown, the secretory protein is in fact transported from the cisternae of the endoplasmic reticulum to the Golgi complex in vesicles.

To establish this they overcame the difficulties which make it impossible to give a short pulse of labelled amino-acid to the pancreas *in vivo*. Instead, they incubated slices of guinea-pig pancreas in a medium containing <sup>14</sup>C leucine for 3 min and then chased the label by incubating in cold medium. The tissue slices were homogenized either immediately after labelling or at intervals during the chase and fractionated by gradient centrifugation. In this way they determined the kinetics of labelling of the rough microsomes (derived from the ER), the smooth microsomes (derived from the periphery of the Golgi complex), and the condensing vacuoles and zymogen granules.

Immediately after giving the pulse the labelled protein is in the rough microsomes, but during a 7 min chase it is transferred to the smooth microsomes and it then accumulates in the zymogen granules, reaching a maximum concentration after 37 to 57 min. Furthermore, the labelled protein is in the lumen of the microsomes not associated with the bounding membrane and throughout the whole experiment the specific radioactivity of the post microsomal proteins—proteins of the cytoplasmic matrix *in vivo*—remains constant. Clearly, newly synthesized secretory protein is never released into the cell sap but is always kept within membrane bound spaces as it is moved about the cell. On this basis Jamieson and Palade propose a scheme for the intracellular transport of secretory protein that is applicable not only to exocrine pancreas cells but all gland cells specialized for the synthesis, concentration, temporary storage and eventual export of protein.

They envisage that as secretory protein is synthesized it passes immediately across the membrane of the endoplasmic reticulum into the lumina of the cisternae—Sabatini, Tashiro and Palade (*J. Mol. Biol.*, **19**, 503; 1966) suggested how this might occur. Once sequestered within the cisternae the protein can be moved to the transitional zone where the endoplasmic reticulum and Golgi complex meet. Small vesicles then shuttle backward and forward, transporting accumulated protein from the reticulum to the condensing vacuoles of the Golgi complex—there is morphological evidence which suggests this—and in the condensing vacuoles it is concentrated, perhaps by ion pumps; the condensing vacuoles thus become secretory granules. Thus from the very initiation of its synthesis the secretory protein is segregated and directly channelled into a secretory granule.

## Denatured States of DNA

from our Molecular Biology Correspondent

A FRACTIONATION technique of great potential, which has so far received surprisingly little attention, is partition between aqueous polymer two-phase systems.

This method is a product of the Swedish school, and has been developed mainly by Albertsson. Two water-soluble polymers are used, which in appropriate proportions generate two phases, between which water-soluble molecules, such as proteins and nucleic acids, can be distributed. Albertsson showed, for example, that in an aqueous dextran-polyethylene glycol system single and double-stranded DNA will tend to concentrate in opposite phases. A careful exploration by Alberts (*Biochemistry*, **6**, 2527; 1967) of the effect of concentration and proportion of the two polymer components and of salt concentration on the partition coefficients of native and denatured DNA has now produced the spectacular result that these species can be completely separated by a single partition. The conditions in terms of polymer and salt concentration are critical, but, once established, the fractionation involves only thorough mixing and layering of the phases by low-speed centrifugation. The native DNA enters the upper (polyethylene glycol) phase, and is isolated by salting out the polymer; the denatured DNA can be recovered from the lower phase by another extraction under different conditions.

Among several applications described by Alberts is the isolation of a small fraction of "reversible" DNA, present in denatured DNA: it contains cross-links which enable it to return to the fully base-paired state when the denaturing conditions are reversed. It therefore quantitatively enters the upper phase of the partition system. Many uses will surely be found for this interesting technique, such as the separation of single and double-stranded viral nucleic acids, isolation of hybrids, renaturable fractions from animal cells, and so on.

A study of the course of thermal denaturation in  $\lambda$  DNA by electron microscopy is described in an article by Inman (*J. Mol. Biol.*, **28**, 103; 1967). It follows an earlier report that when  $\lambda$  DNA was partially denatured by heating, melted regions ("puddles" in the electron micrographs) appeared preferentially in certain parts of the molecule. The native DNA has a narrow length distribution, clustered around  $18.5\mu$ . By measuring the distances along the chain of the melted segments in a population of molecules which have been exposed to a denaturing temperature for a given time, Inman compiles "denaturation maps". When a plot is constructed of the number of melted regions summed over all the molecules in the field as a function of distance from the end, it is seen that the early stages of denaturation are highly localized. Thus, at the lowest observable degrees of denaturation, a single melted zone is observed, which is followed by two more as the temperature of denaturation is raised. The first zone then sub-divides, apparently into four. The three major zones of melting are then centred  $8.9$ – $11.3\mu$ ,  $13.7\mu$  and  $17.9\mu$  from the end of the molecule, and are thus all situated in one half (the right half) of the chain. As the degree of denaturation is increased, zones appear in the left half of the molecule, though these are less sharply defined than the others. Thereafter the denaturation spreads and the pattern vanishes. The extent of the denaturation is determined both by the temperature of heating and the time it is allowed to remain there.

One of the conclusions is that all  $\lambda$  DNA molecules are similar in sequence. It must be supposed that the

labile regions are much richer in A-T than the remainder. In 1962 Felsenfeld and Sandeen noted, however, that T4 DNA showed only a very small spread of base composition of regions melting at different temperatures—much smaller than the highly heterogeneous calf thymus DNA. The present results invite a similar spectrophotometric analysis. They appear in any event to reflect much more non-co-operative melting than has previously been expected.

## The Physics of Quasars

from a Correspondent

FOLLOWING closely on the General Assembly of the International Astronomical Union at Prague, the Conference on the Physics of Quasars at Manchester allowed a more detailed look at recent observations and theory than could be afforded in the unwieldy milieu of the big assembly. The conference was arranged by the Institute of Physics and the Physical Society, with the Royal Astronomical Society, primarily to bring more physicists into contact with astronomers. The astronomers already immersed in the problems of quasars are not, however, averse to arguing between themselves, since neither the optical nor the radio observations provide an unequivocal picture of the facts which physical theory must explain.

The optical evidence, summarized by Professor Maarten Schmidt, has not changed dramatically in recent months (see F. G. Smith, *Nature*, **213**, 967; 1967). A quasar is still defined as a radio source of stellar appearance, intense in blue and ultra-violet light, often optically variable, and with emission lines shifted very far to the red. There seem to be at least  $10^5$  of these detectable in the whole sky, if we may include some which are not intense radio emitters. Some oddities remain about their distribution over the sky, but most observers do not place much emphasis on this, and consider instead their distribution with distance. Quasars are evidently not uniformly spaced through the observable universe, but increase in numbers at increasing distances, as indeed has been indicated for some time by the counts of radio sources. Further analysis of the relation between red-shift and optical and radio magnitudes was described by Longair and Scheuer and by Daintree and Horton (*Nature*, **215**, 917; 1967), but none of these analyses can yet say anything about the controversial question of the actual distances, or choose between cosmological models if the red-shifts are interpreted according to Hubble. They cannot even indicate whether part of the red-shift is caused by a strong gravitational field, which is the contention of those who support the smaller distance scales.

Variability, both optical and radio, has been detected in about a dozen quasars. Cannon and Penston are continuing a particularly useful series of optical observations at Herstmonceux, and regular radio observations are now being made at many observatories. It seems inescapable that the rapid fluctuations indicate that the main light source of these quasars is very small, possibly only about the size of the solar system. The optical emission lines must come from a larger region, a few parsecs across, resembling the planetary nebulae. Radio waves could not penetrate a uniform plasma of this type, so they must escape



from the centre through irregularities, or else they must be generated outside. The total emission represents an enormous power, whether the quasars are placed at some tens or some thousands of megaparsecs; evidently the central bright object contains a mass comparable with that of an entire galaxy, much of which is being converted to radiated energy via high energy electrons and a magnetic field. Theoretical arguments by Burbidge, Scheuer and Rees could only refer to this as "the horrid object X", although they did show that the observed properties of the radiation could be accounted for by a combination of synchrotron and inverse Compton radiation.

Although a rough physical picture of the radiation processes could be presented, no agreement was reached on the burning question of distance. Hoyle pointed out that such massive objects, with such small size, would undoubtedly have very large gravitational fields in the emitting regions. It might be quite wrong to apply our familiar physics, or to interpret red-shifts simply as recession velocities under such unusual circumstances.

In spite of the fog surrounding all attempts at interpretation, everyone agreed that the current pitch of excitement in the quasar story could only be matched by the era forty years ago, when Hubble discovered the law of recession of the nebulae. Two kinds of information are expected to emerge. Cosmologically, the quasars are important whatever their distance may be, since either they provide a searching test of cosmological models or they represent a local example of a cataclysmic event on a scale far greater than any previously envisaged in the evolution of galaxies. Physically, the release of energy on such a scale, possibly amounting to  $10^{62}$  ergs, equivalent to the conversion into energy of the whole mass of a small galaxy, and the existence of very strong gravitational

fields, represent conditions unthinkable in any terrestrial laboratory.

Experimental work also divides into two lines. The detailed physics of quasars can only be elucidated by further work on their characteristics, such as their variability, spectral lines, red-shift and polarization. Their place among other celestial objects, and particularly the radio galaxies, needs sky surveys and detailed maps of as many objects as possible. Some observers, particularly Ryle and Sandage, regard the quasars and radio galaxies as one continuous evolutionary series, while others, notably Hoyle, do not believe this "continuity" exists. Family resemblances are emerging, nevertheless: some optically normal quasars have the radio appearance of a radio galaxy, while some optically ordinary galaxies have a radio object like a quasar within them. Again, Oke has reported a radio galaxy, 3C 371, which has an optically variable nucleus; by contrast the radio measurements of diameters show that at least one-third of radio galaxies contain small bright radio condensations, again like quasars. No one can yet suggest an evolutionary sequence, or guess at the proportion of galaxies which are connected with quasars at some stage of their existence, but quasars are evidently not just an uncommon freak.

Perhaps the best conferences cannot be expected to provide answers, but only to highlight some particularly interesting but difficult problems. If so, this was a very good conference. It was certainly an enjoyable and comfortable conference, with the new Schuster Physics Laboratory at our disposal, and with accommodation in the Owens Park student residence. The final meeting was held at Jodrell Bank, where a tour of experiments in progress displayed results on an occultation of a quasar and some long baseline interferometry obtained while the conference was in progress.

## Retrospect

by

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Sir John Cockcroft, until recently a part-time member of the United Kingdom Atomic Energy Authority, reviews the authority's past achievements and its future.

THE late 1940s and early 1950s was the Golden Age of atomic energy development in Britain. We had by then equipped ourselves with the essential technological tools at Harwell—the research reactors and nuclear accelerators; the chemical, metallurgical and physical laboratories. Technological information had been developed to enable the Risley Industrial Group to build the Windscale plutonium production reactors and the chemical separation plant.

So, from 1948, we were able to turn our attention to the fascinating challenge of producing nuclear power. We had to increase the operating temperature of uranium fuel elements to the point when steam could be produced at temperatures and pressures sufficient to produce a reasonable thermal efficiency for the generation of electricity. A choice had to be made between the competing varieties of reactor. The fast breeder reactor was an early favourite, but it was seen that the quantities of plutonium or uranium-235 required for commercial power stations

would not be available in the 1950s. From the three possible types of thermal reactors we chose graphite moderated, natural uranium fuelled reactors because the alternatives required enriched uranium and heavy water which were unlikely to be available in Britain in sufficient quantities in the 1950s.

There were two main classes of problems. The first was to design a nuclear power station which would have a reasonable capital cost per kilowatt. Our rough estimates, made between 1948 and 1953, fluctuated by a factor of 2 but encouraged us to believe that nuclear power could be generated for about 1d. per unit—with the 4 per cent interest rates then prevailing. These cost estimates became firmer as the Risley Group took over the design of "Project PIPPA" and developed it into the Calder Hall nuclear power station. The second group of problems were technological. We had to prove that the graphite and uranium fuel would not be seriously affected by radiation damage; that the  $\text{CO}_2$  heat transfer gas would not



react too violently with the graphite in the presence of radiation; that the heat transfer problems could be solved; that the nuclear parameters of the reactor were correct. All these points were checked by experiments and this took about five years—a quite reasonable period for such development.

The Risley engineers showed that it was possible to design large scale nuclear plants within the estimates and within the time schedule. This was achieved by rigorous cost control and by an efficient progressing system which overcame all the bottlenecks. Harwell was successful in foreseeing most of the technological problems which had to be studied and solved. A good pattern of collaboration between designers and technologists was established and collaboration with industry and the Electricity Generating Board was very helpful.

When the generating boards embarked on the first stage of their nuclear power programme the role of the authority changed, so far as "magnox" stations were concerned, to that of an adviser on design, but it still contributed to the technology through the development of fuel elements using the facilities of the materials testing reactors and Calder Hall. The authority also retained responsibility for the development and construction of prototype reactors and for the trading activities of fuel element production and processing, and production of radioactive isotopes. This trading activity, which now has an annual turnover of the order of £30 million and makes a reasonable commercial profit, will be appreciably assisted if Britain joins the European Economic Community.

There has been a large scale, fruitful interaction with industry in addition to nuclear power station construction. Harwell has placed about 1,400 development contracts with industry since 1946 (*Nature*, 214, 343; 1967). Many of these were for the development of instruments which have had a wide application in industry and in universities—scalars, thickness gauges, polarographs, for example. The research reactors DIDO and PLUTO have been replicated by industry at Dounreay and in Australia, Denmark and Germany, and the electron linear accelerators pioneered by the Atomic Energy Research Establishment have been commercially developed for radiotherapy, radiation sources and university research. The production of 100,000 c. cobalt-60 sources in the Calder Hall reactors enabled Wantage to establish a new radiation application industry for sterilization of medical supplies and British companies have built or secured orders for four plants in Britain, one in Australia and five in Europe.

It was natural for Harwell to establish close relations with the universities. Nuclear and solid state physicists were encouraged to use the Harwell accelerators and research reactors. More than 50 graduate students and many university staff worked at a particular time in this way, anticipating the Sutherland report by more than a decade, and neutron crystallography developed out of this association. The early Harwell interest in helium-3 separation led to Dr London's helium-3 cycle for producing temperatures down to 0.01° K. But undoubtedly the greatest contribution of Harwell and the Radiochemical Centre to university research has been the production and speedy supply of radioactive isotopes and their incorporation into organic compounds for biological research throughout the world.

The associated Medical Research Council Radio-biological Laboratory at Harwell has a similar record of achievement. One of its most important discoveries was the method of making visible human chromosomes. Almost at once this showed that chromosome abnormalities led to specific human defects—the triplication of a chromosome to a mongol child, other abnormalities to sex disorders, and yet others to mental disabilities.

The establishment of research fellowships at Harwell tenable for up to 3 years brought to the establishment many promising young scientists from the United Kingdom



and abroad, who would not otherwise have been attracted by a Civil Service career. We took the first step to reduce the "brain drain" by sending out each year a "recovery mission" to interview scientists from the United Kingdom working in the United States and Canada on fellowship appointments and, by offering research fellowships or permanent appointments, we were successful in inducing many to return. The Atomic Energy Authority has since been joined by the Central Electricity Generating Board and the Civil Service Commission in this annual recovery operation. Harwell staff have been appointed to about forty university professorships, twenty-nine of them in Britain, and through its Isotope School and Postgraduate Education Centre the establishment carries out important educational functions.

In spite of the achievements of competitive nuclear power in the advanced gas reactor series of nuclear power stations, the authority still has an important role in development. Not all the development problems of fast reactor power stations or steam generating heavy water reactor power stations are solved. The technology developed for the Dragon reactor project at Winfrith may lead to power stations of still higher efficiency working perhaps with high power gas turbines, and its coated particle fuels may be used in future advanced gas-cooled reactor stations. The high "availability" of our nuclear power stations (85 per cent) reported by the chairman of the Central Electricity Generating Board reflects the strong research and development effort of the past.

Nevertheless the research and development effort in atomic energy is being reduced and transferred partly to other non-atomic energy problems. Harwell's interdisciplinary strength is to be used to establish a ceramics centre and a non-destructive testing centre, and a recent Harwell conference showed the possibility of contributing to the exploitation of the resources of the ocean. The Minister of Technology recently spoke with admiration of the role of US research institutes such as Battelle. Part of the authority's great technological strength could be used for similar purposes.

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# Peptide Synthesis from Hydrogen Cyanide and Water

by

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Did primitive proteins arise directly from simple inorganic molecules? Experiments show hydrogen cyanide polymers react with water to produce peptides containing at least twelve  $\alpha$ -amino-acids.

It has long been known that base-catalysed polymerization of hydrogen cyanide yields mixtures of solid products which include a tetramer—diaminomaleonitrile—and black intractable polymers believed to have fused tetrahydropyridine structures<sup>1</sup>. Recently, adenine and related imidazoles have also been detected<sup>2-4</sup>. In this paper we describe the synthesis from hydrogen cyanide of yet a further class of compounds—polymers that are readily converted by water to peptide like solids<sup>4</sup>.

In a typical experiment, freshly distilled hydrogen cyanide (500 g) and anhydrous liquid ammonia (5 g) were introduced into an evacuated 1 l. 'RB' flask capped with a ground glass joint connected through a vacuum stopcock to an exit tube. On standing at room temperature<sup>5</sup>, the colourless liquid slowly darkened, to become yellow, orange and then brown-black as a solid gradually deposited. After 4 weeks unreacted cyanide and ammonia were removed under vacuum, leaving a dark solid residue (270 g). A sample removed for elemental analysis gave carbon, 44.48; hydrogen, 3.99; nitrogen, 52.47 per cent. Theoretically hydrogen cyanide should contain carbon, 44.44; hydrogen, 3.73; nitrogen, 51.83 per cent. After stirring a portion of the dark solid (38 g) with cold water (250 ml.) and filtering off a solid black residue on a layer of filter paper pulp, the dark filtrate was freeze-dried to a brown solid.

This material (2.0 g) was dissolved in water, further clarified by filtration through a short column of 'Sephadex' resin (G-15, 4.5 × 20 cm), and freeze dried to a yellow-brown solid (1.3 g). Separation of this mixture was carried out using a longer 'Sephadex' column (G-15, 2.5 × 90 cm) with ammonium acetate buffer (ionic strength 0.01). Portions of the eluted solution (3 ml.) were collected

automatically, the concentration of material in each sample was followed spectrophotometrically at 230 m $\mu$  and recorded in the form of an elution diagram (Fig. 1). The collected solutions were combined into three fractions which were freeze dried to give yellow brown solids 4A (220 mg), 4B (120 mg) and 4C (65 mg). 4B was shown to be essentially pure diaminomaleonitrile by its infra-red spectrum, by recrystallization to give yellow crystals (melting point 182°–184° C) and by the chromatogram obtained following automatic peptide analysis<sup>6</sup>. The comparable chromatograms of 4A and 4C (Fig. 4) showed that each solid contained a mixture of peptidic material and virtually no free  $\alpha$ -amino-acids. For example, the peaks on the upper curve of chromatogram 4A at 2, 2.6, 2.8, 3.8 and 5.2 h show greater absorptions than the corresponding points on the lower curve, indicating the presence of peptides<sup>6</sup> in 4A. Colour tests<sup>7</sup> established that no hydrogen cyanide tetramer was present. The infra red spectra of 4A and 4C exhibited peaks at 2,250 cm<sup>-1</sup> and 1,660 cm<sup>-1</sup>, showing the presence of nitrile and imino/ketone groups. Elemental analysis for 4A gave carbon, 39.1; hydrogen, 4.77; nitrogen, 38.35 per cent, and for 4C gave carbon, 40.7; hydrogen, 4.58; nitrogen, 43.45 per cent. The weight average molecular weight of 4A was 620. Hydrolysis (6 normal hydrochloric acid at 100° C for 24 h) of 4A, 4B and 4C followed by automatic  $\alpha$ -amino-acid analysis showed that each hydrolysate consisted of ammonia and glycine with lesser amounts of several other  $\alpha$ -amino-acids; at least ten amino-acids were detected in 4C (Table 1).

In a variation of the anhydrous polymerization reaction, the initial black solid product was boiled with water for 24 h and then worked up as before. Solutions corre-

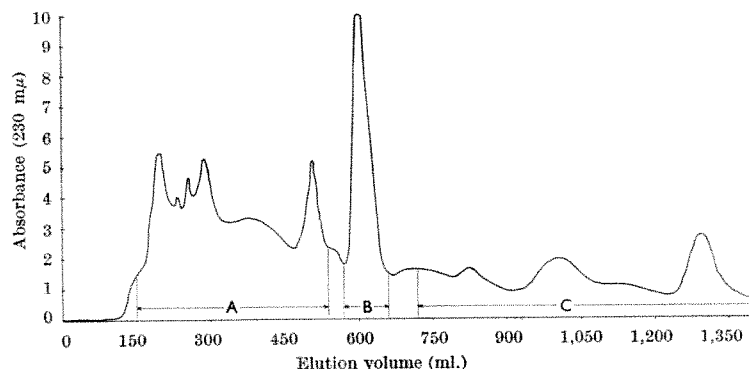


Fig. 1. Elution diagram ('Sephadex G-15' column: ammonium acetate buffer, ionic strength 0.01) for fractionation of peptidic products (A-C) obtained from HCN-NH<sub>3</sub> polymerization followed by treatment with cold water (25°).

sponding to seven major peaks in the resulting elution diagram (Fig. 2) were freeze dried to give six yellow brown solids (5A-5G; samples 5F and 5G were combined). Automatic peptide analysis (Fig. 5) showed that each solid contained mixtures of peptidic material and no diaminomaleonitrile. Appreciable amounts of glycine were also present in 5A and 5C (see parallel peaks at 5 h), as had been indicated by prior ninhydrin tests using thin-layer chromatography. Hydrolysis (6 normal hydrochloric acid at 100° C for 24 h) of the six solids yielded mixtures of glycine and several other  $\alpha$ -amino-acids (Table 1). Clearly, boiling had modified the peptidic products and had converted diaminomaleonitrile to other peptide like solids and to glycine. Parallel experiments<sup>8</sup> showed that when pure diaminomaleonitrile was boiled in water for 24 h and subjected to the separation procedures previously used, four yellow brown solids were obtained, each containing peptidic mixtures, with glycine also being found in two of the samples. Cold water had no effect on the tetramer.

In another series of experiments, hydrogen cyanide (1.5 mole, 41 g) was added to an aqueous solution of ammonia (1.0 molar, 100 ml.) in a stoppered 1 l. 'RB' flask cooled by an iced water bath. The darkening mixture was stirred magnetically for 4 h and was then packed in ice and kept in a refrigerator for 4 days. The resulting black slurry was diluted with water, flushed with nitrogen for 4 h to remove unreacted hydrogen cyanide and ammonia, and then filtered to remove a black residue. The black filtrate was worked up as before to give four solutions (Fig. 3) which were freeze dried to yellow brown solids (6A-6D). 6D was shown to be diaminomaleonitrile. Automatic peptide analysis (Fig. 6) showed that 6A, 6B and 6C contained mixtures of amino-acid polymers

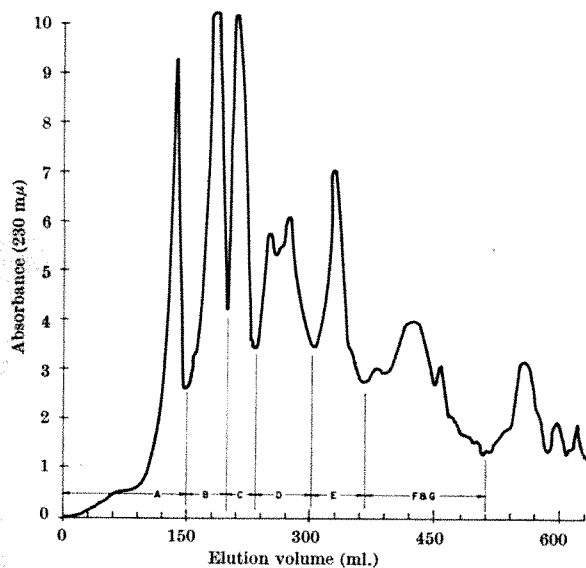


Fig. 2. Elution diagram ('Sephadex G-15' column: ammonium acetate buffer, ionic strength 0.01) for fractionation of peptidic products (A-G) obtained from HCN-NH<sub>3</sub> polymerization followed by treatment with boiling water.

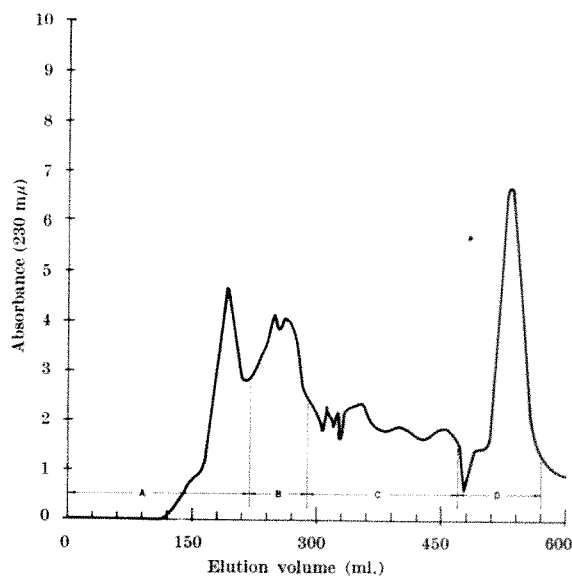


Fig. 3. Elution diagram ('Sephadex G-15' column: ammonium acetate buffer, ionic strength 0.01) for fractionation of peptidic products (A-D) obtained from HCN-NH<sub>3</sub>-H<sub>2</sub>O polymerization followed by treatment with cold water (25°).

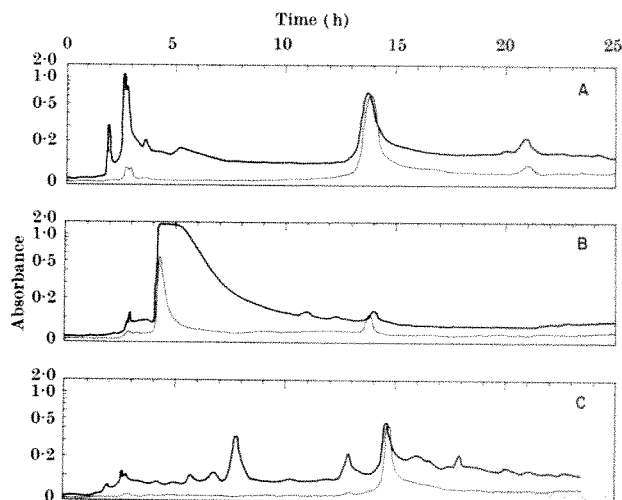


Fig. 4. Chromatograms of separated peptidic products (A-C) obtained from HCN-NH<sub>3</sub> polymerization followed by treatment with cold water (25°), recorded before (lower curves) and after (upper curves) alkaline hydrolysis.

and virtually no free  $\alpha$ -amino-acids. Hydrolysis (6 normal hydrochloric acid at 100° C for 24 h) of each of the four solids gave mixtures of up to twelve  $\alpha$ -amino-acids (Table 1).

Control experiments with 'Sephadex' columns showed that many cyano compounds are strongly retained by the resins. As a result, elution of the complex peptide-like mixtures does not take place in a systematic way related to molecular size. The peptidic solids apparently remain

Table 1. AUTOMATIC  $\alpha$ -AMINO-ACID ANALYSES OF HYDROLYSATES OBTAINED FROM HYDROGEN CYANIDE POLYMERS 4A-4C, 5A-5F AND 6A-6D

Amino-acid	4A	4B	4C	5A	5B	5C	5D	5E	5F+5G	6A	6B	6C	6D
Lysine	1	1	3	0.2	11	5	0.7	6	—	1	0.9	3	—
Histidine	5	7	5	1	0.4	1	1	6	67	13	18	5	2
Ammonia	5,525	2,630	3,942	1,759	2,159	1,088	2,072	4,926	1,529	2,417	1,851	1,338	5,263
Arginine	—	—	12	—	—	—	—	—	3	—	—	7	9
Aspartic acid	17	3	7	187	24	60	25	26	14	38	59	27	0.5
Threonine	11	0.4	0.5	2	3	3	0.7	2	0.5	0.3	0.6	1	—
Serine	3	3	4	—	—	12	1	3	1	0.3	—	5	3
Glutamic acid	0.5	—	0.8	—	0.4	0.1	0.2	0.8	0.2	57	31	0.9	—
Glycine	3,541	2,305	665	75	108	887	574	733	29	207	259	480	2,159
Alanine	21	—	5	21	16	—	27	17	25	20	21	12	—
Valine	—	—	—	0.3	—	—	—	—	37	0.7	0.9	0.2	—
Isoleucine	7	—	0.4	—	3	1	—	—	—	—	—	0.7	—
Leucine	0.5	—	0.4	—	0.2	0.8	—	—	—	0.2	0.3	0.7	—

All results have been adjusted to read  $\mu$ moles  $\alpha$ -amino-acid/g hydrolysate.



admixed with other products, as shown by the fact that, after hydrolysis, yields of  $\alpha$ -amino-acids are not quantitative. The several kinds of evidence that have already been discussed, however, are consistent with our conclusion that the polymers (or segments of the polymers) have peptidic structures. Thin-layer chromatographic ninhydrin tests<sup>4</sup> indicate the absence of monomers such as  $\alpha$ -amino-acids and related amides and amidines, dinitrophenylation experiments<sup>4</sup> point to the presence of peptide linkages and automatic  $\alpha$ -amino-acid analysis shows that mixtures of  $\alpha$ -amino-acids are present after hydrolysis. Automatic peptide analysis suggests that mixtures of peptides are present before hydrolysis, while molecular weight determinations confirm the polymeric nature of the products.

These experiments show that base-catalysed polymerization of hydrogen cyanide yields polymers which are converted by water to peptidic solids. The water can be added to the anhydrous products or can be introduced as a solvent for the reaction. Other products<sup>1-4</sup> present include diaminomaleonitrile, adenine and azulmin—black, intractable polymeric material. Current ideas of the structure of the black polymer need to be reconsidered because previously studied samples of azulmin formed in non-aqueous solvents must have contained polymeric peptidic precursors. As a result, nitrile groups of these peptidic polymers, detected by infra-red spectroscopy, have been wrongly attributed to the black solids. Direct synthesis of  $\alpha$ -amino-acids does not take place. Their presence under certain conditions is due to hydrolysis of primary reaction products including diaminomaleonitrile, adenine<sup>9</sup> and the peptidic solids.

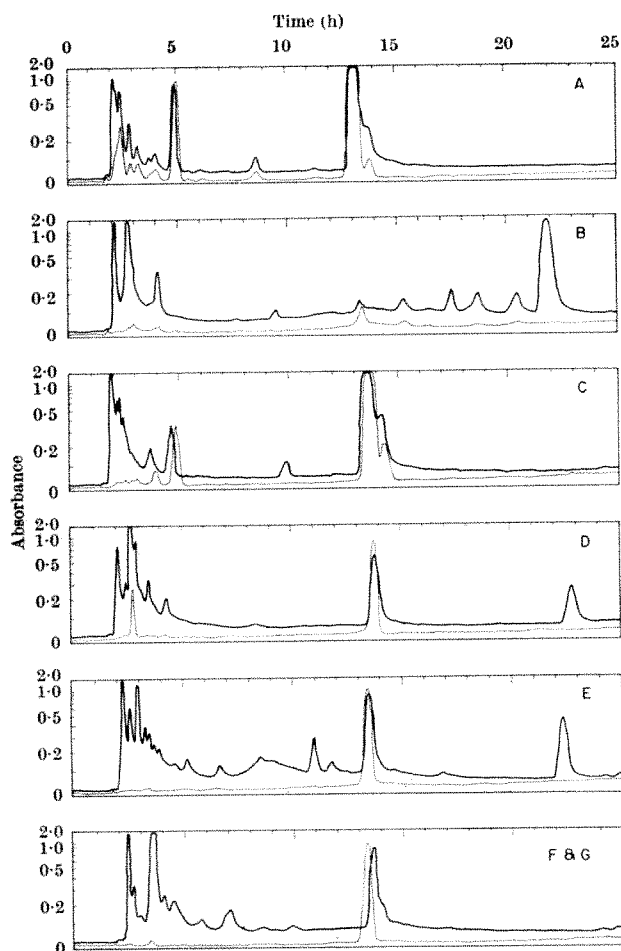


Fig. 5. Chromatograms of separated peptidic products (A-G) obtained from  $\text{HCN-NH}_3$  polymerization followed by treatment with boiling water, recorded before (lower curves) and after (upper curves) alkaline hydrolysis.

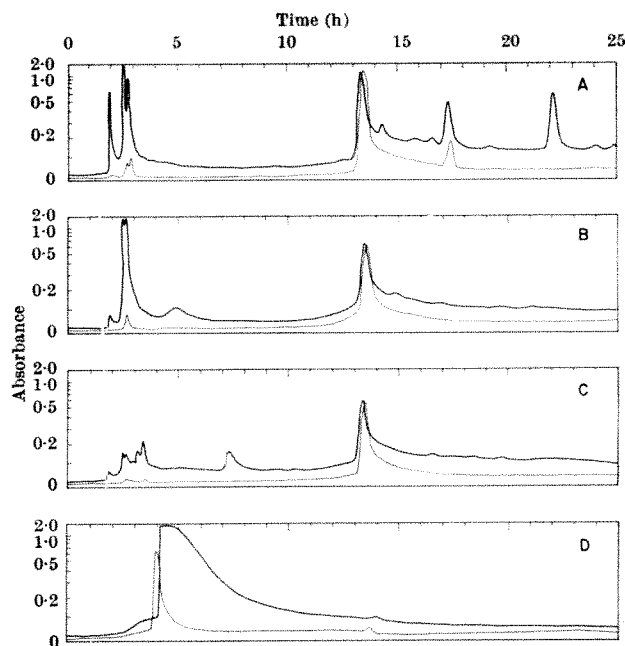


Fig. 6. Chromatograms of separated peptidic products (A-D) obtained from  $\text{HCN-NH}_3\text{-H}_2\text{O}$  polymerization followed by treatment with cold water (25°), recorded before (lower curves) and after (upper curves) alkaline hydrolysis.

Total hydrolysis of the peptidic solids yields up to fourteen known  $\alpha$ -amino-acids, assuming that the amide precursors of aspartic and glutamic acid were also formed. A few other ninhydrin reactive compounds present in trace amounts have not been characterized. So far, detection of proline, tyrosine, phenylalanine and tryptophan remains uncertain. The aromatic  $\alpha$ -amino-acids are not expected products of these reactions in the absence of acetylene or acetylene derivatives<sup>4</sup>. Of the twenty  $\alpha$ -amino-acid residues commonly found in proteins, lysine, histidine, arginine, aspartic acid, threonine, serine, glutamic acid, glycine, alanine, valine, isoleucine, leucine, probably asparagine and glutamine and possibly proline are thus products of spontaneous reactions involving only hydrogen cyanide and water<sup>10</sup>.



Fig. 7. Polypeptide synthesis from hydrogen cyanide and water. *R* represents 15 different  $\alpha$ -amino-acid side chains.

To account for the overall synthesis leading to peptides (Fig. 7) we propose<sup>4,11</sup> the following reaction sequence, starting with the dimerization<sup>10</sup> of hydrogen cyanide to aminocyanocarbene, a ground state singlet compound with considerable dipolar character<sup>12</sup> (Fig. 8).

Spontaneous polymerization of the dimer, followed by tautomerization and addition of hydrogen cyanide to the backbone double-bonds, then led to the formation of polyaminomalononitrile<sup>4</sup>. The activated nitrile groups of this polyamidine became further modified by cyanide attack<sup>10</sup> to give more complex side chains. Interaction of water with various projecting imino and cyano groups followed by decarboxylation finally yielded the peptide structures<sup>4</sup> (Fig. 9).

Alternatively, peptide synthesis in solution may have proceeded by anionic polymerization following the formation of an ambident anion<sup>13</sup> from aminocyanocarbene and a base (*B*) (Fig. 10).

Successive attack by the positively charged ends of aminocyanocarbene molecules on the unhindered<sup>13</sup> ketimine ends of growing anions then led to the polyketenimine

precursor of polyaminomalnonitrile (Fig. 10). The formation of this latter polymer by direct polymerization of aminomalnonitrile, a trimer of hydrogen cyanide highly unstable in water<sup>3</sup>, seems less probable than either of the two routes outlined here. That aminomalnonitrile decomposes to yield peptidic solids which can be further hydrolysed to mixtures of  $\alpha$ -amino acids<sup>14</sup> suggests that polymerization proceeds through hydrogen cyanide and its dimer rather than by way of the trimer.

In the context of studies of chemical evolution concerned with the origin of proteins<sup>15-18</sup> we conclude that in the many simulation experiments that have been carried out involving hydrogen cyanide as a starting material or reaction intermediate, peptide synthesis occurred spontaneously following the formation and modification of a hydrogen cyanide polymer with the structure of polyaminomalnonitrile. We therefore propose the following reinterpretations of the results of several types of pioneering experiments.

Mixtures of methane, ammonia and water (or comparable reducing mixtures) subjected to high energy radiations have yielded  $\alpha$ -amino-acids<sup>15-21</sup> and, in some cases, materials like peptides<sup>20,21</sup>. It is usually assumed that the peptides are condensation products of  $\alpha$ -amino-acids that were first formed by the well known Strecker route from  $\alpha$ -aminoacetonitriles. It appears far more likely to us that the hydrogen cyanide formed from the reactants gave rise to polyamides which reacted with water to yield peptides. Under the extreme conditions used, these became wholly or partially hydrolysed to  $\alpha$ -amino-acids. To support this view we have shown<sup>4</sup> that polymeric peptide precursors, synthesized by applying electric discharges to mixtures of methane and ammonia, are converted by water to peptide like solids.

It has been proposed<sup>22-24</sup> that the formation of triglycine from aminoacetonitrile (by heating with kaolinite and extracting with water)<sup>22</sup> shows how proteins could have originated from polyamides assumed to have been

synthesized by condensation between amine and nitrile groups. It seems more probable to us that in these experiments this unstable nitrile decomposed rapidly to hydrogen cyanide and ammonia. Polymerization and contact with water then yielded peptides. Even at room temperature, aminoacetonitrile falls apart rapidly in air or in water to give a black solid and other products which we have shown (unpublished results) can be hydrolysed to a mixture of  $\alpha$ -amino-acids.

Reactions of hydrogen cyanide in water catalysed by bases, investigated under various conditions by a number of workers<sup>1,25-27</sup>, have been reported to yield  $\alpha$ -amino-acids. We think that these products were formed by hydrolysis of peptides and hydrogen cyanide oligomers (diaminomaleonitrile and adenine) during the course of the reactions.  $\alpha$ -Amino-acid polymers detected in ammoniacal cyanide solutions at 90° C were assumed to have condensed from free  $\alpha$ -amino-acids, particularly as added labelled methionine was incorporated into the polymeric materials<sup>26</sup>. While some condensation would be expected at these high temperatures<sup>23</sup>, the results of our anhydrous and aqueous polymerization studies led us to conclude that most of the peptidic products arose directly from hydrogen cyanide, supplied both as a starting material and by decomposition of diaminomaleonitrile<sup>8</sup>. We consider<sup>4,11</sup> polycondensation of  $\alpha$ -amino-acids under high temperature, anhydrous or acidic conditions<sup>15-18</sup> to be irrelevant to the question of the origin of proteins.

In the reducing environment of primeval times direct synthesis of polypeptides would have been highly favoured as hydrogen cyanide was certainly a major component of the atmosphere<sup>11</sup>. We suggest that after polymerization and modification in the presence of other reactive molecules such as hydrogen sulphide and acetylene<sup>4</sup>, the prototypes of today's proteins were formed after the macromolecules settled into the cold<sup>3,11</sup> oceans. As the process of molecular evolution continued, this protein-dominated material gave rise to protein-directed life.

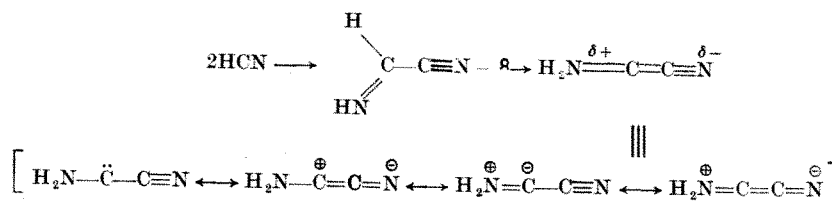


Fig. 8. Formation and structure of aminocyanocarbene (HCN dimer).

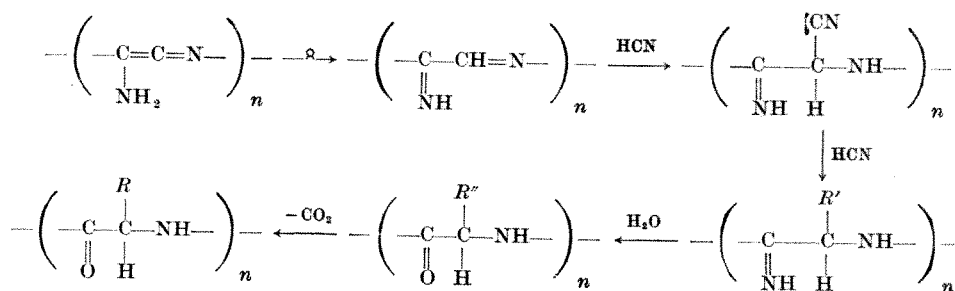


Fig. 9. Polypeptide formation from HCN and H<sub>2</sub>O. R represents 15 different  $\alpha$ -amino-acid side chains whose precursors are R' and R".

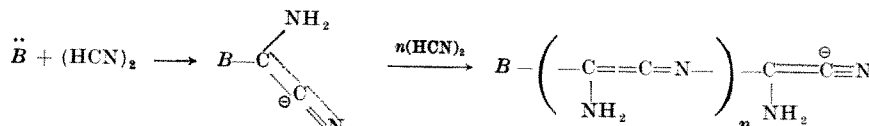


Fig. 10. Anionic polymerization of aminocyanocarbene (HCN dimer).

We thank H. L. Arons and A. R. Claggett for experimental assistance, R. F. Jansen for the peptide analyses, R. A. Martin for the  $\alpha$ -amino-acid results and N. Cirulis for the molecular weight determinations.

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<sup>2</sup> Oró, J., and Kimball, A. P., *Arch. Biochem. Biophys.*, **94**, 217 (1961).

<sup>3</sup> Sanchez, R., Ferris, J., and Orgel, L. E., *Science*, **153**, 72 (1966).

<sup>4</sup> Matthews, C. N., and Moser, R. E., *Proc. US Nat. Acad. Sci.*, **56**, 1087 (1966).

<sup>5</sup> One of our mixtures of hydrogen cyanide and base (potassium cyanide) polymerized explosively. For safety, all such reactions should be carried out in an isolated and well-ventilated location.

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<sup>7</sup> A spray solution of 1 ml. of saturated aqueous cupric nitrate and 0.2 ml. of 10 per cent nitric acid in 100 ml. of 96 per cent ethanol was used to detect diaminomaleonitrile on silica gel thin-layer chromatography plates. A characteristic purple spot appears with as little of the tetramer as 0.1  $\mu\text{g}/\text{cm}^2$ .

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<sup>9</sup> Cavalieri, L. F., Tinker, J. F., and Brown, G. B., *J. Amer. Chem. Soc.*, **71**, 3973 (1949).

<sup>10</sup> We assume that the various hydrogen cyanide reactions discussed in this paper are subject to general base catalysis or initiation, the most likely bases involved being cyanide ion, ammonia and hydroxide ion. Photochemical activation would also be feasible.

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## Regulation of Nitrate Reduction in *Aspergillus nidulans*

by

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Production of nitrate reductase may be under the control of a regulator gene with a product which can exist as an inducer or a repressor.

NITRATE is readily utilized as a source of nitrogen by plants and many kinds of micro-organisms. It is generally accepted that the initial stages of utilization are the reduction of nitrate to ammonium as follows



The enzymes known to be involved in this pathway in many organisms are nitrate reductase, nitrite reductase and hydroxylamine reductase. The identity of the intermediates between nitrite and ammonium and the enzymes responsible for the reactions is not known for certain. It is probable that in some higher plants<sup>1</sup> and in *Aspergillus nidulans* only two enzyme proteins are involved. These are nitrate reductase and nitrite reductase, which in *A. nidulans* probably also exhibits hydroxylamine reductase activity<sup>2</sup>. These enzyme activities in *A. nidulans* are induced by nitrate and nitrite and repressed by ammonium<sup>2–4</sup>. The following relevant types of mutants affecting the utilization of nitrate are known in *A. nidulans*<sup>2</sup>. The *nia D* locus is probably the structural gene for nitrate reductase; *nia D* mutants lack normal nitrate reductase activity and cannot utilize nitrate as a nitrogen source. The *nii A* locus is probably the structural gene for nitrite and hydroxylamine reductase; *nii A* mutants possess normal inducible nitrate reductase, lack nitrite and hydroxylamine reductase activity and cannot utilize nitrate or nitrite. The *nii B* gene is probably a regulator gene concerned with the induction of the nitrate reducing enzymes; *nii B* mutants lack nitrate, nitrite and hydroxylamine reductase activities and cannot utilize nitrate or nitrite.

The *nii A* mutants possess inducible nitrate reductase and lack nitrite reductase activity, and so when *nii A* strains are grown on urea as a nitrogen source and with nitrate also present, nitrite accumulates in the mycelium and is also excreted into the medium (unpublished work

of Pateman). This property of the *nii A* mutants permits recognition of mutants which are constitutive with respect to the synthesis of nitrate reductase. The essentials of the method are as follows. Conidia from an *nii A* strain are treated with a mutagen—nitrosoguanidine was used in the experiments reported here—and plated on agar medium containing 0.08 per cent sodium deoxycholate with urea as the nitrogen source. The resultant colonies are replicated<sup>5</sup> onto a second set of medium containing urea as the sole nitrogen source.

The resultant replica colonies, while they are still actively growing, are treated with a solution of sodium nitrate for 5–10 min. The colonies are tested for the presence of nitrite using a modification of the colorimetric method of Nason and Evans<sup>6</sup>, which provides a very sensitive test for nitrite, the presence of which is indicated by a purple colour. Normal *nii A* colonies do not stain purple in these conditions. If any of the replica colonies turn purple, indicating the presence of nitrite in the mycelium, the original colony on the master plate can be traced and is available for investigation.

The rationale for the technique is based on the observation that *nii A* colonies in appropriate conditions require a total of 20–25 min for nitrate to be taken up, the induction of nitrate reductase and subsequent production of a detectable quantity of nitrite. Consequently an *nii A* mutant constitutive for nitrate reductase should produce detectable nitrite after a shorter period of nitrate than is required by an inducible *nii A* strain. This proves to be the case and a number of constitutive *nii A* mutants have been isolated by this method. We report here some of the biochemical and genetical characteristics of one of those constitutive mutations designated *nir*<sup>c</sup> to indicate a constitutive mutation in a regulator gene for the nitrate reductase pathway.

The strains were grown for enzyme assay in shaken culture at 25° (ref. 7). The procedures for the various enzyme assays have been given before<sup>2</sup>. *nii A* *nir*<sup>c</sup>: The

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Table 1. ENZYME ACTIVITIES OF MYCELIUM OF VARIOUS GENOTYPES GROWN IN THE PRESENCE AND ABSENCE OF NITRATE

Genotype	Enzyme activities of mycelium grown on urea			Enzyme activities of mycelium grown on urea + sodium nitrate		
	Nitrate reductase	Nitrite reductase	Hydroxylamine reductase	Nitrate reductase	Nitrite reductase	Hydroxylamine reductase
<i>nir<sup>+</sup></i>	4.2	2.2	2.6	75	136	146
<i>nir<sup>+</sup> niiA<sup>-</sup></i>	1	3	9	76	2	21
<i>nir<sup>c</sup> niiA<sup>-</sup></i>	61	1	5	52	1	10
<i>nir<sup>c</sup></i>	124	60	100	125	237	348
<i>nir<sup>c</sup>/nir<sup>c</sup></i>	107	71	108	140	267	451
<i>nir<sup>c</sup>/nir<sup>+</sup></i>	41	8	29	121	85	119
<i>nir<sup>+</sup>/nir<sup>+</sup></i>	4	1	18	80	192	204
<i>nir<sup>c</sup>/niiB<sub>1</sub></i>	58	30	47	108	109	149

All enzyme activities are expressed as  $\mu$ moles of substrate transformed/min/mg of protein.

constitutive mutation *nir<sup>c</sup>* was induced in an *niiA<sup>-</sup>* strain, so that it possessed nitrate reductase but not nitrite or hydroxylamine reductase activity. The *niiA<sup>-</sup> nir<sup>c</sup>* strain was grown on various nitrogen sources and the nitrate reductase activity determined. The results are given in Table 1. The *niiA<sup>-</sup> nir<sup>c</sup>* strain is fully constitutive for nitrate reductase and when grown on urea or uric acid produces concentrations of nitrate reductase equivalent to those produced on urea plus the co-inducer nitrate. In the prototroph *niiA<sup>+</sup> nir<sup>+</sup>* and the mutant *niiA<sup>-</sup> nir<sup>+</sup>* nitrate reductase is only produced in a high level concentration after growth in the presence of nitrate. The constitutive mutant *niiA<sup>-</sup> nir<sup>c</sup>* no longer requires nitrate for induction but it is still subject to repression by ammonium in a similar fashion to a strain wild type with respect to its nitrogen metabolism and the *niiA<sup>-</sup> nir<sup>+</sup>* mutant.

A strain of *nir<sup>c</sup>* was obtained from a cross between the wild type and the *niiA<sup>-</sup> nir<sup>c</sup>* mutant. The *nir<sup>c</sup>* strain is wild type with respect to all the structural genes concerned with nitrate utilization but carries the constitutive mutation at the *nir* locus. This strain, together with the wild type, was grown on various nitrogen sources and the nitrate, nitrite and hydroxylamine reductase activities were determined. The results are given in Table 1. The *nir<sup>c</sup>* mutant is constitutive for nitrate reductase. The levels of activity of this enzyme in *nir<sup>c</sup>* are similar in mycelium grown on urea or on urea plus nitrate and rather higher than those of *nir<sup>+</sup>* grown on urea plus nitrate. The situation with respect to nitrite and hydroxylamine reductase activity is different. When *nir<sup>c</sup>* is grown in the absence of the co-inducer nitrate, the levels of nitrite and hydroxylamine reductase activity are about 50 per cent of those of *nir<sup>+</sup>* when it is grown in the presence of nitrate. When *nir<sup>c</sup>* is grown on urea plus nitrate the nitrite and hydroxylamine reductase activities are typically two to three times higher than those of *nir<sup>+</sup>* grown on urea plus nitrate. Thus the constitutive mutation in the *nir* gene results in semi-constitutivity in the absence of co-inducer and super-inducibility in the presence of co-inducer for nitrite and hydroxylamine reductase activity.

To investigate the dominance relationship of the *nir<sup>c</sup>* and *nir<sup>+</sup>* alleles the following diploid strains were made: the homozygous diploid constitutive strain *nir<sup>c</sup>/nir<sup>c</sup>*; the heterozygous diploid carrying both *nir<sup>c</sup>* and *nir<sup>+</sup>* alleles, *nir<sup>c</sup>/nir<sup>+</sup>*; and the homozygous diploid wild type *nir<sup>+</sup>/nir<sup>+</sup>*. These diploid strains were grown on various nitrogen sources, and their enzyme activities were determined. The results are given in Table 1. The *nir<sup>c</sup>/nir<sup>c</sup>* diploid is similar to the haploid *nir<sup>c</sup>* and the *nir<sup>+</sup>/nir<sup>+</sup>* diploid is similar to the haploid *nir<sup>+</sup>*. The nitrate reductase

activity of the heterozygous diploid *nir<sup>c</sup>/nir<sup>+</sup>* when grown on urea alone is about 50 per cent of that possessed by the haploid *nir<sup>+</sup>* or diploid *nir<sup>+</sup>/nir<sup>+</sup>* prototrophs when grown on urea plus nitrate. That is, the *nir<sup>c</sup>* allele is functionally semi-dominant with respect to the regulation of the synthesis of nitrate reductase. The nitrite and hydroxylamine reductase activities of *nir<sup>c</sup>/nir<sup>+</sup>* when grown on urea alone are very low, but are significantly higher than those of the wild type *nir<sup>+</sup>/nir<sup>+</sup>* on urea. Thus the *nir<sup>c</sup>* allele is almost completely recessive with respect to the regulation of nitrite and hydroxylamine activity. The level of activity of the two enzymes in *nir<sup>c</sup>/nir<sup>+</sup>* is less than that of the wild type diploid when both are grown on urea plus nitrate. This result was obtained with all three separate heterozygous diploids tested; its significance, if it is a genuine characteristic of such diploids, is not clear.

Genetic evidence—see following—suggests that the *nir<sup>c</sup>* mutation is in the *niiB* gene. Consequently, a diploid *nir<sup>c</sup>/niiB<sub>1</sub>* was made, grown on various nitrogen source and assayed for the nitrate utilizing enzymes. The results given in Table 1 are similar to those obtained for the *nir<sup>c</sup>/nir<sup>+</sup>* diploid.

The presence of ammonium represses the synthesis of the nitrate utilizing enzymes in the wild type. The haploid constitutive strain was grown on three types of medium containing, respectively, 0.015 molar ammonium tartrate, 0.015 molar ammonium tartrate plus 0.01 molar sodium nitrate and 0.015 molar ammonium tartrate plus 0.1 molar sodium nitrate. The enzyme activities of the *nir<sup>c</sup>* and *nir<sup>+</sup>* strains grown in these conditions are given in Table 2. The constitutive strain is strongly repressed by ammonium but to a lesser degree than the wild type. Increasing the concentration of the co-inducer nitrate relieves the ammonium repression to some extent as it does with the wild type.

The original *niiA<sup>-</sup> nir<sup>c</sup>* mutant strain was detected because in the appropriate conditions an *niiA<sup>-</sup> nir<sup>c</sup>* colony accumulates nitrite and can be stained purple by the chemical test for nitrite. It was subsequently found that this staining procedure could be used to detect the presence of the *nir<sup>c</sup>* allele in the absence of the *niiA<sup>-</sup>* allele. In short, after growth on urea, and a brief treatment with nitrate, followed by the nitrite staining procedure, *niiA<sup>-</sup> nir<sup>c</sup>* colonies are a deep purple pink, *niiA<sup>+</sup> nir<sup>c</sup>* colonies are a faint pink and *niiA<sup>+</sup> nir<sup>+</sup>* colonies are white. Classification of these genotypes by a simple chemical test permits the genetic analysis of the segregation of the *nir* gene. The following crosses were set up. (a) *niiA<sup>+</sup> nir<sup>c</sup>*  $\times$  *niiA<sup>-</sup> nir<sup>+</sup>*. Only one of the recombinant genotypes, *niiA<sup>+</sup> nir<sup>+</sup>*, can be readily classified in this cross. A total of twelve *niiA<sup>+</sup> nir<sup>+</sup>* was present in a sample of seventy progeny. This indicates that the *niiA* and *nir* genes are about 35 cM apart on chromosome VIII. (b) *niiA<sup>-</sup> nir<sup>c</sup>*  $\times$  *niiA<sup>+</sup> nir<sup>+</sup>*. Both of the recombinant types, *niiA<sup>-</sup> nir<sup>+</sup>* and *niiA<sup>+</sup> nir<sup>c</sup>*, can be classified in this cross. A total of fifty-five recombinants was found in a sample of 123 progeny. This indicates that the *niiA* and *nir* genes are about 45 cM apart on chromosome VIII. (c) *niiB<sup>+</sup> nir<sup>c</sup>*  $\times$  *niiB<sup>-</sup> nir<sup>+</sup>*. Only the recombinant type *niiB<sup>+</sup> nir<sup>+</sup>* can be readily classified in this cross. A total of 627 progeny were analysed from this cross and two of them were the *niiB<sup>+</sup> nir<sup>+</sup>* recombinant type. It is possible, although unlikely, that these two apparent recombinants were contaminants. The data demonstrate, however, that the *nir*

Table 2. ENZYME ACTIVITIES OF MYCELIUM OF THE WILD TYPE AND A CONSTITUTIVE MUTANT GROWN IN THE PRESENCE OF VARIOUS CONCENTRATIONS OF AMMONIUM AND NITRATE

	0.015 molar ammonium tartrate			0.015 molar ammonium tartrate 0.01 molar sodium nitrate			0.015 molar ammonium tartrate 0.1 molar sodium nitrate		
	Nitrate reductase	Nitrite reductase	Hydroxylamine reductase	Nitrate reductase	Nitrite reductase	Hydroxylamine reductase	Nitrate reductase	Nitrite reductase	Hydroxylamine reductase
<i>nir<sup>+</sup></i>	<1	<1	9	4	2	18	21	15	25
<i>nir<sup>c</sup></i>	7	6	32	27	38	76	31	42	126

All enzyme activities are expressed as  $\mu$ moles of substrate transformed/min/mg of protein.



gene is either identical with the *nii B* gene or, if there are two genes, they are closely linked.

The experiments reported here show: (1) the *nir*<sup>c</sup> mutation affects all the known enzyme activities thought to be concerned with nitrate reduction in *A. nidulans*; (2) the *nir*<sup>c</sup> mutation is semi-dominant with respect to the induction of nitrate reductase and recessive with respect to the induction of nitrite and hydroxylamine reductase activities in the heterozygous diploid of genotype *nir*<sup>c</sup>/*nir*<sup>+</sup>; and (3) the *nir*<sup>c</sup> mutation is located in or very close to the *nii B* locus. We propose the following hypothesis on the basis of these observations and our previous work<sup>2,3,7,8</sup>.

There is a regulator gene which controls the induction of the enzymes responsible for nitrate reduction. The product of this regulator gene can exist in two forms, one of which represses, while the other is necessary for the synthesis of enzyme. In wild type strains, in the absence of co-inducer, the regulator product is in its repressor form. The co-inducer, which may be either nitrate or nitrite, combines with the regulator product, converting it to its other form, which we shall call an inducer. Mutation in the regulator gene can result in various different altered regulator substances. First, mutation could lead to the complete inability to make a regulator substance. Second, a regulator substance could be produced, which cannot be converted to its inducer form by the co-inducer. The *nii B*<sup>-</sup> mutants, of which some eighteen are known, are probably examples of these first two types of mutations. Third, an altered regulator product could be made, which even in the absence of co-inducer has some of the properties of the inducer. Mutant strains producing such a regulator substance would be constitutive and it is proposed that *nir*<sup>c</sup> is such a strain. There are a number of points concerning this hypothesis which merit further discussion.

In contrast to the classical case of the *i* gene of the  $\beta$ -galactosidase system of *E. coli*<sup>9</sup>, the product of the *nir* gene is assumed to play not only a negative but also a positive role in the induction of nitrate and nitrite reductases. This positive role is inferred from the dominance of the *nir*<sup>c</sup> allele. It is also possible to explain dominant constitutive mutations without invoking a

positive control system by constructing elaborate cascade models, but such a hypothesis would need to be considerably more complex than the one proposed to explain all the findings in this system. Other examples are known of regulator genes which exhibit some degree of dominance; these include a regulator gene for xanthine dehydrogenase in *Aspergillus nidulans*<sup>10</sup>, and the *C* gene in the L-arabinose system of *E. coli*<sup>11</sup>.

We propose that the product of the *nir*<sup>+</sup> gene plays a negative as well as a positive regulatory role, in order to accommodate the finding that the *nir*<sup>c</sup> allele is not fully dominant. The situation is best envisaged as follows. The product of the *nir*<sup>+</sup> allele acts as a repressor, but its inducer form, that is, when it is combined with co-inducer, is necessary to actively initiate enzyme synthesis. The product of the *nir*<sup>c</sup> allele is, however, able to initiate synthesis, even in the absence of co-inducer. In the *nir*<sup>+</sup>/*nir*<sup>c</sup> diploid in the absence of co-inducer, there is competition between the *nir*<sup>+</sup> product, which functions as a repressor, and the *nir*<sup>c</sup> product, which functions as an inducer, and consequently enzyme activities intermediate between the two homozygotes are found.

It must be supposed that the interaction between the structural gene and the regulator gene product is somewhat different for the nitrate and nitrite reductase structural genes. The *nir*<sup>+</sup>/*nir*<sup>c</sup> heterozygous diploid produces in the absence of co-inducer about 40 per cent of the nitrate reductase but only 10 per cent of the nitrite reductase activity which it produces in the presence of co-inducer. It therefore seems that the affinity of the *nir*<sup>c</sup> product for the recognition site of the nitrite reductase structural gene is less than its affinity for the recognition of the nitrate reductase structural gene. Furthermore, the increased synthesis of nitrite reductase in the presence of the co-inducer nitrate, compared with that in its absence, can be interpreted as indicating that the *nir*<sup>c</sup> allele product can interact with nitrate to become a better inducer substance. The principal features of the hypothesis proposed are represented diagrammatically in Fig. 1. Because the structural genes, *nii D* and *nii A*, are 10 cM apart<sup>2</sup>, there can be no operon organization for the nitrate reductase pathway. The situation with regard to the control of these enzymes, however, must be more complex. This

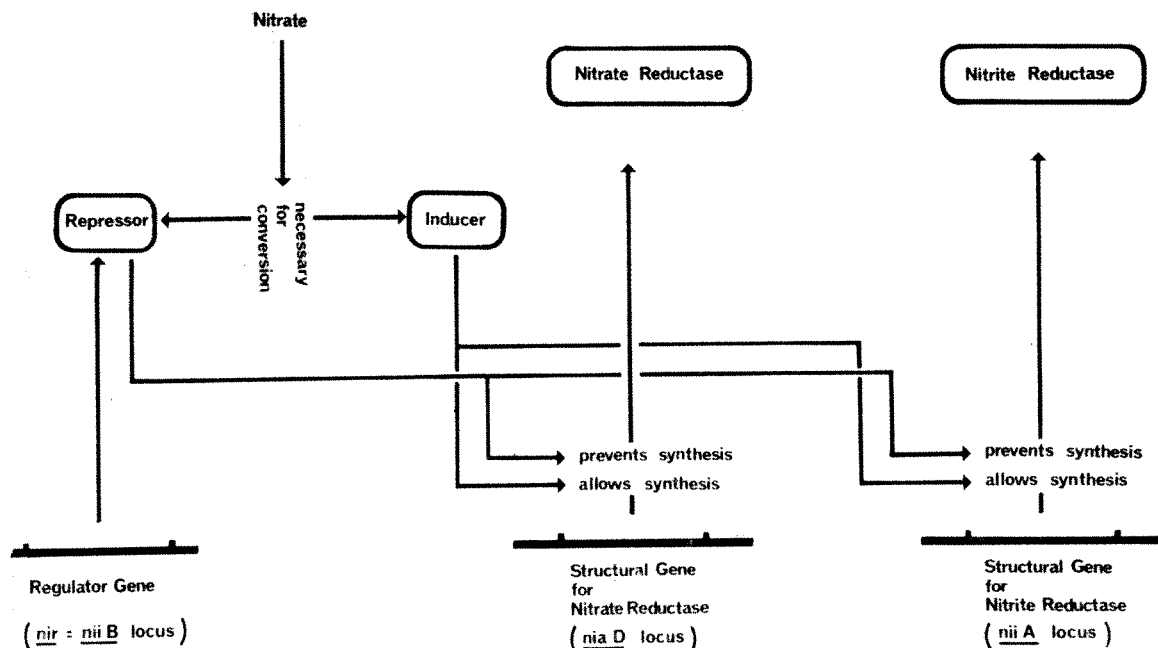


Fig. 1. A model illustrating the simplest hypothesis to account for the action of the regulator gene *nir*. The product of the regulator gene *nir* can exist in a repressor or inducer form depending on the presence of the co-inducer nitrate. When the regulator substance is in the inducer form, it has a positive action necessary for the initiation of enzyme synthesis. In the repressor form it prevents enzyme synthesis.

model does not prejudge at what stage gene action is controlled and takes no account of the repression of nitrate and nitrite reductase synthesis by ammonium, which can largely overcome the action of the co-inducer nitrate. Also there is a considerable body of evidence which suggests that nitrate reductase is itself a component in the control of the pathway. Some of the evidence for this and the resultant necessary modifications will be described elsewhere.

If the foregoing hypothesis is largely correct and the mutations, previously known as *nii B*<sup>-</sup>, do result in the production of a defective regulator substance, the following predictions can be made. In some cases mutation will result in the production of a regular substance which no longer acts as an inducer but which still functions as a repressor. This mutant repressor could still be inactivated by the co-inducer. In the heterozygous diploid containing such an allele and the *nir*<sup>c</sup> allele, the concentrations of enzyme in the presence and absence of the co-inducer will be similar to those of the *nir*<sup>c</sup>/*nir*<sup>+</sup> heterozygous diploid. The diploid between *nir*<sup>c</sup> and *nii B*<sup>-</sup> reported in this

article is an example of this. It should also be possible for mutation at the *nii B* locus to result in the absence of regulator substance or a regulator substance which is defective in both inductive and repressive functions. Such a mutation, when present in a heterozygous diploid with the *nir*<sup>c</sup> allele, should be completely recessive and the diploid should be completely constitutive in phenotype. At present a survey is being made of the known *nii B*<sup>-</sup> mutations in order to discover if any are completely recessive to *nir*<sup>c</sup>.

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## Half-lives of Peptides and Amines in the Circulation

by

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The blood-bathed organ technique, which allows the continuous bioassay of substances in the circulating blood, has been used to measure the half-lives of several substances in the circulation.

THE half-life of a hormone in the circulating blood has usually been measured by one of two methods. In one, blood samples are taken after injection or infusion of the substance, and its concentration in the plasma is then estimated chemically or by bioassay<sup>1-4</sup>. This sampling technique is limited by its intermittent nature, the large doses of the substance that have to be given to achieve measurable concentrations in a small blood sample and the possibility that the removal of blood will itself affect the concentration of the substance or lead to a progressive alteration of the animal's physiology. In the other method, the physiological response to the hormone is used as an indicator of its concentrations in the circulation<sup>5</sup>. This method is limited by the assumption that the response will persist as long as the hormone is present in the circulating blood. Two illustrations show that this assumption is questionable. First, in the rat, the blood pressure response to noradrenaline declines during its continuous infusion; by contrast the vasoconstrictor response in the hind quarters is maintained even 1 h after the end of infusion<sup>6</sup>. Second, the anti-diuretic effects of analogues of vasopressin outlast the presence of the peptides in the circulation<sup>7,8</sup>.

A technique which employs a blood-bath organ<sup>9</sup> was developed to overcome some of these limitations. It permits continuous assay of many hormones at concentrations found after their release into the circulation<sup>10-12</sup>. We have applied this method to the determination of the half-lives of several substances in the circulation of cats and dogs.

Cats of either sex weighing 2-5 kg were anaesthetized with ethyl chloride and ether; anaesthesia was then maintained with chloralose (80 mg/kg intravenously). Dogs of either sex weighing 5-20 kg were anaesthetized

with halothane delivered from a Goldman vaporizer; anaesthesia was maintained with chloralose (100 mg/kg intravenously) and supplemented when necessary with pentobarbitone (5-10 mg/kg intramuscularly or intravenously). The trachea was cannulated and the lungs were ventilated mechanically. Polyethylene cannulae were tied into a femoral or carotid artery and into a femoral or jugular vein for removal and replacement of blood. Mean arterial blood pressure was recorded on a Beckmann-Offner dynograph using a Statham pressure transducer (type P23Db) attached to a side arm of the arterial cannula.

The following organs were used to assay the different substances: for bradykinin, strips of cat jejunum<sup>13</sup>; angiotensin, the rat colon<sup>14</sup>; 5-hydroxytryptamine, adrenaline and noradrenaline, the rat stomach strip<sup>15</sup>. All of these isolated organs contracted in response to the test substance except the rat stomach strip which relaxed to adrenaline and noradrenaline. They were suspended in polypropylene chambers and superfused<sup>16</sup> in cascade with Krebs solution while the animal was being prepared; their movements were transduced by Ether strain gauges attached to auxotonic<sup>17</sup> levers and recorded on the dynograph. The initial load on the assay organs was 1-3 g.

Heparin (1,000 IU/kg) was injected intravenously and the assay organs were then superfused with arterial blood delivered by a roller pump at 10-15 ml/min. The blood was collected in a reservoir and returned to the animal either by gravity or through a second channel or roller pump. The assay organs were calibrated by infusing the substances directly into the blood stream just after it had left the animal; for example, an infusion of 10 ng/min into a flow of 10 ml/min gave a rise in concentration of 1 ng/ml.

Constant intravenous infusions of the substances produced responses of the isolated blood-bathed organs which increased during the first 1 or 2 min to a plateau,

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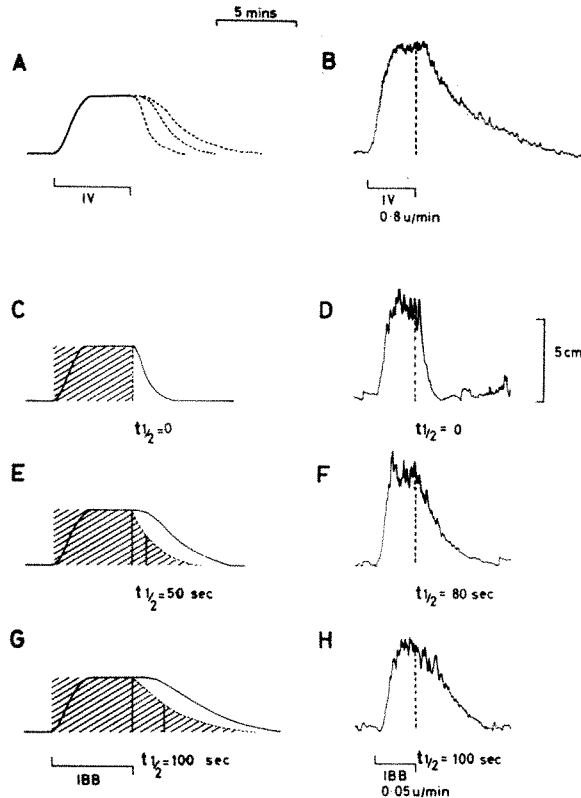


Fig. 1. The left hand side shows a diagram of the technique and the right hand side an actual tracing obtained using it. (A) Some possible patterns of relaxation of an assay organ after stopping an intravenous infusion. (C, E and G) Effects of infusions when given directly to the assay organs. (C) Infusion (shaded area) was stopped abruptly ( $t_{1/2} = 0$ ); (E) infusion was stopped with a half-time of 50 sec, and (G) with a half-time of 100 sec. The continuous line in each of these diagrams represents the contraction of the isolated organs. The right hand side of the figure is taken from an experiment in a cat in which a strip of cat jejunum was bathed with arterial blood. Kallikrein ( $0.8 \text{ u/min}$ ) was infused intravenously (B) or directly to the tissues with the infusion ( $0.05 \text{ u/min}$ ) ending with a half-time of 0 (D), 80 sec (F) and 100 sec (H). The decay of the contraction after intravenous infusion was longer lasting than after the calibrating infusion with a half-time of 100 sec.

which was then maintained for the duration of the infusion. When the infusion was abruptly stopped the response declined gradually (Fig. 1A and B). If the rate of decline of the effect depended only on the rate of decrease of drug concentration the half-life of the drug in the circulation could have been determined directly from the decay curve. The assay organs themselves, however, had a finite response time. An example of this is shown in Fig. 1C and D. In general, the return to baseline of the assay organs themselves had a half time varying from 5 to 50 sec, depending on the organ and on the drug.

After drugs are injected or infused, their concentration in the circulation tends to decline exponentially<sup>3,5,18,19</sup>. We therefore devised a method for infusing the substance directly into the assay system to produce an exponential decline in concentration. Infusions of the test substances were made into the blood bathing the assay organs with a roller pump<sup>20</sup>. The speed of the pump, and therefore the rate of infusion, was controlled by a 'Servomex MC 43' motor controller. Usually the speed of the motor controller of this system is regulated by a negative feedback voltage which can be set at any desired level. Our controller circuit was modified so that the feed-back voltage could be supplied, when desired, by a condenser discharging through a resistance so that the speed of the motor decayed exponentially. Condensers of different capacities were chosen to give exponential decays with half-times ( $t_{1/2}$ ) of 10, 15, 20, 45, 60, 85 and 100 sec.

Infusions were made into the blood bathing the assay organs, after it had left the animal, for long enough to

give a plateau response of the isolated organs. The infusions were stopped either abruptly ( $t_{1/2} = 0$ , Fig. 1D) or at different exponential rates (Fig. 1E, F, G and H) by the use of the condenser discharge system. The return to baseline of the assay tissues was progressively slower as the half-times of the exponential decay in the infusion rates were increased. To compensate for any variation in the height of the plateau response the rates of decline of the contractions of the assay organs were plotted as a percentage of the plateau response against time. This gave a family of curves which allowed discrimination between all the half-times used except 0 and 10 sec. The decline of the contraction of the assay organs after an intravenous infusion (Fig. 1A and B) was plotted in the same way. The resultant curve was compared with the family of curves to find the one which produced the closest match. The half-time of this curve was taken to be the half-life of the substance in the circulation. The figures show that the shapes of the curves were strikingly similar, confirming the validity of the underlying assumption that the decline in concentration after intravenous infusion was exponential.

The design of the experiments to find the half-life of bradykinins in the brain of the cat was as follows. First infusions of bradykinin were made into the bathing blood and intravenously to find infusion rates which gave similar contractions of the cat jejunum. In Fig. 2, the responses are to infusions of bradykinin ( $3 \text{ ng/ml}$  in the bathing blood) stopped instantaneously ( $t_{1/2} = 0$ , Fig. 2B), exponentially, with a half-life of 10 sec ( $t_{1/2} = 10$ , Fig. 2D) or of 15 sec ( $t_{1/2} = 15$ , Fig. 2A). Fig. 2C shows the effect of an intravenous infusion of bradykinin ( $5 \text{ ng/min}$ ). There was no change of blood pressure during infusion. In this and all other experiments, the infusions were given in random order. The graphs obtained by plotting the results of this experiment are shown in Fig. 2E. The decline of the response to a 10 sec half-time was not distinguishable from that of an instantaneous cessation of infusion, but the response to a 15 sec half-time was clearly

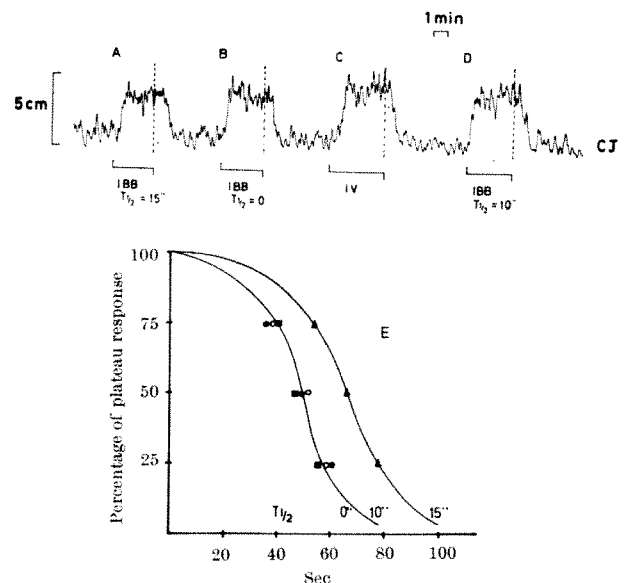


Fig. 2. The upper part of the figure shows contractions induced by infusing bradykinin either directly ( $3 \text{ ng/ml}$  IBB) to the cat jejunum strip bathed in arterial blood from a cat, or intravenously ( $5 \text{ ng/min}$ ) into the cat. The first effect (A) is a response to an IBB infusion which ends with a half-time of 15 sec, the second (B) with a half-time of 0 and the fourth (D) with a half-time of 10 sec. The third effect (C) is after the intravenous infusion. The vertical dotted lines are superimposed to show more clearly the end point of the infusion. The return to baseline of the contraction was plotted as a percentage of the plateau response in the graph below. It was difficult to separate the lines for  $t_{1/2} = 0$  (■) and  $t_{1/2} = 10$  sec (●) but  $t_{1/2} = 15$  sec (▲) was clearly distinguishable. The half-life of the intravenous infusion (○) lay within the 0 to 10 sec half-time range, showing that the half-life of bradykinin in this cat was less than 15 sec.

distinguishable. The intravenous infusion of bradykinin gave a curve which approximated to the instantaneous and 10 sec curves, showing that the half-life was certainly less than 15 sec. This was confirmed in two other experiments. Thus the half-life of bradykinin in the circulation is less than one circulation time. In man bradykinin has a half-life of less than 30 sec (ref. 3).

It should be noted that this technique detected changes in concentration of 1–5 ng/ml. To obtain such concentrations with intravenous infusions, infusion rates of 5  $\mu$ g/min had to be used, again suggesting that the removal of bradykinin from the circulation is very rapid.

Bradykinin is formed in the circulation by kallikrein, activated in the plasma or originating from tissues<sup>23,24</sup>. Although the half-life of bradykinin itself is short, it may continue to be formed in the circulation if kallikrein persists there in an active form. The blood-bathed organ technique was used to measure the half-life not only of the polypeptide but also of one of the enzymes which can liberate bradykinin. Kallikrein ('Glumorin', Farbenfabriken Bayer AG Leverkusen, Germany) was infused either directly into the bathing blood or intravenously into the cat in the same way as bradykinin. At the infusion rates used, kallikrein did not contract the cat jejunum bathed in Krebs solution, showing that the enzyme had no activity on this preparation. When bathed in blood, however, the cat jejunum contracted to infusions of kallikrein because of the generation of bradykinin. Thus the formation of bradykinin could be used to assess the presence of kallikrein. The contractions of a preparation of cat jejunum in response to infusions of kallikrein from one of four experiments in cats are shown in Fig. 1 and the curves plotted from these effects in Fig. 3. The half-life of kallikrein was slightly longer than that of the largest condenser discharge available (100 sec), and was estimated to be about 120 sec in this experiment. In two other experiments with the cat, the half-life was 100 sec and in the fourth it was many minutes. The half-life of kallikrein did not depend on its concentration in the plasma, for the same estimate was obtained after an infusion of 0.4 U/min given intravenously as was obtained after an infusion of 0.8 U/ml. given intravenously, although the faster infusion rate generated much more bradykinin. These rates of infusion of kallikrein were too slow to result in a decrease of blood pressure.

Angiotensin has a half-life in the circulation of the dog of less than 3 min<sup>10</sup>; its removal from the circulation is not caused by destruction in the blood stream, but it disappears in peripheral vascular beds<sup>25</sup>. This disappearance varies from 40 per cent in the hind quarters to 75 per cent in the kidneys, suggesting an overall value of about 50 per cent, or a half-life of angiotensin in the circulation of about one circulation time. In order to compare in the same animal the method of measuring the rate of disappearance with the method of measuring half-life the following experiment was carried out on a dog. The overall disappearance of angiotensin in one passage through the peripheral circulation was first established. A coaxial polyethylene catheter was passed down the right carotid artery and advanced retrogradely into the ascending aorta so that the tip of the inner catheter (external diameter 2 mm) lay just above the aortic valves. The outer catheter (external diameter 3 mm) was about 2 cm shorter than the inner one and therefore opened downstream with respect to the inner catheter. Blood for supplying the assay circuit was withdrawn through the inner catheter, so that it sampled the cardiac output. Infusions of angiotensin were made through the outer catheter, so that they mixed with the blood in the ascending aorta just after it had been sampled. Thus angiotensin reached the assay circuit only after it had passed once through the peripheral tissues. The blood-bathed rat colon was used to detect angiotensin, and the effects of infusions given through the arterial catheter were compared with the effects of infusions given intravenously.

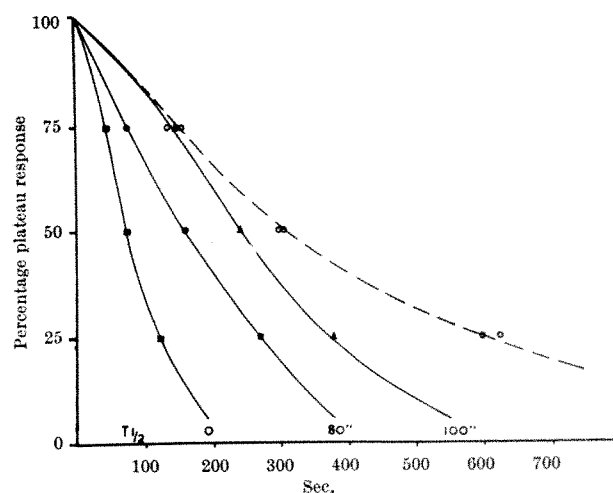


Fig. 3. The lines are plotted from the return to base line of a cat jejunum preparation bathed in arterial blood from a cat, after kallikrein was infused either intravenously (for tracing, see Fig. 1B) or in calibrating doses IBB (Fig. 1, D, F and H). The return to base line after the infusions which ended with  $t_{1/2} = 0$  (■), 80 (●) and 100 (▲) sec were clearly distinguishable. The intravenous infusion (O) had a half-life of longer than 100 sec. In this experiment, two rates of intravenous infusion of kallikrein were used (0.4 and 0.8 U/min) but the curves for both lay on the same line.

The validity of comparison depends on the fact that no angiotensin disappears in the pulmonary circulation<sup>25</sup>. An arterial infusion of 0.5  $\mu$ g/min of angiotensin gave a plateau response of the rat colon which matched that produced by an intravenous infusion of 0.25  $\mu$ g/min: thus 50 per cent of the angiotensin infusion disappeared in passage from the arterial to the venous side of the circulation. In the same dog, infusions with different exponential declines were then made into the blood bathing the rat colons. The curves which were plotted were compared with those from the intravenous infusion (Fig. 4). The curve from the intravenous infusion matched almost exactly that with a 20 sec half-life, showing that in this dog the half-life was, indeed, about one circulation time. This experiment therefore demonstrates that the two methods give similar results and confirms the suggestion<sup>25</sup> that the half-life of angiotensin in the dog is about one circulation time.

The half-life of renin in the circulation is very much longer than that of angiotensin<sup>10,11,26</sup>, and probably exceeds 15 min. The present method could not be used to determine such a long half-life because condenser discharges of such duration could not be achieved. The error introduced by the delayed response of the assay organs, however, in comparison with such a long half-life is probably insignificant. Thus it may be possible to calculate the half-life of renin in the circulation of the dog simply by plotting the curve for the relaxation of the rat colon; this is being done (Ng and Vane, unpublished work).

Adrenaline is removed from the circulation by peripheral vascular beds<sup>27–29</sup>; for example, more than 90 per cent disappears in one circulation through the hind quarters<sup>27</sup>. A similar figure was obtained for noradrenaline<sup>27</sup>. Thus it might be expected that the half-life in the circulation would be less than one circulation time. This was confirmed in a cat (Fig. 4). An intravenous infusion of adrenaline gave a curve which matched the 10 sec half-life curve and an infusion of noradrenaline gave a curve which lay between the 0 and 15 sec half-life curve. A half-life of less than one circulation time was also obtained in a dog.

Unlike the catecholamines, 5-hydroxytryptamine is chiefly removed from the circulation by the lungs<sup>30–32</sup> and 90–98 per cent of an intravenous infusion disappears in one passage through the pulmonary circulation (Thomas and Vane, unpublished work). That 5-hydroxytryptamine



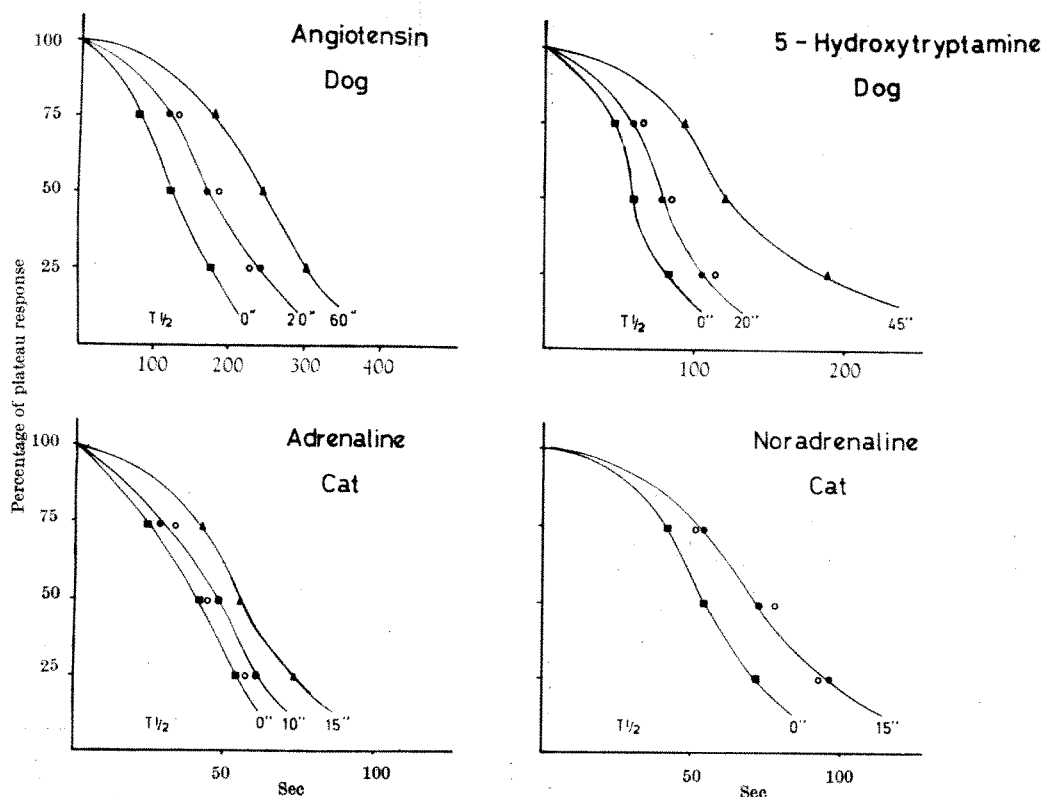


Fig. 4. The graphs are plotted from experiments with angiotensin, 5-hydroxytryptamine, adrenaline and noradrenaline. The half-lives of intravenous infusions (○) of these substances were 20 sec for angiotensin in the dog, 20 sec for 5-hydroxytryptamine in the dog, between 0 and 10 sec for adrenaline and 15 sec for noradrenaline in the cat.

has a half-life in the circulation of less than one circulation time was confirmed in a dog experiment (Fig. 4D).

To summarize our results: the way in which the contractions or relaxations of the blood-bathed assay organs return to baseline after an intravenous infusion can be exactly mimicked by stopping an infusion directly to the organs in an exponential fashion. This must mean that when an intravenous infusion is terminated, the concentration of hormone declines exponentially, even though the relaxation of the assay organ itself does not describe an exponential curve, presumably because it represents the addition of two exponential functions. For bradykinin, angiotensin, adrenaline, noradrenaline and 5-hydroxytryptamine, we found that the half-life in the circulation was less than or about equal to one circulation time. Thus when any of these substances are released or infused into the blood stream, the concentration will be reduced to about 10 per cent within three circulations. It is interesting that all of these naturally occurring substances, some of which have important hormonal functions, have such a short half-life in the circulation, although their method of removal from the circulation differs.

The enzymes kallikrein and renin have much longer half-lives; thus, just as for the renin-angiotensin system, it is likely that for bradykinin to have any importance as a circulating substance in physiological or pathological conditions the generating enzyme itself must be released into and circulate in the vascular system.

Finally, we must stress that we have measured the half-lives of these substances in the circulation; this may not reflect the half-lives of their activity in particular vascular beds. It is possible that each substance is inactivated by a different mechanism at a different rate, once it has left the circulation. As far as their effects on the pre-capillary resistance vessels are concerned, however, this factor may not be important.

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# Haemoglobin Synthesis in $\alpha$ -Thalassaemia (Haemoglobin H Disease)

by

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In haemoglobin H disease, the rate of synthesis of the  $\alpha$ -chains of haemoglobin is depressed, while that of the  $\beta$ -chains remains normal. It is shown that part of this excess of  $\beta$ -chains forms a pool capable of further participation in haemoglobin synthesis. The  $\beta$ -chains of haemoglobin H can exchange reversibly with those of haemoglobin A in the intact red cell.

THE thalassaemias are a group of inherited anaemias resulting from a reduced rate of synthesis of the peptide chains of haemoglobin. Normal adult haemoglobin A has two  $\alpha$ - and two  $\beta$ -peptide chains which are under independent genetic control. There are therefore two principal groups of thalassaemias, one resulting from defective  $\alpha$ -chain synthesis, called  $\alpha$ -thalassaemia, and the other resulting from defective  $\beta$ -chain synthesis,  $\beta$ -thalassaemia<sup>1</sup>.

In  $\beta$ -thalassaemia the reduced rate of  $\beta$ -chain synthesis can be compensated in part by increased production of the other normal haemoglobin components, haemoglobin F, which has two  $\alpha$ -chains and two  $\gamma$ -chains, and haemoglobin A<sub>2</sub>, which has two  $\alpha$ -chains and two  $\delta$ -chains. Because the  $\alpha$ -chains of haemoglobins A, F and A<sub>2</sub> are controlled by a single genetic locus, however, synthesis of all these haemoglobins is depressed in  $\alpha$ -thalassaemia. In this disorder, haemoglobin H ( $\beta_4$ ) and Bart's ( $\gamma_4$ ) are found in varying amounts. It has been suggested that these abnormal haemoglobins arise by aggregation of excess  $\beta$ - and  $\gamma$ -chains which are present in the cell because of the overall deficit in  $\alpha$ -chains<sup>1</sup>.

The homozygous state for  $\alpha$ -thalassaemia is characterized by intra-uterine death with the clinical picture of hydrops foetalis associated with very high proportions of haemoglobin Bart's and very little haemoglobin A or F (ref. 2). The heterozygous state is very difficult to recognize in adult life and is unassociated with anaemia or a raised reticulocyte count, suggesting that one normal  $\alpha$ -chain gene is able to compensate almost completely for lack of  $\alpha$ -chain production by the abnormal allele.

Haemoglobin H disease is a form of  $\alpha$ -thalassaemia of intermediate severity, characterized by the presence of 5–25 per cent haemoglobin H, and a moderately severe haemolytic anaemia. It is currently believed that this condition results from heterozygosity for 2 different  $\alpha$ -thalassaemia genes, the severe type which results in hydrops foetalis in homozygotes (the  $\alpha_1$ -thalassaemia gene), and a "milder" gene (the  $\alpha_2$ -thalassaemia gene) (ref. 3). Because of the associated anaemia and reticulocytosis, blood samples from patients with haemoglobin H disease can be used to study *in vitro* haemoglobin synthesis in  $\alpha$ -thalassaemia. Studies of the haemoglobin patterns of stillborn infants apparently homozygous for the  $\alpha_1$ -thalassaemia gene suggest that it is completely ineffective in directing  $\alpha$ -chain production<sup>4</sup>. Thus the  $\alpha$ -chain synthesized in haemoglobin H disease will be almost entirely due to the activity of the milder  $\alpha_2$ -thalassaemia gene.

Previously, it was shown that in haemoglobin H disease the rate of  $\beta$ -chain synthesis exceeded that of  $\alpha$ -chain synthesis by a factor of 1.5 to 3 times (ref. 5). It was suggested that the excess of  $\beta$ -chain produced a large intracellular pool capable of contributing  $\beta$ -chains to

newly synthesized haemoglobin A. The present study confirms this suggestion and provides a more complete picture of the kinetics of  $\alpha$ - and  $\beta$ -chain production in haemoglobin H disease.

Approximately 5 ml. of red cells, obtained from patients with haemoglobin H disease, were washed in saline and incubated at 37° C under the conditions described by Lingrel and Borsook<sup>6</sup>. After incubation for 15–30 min, 50–250  $\mu$ c. of tritiated leucine was added and the incubation allowed to continue for periods of time ranging between 10 and 60 min. The cells were then washed and lysed, and the lysate divided into two parts. Globin was prepared from one fraction, without any further purification, by precipitation with cold 2 per cent acid-acetone. This "globin" thus consisted of material both from the soluble haemoglobin fraction, and from any haemoglobin bound to insoluble stromal matter together with the insoluble cell debris. The second fraction was centrifuged at 4° C to remove the stroma, and then either dialysed for 12 h at 4° C against the phosphate buffer to be used in a subsequent chromatographic separation of haemoglobins A and H, or immediately passed through a 'Sephadex G-25' column, equilibrated against the same buffer. The haemoglobins were then separated on 'Amberlite IRC-50' columns using developer No. 2 of Allen *et al.*<sup>7</sup>. Under these conditions, haemoglobins H and Bart's appeared in the "breakthrough" peak at 4° C. The haemoglobin A fraction, which was retained by the column at 4° C, was later eluted at 24° C after all the haemoglobin H and Bart's had been eluted. The two fractions were then concentrated at 4° C by pressure dialysis, and converted into globin by the acid-acetone method. The  $\alpha$ - and  $\beta$ -chains were isolated on CM cellulose columns in 8 molar urea/2-mercaptoethanol buffers as previously described<sup>8</sup>. The purified chains were freed of urea and salts and the incorporated radioactivity then determined. The relative amounts of haemoglobins A, Bart's and H were estimated by starch block or cellulose acetate electrophoresis, or by chromatography on 'Amberlite IRC-50'.

Radioactive  $\alpha$ - and  $\beta$ -chains were isolated from unfractionated whole cell lysates made from cells of non-thalassaemic patients with a variety of haemolytic anaemias. In all cases the amount of radioactivity incorporated into the  $\alpha$ - and  $\beta$ -chains was closely similar, as were the specific activities of the chains, suggesting that  $\alpha$ - and  $\beta$ -chains are produced at the same rate in non-thalassaemic reticulocytes. In similar experiments, using cells from individuals with haemoglobin H disease, however, the amount of radioactivity incorporated into the total  $\beta$ -chain fraction (that is, the  $\beta$ -chain of HbA and HbH) exceeded that found in the  $\alpha$ -chain, by ratios ranging from 2.7/1 to 1.9/1 (Fig. 1a, Table 1). The red

Table 1. RADIOACTIVITY OF THE  $\alpha$ - AND  $\beta$ -CHAINS ISOLATED FROM UNFRACTIONATED WHOLE CELL LYSATES PREPARED FROM THE CELLS OF PATIENTS WITH HAEMOGLOBIN H DISEASE AND PATIENTS WITH A RAISED RETICULOCYTE COUNT ASSOCIATED WITH NON-THALASSAEMIC DISORDERS

Diagnosis	Incubation time (min)	Total radio-activity in $\alpha$ -chain (c.p.m.)	Total radio-activity in $\beta$ -chain (c.p.m.)	Ratio $\alpha/\beta$
Haemoglobin H	10	4,180	7,972	0.5/1
Haemoglobin H	60	4,720	11,150	0.4/1
Haemoglobin H	60	8,070	22,560	0.3/1
Haemoglobin H	6	130	340	0.4/1
Immune haemolytic anaemia	10	2,059	2,085	1/1
Post-haemorrhagic anaemia	60	3,050	3,075	1/1

cells in haemoglobin H disease are hypochromic and have a marked reduction in mean corpuscular haemoglobin concentration. These findings therefore provide clear evidence that the rate of  $\alpha$ -chain synthesis is reduced in this condition, and do not support the hypothesis that haemoglobin H disease is due primarily to an overproduction of  $\beta$ -chain in the presence of normal levels of  $\alpha$ -chain synthesis. In addition to an excess of radioactivity in the  $\beta$ -chain fraction relative to that in the  $\alpha$ -chain, there is also an excess of protein as measured by the optical density at 280 m $\mu$ . This represents the  $\beta$ -chain of haemoglobin H (Hb  $\beta_4$ ), which appears with the  $\beta$ -chain peak on chromatography of globin made from the crude whole cell lysates. The excess of protein in the  $\beta$ -chain peak was proportional to the level of haemoglobin H in each case.

The presence of such an obvious excess of  $\beta$ -chain in the haemoglobin H disease cells raised the question of its role

in the overall pattern of haemoglobin synthesis, and, indeed, of its exact nature, for it was conceivable that some or all of the haemoglobin H seen in cell lysates forms by aggregation of  $\beta$  subunits on cell lysis. It is possible that it exists in a large labile pool of free  $\beta$ -chain monomer, capable of supplying  $\beta$ -chains to combine with newly synthesized  $\alpha$ -chains, as these become available to form haemoglobin A. If this were the case, however, virtually no labelled  $\beta$ -chain would be expected to appear in haemoglobin A during these experiments, for the relatively small number of  $\beta$ -chains synthesized would be greatly diluted by such a vast intracellular pool of unlabelled  $\beta$ -chain.

Table 2. RADIOACTIVITY OF THE  $\alpha$ - AND  $\beta$ -CHAINS PREPARED FROM PURIFIED HAEMOGLOBIN A FROM LYSATES OF THE RED CELLS OF FIVE PATIENTS WITH HAEMOGLOBIN H DISEASE AND A PATIENT WITH A RAISED RETICULOCYTE COUNT DUE TO A NON-THALASSAEMIC DISORDER

Diagnosis	Incubation time (min)	Specific activity of $\alpha$ -chain (c.p.m./mg)	Specific activity of $\beta$ -chain (c.p.m./mg)	Ratio $\alpha/\beta$
Haemoglobin H	10	378	218	1.7/1
Haemoglobin H	10	266	57	4.6/1
Haemoglobin H	60	375	47	7.9/1
Haemoglobin H	60	507	54	9.3/1
Haemoglobin H	300	603	191	3.1/1
Immune haemolytic anaemia*	60	630	658	0.95

\* The  $\alpha/\beta$  ratio is almost unity as previously reported<sup>3</sup>.

To clarify these points, haemoglobin A was separated from haemoglobin H, the  $\alpha$ - and  $\beta$ -chains isolated and the incorporated radioactivity determined (Fig. 1*b*; Table 2). In each case, the specific activity of the  $\alpha$ -chain exceeded that of the  $\beta$ -chain, the ratio of  $\alpha/\beta$  ranging from 9.3/1 to 1.7/1. The distribution of radioactivity, in the completed haemoglobin A, could only have occurred if the excess of  $\beta$ -chains released into the red cells had formed a pool capable of providing  $\beta$ -chains to combine with newly made  $\alpha$ -chains. Thus, during the period of incubation with tritiated leucine, newly made and therefore labelled  $\alpha$ -chain would combine with unlabelled  $\beta$ -chain from this pool. As seen in Fig. 1*b* and Table 2, however, there was always a considerable amount of radioactivity associated with the  $\beta$ -chain peak of the purified haemoglobin H, suggesting that if a usable pool of free  $\beta$ -chain is present it must be quite small. Alternatively, it was possible that the radioactivity in the  $\beta$ -chain of haemoglobin A was derived from haemoglobin H by some unspecific process of exchange of  $\beta$ -chain subunits. Such exchange might occur after lysis of the cells before complete chromatographic separation of haemoglobin A and haemoglobin H had been achieved, or intracellularly during the period of incubation.

To test the first hypothesis, cells from a patient with haemoglobin H disease were incubated for 45 min with tritiated leucine, lysed in cold water, and the stroma removed by centrifugation at 4° C. The lysate was then divided into two parts. One fraction was immediately passed through a 'Sephadex G-25' column equilibrated with developer No. 2, pH 7.18, which was also used to equilibrate an 'Amberlite IRC-50' column, both columns being maintained at 4° C. Immediately after passage through 'Sephadex', the lysate was applied to the 'Amberlite' column and haemoglobin H immediately eluted at 4° C. Haemoglobin A was then subsequently eluted at 24° C. Complete separation of haemoglobins A and H was completed within 1 h. The second fraction was dialysed against the same phosphate buffer for 48 h at 4° C, before application to the 'Amberlite' column, and the haemoglobins A and H were then separated as before. The  $\alpha$ - and  $\beta$ -chains from the purified haemoglobin A from each fraction were separated and their specific activities determined. The specific activity of the  $\beta$ -chain of haemoglobin A prepared from the sample dialysed for 48 h was 57 c.p.m./mg, while that of the sample which was separated immediately was 58 c.p.m./mg (Table 3). Thus no significant exchange of  $\beta$ -chain subunits between haemoglobin H and A occurs on prolonged dialysis under these conditions, and therefore this mechanism cannot account for most of the radioactivity found in the  $\beta$ -chain of haemoglobin A in our

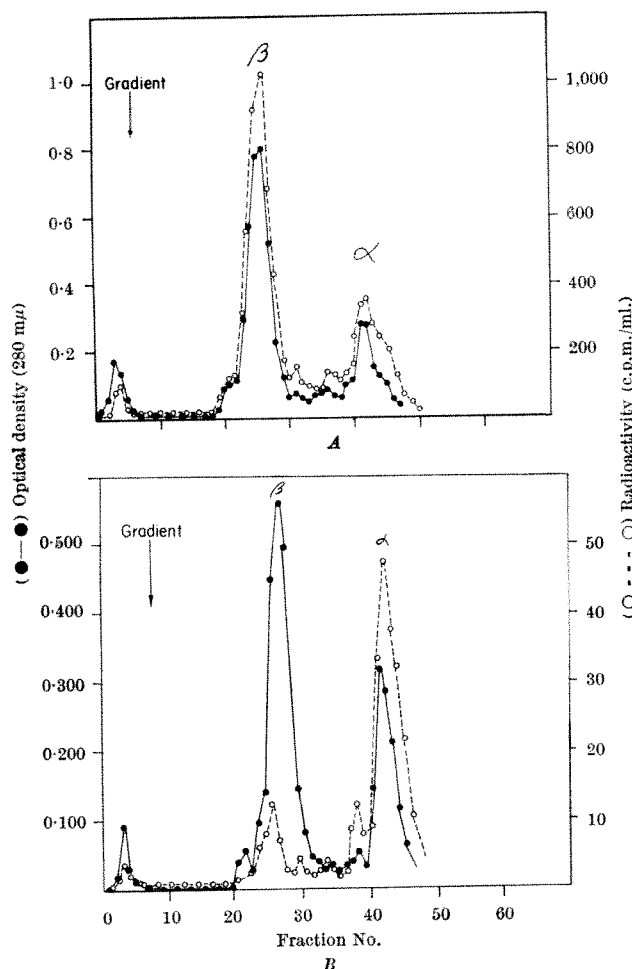


Fig. 1. A, Distribution of radioactivity in the  $\alpha$ - and  $\beta$ -chains of a lysate prepared from the red cells of a patient with haemoglobin H disease, after 60 min incubation with tritiated leucine. Globin was precipitated after lysis of the cells without further purification. B, Distribution of radioactivity in the  $\alpha$ - and  $\beta$ -chains of purified haemoglobin A prepared from the red cells of a patient with haemoglobin H disease, after 60 min incubation with tritiated leucine.

experiments. Furthermore, the period of dialysis was about four times greater than that usually used in these experiments.

Table 3. INVESTIGATION OF THE POSSIBILITY OF EXCHANGE OF SUBUNITS BETWEEN HAEMOGLOBINS A AND H AFTER LYSIS OF THE RED CELLS

Procedure	Specific activity of $\alpha$ -chain (c.p.m./mg)	Specific activity of $\beta$ -chain (c.p.m./mg)	Proportion of haemoglobin H (per cent)
Haemoglobin H separated by immediate chromatography	266	58	20
Haemoglobin H separated after 48 h dialysis at 4° C	262	57	20

Two experiments were performed to test the possibility of intracellular exchange of  $\alpha$ -chain subunits between haemoglobins A and H. 2 ml. of red cells from a patient with haemoglobin H disease were incubated for 1 h with tritiated leucine and further protein synthesis then stopped by the addition of puromycin to a final concentration of mmole. After incubation for 10 min with puromycin a sample was removed and the cells were washed in the cold and then frozen. Incubation of the remaining cells in the presence of puromycin was allowed to continue for a further 6 h, and the cells were then washed and frozen. Next, both lysates were thawed, centrifuged in the cold to remove the stroma, and haemoglobins A and H quickly purified by passage through 'Sephadex' and 'Amberlite' columns at 4° C as before. The  $\alpha$ - and  $\beta$ -chains of haemoglobin A and the  $\beta$ -chains of haemoglobin H were isolated by CM-cellulose chromatography and the specific activity of each determined (Table 4). The specific activity of the  $\beta$ -chain of haemoglobin A had increased by more than 50 per cent after 6 h, an equivalent loss of radioactivity in the  $\beta$ -chain of haemoglobin H being noted. In a second experiment (Table 4), synthesis was stopped by puromycin after incubation for 1 h with isotope, and samples were removed and frozen at intervals up to 20 h. There was a steady increase in radioactivity in the  $\beta$ -chain, the specific activity having more than doubled after 20 h. A reciprocal fall in radioactivity in the  $\beta$ -chain of haemoglobin H was again noted.

Table 4. INTRACELLULAR EXCHANGE OF  $\beta$ -CHAINS BETWEEN HAEMOGLOBIN H AND HAEMOGLOBIN A

Hours of incubation after inhibition with puromycin	Specific activities of peptide chains of haemoglobin A (c.p.m./mg)	$\beta$	Specific activity of $\beta$ -chain of haemoglobin H (c.p.m./mg)	Proportion of haemoglobin H (per cent)
Experiment 1				
0	507	54	1,600	21
7	485	83	1,545	22
Experiment 2				
0	312	36	1,160	23
6	324	58	1,090	22
20	312	78	1,050	22

The results of these experiments provide clear evidence that intracellular exchange of subunits does occur. The amount of this exchange is, however, insufficient to explain the degree of labelling of the  $\beta$ -chain which occurs during incubation for 60 min of red cells from persons with haemoglobin H disease. Thus about 10 per cent of the radioactivity present in the  $\beta$ -chain of haemoglobin A after incubation for 60 min probably arises by intracellular subunit exchange during the incubation. Unless some very unusual exchange process takes place immediately on lysis, the remaining 90 per cent of the counts must represent genuine *de novo*  $\beta$ -chain incorporation during the synthesis of the haemoglobin A.

As far as we are aware, these experiments demonstrate for the first time that subunit exchange between haemoglobins can occur intracellularly. Extracellular exchange of haem has been recently reported, but exchange of globin

subunits was not demonstrated<sup>9</sup>. Apparently extracellular exchange of globin subunits does occur, however, under rather extreme conditions<sup>10</sup>.

From these experiments, a fairly clear picture of the kinetics of  $\alpha$ - and  $\beta$ -chain synthesis in haemoglobin H disease can be built up. There is a reduced rate of synthesis of  $\alpha$ -chains resulting in an excess of  $\beta$ -chains, which are freely released into the red cell. Most of these chains probably form tetramers (haemoglobin H), and in that state are no longer available for combination with newly made  $\alpha$ -chain to form haemoglobin A. This concept is in keeping with the fact that the  $\beta$ -chains of haemoglobin A contain considerable amounts of radioactivity which cannot be accounted for by exchange of subunits between haemoglobins H and A, either within the cells or after lysis of the cells. These observations provide very strong evidence for the existence of a relatively small pool of free  $\beta$ -chain in haemoglobin H disease. Whether this exists as  $\beta$ -chain monomer or  $\beta_2$  dimer is uncertain, but because haemoglobin probably passes through the  $\alpha\beta$  subunit during synthesis, and because  $\beta_4$  subunits would probably tend to form rapidly the  $\beta_4$  tetramer, the usable pool may well be principally in the monomeric state. The situation may be compared with that found in normal reticulocytes, where a small pool of  $\alpha$ -chain, probably on the ribosomes, is responsible for the unequal labelling of  $\alpha$ - and  $\beta$ -chains at very short times of incubation<sup>5,11</sup>.

These experiments also confirm the destruction and loss of haemoglobin H from the circulating red cells. Thus the specific activity of the total  $\beta$ -chain fraction is always significantly greater (by a factor of 10–20 per cent) than that of the  $\alpha$ -chain of haemoglobin A, confirming that  $\beta$ -chain is not present in the red cell population at the same level as it is being synthesized. This is probably a reflexion of the fact that a heterogeneous population of cells is being studied, with the cells active in synthesis producing haemoglobin H at a rate greater than that which is compatible with the level of haemoglobin H in the total cell population. This discrepancy is due to the fact that haemoglobin H tends to precipitate as the red cell matures, aged red cells containing the resulting inclusion bodies being removed by the spleen<sup>12</sup>. The removal of cells containing precipitated haemoglobin H by the spleen has been previously suggested by *in vivo* labelling of haemoglobin<sup>13</sup>.

These experiments unfortunately provide no insight into the nature of the defect in  $\alpha$ -chain synthesis, but they do at least suggest that the next obvious step is to examine in detail the assembly of the  $\alpha$ -chain at the ribosomal level. What meagre genetic evidence there is points to a locus very close to, or at, the  $\alpha$ -chain locus, suggesting that a functionally defective mRNA may be involved. Alternative mechanisms involving regulatory genes affecting the production of mRNA, while no less plausible, would not at present prove as amenable to experiment in human material.

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# Pattern Recognition in Bees

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Experiments show that there is a mechanism which enables bees to measure different angles of stripes on a vertical screen, and what kinds of stimuli may be decisive as sensory input.

THE way in which animals recognize shapes can be studied by their reactions to simple geometrical figures. We have carried out experiments to identify the specific parameters which are necessary for the visual system of animals to analyse figures. Even as simple a geometrical configuration as a black stripe can be described by many parameters, such as its size, the contrast between the stripe and the background, its outlines—which may be sharp or diffuse—or the orientation of the stripe on a vertical screen. With regard to the sensory input of form perception, these parameters need not be of equal importance. In the classical papers on form perception in bees<sup>1-9</sup> the total amount of black and white contours was considered the most decisive criterion enabling insects to discriminate between different geometrical figures. Some recent findings on arthropods taken from the ethological as well as from the electrophysiological point of view, however, show a more complex system of form perception.

The existence of retinal receptive fields has been proved for arthropods as well as vertebrates. One of the most common properties of these receptive fields is a specific angular size or the subdivision into excitatory

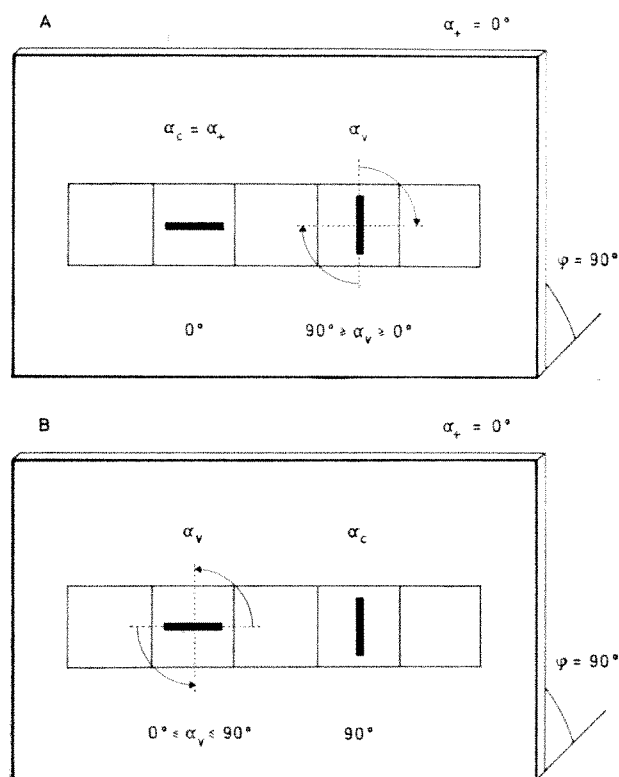


Fig. 1. Schematic representation of the apparatus. In the critical tests two angles of a stripe pattern (here single stripes) are presented on a vertical screen. The bees were formerly trained to a horizontal stripe ( $\alpha_+ = 0^\circ$ ). A shows the arrangement of stripes in the critical tests in order to determine the accuracy of goniometry. Size of  $\alpha_+$  could be varied from  $0^\circ$  to  $90^\circ$ . B shows the arrangement in the transfer tests (for further explanation see text). The position of patterns  $\alpha_+$  and  $\alpha_v$  on the screen was changed periodically.

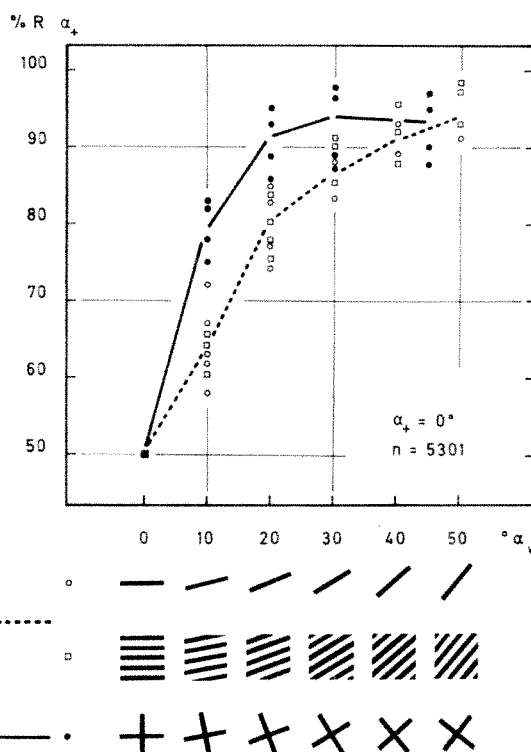


Fig. 2. The discrimination of differently sloped stripe patterns from the patterns in the horizontal position ( $\alpha_+ = 0^\circ$ ).  $\alpha_+$  = training pattern. The angle of the control pattern,  $\alpha_v$ , varied from  $0^\circ$  to  $50^\circ$ .  $R$  = reaction intensity, that is, percentage of visiting flights to  $\alpha_+$ . The symbols represent the mean values of the single tests carried out with  $30 \pm 5$  bees. The curves always join the total average of all tests.

and inhibitory areas. One type of receptive field, however, is strikingly characterized by sensitivity to the specific direction of a light slit, a dark stripe or a moving point of light. These directionally selective units, previously found in vertebrates, crustaceans and insects, only respond to a certain direction of visual stimuli in the inherent receptive field. In the past few years, electrophysiological recordings have been made from these units in the tectum opticum of frog<sup>10</sup> and pigeon<sup>11</sup>, the lateral geniculate body<sup>12</sup> and the retina<sup>13-16</sup> of the rabbit, the visual cortex of the cat<sup>17-20</sup>, the eyestalks of the crab *Podophthalmus*<sup>21</sup>, the optic nerve of the crayfish<sup>22-24</sup> and the medulla externa of the locust<sup>25</sup>. Also there is evidence that the directionally selective units occur in the octopus<sup>26,27</sup>.

The experiments reported here were designed to find out whether bees are able to measure the slope of black stripes presented on a vertical screen, how precisely such a mechanism of goniometry works and what kind of stimuli are necessary as sensory input. The apparatus consisted of a vertical plane ( $165 \times 100$  cm) to which three types of stripe patterns were applied: single stripes; sets of parallel black and white stripes equidistant apart;

and a cross-like configuration of two stripes. In all three types, the orientation of the stripes varied from the horizontal ( $\alpha = 0^\circ$ ) to the vertical ( $\alpha = 90^\circ$ ). During each experiment a group of about 30 ( $\pm 5$ ) individually marked bees was trained to a certain slope of the stripes called the "positive" angle  $\alpha_+$ . The bees had to crawl through an aperture 10 mm in diameter in the centre of the stripe and then enter a dark box in which they found a solution of saccharose (0.2–2 molar). The training to  $\alpha_+$  lasted for at least 10 h so that the bees would make 100–150 approach flights to the  $\alpha_+$  pattern. (For further details see ref. 28.)

Once the bees had been trained to a certain slope,  $\alpha_+$ , of the stripes, a series of critical tests was carried out. In all these tests the bees had to discriminate between two directions of the same pattern: the positive angle  $\alpha_+$  remained constant during all tests, while the other pattern orientated at  $\alpha_v$  was varied throughout the whole range from  $0^\circ$  to  $90^\circ$  ( $0^\circ \leq \alpha_v \leq 90^\circ$ ) (Fig. 1A). The percentage of bees that chose  $\alpha_+$  was noted in each test. The results for  $\alpha_+ = 0^\circ$ , that is for a horizontally arranged stripe or stripe pattern, are given in Fig. 2. The curves rise steeply as the difference between the constant training pattern,  $\alpha_+$ , and the varying control pattern,  $\alpha_v$ , increases, and finally they reach a plateau. The so-called "similarity range"  $\Delta\alpha_0$  encloses by definition all directions  $\alpha_v$  which are not optimally distinguished by the bees from the training angle  $\alpha_+$ . It represents therefore the ascending part of the curves. Using the parameter  $\Delta\alpha_0$  one concludes from Fig. 2 ( $\alpha_+ = 0^\circ$ ) and Fig. 3 ( $\alpha_+ = 45^\circ$ ) that there is no statistically significant difference between the values for a single stripe or a pattern of parallel stripes and that the cross-like patterns enable bees to measure angles more accurately ( $\Delta\alpha_0 = 20^\circ$ ) than do single or parallel stripes ( $\Delta\alpha_0 = 30^\circ$ – $40^\circ$ ).

In a special series of experiments<sup>28</sup>, it was found that bees managed to discriminate between the trained upright cross  $\alpha_+$  and an oblique cross  $\alpha_v$  at a difference of inclination of only  $4^\circ$  when they were simultaneously presented. A single stripe or a set of parallel stripes must be rotated

by  $10^\circ$  before it can be distinguished significantly from the horizontal one.

Bees were not only trained to horizontal stripes as described so far, but also to  $45^\circ$  and  $90^\circ$  inclination of the same stripe patterns. For  $\alpha_+ = 90^\circ$ , that is to say, after training to the vertically orientated stripes, there results a similar but mirror shape, for the curves compared with those obtained for  $\alpha_+ = 0^\circ$ . For  $\alpha_+ = 45^\circ$ , however, symmetrical curves as shown in Fig. 3 are typical. This means that it does not matter whether the control pattern  $\alpha_v$  presented in the tests simultaneously with the training pattern  $\alpha_+$  is inclined upward or downward from  $\alpha_+ = 45^\circ$ . This is in agreement with the previous finding that bees do not show an innate preference for horizontal or vertical stripes.

In order to decide whether bees can tell the slope of a stripe pattern more accurately from the training slope  $\alpha_+ = 0^\circ$  (or  $90^\circ$ ) than from  $\alpha_+ = 45^\circ$ , the following method can be used: the difference in angle between  $\alpha_+$  and  $\alpha_v$  at 60 per cent visiting flights to  $\alpha_+$  is read off the curves such as shown in Figs. 2 and 3. It can be seen that adjacent inclinations of stripes are more precisely discriminated within the range from  $0^\circ$  to  $90^\circ$  than in that about  $45^\circ$ . This is true for all types of stripe patterns used in the experiments (see Table 1).

The mechanism which enables bees to discriminate between two different slopes of the same stripe pattern can also be examined by another method. In critical tests two patterns are offered, neither of which is inclined at the training angle, Fig. 1B, one of them ( $\alpha_c$ ) remains constant throughout all tests whereas the other varies within the range of  $0^\circ \leq \alpha_v \leq 90^\circ$ . The curve in Fig. 4 is drawn from the results of a series of similar experiments, in which the bees were trained to a horizontal stripe ( $\alpha_+ = 0^\circ$ ) but when the same stripe was presented at an angle of  $45^\circ$  or  $90^\circ$ , the bees mostly refused to approach. When  $\alpha_v$  simultaneously offered with  $\alpha_c$  was greater than  $40^\circ$  most of the bees accepted neither, but returned to the hive. Bees only approached stripes which differed

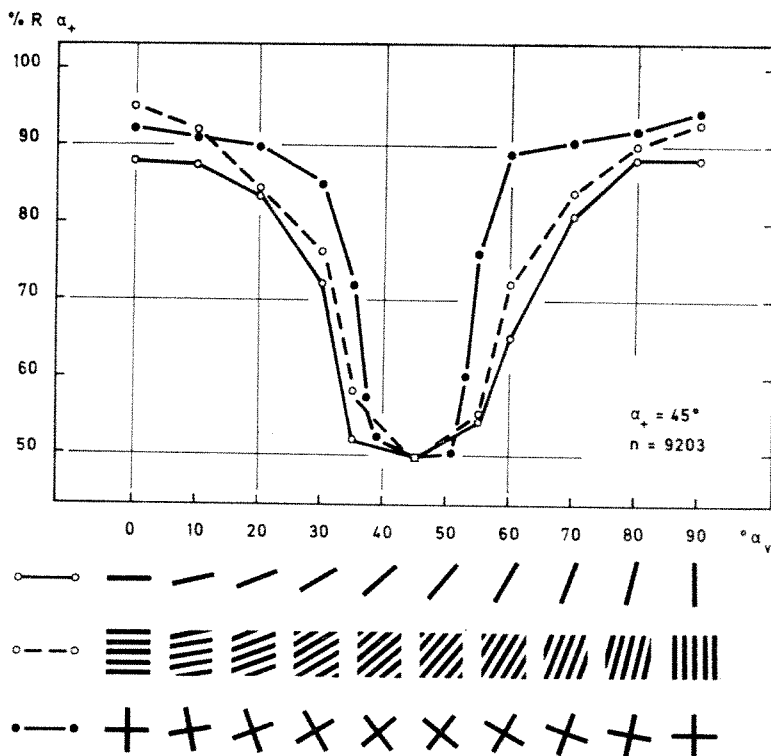


Fig. 3. The discrimination of differently sloped stripe patterns from the patterns in the oblique position ( $\alpha_+ = 45^\circ$ ). For explanation see Fig. 2. The mean values of all tests are graphed. The true values for the single tests maximally scatter in the range of 20 per cent (in the case of  $\alpha_v = 30^\circ$  and  $60^\circ$ ).

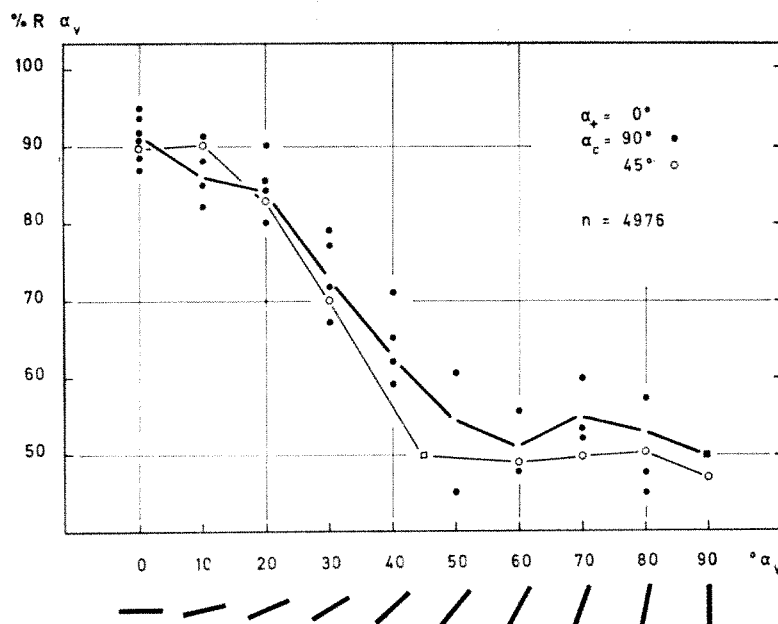


Fig. 4. Transfer of the bees trained to horizontal stripes to other inclinations of single stripes. In the critical tests all angles were simultaneously presented with the constant control pattern  $\alpha_c = 90^\circ$  (●) or  $\alpha_c = 45^\circ$  (○). In the range  $\alpha_c > 50^\circ$  the bees scarcely visited either angle of the two stripes, and the few approach flights were distributed at random to both angles. In the (●) curve the mean values of the single tests are given, while the (○) curve only shows the total average values of all tests.

no more than  $30^\circ$ – $40^\circ$  from the training pattern  $\alpha_+ = 0^\circ$ . This means that the more horizontal of two stripes is exclusively preferred in the so-called "transfer range",  $\Delta\alpha_T = 30^\circ$ – $40^\circ$ , and thus the transfer range is identical with the "similarity-range" already described; in short,  $\Delta\alpha_T = \Delta\alpha_0$ . We may therefore conclude that only those stripes which are close to the training angle, and which are not optimally discriminated from the training angle, that is within  $30^\circ$ – $40^\circ$ , are acceptable to the bees. The same results are inferred from experiments with  $\alpha_+ = 45^\circ$  and  $90^\circ$ .

In another series of tests, the slope  $\alpha_c$ , which remains constant in all tests, was kept within the variation range:  $\alpha_c = 20^\circ$  and  $30^\circ$ , see Fig. 5. Now the bees already trained to the horizontal sets of parallel stripes ( $\alpha_+ = 0^\circ$ ) also accepted the control pattern  $\alpha_c$ . They always preferred the more horizontal of the two inclinations even when the angles differed by only  $10^\circ$ .

In all the experiments so far described the bees had to fly to a vertical screen. This means that the patterns offered on the screen were projected to the frontal part of the eye. The slope of the screen was now varied from the vertical position ( $\psi = 90^\circ$ ) to the horizontal one ( $\psi = 0^\circ$ ). In each position the bees had to discriminate between two stripe patterns: one at an angle of  $45^\circ$  ( $\alpha_+$ ), the other at an angle of  $135^\circ$  ( $\alpha_v$ ) to the horizontal direction. The longer the bees were trained the better they could distinguish between the two stripe patterns  $\alpha_+$  and  $\alpha_v$ . The "learning-curves" obtained show the percentage of approach flights to  $\alpha_+$  as a function of the number of training flights. The rate of rise and the plateau of these curves decrease

Table 1. THE DIFFERENCES IN THE ACCURACY OF GONIOMETRY FOR SINGLE STRIPES (SS), SETS OF PARALLEL BLACK AND WHITE STRIPES (PS) AND RECTANGULARLY CROSSED STRIPES (CS), EXAMINED FOR DIFFERENT TRAINING INCLINATIONS OF THE STRIPES ( $\alpha_+ = 0^\circ, 45^\circ, 90^\circ$ )

	$0^\circ$	$45^\circ$	$90^\circ$
SS	7.1	10.7 12.8 12.0 12.5	6.0
PS	7.8	10.6 11.8	6.6
CS	3.6 2.8	8.5 8.0	(See $0^\circ$ )

The values represent the angle difference between  $\alpha_+$  and the  $\alpha_c$  inclination which is distinguished from  $\alpha_+$  by 60 per cent of the trained bees. The curves which the considered values are read off are based on 28,617 approach flights of the trained bees. Each value graphed in the table is taken from one specific curve.

when the pattern plane is gradually brought from the vertical to the horizontal position as shown in Fig. 6. The mean values for  $\psi = 45^\circ$ – $90^\circ$  are significantly different from those for  $\psi = 0^\circ$ – $10^\circ$ .

The following conclusions are drawn from further experiments<sup>22</sup>: the pattern plane can be inclined out of the vertical position for a wider range than out of the horizontal position for the discrimination between the

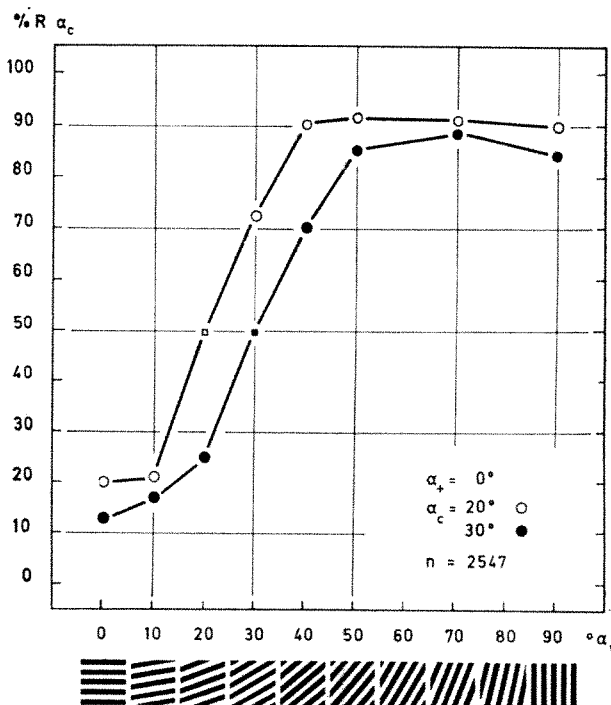


Fig. 5. Transfer of the bees trained to the horizontal pattern to other angles of an equidistant stripe pattern. The testing situation is the same as that in Fig. 4, but the constant control pattern is now orientated at an angle  $\alpha_c = 20^\circ$  (○) and  $\alpha_c = 30^\circ$  (●). The mean values of all tests carried out represent the percentage of approach flights to  $\alpha_c = 20^\circ$  and  $30^\circ$  respectively.

two stripe patterns to be preserved. Measurement of the direction of stripes by the bees is more accurate if the stripe patterns are presented in the vertical than in the horizontal plane.

Offhand, it would seem possible that bees measure the slope of a black stripe on a vertical screen by means of terrestrial or celestial orientation marks in the surroundings of the plane to which the patterns are applied. To test this the bees were trained to a  $45^\circ$  stripe which ran from left-below to right-above or—with regard to the celestial regions—from east-below to west-above. During the experiment, the screen was rotated through  $180^\circ$  so that the pattern plane now looked south instead of north, its former orientation. Two stripes were presented in the test: one at an angle of  $45^\circ$ , the other at an angle of  $135^\circ$ . In the new position of the screen only a few bees chose one of the two stripes offered, but they all flew to the  $45^\circ$ -inclined stripe which now ran from west-below to east-above. This means that bees measure the angle of a stripe presented on a vertical screen by means of its orientation in the visual field, not by its position in space. This, however, does not apply to stripes offered in the horizontal plane<sup>29</sup>.

Another question is what parameter of sensory input is decisive in measuring the angle of a stripe. The total amount of black and white contours irrespective of their direction, cannot be the parameter necessary for goniometry; for a  $45^\circ$  and a  $135^\circ$  inclined stripe pattern are unequivocally distinguished, although both patterns have identical amounts of contour. The orientation of the contours is also not as important as may be supposed at first. The stripe contours can be dissected in a sawtooth like pattern without affecting the orientation of bees to the inclination of the long axis of the black stripe. The bees discriminate between the cross-shaped patterns in the upright and in the oblique position even when all the contours extend in a direction of  $45^\circ$  to the stripe axes (Fig. 7). It is only necessary then that the axes of the stripes and therefore the main extent of the black area coincide with the positive inclination to which the bees have been trained.

The results reported here prove the existence of a mechanism which enables bees to measure the different angles of a stripe or a stripe pattern presented on a vertical

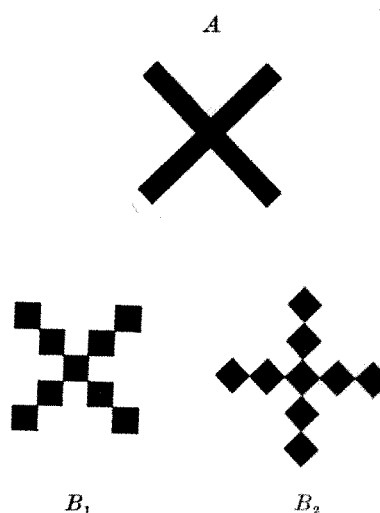


Fig. 7. The patterns offered in order to decide whether edges or areas are decisive for goniometry. The bees were trained to an oblique cross A. In the critical tests the patterns  $B_1$  and  $B_2$  were presented. In all the tests in which the number as well as the size of the squares differed, 81–92 per cent of the trained bees chose the pattern of type  $B_1$  ( $n=2,135$ ).

screen. Similar training experiments have been carried out on cephalopods<sup>26</sup>, fishes<sup>20</sup>, birds<sup>31</sup> and mammals<sup>32,33</sup>. The number of ants running spontaneously to a vertically striped pattern greatly decreases when the stripe pattern is declined by more than  $20^\circ$  from the vertical<sup>34</sup>.

When bees are trained to a certain inclination  $\alpha_+$  of a stripe or pattern consisting of parallel black and white stripes they optimally discriminate from  $\alpha_+$  all inclinations varying from  $\alpha_+$  by more than  $30^\circ$ – $40^\circ$ . For comparison Hubel and Wiesel<sup>20</sup> have found with electrophysiological technique in the cat that cortical neurones representing directionally selective units show maximal spike frequencies when a light slit projected on the retina differs by no more than  $30^\circ$  from the direction for which the unit is selective. Such a mechanism of goniometry requires that the eyes of flying bees are always kept in the same position by proprioceptive control of the position of head and

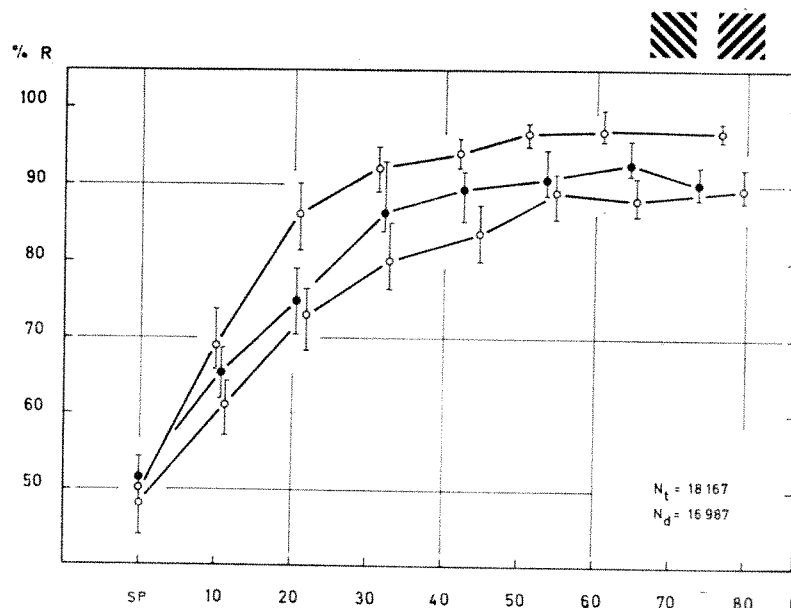


Fig. 6. The discrimination of two oppositely orientated stripe patterns ( $\alpha_+ = 45^\circ$  and  $\alpha_- = 135^\circ$ ) as a function of the number of training flights ( $F$ ) and the inclination  $\psi$  of the plane of the patterns. Upper ( $\circ$ ) curve:  $\psi = 45^\circ$  to  $90^\circ$ ; ( $\bullet$ ) curve:  $\psi = 20^\circ$  to  $30^\circ$ ; lower ( $\circ$ ) curve:  $\psi = 0^\circ$  to  $10^\circ$ . Each of the curves represents the average of 5–6 curves obtained in a series with  $15 \pm 3$  bees. These 5–6 curves scatter in the range graphed for each mean value.  $N_t$  = No. of approach flights in the critical tests,  $N_d$  = No. of training flights. From Wehner, R., *Z. Vergl. Physiol.* (Springer-Verlag, Berlin, 1967).



thorax. By this the training inclination of stripes is always projected in the same way to the ommatidia. Wiersma and Yamaguchi<sup>22-24</sup> recently found, however, in the eyestalks of the crayfish some "space constant fibres" detecting a certain direction of visual stimuli irrespective of the position of the eyes.

The most important parameter for measuring the angle of stripes is the orientation of the long axes of the black stripes, but not the direction of the black and white contours. Even the optokinetic reactions of *Carcinus* and *Locusta* refer not only to the contours of a rotated stripe pattern, as believed so far, but also to the black areas<sup>25,26</sup>. In addition, further experiments lead to the conception—although not yet fully examined—that the orientation of bees to the direction of a vertically presented stripe is independent of the size of the stripe<sup>27</sup> and of the contrast in brightness between the dark stripe and its light background.

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## Three Stage Electron Transfer in Aqueous and Alcoholic Solutions

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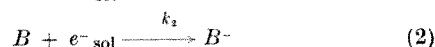
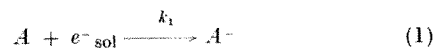
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Molecules of high electron affinity may be able to enhance radiobiological damage by increasing the effective diffusion radius of hydrated electrons produced by radiation. They do this by acting as electron carriers and electron transfer agents.

BECAUSE of ground state electron delocalization, conjugation between electron acceptor groups greatly enhances the electron affinity of organic compounds<sup>1,2</sup>. Attachment of electrons to such molecules frequently produces relatively long lived, radical anions, particularly in solutions of high pH. Correlations between these electrophilic structures and radiobiological sensitization<sup>3-5</sup> have led to the characterization of a new class of radiosensitizers. These include the conjugated compounds, benzo- and acetophenone, quinones, dicarbonyl compounds, diethyl oxalate, derivatives of maleic, fumaric, phthalic and pyruvic acids, and also indane trione. The hypothesis was based originally on the postulate that molecules of high electron affinity, acting as electron carriers and electron transfer agents, enhanced radiobiological damage by increasing the effective diffusion radius of hydrated electrons produced by the radiation. Although other related models may be constructed, the empirical relationship between electron-affinity and radiobiological sensitization is reasonably well established.

Several suggestions have been made independently that the relative efficiency of two scavengers competing

for electrons of thermal energy, including solvated electrons, may depend on differences in electron affinity rather than on differences in reaction rate constants. This has been discussed in terms of the chemistry of the model referred to here<sup>3-5</sup>; the radiation chemistry to aqueous solutions<sup>4,6-8</sup>, organic liquids<sup>9</sup> and gas phase mixtures<sup>10</sup>:



The initial fate of the electron is governed clearly by the relative magnitude of the two terms  $k_1[A]$  and  $k_2[B]$ . If  $A$  and  $B$  differ in electron affinity, however, anionic transfer may occur



In pulse radiolysis studies, Baxendale *et al.*<sup>11</sup> have measured the rate of electron transfer from unstable metal ions, for example  $\text{Cd}^+$ ,  $\text{Zn}^+$  and  $\text{Pb}^+$ , to stable oxidizing metal ions and, in some cases, have determined the relative potentials of the couples  $M^+/M^{2+}$ . Electron

transfer from the electron adduct of nitromethane to tetranitromethane has been reported<sup>6</sup>. Similar phenomena have been observed for arene radical-ions in alcohols<sup>7</sup>.

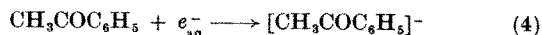
In stationary state radiolyses, this type of competition has been observed in aqueous mixtures<sup>7,8</sup> and also gas mixtures<sup>10</sup>.

In this report, we discuss experiments in which pulse radiolysis has been used to explore the radiation chemical behaviour of some compounds of high electron affinity. The following acceptors have been used: acetone, acetophenone, benzophenone, fluorenone, N-ethylmaleimide, dimethylfumarate, nitrous oxide and oxygen.

Solutions in either triply distilled water or highly purified ethanol were made alkaline with potassium hydroxide and sodium ethoxide respectively. Samples were irradiated with a 0.2  $\mu$ sec pulse of 1.8 MeV electrons from a linear accelerator, and the transient changes in optical density were recorded spectrophotometrically. Experimental details of the pulse radiolysis equipment have been published previously<sup>12</sup>.

Transient spectra from several deoxygenated, aqueous, alkaline solutions of acetophenone, benzophenone and acetone are shown in Fig. 1. In Fig. 1 (a), curve (i) is the transient spectrum observed 1  $\mu$ sec after the pulse, from 3.34 mM solution of acetophenone at about pH 13. Curve 1 (a) (ii) is the spectrum from a solution containing 166 mM acetone and 0.84 mM acetophenone.

The absorption maximum at 4400 Å is assigned to the electron adduct of acetophenone



while the peak at 3700 Å, characteristic of irradiated aqueous solutions of aromatic compounds, is, with little doubt, caused by the OH adduct, a substituted cyclohexadienyl radical. In the presence of a high concentration of acetone (curve (ii)), the latter peak is suppressed because the precursors, OH radicals, are scavenged by this solute, but the peak at 4400 Å is virtually unaffected. Both solutes react with  $e_{\text{aq}}^-$  with almost diffusion-controlled rate constants, and, because the concentration of

acetone greatly exceeds that of acetophenone, the former solute will scavenge all hydrated electrons. The maximum at 4400 Å in curve (ii) is due, we believe, to the electron transfer reaction

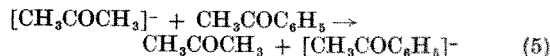
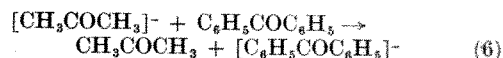


Fig. 1 (b) shows a set of similar data for acetone and benzophenone. The spectrum (curve 1 (b) (i)) is produced 1  $\mu$ sec after irradiation of 165  $\mu$ M benzophenone at about pH 13. The peak near 6100 Å is clearly produced by the ketyl radical anion previously observed by both flash photolysis<sup>13</sup> and pulse radiolysis<sup>14</sup>, and that at 3700 Å has been assigned to the OH adduct<sup>14</sup>. In the presence of 44 mM acetone, the latter peak is suppressed, while the ketyl radical anion is unaffected. In this system, too, the acetone concentration is sufficient to prevent any direct electron scavenging by benzophenone, and, because in pulse radiolysis the ketyl radical anion can be formed only by electron attachment or ionization of the neutral, ketyl radical<sup>14</sup>, we conclude that electron transfer is again responsible.



The data from an experiment combining the two systems in Figs. 1 (a) and 1 (b) are shown in Fig. 2. The oxygen free solution contained 6 per cent acetone, 3.4 mM acetophenone, 72  $\mu$ M benzophenone and 0.1 N potassium hydroxide. The spectrum (i) with a maximum at 4400 Å was produced during the pulse and is identical to that of the acetophenone ketyl anion shown in Fig. 1 (a). Within 50  $\mu$ sec after the pulse, however, this spectrum decays and is replaced by another spectrum with a maximum near 6100 Å identical to that of the benzophenone ketyl anion. The oscillogram inset illustrates the kinetic behaviour. The rise in the optical density at 6100 Å is exponential, and occurs over a similar time scale to the decay of the 4400 Å peak. The latter decay is complicated slightly by a small, longer lived component, due probably

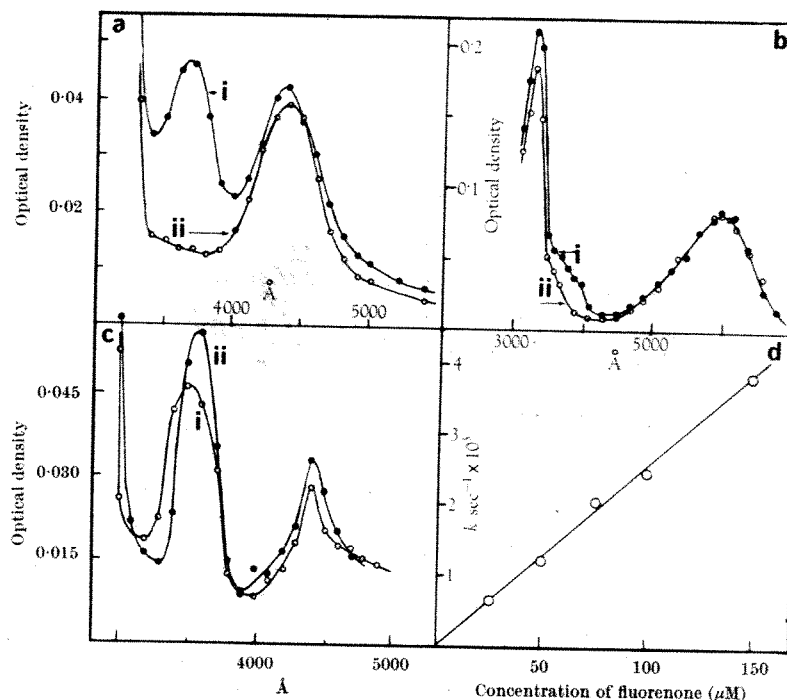


Fig. 1. Electron transfer spectra. a, Transient spectra from 3.3 mM acetophenone in aqueous  $10^{-1}$  N potassium hydroxide. (i) No acetone; (ii) 0.17 M acetone. b, Transient spectra from 0.2 mM benzophenone in aqueous  $10^{-1}$  N potassium hydroxide. (i) No acetone; (ii) 44 mM acetone. c, Transient spectra from 44  $\mu$ M fluorenone in aqueous  $10^{-1}$  N potassium hydroxide. (i) No nitrous oxide; (ii) nitrous oxide saturated. d, Kinetics of electron transfer from  $\text{N}_2\text{O}^-$  to fluorenone in ethanol containing  $10^{-2}$  N sodium ethoxide.

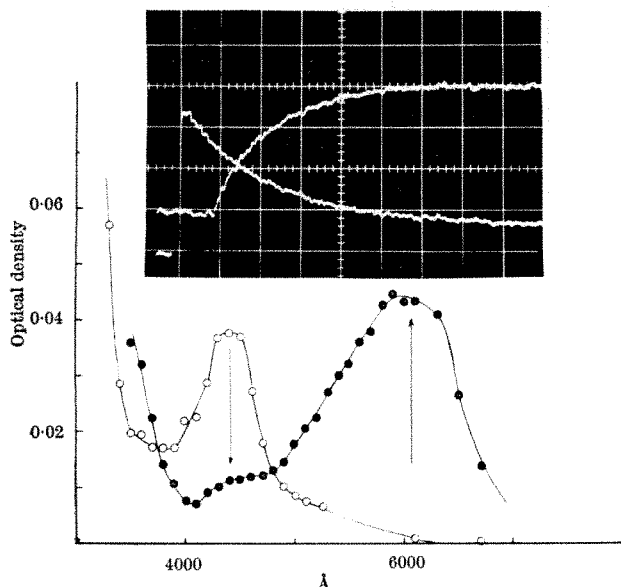


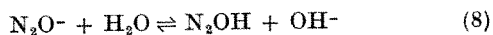
Fig. 2. Transient spectra from an aqueous solution containing 0.82 M acetone, 3.34 mM acetophenone, 72  $\mu$ M benzophenone and  $10^{-1}$  potassium hydroxide (nitrogen saturated). Spectra taken 2  $\mu$ sec (○) and 50  $\mu$ sec (●) after the pulse. Inset, oscillogram: build-up, absorption at 6100 Å; decay, absorption at 4400 Å. Ordinate: absorption, 2.1 per cent per division; abscissa: time, 10  $\mu$ sec/division.

to a species produced by reaction between OH and acetone, with a maximum at lower wavelengths.

In this system, acetone must scavenge all hydrated electrons, and therefore the sequential formation of the two ketyl anions indicates the occurrence of a simple three-stage chain electron transfer process



Henglein and co-workers<sup>8</sup> have shown that, in neutral solution and in the presence of excess nitrous oxide, the formation of the electron adduct of nitromethane is prevented. At a high pH, however, nitrous oxide appeared to be ineffective in competing for solvated electrons. They suggested that the equilibrium



was involved in the phenomena.

At low pH, the equilibrium lies to the right, and  $\text{N}_2\text{OH}$  decomposes rapidly to nitrogen and OH. In alkaline solution,  $\text{N}_2\text{O}^-$  is stabilized and is sufficiently long lived to permit electron transfer to nitromethane.

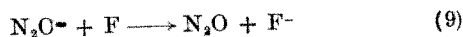
Pulse radiolysis studies<sup>15</sup> have shown that at natural pH, where equilibrium in equation (8) lies far to the right, the lifetime of  $\text{N}_2\text{O}^-$  is of the order of  $10^{-9}$  sec.

In the stationary state radiolysis of aqueous alkaline solutions of isopropanol it was suggested<sup>16</sup> that nitrous oxide reacts with the isopropanol radical ion to produce an oxidizing radical (OH). This would be in keeping with the mechanism of electron transfer to nitrous oxide.

The data in Fig. 1(c) provide some support for the relative stability of  $\text{N}_2\text{O}^-$  in alkaline solution.

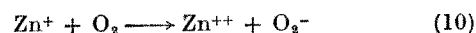
Curve 1(c) (i) shows the transient spectrum immediately after the pulse in an aqueous solution containing 44  $\mu$ M fluorenone at pH 13. The maximum at 4400 Å is similar to that in acetophenone solutions, and is assigned to the fluorenone radical anion ( $\text{F}^-$ ). When the solution is saturated with nitrous oxide, before irradiation, the absorption of  $\text{F}^-$  is still observed.

We believe this effect, similar to that observed with nitromethane<sup>8</sup>, is due to electron transfer from the stabilized radical anion,  $\text{N}_2\text{O}^-$ .

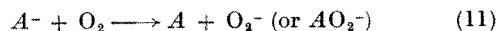


Similar phenomena are observed in ethanolic solutions containing sodium ethoxide. Absolute confirmation of this  $\text{N}_2\text{O}$  mechanism does, however, require the elimination of the possibility of an  $\text{F}^-$  type spectrum being produced by ionization of an OH-adduct or by electron transfer from  $\text{O}^-$ .

We have been informed by Dr Baxendale that electron transfer to oxygen has been observed. Pulse radiolysis of solutions of  $\text{Zn}^{++}$  produces, by reduction, the transient ion,  $\text{Zn}^+$ , which decays by second order kinetics. In the presence of small but excess amounts of oxygen, the absorption decays first order in oxygen<sup>11</sup>



We find that in the presence of oxygen, the radical anions of fluorenone, benzophenone and the other acceptors, produced by electron attachment or by chain electron transfer, decay by an exponential process. In all cases the half times are inversely proportional to oxygen concentration indicating that reaction (11) takes place.



We have found no evidence for peroxyanion-radical formation which would favour the mechanism of electron transfer rather than oxygen addition for reaction (11).

Fig. 1(d) shows that, in alkaline ethanolic solutions saturated with nitrous oxide, the first order rate constants for electron transfer to fluorenone are proportional to fluorenone concentration. From the slope, the second order rate constant was calculated and is given in Table 1.

Table 1

Donor	Acceptor	Solvent	Potassium hydroxide or sodium ethoxide (mM)	Rate constant ( $\text{M}^{-1} \text{sec}^{-1}$ )
Acetone-	Fluorenone	Ethanol	16	$2.0 \times 10^9$
Acetone-	Acetophenone	Water	100	$7.8 \times 10^8$
Acetophenone-	Benzophenone	Water	100	$7.8 \times 10^8$
$\text{N}_2\text{O}^-$	Benzophenone	Water	100	$1.3 \times 10^9$
$\text{N}_2\text{O}^-$	Fluorenone	Water	100	$1.2 \times 10^9$
$\text{N}_2\text{O}^-$	Fluorenone	Ethanol	100	$2.5 \times 10^9$
Fluorenone-	Oxygen	Ethanol	10	$8.9 \times 10^8$
Fluorenone-	Oxygen	Ethanol	10	$9.8 \times 10^8$
( $\text{N}_2\text{O}$ satd.)				
Dimethylfumarate-	Oxygen	Water	1	$5.3 \times 10^9$
Dimethylfumarate-	Oxygen	Ethanol	10	$1.5 \times 10^9$

Data for various electron transfer reactions are also given in Table 1 and were obtained from the slope of similar plots.

We wish to thank Professor J. J. Weiss and Dr G. Scholes, of the University of Newcastle, for their helpful interest.

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# Electron Thermodynamic Nonequilibrium in $p$ - $n$ Junctions

by

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The concepts of the recently proposed "generalized field theory"—a theory which attempts to differentiate completely between active and passive transport processes—are applied to forward conduction in  $p$ - $n$  junctions. The predictions of the theory agree very closely with the results of experiments.

In some heterogeneous systems, thermodynamic equilibrium and nonequilibrium can be accounted for by a new phenomenological theory<sup>1,2</sup> which has the property of making a strict distinction between reversible (active) and irreversible (passive) transport processes. One consequence of this generalized field theory is that in equilibrium each irreversible transport process vanishes, and each reversible transport process is conservative. These conditions, which apply to each constituent in multi-component systems, are necessary and sufficient for equilibrium. Further consequences of this theory include new interpretations of the principle of detailed balancing, and the fundamental law of conservation of energy as applied to transport processes. What follows is a new theory of forward conduction in  $p$ - $n$  junctions based on the generalized field theory.

## The Generalized Field Theory

In order to state the hypotheses of the generalized field theory for the case of conduction electrons in solids, for example, two definitions are necessary. First, passive or irreversible processes are defined as those which involve flow and which invariably increase the internal energy of the host medium, usually as a result of positive entropy production. An obvious example is that of ohmic conduction in a resistor. A less obvious example is the diffusion which occurs only during conduction in the homogeneous  $n$  and  $p$  regions of a  $p$ - $n$  junction.

Active or reversible transport processes, by contrast, are defined as those which can exist in equilibrium. According to the direction of current flow, there may be an increase or a decrease of the internal energy of the host medium. One obvious example is that associated with the electromotive force of a battery. Less obvious examples are the electrostatic and diffusion processes in the interface regions between two solids—the transition region of a  $p$ - $n$  junction, for example.

It is now possible to state the three following hypotheses.

(1) Associated with every physical transport process involving conduction electrons in solids there exists a force per electron, statistical or otherwise. This force constitutes a vector field, which is mathematically defined throughout the solid, regardless of whether this process is reversible, irreversible, conservative or non-conservative.

(2) If conduction electrons are involved in any irreversible transport processes, which are varying sufficiently slowly in time, so that inductive and capacitive effects are negligible, then near equilibrium the electron current density  $\mathbf{J}_n$ , at any point  $Q$ , is related to the generalized irreversible force  $\mathbf{f}_i$  per electron, associated with all the processes at that point, by

$$\mathbf{J}_n = -\mu_n n \mathbf{f}_i \quad (1)$$

where  $n$  and  $\mu_n$  are the electron concentration and mobility at  $Q$ , respectively. For the reversible generalized force  $\mathbf{f}_r$ , however, the preceding relation will not hold.

(3) Around every possible closed loop,  $C$ , within any solid medium, under the conditions stated for the second hypothesis, the reversible and irreversible generalized forces per electron associated with all the processes are related by

$$\oint_C (\mathbf{f}_r - \mathbf{f}_i) \cdot d\mathbf{l} = 0 \quad (2)$$

where  $d\mathbf{l}$  is an increment of length along the closed loop.

Similar statements may be made for holes in semiconductors.

The thermodynamic field, to be designated by  $\mathbf{f}_t$ , is defined as the statistical force per electron associated with the gradient of the electron concentration  $n$  and of the temperature  $T$ . The mathematical existence of  $\mathbf{f}_t$  at every point in a system has been specified by the first hypothesis. Furthermore, it can be shown<sup>1</sup>, on the basis of the first and second law of thermodynamics, that

$$\mathbf{f}_t = \mathbf{f}_T + \mathbf{f}_d, \quad \mathbf{f}_T = s \nabla T, \quad \mathbf{f}_d = -(1/n) \nabla P \quad (3)$$

where  $\mathbf{f}_T$  is the virtual or effective thermal force per electron,  $\mathbf{f}_d$  is the diffusion force per electron,  $s$  is the absolute entropy per electron, and  $P$  is the electron internal pressure, which is strictly expressed by the equation of state<sup>3</sup>.

When the proposed hypotheses of nonequilibrium are interpreted from the point of view of a few fundamental laws of nature, one can deduce<sup>2,3</sup> the new generalized conditions of equilibrium stated earlier, and the new generalized principle of detailed balancing, which states: at any point in a thermodynamic system in equilibrium, for every particle of a specific constituent which is crossing a given area per unit time in one direction, under the action of each process, there is another particle of the same constituent crossing in the opposite direction. This is in contrast with the conventional interpretation of the balancing considered to be caused necessarily by all the processes combined.

It can be shown<sup>2,3</sup> that Ohm's Law and Fick's Law can be derived from the second hypothesis. Thus Ohm's law and Fick's law represent purely irreversible phenomena and, according to the generalized field theory, cannot be used to explain the completely reversible conditions of equilibrium, such as those prevailing in the transition region of a  $p$ - $n$  junction.

## Theory of Forward Conduction in $p$ - $n$ Junctions

In 1949 the first theory of conduction in  $p$ - $n$  junctions was presented by Shockley<sup>4</sup>. It was assumed then that there was no carrier recombination in the transition region, and that conduction was caused by the injection of



minority carriers, which would recombine and diffuse in the bulk regions. Using the conventional hypotheses of thermodynamic equilibrium and nonequilibrium, Shockley has shown<sup>4</sup> that the diode forward applied d.c. voltage  $V$  and current  $I$  are related by an equation equivalent to

$$V = V_T \ln(1 + \alpha'), \quad \alpha' = I/I_s \quad (4)$$

$$V_T = kT/q \quad (5)$$

where  $k$  is Boltzmann's constant,  $q$  is the magnitude of the electron charge and  $I_s$  is the reverse-saturation current.

Equation (4) has been found to be in agreement with experiment only at low injection levels in germanium diodes<sup>5</sup>. Since 1949 there has been more than seven different theories proposed<sup>6-9</sup> to explain quantitatively or qualitatively portions of the problem of forward conduction in germanium, silicon and gallium-arsenide  $p$ - $n$  junctions. Most theories have assumed processes leading to new current mechanisms, which make theory and experiment agree under strongly restricted conditions.

By contrast, analysing the conduction processes proposed by Shockley<sup>4</sup> in 1949, on the basis of the generalized field theory, seems to lead to results in agreement with most published experimental data on (non-tunnelling)  $p$ - $n$  junctions regardless of material, temperature and injection levels. Specifically, if one neglects the irreversible electrostatic process (ohmic conduction) and assumes that the injection is carried by one type of carrier, such as holes, then the forward voltage-current characteristics can be shown<sup>10</sup> to be given by

$$V = V_T \ln(1 + \alpha) + V_T[1 - (1/\alpha) \ln(1 + \alpha)] + \delta\psi \quad (6)$$

$$\alpha = (II), \quad p_e = (1 + \alpha)p_n \quad (7)$$

where  $I^*$  is defined as the reverse-saturation current, and  $p_e$  and  $p_n$  are the concentration of minority holes at the  $n$ -region boundary and sufficiently far from the junction, respectively.

The quantity  $\delta\psi$  represents the increase in the junction

reversible electrostatic voltage caused almost entirely by the deviation  $\delta p(y)$  of the distribution of hole concentration  $p_1(y)$  from its equilibrium value  $p_0(y)$ , where  $y$  is the distance between any point within the transition region and the  $p$ -region boundary. Electron effects are negligible for the bulk concentration of majority holes  $p_p$  is much higher than that of majority electrons  $n_n$ . Using Poisson's equation of electrostatics,  $\delta\psi$  can be found by assuming an exponential distribution for  $p_0(y)$  and  $p_1(y)$ . Thus

$$d^2[\delta\psi(y)]/dy^2 = -(q/\epsilon)p_p[e^{-a_1y} - e^{-a_0y}] \quad (8)$$

where  $\epsilon$  is the crystal dielectric constant, and  $a_0$  and  $a_1$  are constants determined from the boundary conditions that  $p_0(w) = p_n$ , and  $p_1(w) = p_e$ . Here  $w$  is the width of the junction. For most cases of interest,  $p_p \gg p_e$ . Consequently, it can be shown that, regardless of the injection intensity,  $w$  will remain nearly constant; and  $\delta\psi$  will be almost entirely contributed by the positive portion of the junction dipole charge, which will be much wider than the negative portion. These two properties considerably simplify solving equation (8) for  $\delta\psi$ , which may be written as

$$\delta\psi \simeq \delta\psi^* [\ln^{-2}(p_p/p_e) - \ln^{-2}(p_p/p_n)] \quad (9)$$

$$\delta\psi^* = qp_p w^2/\epsilon \quad (10)$$

The arbitrary constant encountered in solving the differential equation has been evaluated from the condition that the reversible electrostatic field vanishes at the  $n$ -region boundary.

Fig. 1 represents sets of theoretical and experimental forward  $V$ - $I$  characteristics for germanium, silicon and gallium-arsenide alloy  $p$ - $n$  junctions. The theoretical characteristics are either plots of equations (4) or (6). Table 1 shows all the constants for the germanium and silicon diodes used in plotting equation (6).

The generalized field theory and experiment appear to be generally in good agreement. The experimental

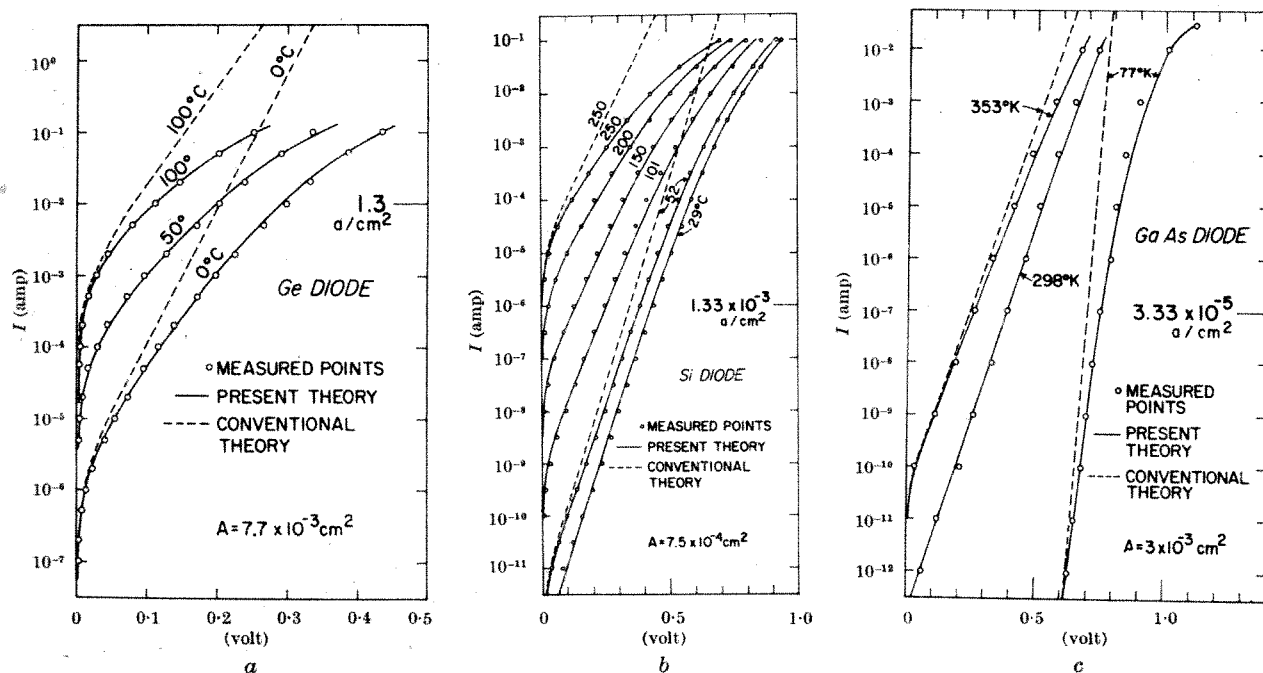


Fig. 1. Theory and experiment are compared for three different forward-biased alloy  $p$ - $n$  junctions. The theoretical curves are based on Shockley's 1949 conduction mechanisms<sup>4</sup> and either the generalized field theory or the conventional concepts of equilibrium and nonequilibrium. The germanium diodes are made by G.E.C., USA (Type 1N91). Experiments on the silicon and gallium-arsenide diodes were conducted by Sah<sup>6</sup>, and by Rediker and Quist<sup>8</sup>, respectively.

Table 1. PHYSICAL CONSTANTS FOR THE GERMANIUM AND SILICON DIODES

$T (^{\circ}\text{C})$	$p_n(\text{cm}^{-3})p$	$I^*$ (amp)	$\delta\psi^*$ (volt)
Germanium diode			
	$p_p = 5 \times 10^{18}/\text{cm}^3$ , $n_n(28^{\circ}\text{C}) = 9.13 \times 10^{12}/\text{cm}^3$		
0	$2.74 \times 10^{11}$	$3 \times 10^{-4}$	7.68
50	$3.79 \times 10^{11}$	$1.1 \times 10^{-4}$	3.93
100	$5.55 \times 10^{11}$	$1.5 \times 10^{-3}$	2.9
Silicon diode			
	$p_p = 3.75 \times 10^{20}/\text{cm}^3$ , $n_n(28^{\circ}\text{C}) = 8 \times 10^{14}/\text{cm}^3$		
29	$2.18 \times 10^5$	$7.5 \times 10^{-13}$	23.5
52	$6.72 \times 10^5$	$8 \times 10^{-12}$	17.5
101	$3.12 \times 10^9$	$1.8 \times 10^{-9}$	15.0
150	$5 \times 10^{11}$	$8 \times 10^{-8}$	11.0
200	$1.83 \times 10^{12}$	$2.0 \times 10^{-6}$	10.4
250	$5 \times 10^{14}$	$2.1 \times 10^{-4}$	8.16

points for the gallium-arsenide diodes seem to be, however, somewhat higher than the theoretical ones between  $10^{-6}$  amp  $< I < 10^{-2}$  amp. This is believed to be caused by the measuring techniques. According to Rediker and Quist<sup>9</sup>, direct current was used in the experiment except for the highest one or two points. For these points a pulsating current was used specifically to reduce heat generation. Conceivably some heating gradually developed when the d.c. current exceeded  $10^{-6}$  amp. This effect was clearly observed in our measurements on the germanium diodes, and adequate precautions had to be taken.

## Discussion

Conduction electrons and valence holes in  $p$ - $n$  junctions represent particularly interesting examples of heterogeneous thermodynamic systems. The degree of carrier heterogeneity in such semiconductor structures can, in some cases, rarely be exceeded in other systems. For example, in the silicon diodes, the characteristics of which are shown in Fig. 1b, in equilibrium and at  $29^{\circ}\text{C}$ , the hole concentration varies from  $p_p = 3.75 \times 10^{20}/\text{cm}^3$  to  $p_n = 2.18 \times 10^5/\text{cm}^3$  through a junction width of only a few hundred angstroms. When the forward current increases from  $I = I^* = 7.5 \times 10^{-13}$  amp to  $I = 10^{-1}$  amp, the concentration of injected minority holes increases gradually from  $(2 p_n)$  to more than  $(10^{11} p_n)$ . By increasing the temperature from  $29^{\circ}$  to  $250^{\circ}\text{C}$ ,  $p_n$  increases by a factor exceeding  $10^9$ . Thus analysing conduction in  $p$ - $n$  junctions on the basis of the conventional and proposed concepts of thermodynamic nonequilibrium provides an especially interesting means for checking some important consequences of these concepts directly by experiment under widely varying conditions in strongly heterogeneous systems.

Equation (6), derived on the basis of the generalized field theory, involves three fundamental quantities. The first,  $kT \ln(1 + \alpha) = qV_1$ , is the closed-loop line integral of the rotational reversible diffusion force  $\mathbf{f}_d$ . This force exists only in the heterogeneous interfaces. It must become irrotational everywhere in equilibrium. Thus under such conditions,  $V_1$  must vanish, as may be observed when  $\alpha$  tends to zero. The second quantity in equation (6) is  $kT[1 - (1/\alpha)\ln(1 + \alpha)] = qV_2$ . This term equals the closed-loop line integral of the irreversible diffusion force  $\mathbf{f}_d$ . This force only exists in the excess-charge (homogeneous)  $n$  region. Because in equilibrium there can be no irreversible transport processes, this force must then vanish, and also its line integral,  $qV_2$ , as may be seen when  $\alpha$  tends to zero. The third quantity is  $(q\delta\psi)$  which equals the closed-loop line integral of the rotational reversible electrostatic force  $\mathbf{f}_e$ . This force only exists in the heterogeneous interfaces. In equilibrium  $\mathbf{f}_e$  must become irrotational everywhere so that  $\delta\psi$  must vanish. This conclusion is confirmed by equation (9). Thus each one of the reversible and irreversible transport processes in the system verifies the new generalized conditions of equilibrium<sup>1,2</sup>. Furthermore, the new generalized principle of detailed balancing is clearly satisfied.

In a recent article, Professor McLellan<sup>11</sup> has stated that the diffusion force  $\mathbf{f}_d$ , used in the generalized field theory, would not account for the energy caused by the change of volume when particles are transported in a heterogeneous isothermal system. He has therefore

concluded that the basic arguments presented in references 1, 3 and 12, the proposed concepts of nonequilibrium and the consequent  $p$ - $n$  junction theory, would not be valid. There are at least two reasons supporting the statement that, under isothermal conditions,  $\mathbf{f}_d$  indeed accounts for whatever relates to the process of a thermodynamic nature, and only that process—not the electrostatic one, for example. Specifically,  $\mathbf{f}_d$  accounts for the work associated with the change in the particle transport potential energy and thermal kinetic energy. These new transport concepts do account fully for the energy change caused by the change in volume. The first reason actually follows from a careful understanding of the subtle arguments, and the derivations of  $\mathbf{f}_d$  and  $\mathbf{f}_e$  presented in references 1, 3 and 12. The second and more conclusive reason is clearly implied by the precise, general agreement of the new  $p$ - $n$  junction theory and experimental measurements, as may be observed in Fig. 1. In this figure, it can be also seen how the conventional  $p$ - $n$  junction theory which is directly based on the conventional concepts of nonequilibrium compares with experimental observations. Comparison of equations (4) and (6) clearly shows that the generalized field theory has introduced, rather than overlooked, two significant energy terms, which cannot possibly be accounted for through the conventional concepts of equilibrium and nonequilibrium and the basic conduction mechanisms assumed by Shockley<sup>4</sup> in 1949. These conduction mechanisms, however, have proved conclusively here to be fully satisfactory, and need none of the numerous drastic changes introduced in the past eighteen years in order to bring about, but severely restricted, qualitative or quantitative piecewise agreement between theory and experiment.

As predicted by the generalized field theory, and clearly observed in Fig. 1, the deviation between the conventional theory and experiment becomes more serious at higher current densities for more heterogeneous junctions, that is, wider semiconductor energy gaps and lower temperatures. Thus the most extreme deviation in the shown characteristics occurs in the gallium-arsenide diode at  $T = 77^{\circ}\text{K}$ , and  $V \approx 1.1$  volt. Under such conditions the conventional theory overestimates the experimentally measured current by about  $10^{20}$  times.

In conclusion, predictions of the generalized field theory should be expected in general to deviate more seriously from those of the conventional concepts of thermodynamic nonequilibrium in systems with stronger reversible transport processes. Such processes become more intense with increasing heterogeneity. In isothermal homogeneous systems, however, predictions of the new and conventional concepts become, for the most part, identical.

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# Structure and Organization of Actin in a Molluscan Smooth Muscle

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X-ray diffraction shows that some degree of three-dimensional organization of actin exists in a molluscan smooth muscle. From X-ray patterns from this muscle and the toad sartorius, both in the living relaxed state, it can be deduced that the actin structure repeats at intervals of about 360 Å. In this respect the structure of actin may be similar in all muscles.

ELECTRON microscopy has shown that actin filaments are double helical structures composed of globular units spaced about 55 Å apart<sup>1</sup>. This structural picture is in agreement with earlier X-ray diffraction studies<sup>2</sup> on certain dried molluscan muscles. Analysis of moderate-angle patterns suggested two alternative helical actin models but the authors could not decide between them. One structure has thirteen units, the other fifteen units, in each turn of helix, giving a helical repeat of either 350 Å or 410 Å. Electron microscopy has not yet provided a consistent value for the helical repeat in isolated natural or synthetic actin filaments<sup>3</sup>. For an understanding of the contractile mechanism it is important to determine the helical repeat and, particularly, to establish whether any changes occur in the actin structure during normal contraction<sup>1,4</sup>. X-ray diffraction studies of muscles in various states have attempted to deal with these problems<sup>5-11</sup>.

We have studied actin structure in several molluscan smooth muscles; particularly the anterior byssus retractor of *Mytilus edulis* (ABRM) by X-ray diffraction. Our diffraction patterns from the ABRM in the living relaxed state show certain features which have not been seen before and which provide new information about the organization of actin filaments in this muscle.

The ABRM was studied in the living relaxed state, as well as in the dried state. The living muscle was kept taut by means of a small weight and irrigated with natural sea water at just above freezing point. We have also examined dried specimens prepared from the smooth adductor of *Pecten chlamys*, and from the pharynx retractor of *Helix pomatia*.

The X-ray source was a Hilger and Watts semi-micro-focus tube operated at 45-50 kV and 2-3 mamp. Our camera was designed by Dr A. Elliott<sup>12</sup>, and had a toroidal mirror with a nickel surface which will be described elsewhere, as well as modifications to give improved low-angle resolution. Diffraction patterns were obtained in air or in an evacuated chamber. We are indebted to Dr H. E. Huxley for suggesting the use of the latter for recording the 51 Å layer line in the actin pattern.

We have taken as the first approximation to the helical repeat of the actin structure the value obtained from measurements of the spacings of the 59 Å and 51 Å layer lines, seen most clearly in dried specimens (Fig. 1). For the living relaxed ABRM we obtain a repeat value of 369 Å. This value is the mean of measurements on ten muscles; the values range from 350 Å to 385 Å, and the standard deviation of the mean is 15 Å. For the dried ABRM, *Pecten* and *Helix* muscles, the mean values and deviations from a similar number of measurements are respectively  $356 \pm 16$  Å,  $367 \pm 18$  Å and  $373 \pm 25$  Å.

To improve the accuracy of these values for the repeat, the indices of the observed layer lines are required. With reference to the two integral helix models of Selby and Bear<sup>2</sup>, we find that for the ABRM in both the living relaxed and dried state it is possible (within the limits of accuracy stated) to index the 59 Å and 51 Å layer lines, as well as the 27 Å meridional reflection as orders 6, 7 and 13 of the repeat; but not as orders 7, 8 and 15. Thus if the former indices are correct, the values for the repeat in living relaxed and dried ABRM would be  $358 \pm 2$  Å and  $356 \pm 2$  Å, respectively. For the dried

*Pecten* and *Helix* muscles the same indexing is possible, although the agreement with the thirteen unit model is less satisfactory.

While the fifteen unit model of Selby and Bear with a repeat of 410 Å can thus be excluded for these molluscan muscles, the thirteen unit model should, in our view, only be regarded as an approximation to the true actin structure. We consider it more likely that the actin helix does not contain an integral number of units, and that the structure repeats at intervals of about 360 Å. With the present uncertainties (about 0.5 per cent) in our values for the layer line spacings, however, we cannot distinguish between a number of possible models all of which have higher indices for our layer lines, and repeat at intervals of about 360 Å.

A new feature we have seen in diffraction patterns from the living relaxed ABRM is an intensity maximum on both the 59 Å and 51 Å layer lines (Fig. 2). We take these maxima to indicate the presence of an interference function, resulting from an ordered arrangement of actin filaments, sampling the layer lines at  $1/120 \pm 4$  Å from the meridian. This value is the same as the spacing of a

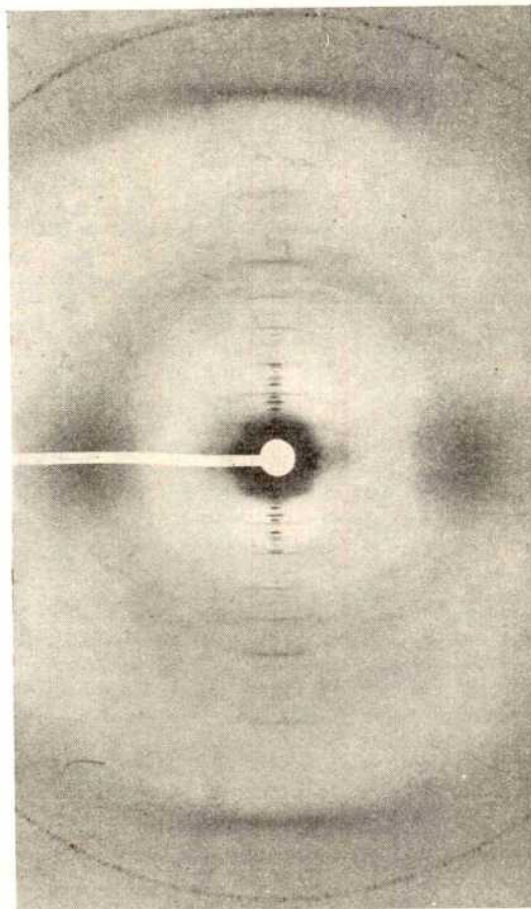


Fig. 1. Dry ABRM. Distance between specimen and film was 8 cm. Exposure was 16 h ( $\times 2$ ).



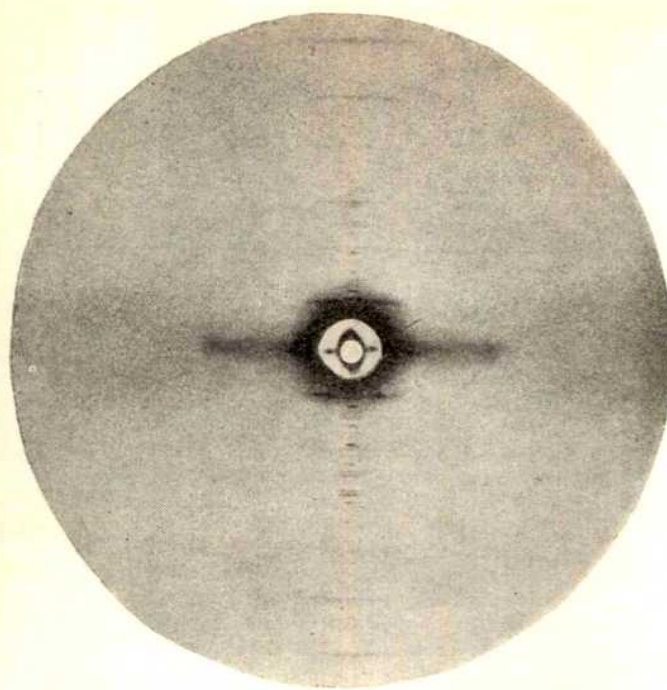


Fig. 2. Living relaxed ABRM. Distance between specimen and film was 8 cm. Exposure was (a) wide angle pattern for 16 h; (b) low angle equatorial pattern (central inset) for 2 h ( $\times 3$ ).

well orientated equatorial reflexion (Fig. 2) which was first seen by Elliott and Lowy<sup>13</sup> and measured as  $122 \pm 5$  Å in the living relaxed ABRM. The equatorial reflexion was seen to streak out when the muscle died. We have confirmed this observation and noted also that the interference maxima on the 59 Å and 51 Å layer lines disappear within 48 h, whereas the equatorial reflexion (together with an increasingly prominent equatorial streak) is still present up to 6 days after the muscle has been isolated from the animal. A day or so later, the equatorial reflexion can no longer be seen clearly. The muscle ceases to contract in response to electrical stimulation 3 or 4 days after isolation.

From these observations we are led to conclude that in the ABRM there exist regions where actin filaments are organized with partial three dimensional order, that this organization degenerates about 2 days after isolation, and that two dimensional order can be maintained for up to three times as long.

Further results which indicate that the equatorial reflexion is caused by the organization of actin filaments come from experiments in which the ABRM was fixed in 70 per cent alcohol. With dried molluscan smooth muscles (similar in structure to the ABRM) such treatment was found to destroy the actin pattern<sup>14</sup>, whereas the paramyosin pattern (caused by thick filaments) shows only a little disorientation. Our experiments with the ABRM have confirmed this and show, in addition, that the equatorial reflexion degenerates at the same time as the actin pattern.

Elliott<sup>15</sup> studied transverse sections of paramyosin filaments by electron microscopy. These showed a lamellar structure, the lamellae being spaced 150–200 Å apart. Accordingly, Elliott and Lowy<sup>13</sup> suggested that the equatorial reflexion is caused by the separation of planar net structures (spaced about 120 Å apart) within the paramyosin filaments. Our results do not support this interpretation.

So far the ABRM is the only muscle in which some degree of three dimensional organization of actin filaments has been found. It will be interesting to see whether such organization also exists in other muscle types, or whether it is uniquely part of the structural specialization associated with "catch" muscles like the ABRM.

We discuss next our results for the repeat of the actin structure in other muscles. For the dried pharynx retractor of *Helix*, our result differs from that of Worthington<sup>5</sup>, who measured the 59 Å and 27 Å layer lines and obtained a value of 410 Å for the actin repeat. Our values for the layer line spacings in the *Helix* muscle cannot be indexed on this repeat.

Millman *et al.*<sup>11</sup> obtained a value of about 400 Å for the actin repeat in the ABRM and the toad sartorius muscle, both in the living relaxed state. For the toad muscle, this result was based on the assignment to the actin structure of six diffuse low-angle layer lines with spacings greater than 59 Å. Particular reference was made to a layer line near 400 Å (with intensity well off the meridian) which was observed in both the ABRM and the toad muscle, and identified as the first layer line of the actin diffraction pattern.

From measurements of the 59 Å and 51 Å layer lines, Huxley<sup>16</sup> reported a value of about 355 Å for the actin repeat in living relaxed frog sartorius muscle. He considered this to be a better estimate for the actin period than that obtained from the 400 Å layer line which, together with the other diffuse layer lines with spacings between 59 Å and 400 Å, could not straightforwardly be assigned to the actin structure (Huxley, personal communication). Using living relaxed toad sartorius muscle, we have made measurements from the 59 Å and 51 Å layer lines, which give a value for the actin repeat in the range between 360 and 380 Å. Thus, together with our results for the ABRM, our findings do not support the view that the actin period in the living relaxed ABRM and toad sartorius is about 400 Å (ref. 11).

In the living relaxed striated adductor muscle of *Pecten maximus*, Millman and Elliott (quoted in ref. 11) observed a layer line at about 463 Å and suggested that it is caused by the actin structure. In view of the foregoing considerations this interpretation is unlikely to be correct.

The conclusion is that when the same method is used to determine the helical repeat of the actin structure in different muscles examined in the same state, the value obtained is the same (about 360 Å) in muscles as different in structure and function as the toad sartorius and the ABRM. This indicates that the actin structure might be similar in all muscles, though clearly many more types will have to be examined before a firm conclusion can be reached.

In the light of our knowledge that the layer line at about 400 Å is not caused by the actin helix it becomes imperative to determine its real origin. This reflexion constitutes a puzzling feature in the X-ray diffraction pattern, considering that its spacing varies, certainly from one muscle type to another and possibly also with the state of the muscle.

We thank Professor Sir John Randall for provision of facilities, Dr A. Elliott for his help and Mr A. Fasoli for technical assistance. One of us (P. J. V.) is a recipient of an MRC training grant.

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## LETTERS TO THE EDITOR

## ASTRONOMY

## Possible New Evidence bearing on the Lunar Capture Hypothesis

INTEREST in the dynamical history of the Earth-Moon system was stimulated by the calculations of Gerstenkorn<sup>1</sup>, who deduced that the Moon was originally an independent planet in a solar orbit close to that of the Earth and that it was captured into a highly elliptical retrograde terrestrial orbit of small perigee. The orbit was brought closer and its ellipticity was reduced by tidal interaction until a dramatically close encounter with the Earth flipped the Moon over into a prograde orbit, from which it has receded to its present position under the influence of tidal friction. Gerstenkorn's theory has been favourably reviewed by Alfvén<sup>2,3</sup> and very similar dynamical histories are deduced in several more recent calculations<sup>4-6</sup>. An exact estimate of the time since the very close approach of the Moon is not possible because tidal friction is dependent on several factors, including variable geometry of the oceans. Estimates vary between  $2.5 \times 10^9$  yr and  $1.4 \times 10^9$  yr (refs. 1 and 4).

During the close approach, dissipation of tidal energy within the solid part of the Earth would have been intense, greater than the dissipation by marine tides, and certainly sufficient to cause extensive partial melting in the mantle. Once the mantle had been fluidized by partial melting, however, tidal dissipation (and consequently the rate of change of the lunar orbit) would have been greatly reduced, allowing more time for heat to escape and precluding complete melting. Possibly partial melting would have been restricted to the upper mantle (which is nearer to melting than the lower mantle and also has a lower mechanical  $Q$ —that is, greater tidal dissipation<sup>6</sup>), but a dramatic disturbance, at least to the upper mantle, is an inevitable consequence of the lunar capture hypothesis. Absence of geological evidence for such a major disturbance has been regarded as a serious objection to the hypothesis and was one of the reasons which led MacDonald<sup>7</sup> to prefer a lunar origin by accretion in orbit. We draw attention here to recent lead isotope measurements, however, which we interpret as evidence for an upheaval of the kind required by the capture theory.

Published lead isotope measurements on young mantle-derived volcanics<sup>9-14</sup>, together with some unpublished measurements, are represented in Fig. 1 as a plot of lead-206/lead-204 against lead-207/lead-204. There is a clear linear relationship with a gradient distinctly less than that of the single stage zero isochron, shown as a broken line, which would apply if the sources of the volcanics had been mutually isolated since the origin of the Earth  $4.5 \times 10^9$  yr ago<sup>9</sup>. We can therefore impose two boundary conditions on the mantle as a common source for these volcanics: (1) that the lead isotope ratios were uniform at a time geologically long after the origin of the Earth; and (2) that since that time the ratio of uranium to lead has been heterogeneous, so that radiogenic lead isotopes have been added at different rates in different environments.

An estimate of this time dates either the cessation of a long period of homogeneity or a brief event, such as lunar capture, which homogenized a previously heterogeneous mantle. We refer only to homogenization of lead isotope ratios, for which partial melting with some stirring would suffice.

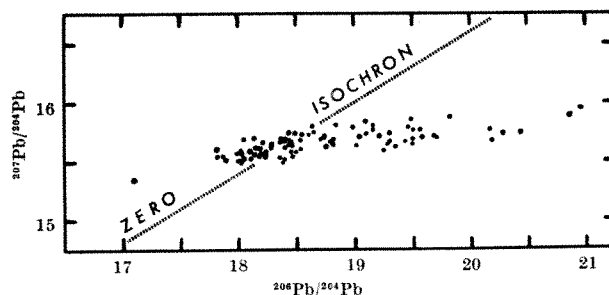


Fig. 1. Plot of lead-206/lead-204 against lead-207/lead-204 for lead isotope measurements on young volcanic material.

We previously estimated the time since mantle lead was isotopically homogeneous at  $2.8 \times 10^9$  yr (ref. 9). Other estimates from different data<sup>11,12</sup> range down to  $0.5 \times 10^9$  yr but the lower values were obtained from rocks with very limited isotopic ranges and are therefore of doubtful validity. Using all the data represented in Fig. 1 and allowing for open system processes, which we have considered previously<sup>9</sup>, we obtain a preferred value of  $2.5 \times 10^9$  yr. This is uncertain by at least  $0.5 \times 10^9$  yr and many more data will be needed to establish or refute our conclusion with any certainty. If the average uranium/lead ratio in the source of the volcanics has remained unaltered during a prolonged multi-stage history<sup>9</sup>, or if a simple two stage model is appropriate<sup>10</sup>, the estimate of age will be reduced. This does not affect our two essential conclusions, however, and the coincidence of the lead isotope event and Gerstenkorn's original estimate of the time of the close approach of the Moon suggests that they were the same event. If this interpretation of the lead isotope data is accepted it not only removes an important objection to the capture theory of the lunar origin but makes the alternative theory of accretion in orbit much less attractive. Accretion at 40 Earth radii, as proposed by MacDonald<sup>7</sup>, would not "switch" on an Earth tide sufficiently violent to account for the lead isotope event, for less than 20 cal/g of rotational energy would be dissipated in the Earth in the following  $10^9$  yr. Accretion at 5 Earth radii would initiate a tidal dissipation of nearly 80 cal/g in  $10^6$  yr, which would be sufficient, but the supposition that lunar material could remain in orbit at 5 Earth radii for  $2 \times 10^9$  yr before accreting (or else suddenly appear in orbit) is difficult to accept. Thus on the present evidence the capture theory of the lunar origin is to be preferred.

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### Far Ultra-violet Spectra of Orion Stars

FAR ULTRA-VIOLET spectra of the brighter stars in the vicinity of  $\epsilon$  Orionis were photographed with an objective spectrograph carried to 165 km by an Aerobee 150 rocket launched from the White Sands Missile Range on September 20, 1966, at 10h 45m U.T. As with previous Princeton flights<sup>1,2</sup> the spectrograph was stabilized by a passive gyro system which limited angular motion in the dispersion direction to about  $\pm 20''$  during a 3 min exposure giving a resolution of about 1 Å.

On this occasion we flew an all reflective spectrograph for which the camera design was based on principles described by K. Schwarzschild<sup>3,4</sup>. The spectrograph is shown in Fig. 1. Light entered through a door in the side of the rocket skin towards a plane grating. The diffracted beam then went along the roll axis to a centrally located convex spherical surface 50 mm in diameter, which in turn reflected the light to a much larger concave spherical mirror with a central hole to pass the initial beam. The second mirror focused the spectra onto a concave piece of film positioned behind the first mirror. The camera was  $f/2$  with a  $12^\circ$  diameter field and the dispersion was about  $50 \text{ Å mm}^{-1}$ . With only reflective optics this system was not impaired by absorption in a transmission element. All three surfaces were specially coated with a thin layer of lithium fluoride<sup>5</sup> immediately after aluminization to improve the reflectivity at wavelengths below 1200 Å. We were able to record spectra to about 1130 Å where the optical efficiency began to drop sharply. It is hoped that more careful protection of the optics from excess humidity will improve the far ultra-violet reflectances for later flights.

Spectra of the hot stars  $\delta$ ,  $\epsilon$ ,  $\zeta$ ,  $\eta$ ,  $\iota$  and  $\sigma$  Orionis were obtained with a 181 sec exposure which is shown in Fig. 2. The Kodak 'Pathé SC5' film was developed for 3 min in D19B at 68° F.  $\gamma$  Ori also appears on the film, but its spectrum is confused by overlapping first and second orders. Very weak images of  $\theta^1$  and  $\psi$  Ori are also present.

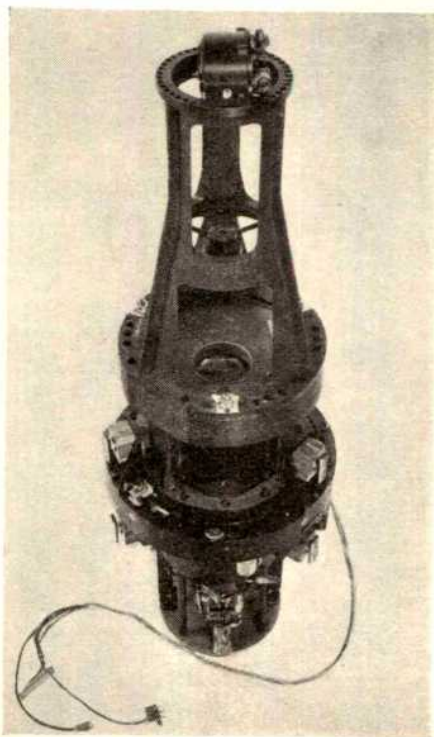


Fig. 1. All reflective rocket spectrograph. In order from the top are the film cassette, the small convex mirror, the large concave mirror, and the plane grating. Underneath is the gyro which provides fine stabilization of the whole spectrograph in the dispersion direction. (Photo, courtesy of Perkin-Elmer Corporation.)

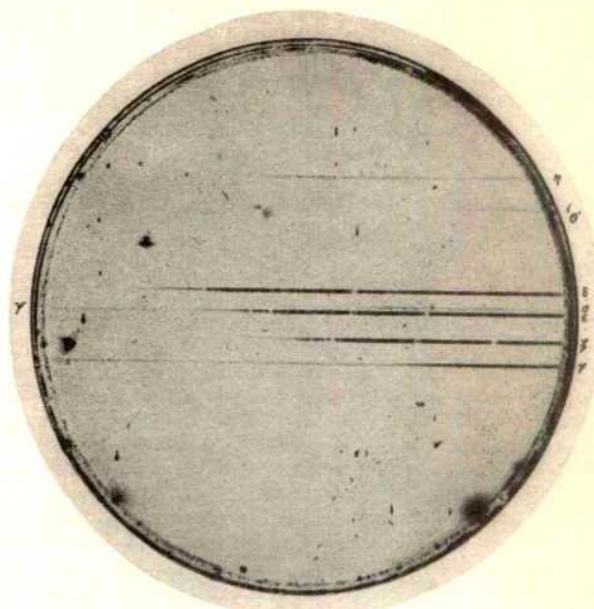


Fig. 2. Far ultra-violet spectra of Orion stars. Wavelengths increase towards the right. In the spectra of  $\delta$ ,  $\epsilon$ , and  $\zeta$  the strongest absorption lines and their laboratory wavelengths are C III (1176 Å), Si III (1207 Å), H I (1216 Å), N V (1240 Å), Si IV (1394 Å, 1403 Å), and C IV (1549 Å). The original film was 22.2 mm in diameter.

About fifty absorption lines have been measured in the well exposed spectra of  $\delta$ ,  $\epsilon$  and  $\zeta$ , but only the strongest lines are clearly visible in the weaker spectra of  $\eta$ ,  $\iota$  and  $\sigma$ . Absolute wavelengths have been determined from zero-order star images on the film. Fine stabilization was maintained only in the dispersion direction; the widening of the spectra to about 5.5 min of arc resulted from the drift and jitter of the Aerobee attitude control system which provided the coarse orientation of the rocket. Near the centre of the film the spectra are a little out of focus probably because part of the double-sided sticky tape used to hold the film against the concave focal surface came loose.

In each of the spectra of supergiants  $\epsilon$  (Bo Ia) and  $\zeta$  (09.5 Ib), and the bright giant  $\delta$  (09.5 II), emission lines of the Si IV and C IV resonance transitions were found near their laboratory wavelengths of 1403 and 1549 Å, respectively. Each emission is accompanied by a strong absorption line shifted to shorter wavelengths by 5–8 Å, corresponding to velocities of 1,000–1,700 km sec<sup>-1</sup> towards us. An earlier Princeton flight<sup>2</sup> had discovered these displaced absorption lines. They show that each star is ejecting mass at remarkably high velocities—up to three times the escape velocity at the stellar surface. The shifted lines are not found at visible wavelengths because the abundant ions in these hot atmospheres have no resonance lines in that spectral region, and the dilute radiation and particle densities in the expanding shell are insufficient to excite the ions to levels from which visual absorptions can occur. In the spectra of cool giants and supergiants, on the other hand, shifts of 10 km sec<sup>-1</sup> have been observed in the resonance lines of lower states of ionization<sup>6,7</sup>.

This last flight confirmed the velocity shifts for Si IV and C IV and provided profiles with much better resolution. This flight further revealed the unresolved N V resonance doublet at 1240 Å with a trace of emission and a wide, shallow absorption profile displaced to shorter wavelengths by about the same velocity. In addition, because of the extra wavelength coverage, this camera recorded for the first time the 1207 Å resonance line of Si III and the 1176 Å excited multiplet of C III, both in absorption. In each star the Si III line has a velocity



comparable with the other resonance lines while C III is displaced somewhat less. This excited line must be formed lower in the atmosphere where the density is higher and the gas is still being accelerated.

Because these three stars are typical of normal OB supergiants and bright giants, it seems very likely that rapidly expanding shells surround all such luminous stars. When a massive star evolves through this phase therefore it will gradually eject some of its matter back into the interstellar medium.

A direct determination of the interstellar atomic-hydrogen abundance between us and the Orion stars may be obtained from the strength of the conspicuous Lyman- $\alpha$  absorption features centred at 1216 Å on the stellar continua. These absorption lines are 8 Å or more wide and very highly saturated, so that radiation damping is by far the most dominant source of line broadening. (Only if turbulent motions are of the order of 1,000 km sec<sup>-1</sup> could they affect the profile.) For such a damping profile the relation of equivalent width to column density is  $N = 1.865 \times 10^{18} W_{\lambda}^2$  atoms cm<sup>-2</sup> (with  $W_{\lambda}$  expressed in Å), a relationship which is independent of any assumptions regarding the physical conditions of the interstellar hydrogen atoms. Lyman- $\alpha$  absorption and emission processes occurring in the star's outer layers or in the

immediate vicinity of the star are expected to have significantly narrower line widths, lying well within the saturate absorption region.

The tracings in Fig. 3 are intended to show the quality of the data obtained in the vicinity of the Lyman- $\alpha$  absorption for each star, as well as to illustrate the successive stages of reduction to equivalent widths. The uppermost profiles for the spectra of  $\delta$ ,  $\epsilon$  and  $\zeta$  Ori show densitometer tracings accompanied by comparison measurements of the background fog level of adjacent unexposed regions on the film. Intensity profiles were derived from the film densities using an H-D curve determined from laboratory test exposures performed several weeks after the rocket flight with the same batch of film and identical developing conditions. Measurements of equivalent widths were made on the lowest curves which are modified intensity profiles normalized to the smooth curve drawn along the continua in the middle illustrations. Adequate allowance was made for an extension of the damping wings on either side of the prominent part of the Lyman- $\alpha$  features.

The much weaker tracings obtained from  $\eta$ ,  $\iota$  and  $\sigma$  Ori do not warrant as detailed a treatment as the strong spectra. The low exposure level may be assumed to lie within a relatively linear region of the H-D curve, and

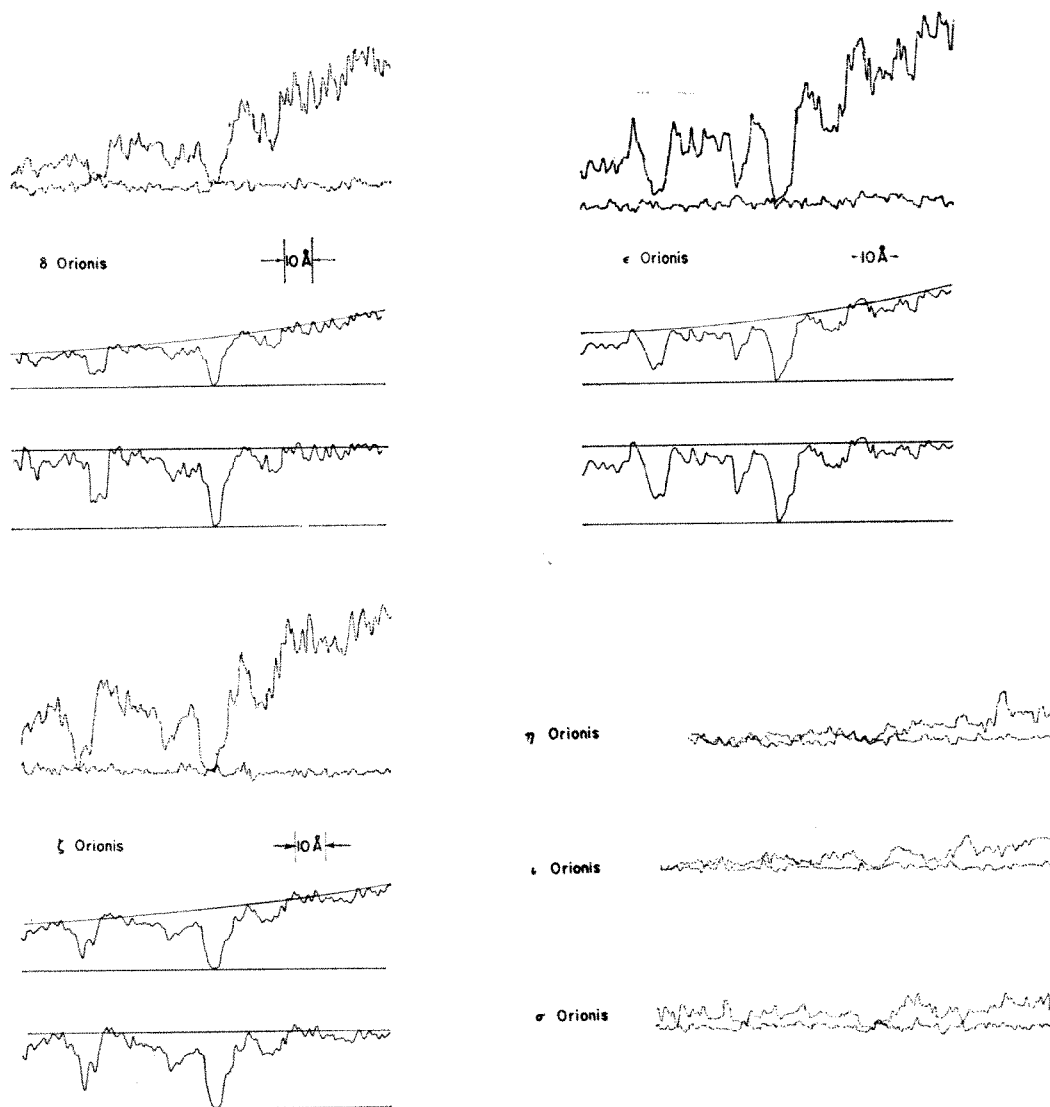


Fig. 3. Spectral scans in the region of Lyman- $\alpha$ . Wavelengths increase towards the right. Shortward of the interstellar line are the stellar lines of C III and Si III, and longward is N V. For  $\delta$ ,  $\epsilon$ , and  $\zeta$  the densitometer trace, the intensity profiles, and the normalized spectra are shown in order from the top. Only the densitometer records are given for the three faint stars.

hence the density tracings justifiably may be treated as intensity profiles. Of course, the errors in the equivalent widths are much larger with the weak spectra.

Measurements of the equivalent widths and estimates of their uncertainties, together with the corresponding number densities, are shown in Table 1. These values are consistent with the Lyman- $\alpha$  widths of about 9 Å found in  $\delta$  and  $\zeta$  Orionis by an earlier Princeton rocket observation<sup>2</sup>. The photometric quality and improved resolution of the new spectra provide much more confidence in the initial results.

Table 1. THE INTERSTELLAR LYMAN- $\alpha$  ABSORPTION LINE

Star	Equivalent width $W_\lambda$ (Å)	Column density $N$ (cm <sup>-2</sup> )
$\delta$ Ori	$8.2 \pm 1$	$1.3 \times 10^{20}$
$\epsilon$ Ori	$8.5 \pm 1$	$1.4 \times 10^{20}$
$\zeta$ Ori	$9.3 \pm 1$	$1.6 \times 10^{20}$
$\eta$ Ori	$12 \pm 6$	$3 \times 10^{20}$
$\iota$ Ori	$9 \pm 3$	$1.5 \times 10^{20}$
$\sigma$ Ori	$12 \pm 3$	$3 \times 10^{20}$

The lack of conspicuous variations in line width from one star to the next suggests that the distribution of hydrogen is not markedly irregular. The average density of hydrogen is about 0.1 atom cm<sup>-3</sup> over an assumed distance of 450 parsec<sup>6</sup> to the Orion stars. In contrast 21 cm emission measurements<sup>9,10</sup> in the Orion region have indicated values around  $1.3 \times 10^{21}$  atoms cm<sup>-2</sup>, which exceed the densities in Table 1 by a factor of ten. Likewise, 21 cm absorption in the continuum of the Orion Nebula<sup>11-13</sup> suggests that column densities in the order of  $1.5 \times 10^{21}$  atoms cm<sup>-2</sup> should be present in front of the nebula. One would not expect a substantial portion of the hydrogen seen in emission to lie beyond the Orion region, which is already about 130 parsec out of the galactic plane.

It is perhaps easier to account for our disagreement with the absorption measurements by saying that most of the hydrogen seen could be associated with the nebula or by assuming that the excitation temperature of the hyperfine transition is a great deal lower than the commonly quoted value of 125° K. Although a direct comparison with the radio data may be confused by such factors as the existence of small scale spatial or temperature inhomogeneities, it should, none the less, be evident that the difficulty in accounting fully for the discrepancy in the measurements may lead to exciting conclusions on the physical nature of the interstellar medium.

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## PLANETARY SCIENCE

### Life in the Clouds of Venus?

WHILE the surface conditions of Venus make the hypothesis of life there implausible, the clouds of Venus are a different story altogether. As was pointed out some years ago<sup>1</sup>, water, carbon dioxide and sunlight—the prerequisites for photosynthesis—are plentiful in the vicinity of the clouds. Since then, good additional evidence has been provided that the clouds are composed of ice crystals at their tops<sup>2,3</sup>, and it seems likely that there are water droplets toward their bottoms<sup>4</sup>. Independent evidence for water vapour also exists<sup>5</sup>. The temperature at the cloud tops is about 210° K, and at the cloud bottoms is probably at least 260–280° K (refs. 4 and 6). Atmospheric pressure at this temperature level is about 1 atm.<sup>7</sup>. The observed planetary albedo falls steeply in the violet and ultra-violet<sup>8</sup>, which accounts for the pale lemon yellow colour of Venus. The albedo decline would not be expected for pure ice particles, and must therefore be caused by some contaminant. Dust, ozone, C<sub>2</sub>O<sub>2</sub> and other gases may possibly explain these data but, whatever the explanation, the ultra-violet flux below the clouds is likely to be low. If small amounts of minerals are stirred up to the clouds from the surface, it is by no means difficult to imagine an indigenous biology in the clouds of Venus. What follows is one such speculation.

A macroscopic organism living in the clouds of Venus must be regulated to live at an essentially fixed altitude. If it is carried, for example by convective downdraughts to the lower atmosphere, it will encounter uncomfortably high temperatures, and if it is carried to the cloud tops it will encounter very little moisture and very low temperatures. We therefore imagine an isopycnic organism constructed as a float bladder<sup>9</sup>. Because the atmosphere is primarily carbon dioxide and nitrogen, a float bladder filled with hydrogen would be very effective. Molecular hydrogen can be produced from water by photosynthesis, as is known in purple bacteria<sup>10</sup>. Although the observed cases are for aerobes there is no reason why photosynthetic production of hydrogen by anaerobes should not occur. We consider such an isopycnic organism near the 0.5 atm. pressure level; the atmospheric density here will be about  $7 \times 10^{-4}$  g cm<sup>-3</sup>, depending somewhat on composition. The organism is essentially a spherical hydrogen gasbag with outer radius  $R_1$ , and inner radius  $R_2$ . For the organism to have a mass equal to the displaced mass of atmosphere, we require

$$5 \times 10^{-5} R_2^3 + \rho(R_1^3 - R_2^3) = 7 \times 10^{-4} R_1^3$$

where  $\rho$  is the density of the outer membrane. For  $\rho \approx 1.1$  g cm<sup>-3</sup>,  $(R_1 - R_2)/R_1 \approx 2 \times 10^{-4}$ . If the minimum skin thickness is about  $1\mu$ , as in terrestrial organisms having a dermal layer one cell thick, the gasbags have a minimum diameter of about 4 cm, about the size of a pingpong ball. Much larger organisms would also be possible. If the skin were a unit membrane thick (about 75 Å), the organism could conceivably be as small as  $75\mu$  in diameter; but this is clearly a lower limit—it is unlikely that the requisite metabolic processes could be contained within a unit membrane.

The postulated photosynthetic organism would reside just below the Venus clouds, or in the lower cloud deck. Water would be collected either as rain or by contact with the droplets, and minerals blown up from the surface would be captured on the sticky underside of the organism, and ingested by pinocytosis. The mineral requirements would be modest, and the ash content would be a very small fraction of the dry weight. Metabolic schemes can be worked out using known terrestrial biochemistry. Much smaller non-isopycnic organisms can also be envisaged. If the Stokes-Cunningham fallout times to reach moderately high temperatures are less than the replication times and if updraughts exist, a stable population of micro-



organisms may be possible<sup>1</sup>. Life at the Venus clouds can be envisaged which operates entirely on known terrestrial principles.

The conditions in the lower clouds of Venus resemble those on Earth more than any other extraterrestrial environment now known. It is possible that life arose under more moderate conditions on the surface of Venus in its early history; for example, the planet may then have been appreciably less degassed than it is today, with an atmospheric greenhouse much less effective than the contemporary one. Outgassing advanced, surface temperatures rose, and the surface became more inclement. Organisms may have then emigrated to the clouds, and may there be awaiting the first biological experiments to be performed in the vicinity of the Venus clouds.

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## Lunar Diurnal Atmospheric Tide

Haurwitz and Chapman<sup>1</sup> have recently described the discovery, analysis and distribution of the lunar semi-diurnal air tide. There is, in addition to lunar semi-diurnal excitation, a smaller lunar diurnal gravitational excitation. Although there must be some atmospheric response to this excitation, it has not yet been found in the data.

The diurnal component of the lunar gravitational potential is given by Bartels<sup>2</sup> as

$$\Omega = \lambda \sin \theta \cos \theta e^{it} \quad (1)$$

where the physical quantity corresponds to the imaginary part of the expression; and  $l = 2\pi(\tau - 2S) + \varphi$ ,  $\theta$  is the colatitude,  $\varphi$  is the longitude,  $\tau$  is the time in sidereal days,  $S = \tau/\text{lunar month}$ , and  $\lambda = -19,706 \text{ cm}^2/\text{sec}^2$ . There is in addition to  $\Omega$  another lunar diurnal component the period of which is one sidereal day. The closeness of the sidereal and solar days would make the separation of this lunar component in the data very difficult.

The approximate analysis of the response of the atmosphere to the diurnal component of lunar gravitational potential is simple, and shows why the response has not yet been detected. It also suggests how one may now proceed to find it. The analysis for a thermal excitation with the same latitude dependence as (1) has been given by Lindzen<sup>3</sup>. If we ignore the difference between  $\tau$  and  $\tau - 2S$ , as it affects the solution of Laplace's tidal equa-

tion, then the extension of the analysis to gravitational excitation is immediate and yields

$$u = -\frac{i\lambda}{a\omega} e^{it} \quad (2)$$

$$v = \frac{\lambda}{a\omega} \cos \theta e^{it} \quad (3)$$

$$\delta p = \delta \rho = \delta T = \omega = 0 \quad (4)$$

where  $u$  is the northerly velocity oscillation,  $v$  is the westerly velocity oscillation,  $a$  is the radius of the Earth,  $\omega$  is the rotation rate of the Earth,  $\delta p$  is the pressure oscillation,  $\delta \rho$  is the density oscillation,  $w$  is the upward velocity oscillation, and  $\delta T$  is the temperature oscillation.

An estimate of the geomagnetic oscillation resulting from  $u$  and  $v$ , as given by (2) and (3), may be obtained from the following approximate equation derived by Baker and Martyn<sup>4</sup>

$$\frac{\partial^2 R}{\partial \theta^2} + \cot \theta \frac{\partial R}{\partial \theta} - \frac{1}{\sin^2 \theta} + \frac{\partial^2 R}{\partial \varphi^2} = \frac{aK_3}{\sin \theta} \left( \frac{\partial}{\partial \varphi} (u H_z \sin \theta) + \frac{\partial}{\partial \varphi} (v H_z) \right) \quad (5)$$

where  $R$  is the electric current function,  $K_3$  is some time and space averaged Cowling conductivity, and  $H_z$  is the vertical component of the Earth's magnetic field.

From Chapman and Bartels<sup>5</sup>

$$H_z \cong C \{ \cos \theta + \tan \theta_0 \sin \theta \cos (\varphi - \varphi_0) \} \quad (6)$$

where  $C \cong -0.6$  gauss,  $\theta_0$  is the colatitude of "equivalent" central magnetic dipole's N-pole  $\cong 11^\circ$ ,  $\varphi_0$  is the longitude of "equivalent" central magnetic dipole's N-pole  $\cong 70^\circ$  W. Substituting equations (2), (3) and (6) into equation (5) and solving for  $R$  one gets

$$R = -\frac{iK_3 C \lambda}{2\omega} \{ \sin \theta e^{it} - \tan \theta_0 \cos \theta e^{i(l - \varphi + \varphi_0)} \} \quad (7)$$

The current intensity is given by

$$U = \frac{1}{a \sin \theta} \frac{\partial R}{\partial \varphi} = \frac{K_3 \lambda C}{2a\omega} e^{it} \quad (8)$$

$$V = -\frac{1}{a} \frac{\partial R}{\partial \theta} = -\frac{iC \lambda K_3}{2a\omega} \{ \cos \theta e^{it} + \sin \theta \tan \theta_0 e^{i(l - \varphi + \varphi_0)} \} \quad (9)$$

where  $U$  is the southward (northerly) intensity, and  $V$  is the eastward (westerly) intensity. The associated variation in magnetic potential is given by

$$\Phi = -\frac{8}{3} \pi R \quad (10)$$

where differences between the distance from the Earth's centre to the dynamo layer and the radius of the Earth have been ignored. From  $\Phi$ , the magnetic field variations are obtained as follows

$$Y = -\frac{1}{a \sin \theta} \frac{\partial \Phi}{\partial \varphi} \cong \frac{8}{3} \pi U \quad (11)$$

$$X = \frac{1}{a} \frac{\partial \Phi}{\partial \theta} \cong -\frac{8}{3} \pi V \quad (12)$$

Due to the obliquity of the Earth's magnetic field there is, in addition to the component of  $R$  following the Moon, another component of  $R$  which is stationary. The same is, of course, true for the fields derived from  $R$ . Schematic representations of the velocity, current and magnetic fields are shown in Fig. 1. From Fig. 1 we see that the lunar diurnal tide may well be the simplest global dynamic system.

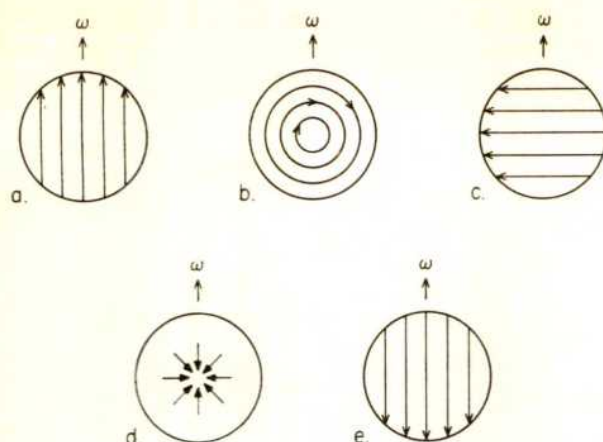


Fig. 1. Schematic representations of various fields for the hemisphere facing the Moon. *a*, Horizontal flow. *b*, Dynamo current (component following the Moon). *c*, Dynamo current (stationary component). *d*, Magnetic field (component following the Moon). *e*, Magnetic field (stationary component). The representations of the stationary components are for  $|\varphi - \varphi_0| < 90^\circ$ , otherwise their directions are reversed.

The observational verification of such a simple system would be gratifying. The failure to find the oscillation in surface pressure data is consistent with equation (4). The analogous result for the lunar diurnal ocean surface height oscillation, obtained by Laplace, is described by Lamb<sup>6</sup>. Whether the oscillation may be found in other fields depends on their magnitudes. Taking

$$a \cong 6.5 \times 10^8 \text{ cm},$$

$$K_3 \cong 0.8 \times 10^{-7} \text{ E.M.U.-cm},$$

$$\text{and } \omega = 2\pi/0.864 \times 10^5 \text{ sec},$$

gives

$$\left| \frac{\lambda}{a\omega} \right| \cong 0.64 \text{ cm/sec} \quad (13)$$

$$\left| \frac{1}{2} \frac{K_3 \lambda C}{a\omega} \right| \cong 1.535 \times 10^{-7} \text{ amp/cm} \quad (14)$$

and

$$\left| \frac{4}{3} \pi \frac{K_3 \lambda C}{a\omega} \right| \cong 0.1285 \gamma \quad (15)$$

From equations (2), (3) and (13) we see that there should be a wind oscillation with an amplitude of 0.64 cm/sec all over the globe. The amplitude should, moreover, be independent of altitude. From Chapman<sup>7</sup> we see that 16 years of data may be sufficient to detect an oscillation with a 1.2 cm/sec amplitude. The lunar diurnal wind oscillation might therefore be found in 64 years of data. Records of this length are available for a number of stations. From equations (11), (12) and (15) we see that both the stationary and travelling components of the magnetic field oscillation are of 0.13 $\gamma$  amplitude or less. The detection of such an oscillation might be barely possible with long data series such as are available for Greenwich Observatory. The detection is, unfortunately, rendered much more difficult by the presence of a larger oscillation with exactly the same period: the luni-solar oscillation due to the interaction of solar daily variations in electrical conductivity and the lunar semidiurnal wind oscillation. The lunar diurnal and the luni-solar oscillations can only be distinguished through their different latitude distributions, and the data necessary to do this are unlikely to be available in the foreseeable future.

In conclusion, the present theory of the lunar diurnal atmospheric tide suggests that, with available data, it will be possible to detect the lunar diurnal tide only in

wind data. Such data is now being processed for this purpose at this laboratory.

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## Pyroclastic Origin of Supposed Microfossils in the Roraima Formation, Guyana

BAILEY has described some microscopic objects of apparent organic origin in material which he considered to be derived from the Roraima Formation in Guyana<sup>1</sup>. The following is an extract from his communication.

"A collection of specimens from stream pebbles and boulders at a locality on the Kako River included some which contain microscopic objects of apparent organic origin. On revisiting the locality on a later occasion an unsuccessful search for the source was made; nevertheless there is little doubt that the material was derived from the Roraima Formation. The fossil-like objects occur in chert and jasper beds. They appear to be the tests of unicellular micro-organisms and sometimes occur abundantly in very narrow bands. Examples from specimen H 265 are shown in Figs. 1 and 2. In Fig. 1 the larger of the objects, which are spherical with a suggestion of tetrahedral symmetry, has a diameter of 0.4 mm with a wall thickness around 0.008 mm. Fig. 2 contains two objects. One is a globular mass about 0.16 mm in diameter with three radiating spine-like projections, one having a length of 0.18 mm. The other object is two-chambered, each chamber being around 0.18 mm diameter with walls about 0.012 mm in thickness. In other sections a variety of forms ranging in diameter from 0.6 mm to 0.1 mm may be seen, some reminiscent of Foraminifera and Radiolaria, but none of which can be identified with any certainty. There can be little doubt, however, of their organic origin."

Because age determinations have shown<sup>2</sup> the Roraima Formation to be not younger than Lower Proterozoic, the presence of organic remains within the formation would be of considerable interest as an occurrence of life in the Pre-Cambrian. Similar microscopic objects have been figured (unpublished work, J. H.

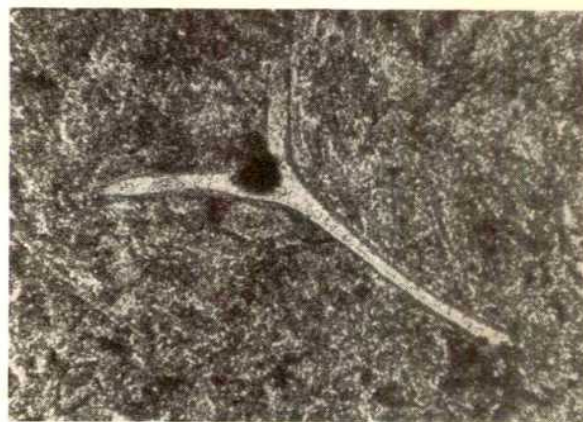


Fig. 1. Photomicrograph of Y-shaped fragment of disrupted volcanic glass in a pebble from the Kako River, Guyana. Collection by P. B. H. Bailey, Geological Survey of Guyana (specimen H 162). (Ordinary light  $\times 172$ .)



Bisschops) subsequently from a tuff in the Roraima Formation of Surinam and "suggest relic structures of possible organic origin, although not one could be classified to date".

The present communication is not intended to comment on any aspect of the Roraima Formation but to correct the impression that the microscopic objects figured by Bailey are of organic origin. Bailey's photomicrographs were taken by me, and I have examined a number of thin sections of the "type-material", including specimen *H* 265. In my view, all the features observed under the microscope are consistent with a pyroclastic origin for these small bodies, which display the characteristic morphology of globular areas of vesiculated volcanic glass in varying stages of disruption. Conditions prevailing at the time of consolidation of the rock, as shown by the absence of "welding" effects, have favoured the preservation of occasional undisrupted bubbles which are infilled with granular quartz, epidote and fine-grained matrix material. The following petrographic observations from a classic paper<sup>3</sup> on ash-flow tuffs are equally applicable to the Guyana material.

"Tuff fragments. One widely occurring type is derived from a vesiculated glass characterized by roughly globular bubbles . . . . This material when explosively disrupted produces curved plates . . . that represent fragments of the walls of these bubbles; cusp- and lune-shaped fragments . . . ; and different forms representing the interstitial glass between several bubbles. These may be tricuspidate fragments bounded by arcs of circles, Y-shaped fragments formed in the same way . . . , and which seem to be one of the most common characteristics of glassy tuff materials. More rarely, forms which represent cross sections of undisrupted spherical bubbles have been observed . . . . The original plastic glass contained bubbles of different size, shape, and thickness of walls, and so there are many variations to the forms mentioned."

Photomicrographs from that paper and, even more convincingly, the plates accompanying an account<sup>4</sup> of a pyroclastic formation of Tertiary age from Oregon, can be matched with the features shown by the microscopic bodies in the Guyana material. A Y-shaped fragment from one of the Kako River specimens is shown in Fig. 1 and is identical in appearance with the Y-shaped shards of volcanic glass that are characteristic of vitric tuffs formed throughout geological time. There is little doubt that similar Y-shaped fragments would result from the breaking away of the three spine like projections from the globular mass in the lower part of Bailey's Fig. 2. A well formed bubble which appears to have originated through the distension of a hollow cylindrical glass body from the same specimen is shown in Fig. 2.

As far as I am aware, no micropalaeontologist has been able to identify the "supposed microfossils" and it

is unfortunate that the material was not examined in the first instance by a petrologist with appropriate experience in the petrography of pyroclastics. The morphology and size of these microscopic bodies are so characteristic of vesiculated volcanic glass that a pyroclastic origin seems to be inescapable.

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### Optical Radar Detection of Backscattering from the Upper Atmosphere

A PREVIOUS communication<sup>1</sup> reported preliminary observations of atmospheric backscattering obtained with an optical radar system at the University of Maryland. The purpose of this communication is to report some of our recent results which indicate enhanced scattering near 80 km, to describe some improvements made in our equipment which have eliminated the high background noise that was evident in our earlier data and to comment on the remarks of Bain and Sandford<sup>2</sup> pertaining to these data.

The system now used is similar to that described previously but includes a rotating disk in front of the photomultiplier tube. The new shutter is synchronized with our original transmitting shutter and protects the photomultiplier during the actual firing of the laser. This protection has completely eliminated the enhanced photomultiplier noise which was caused by the high levels of light present when the laser is fired. Our noise level is now constant in time and equal in magnitude to the expected dark current plus night sky illumination.

Results have been obtained with our improved equipment during three periods since our first results were reported. We have frequently, although not always, seen enhanced scattering from high altitudes, but none as large as that we reported previously has been observed. We agree with Bain and Sandford<sup>2</sup> that the returns shown in our early data were much higher than would be expected and we now conclude that the apparent enhancement should probably be ascribed to the effects of photomultiplier overloading.

Bain and Sandford<sup>2</sup> also suggested that there might be ruby fluorescence in our early data. As we described previously<sup>1</sup>, we use the same optics for transmitting and receiving. The laser pulse is fired through an aperture in a rotating disk which is located in the focal plane of the telescope. The disk is equivalent to the shutter used by Bain and Sandford in their system. Extensive tests on our equipment, which included the use of a third shutter in front of the ruby and the firing of fluorescence only through the system, have shown that there is no fluorescence in our results. Because the original transmit-receive disk acts as the fluorescence block, the third shutter was not used when making our observations.

Fig. 1 is a profile, obtained with our improved system, showing the average return of four consecutive nights of operation. The five lower altitude points are for 50, 80, 150, 220 and 270 laser pulses respectively and the rest for 290. The variation in the number of shots for the first five points is due to the fact that our cut-on altitude depends on the initial setting of the transmit-receive shutter with respect to the laser pulse. This setting can be arbitrarily varied  $\pm 50$   $\mu$ sec when setting up for a night's observations.

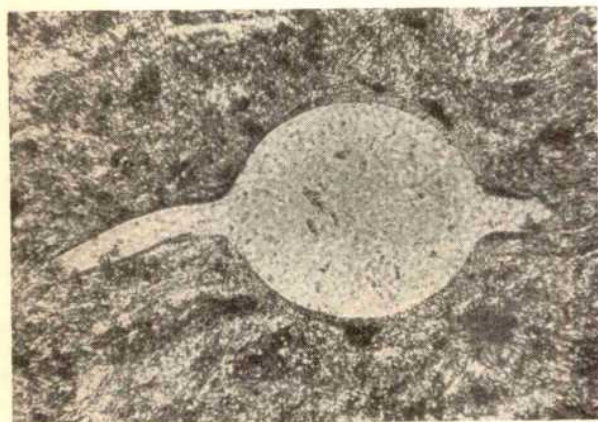


Fig. 2. Photomicrograph of a well formed bubble (specimen *H* 162). (Ordinary light  $\times 172$ .)



Energy output is measured for each pulse. The average energy was about 2 Joules/pulse for the four nights reported here. The temperature of the water that cools the ruby is controlled so that there is no danger of the output wavelength (6943 Å) shifting into nearby atmospheric water vapour absorption lines.

The absolute efficiency of the system was determined by observations of  $\alpha$  UMI (Polaris) and resulted in a value of about  $8 \times 10^{-4}$  which is much lower than expected. The cause was traced to a low quantum efficiency for the EMI 9558 photomultiplier tube used in the experiment by measuring the cathode response at 6943 Å. It is possible that this same problem may be a contributing factor to the large (absolute) discrepancy between the optical radar returns observed by some other groups and the molecular curve that is predicted by the 1962 US Standard Atmosphere for altitudes below 60 km. As can be seen in Fig. 1, the profile based on our measured efficiency lies very close to the expected Rayleigh scattering curve from 45 to 65 km. The discrepancy can easily be explained by the uncertainty in atmospheric transmission and by taking the normal seasonal variation of atmospheric density into account. No attempt has been made in Fig. 1 to fit our data to the predicted molecular curve. An average (one-way) atmospheric transmission of 0.7 (for  $\lambda = 6943$  Å) was assumed when reducing raw data.

The uncertainties for the measured values shown in Fig. 1 were calculated by taking the square root of an accumulated observed pulse count for each altitude interval, and they represent the standard deviation for a Poisson distribution. Our altitude resolution, determined by the on-line computer sample rate, is 2.5 km. The last three points have been averaged over 5 km layers.

The observed value near 80 km may be interpreted as follows. If we consider our total "noise" level to be the sum of system noise and the expected molecular return for this altitude, we would expect 4.8 photoelectron counts. We observed eleven photoelectron counts. Using Poisson statistics, we may therefore conclude that there is only a 1.1 per cent chance that the observed value is the result of statistical fluctuations. If we interpret the observed

value as a real enhancement (99 per cent confidence level) then, when the profile is adjusted for a best fit over the altitude range 45–55 km, our data give a differential back-scattering coefficient of about  $8 \times 10^{-13}$  cm<sup>-1</sup>ster<sup>-1</sup>. Although this conflicts with the observations of Bain and Sandford<sup>3</sup> and Clemesha *et al.*<sup>4</sup>, it is in good agreement with Mikirov<sup>5</sup> and with estimates made by Poultney<sup>6</sup>. The enhanced echoes were not always observed. During our most recent period of access to the 20 in. telescope (January 20 to February 7, 1967), for example, they were present on February 4, 5, 6 and 7 but not on January 22, 28 or 29. The actual source of these echoes has been ascribed to *in situ* aerosols<sup>6</sup>. The alternative possibility that they are "ghost" returns caused by multiple scattering in lower atmospheric aerosols, however, should not be overlooked<sup>7,8</sup>. Variations in the molecular concentration near the mesopause from that of the 1962 US Standard Atmosphere must also be considered.

No average enhancement was observed above 85 km with any statistical certainty and our equipment is not sensitive enough at present to detect molecular scattering at these altitudes. One out of the eight computer print-outs reported in Fig. 1 shows a return from the altitude range 120–130 km. The return from this region was observed on February 7, 1967, at 0700 UT in a print-out based on fifty laser pulses and yielded a differential back-scattering coefficient of about  $5 \times 10^{-12}$  cm<sup>-1</sup>ster<sup>-1</sup>. An attempt to explain returns from this altitude region has been made on the basis of meteoritic fragmentation<sup>9</sup>.

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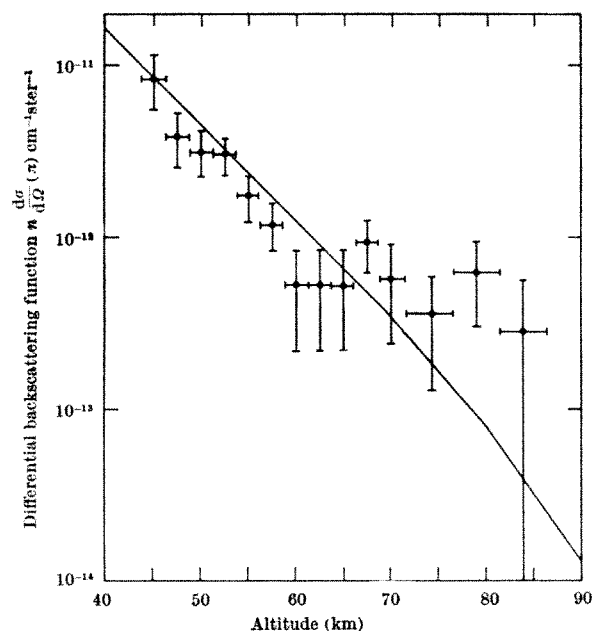


Fig. 1. Optical radar returns for the nights of February 4, 5, 6, and 7, 1967. All data were obtained between 0600 h UT and 0900 h UT. — observed  $n \frac{d\sigma}{d\Omega}$  (π) with standard deviation; — calculated from the 1962 US Standard Atmosphere.

## PHYSICS

### Supposed Anomalies in the Thermal Properties of Water and Aqueous Solutions

Falk and Kell<sup>1</sup> have recently examined some claims which have been made for the existence of "higher order phase transitions", "kinks", and "discontinuities" in the properties of water and aqueous solutions between 0° and 80° C and concluded that in every case the size of the reported anomaly was comparable with the degree of accuracy of the experiments. We have now examined a few additional physical properties for which the reported discontinuities appear at first sight to lie outside the errors of measurement, and obtained new experimental evidence on one of these—the ultra-violet absorption of aqueous solutions of iodide ions.



Briefly, we find that the claims of abnormalities in the surface tension<sup>2-5</sup> and viscosity<sup>6</sup> of water are disproved by the majority of accurate data<sup>7-15</sup>. A recent claim<sup>2-3</sup> that the activity coefficient of sodium chloride in water has a jump between 40° and 60° C is based on measurements<sup>16</sup> that have been superseded by ones that show no anomalies<sup>17-18</sup>. A claim<sup>2-3</sup> that there is a distinct discontinuity in the partial molar volume of potassium iodide in water at 15° C appears to have arisen from a mis-plotting of the results<sup>19</sup> for 12.5° and 15° C.

The most recent claim of evidence for thermal discontinuities in aqueous solutions is that of Blandamer, Fox and Symons<sup>20</sup> concerning shifts of the ultra-violet absorption of iodide ions. These authors found an irregularity at 35°–45° C in the temperature dependence of the position of the 226.3 mμ band, and observed that the irregularity became more pronounced when *t*-butyl alcohol (mole fraction *x*) was added to the system. The excitation energy  $E_{\max}$  decreased evenly with increasing temperature except in a narrow range 40°–45° C, where it was abnormally insensitive to the temperature. The effect is demonstrated by the crosses in Fig. 1, which are taken from the paper of Blandamer, Fox and Symons<sup>20</sup>. A consequence of the anomaly is that the trend of the derivative  $(\partial E_{\max}/\partial x)_T$  as a function of temperature reverses in the region 35°–45° C (ref. 20, Fig. 2).

The effect is so remarkable that we have repeated the measurements, taking careful precautions to be sure of the temperatures of the solutions and the wavelengths of the spectra. The conclusions of Blandamer, Fox and Symons rest on deviations in  $E_{\max}$  of about 0.15 kcal/mole, which corresponds to a shift of the absorption peak by 0.25 mμ. It is usually hard to achieve that accuracy, but the sharpness and high symmetry of the iodide band make it possible. We used solutions of  $1.1 \times 10^{-4}$  molar potassium iodide in 1 cm cells held in a specially constructed cell holder the temperature of which was controlled by a Colara circulating thermostat and which fitted a Beckman DK 2

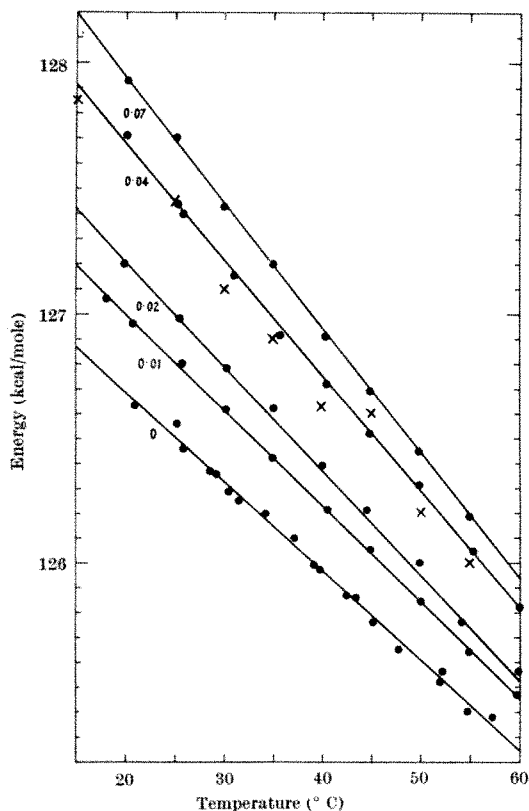


Fig. 1. Transition energy for iodide ions in mixtures of water and *t*-butyl alcohol. The numbers on the curves indicate the mole fractions *x* of *t*-butyl alcohol. The dots are our data and the crosses are those of Blandamer, Fox and Symons<sup>20</sup> for *x* = 0.07.

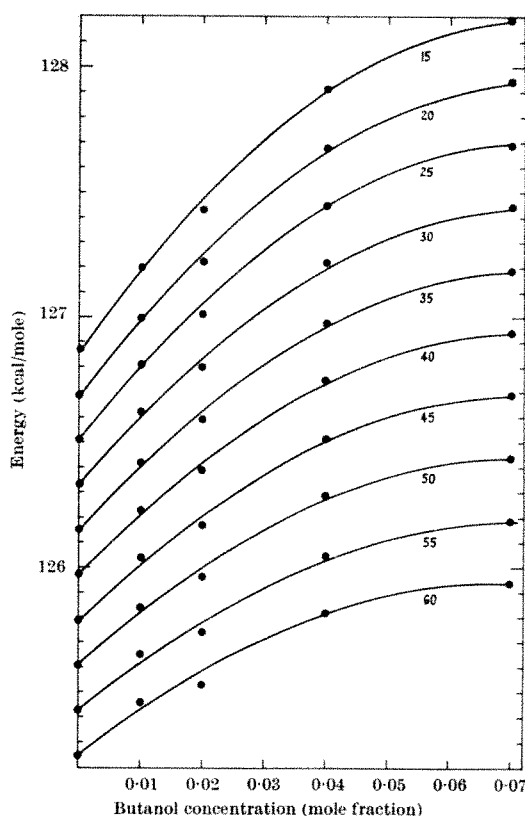


Fig. 2. Transition energy for iodide ions in mixtures of water and *t*-butyl alcohol. The numbers on the curves indicate the temperatures in °C.

spectrophotometer. The temperatures were measured by placing a thermometer directly in the reference cell and removing it only when a record was being made. The temperatures were found to be steady to within 0.1° C during a measurement. To ensure that thermal equilibrium had been established, the records were made 20 min after the temperature in the reference cell had become steady. The wavelength scale was calibrated for every spectrum by immediately afterwards recording the 228.80 mμ emission line of a cadmium lamp (Philips 93107) on the same chart. We found that if the lamp was switched on less than a minute before its emission was recorded, a narrow line was obtained; later the line broadened and became unsuitable for accurate calibration. We consider that our results are accurate to  $\pm 0.1$  mμ, or  $\pm 0.05$  kcal/mole in  $E_{\max}$ , and these limits are considerably less than the magnitudes of the anomalies observed by Blandamer, Fox and Symons. Figs. 1 and 2 show that we found no anomaly of the kind they have reported.

Our work supports the view that there is no good evidence that water has anomalies other than the very shallow minima in its volume at 4° C and in its specific heat at 33° C, which are not discontinuities in the normal sense.

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## THE SOLID STATE

### Crystal Structure of Cubic Deuterium Chloride

In an earlier communication<sup>1</sup> we stated that the X-ray powder diffraction pattern of solid deuterium chloride recorded at 118.5° K and the corresponding neutron powder diffraction pattern recorded at 111.5° K showed the same set of lines, confirming that the face centred cubic unit cell derived from the X-ray diffraction pattern was the true unit cell of the structure. Further analysis of the two diffraction patterns led to the conclusion that the observed face centred cubic structure was the statistical average of a disordered structure. The neutron diffraction pattern available at that time, however, did not provide enough information to determine the exact nature of the disorder.

On continuing this investigation, the angular range of the neutron diffraction pattern was extended to  $\theta = 35^\circ$ —the practical limit of the diffractometer—the scanning time of the diffraction pattern was increased to one week and each diffraction maximum was scanned with at least two boron trifluoride ( $\text{BF}_3$ ) counters in succession, using a bank of five counters mounted at  $10^\circ$  intervals. With these improvements it became possible to derive the integrated intensities of ten diffraction maxima with sufficient accuracy for solving the structure.

Fig. 1 shows the absolute values of the structure factors corresponding to the ten observed diffraction maxima plotted as a function of the diffraction angle  $\theta$ . It can be seen that  $|F|_{\text{obs}}$  usually decreases with increasing  $\theta$ , but this trend is reversed in two cases:  $|F(222)| > |F(113)|$  and  $|F(133)| > |F(004)|$ . These reversals are well reproducible characteristic features of the observed neutron diffraction pattern.

Of the various models tested there was only one which reproduced all details of the observed neutron diffraction pattern. In this model the chlorine atoms form an ordered face centred cubic lattice, but the orientation of the

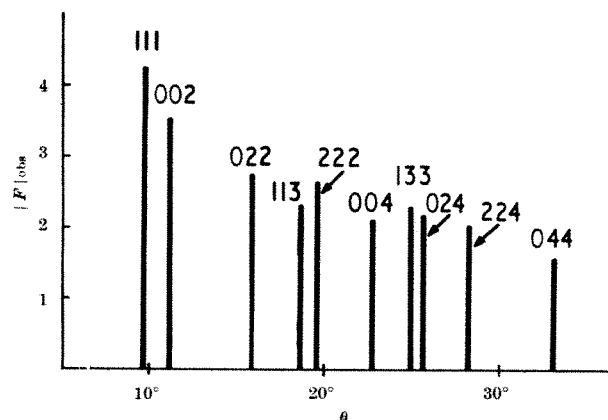


Fig. 1. DCl,  $T = 111.5^\circ \text{K}$ ;  $\lambda = 1.059 \text{\AA}$ .

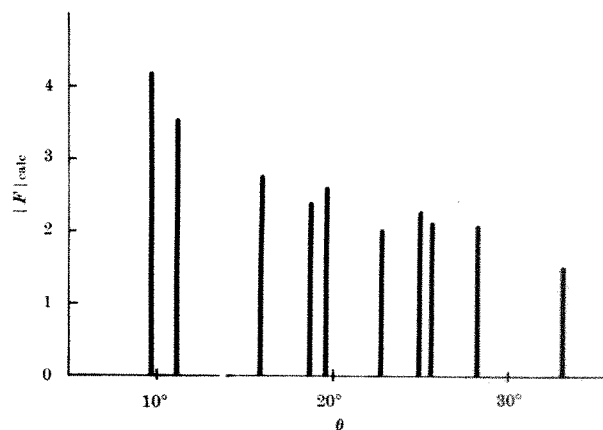


Fig. 2. Twelve-fold disordered model.

deuterium chloride molecules is disordered in the following way. The deuterium atom of each molecule has twelve equally probable equilibrium positions, situated along the lines connecting the chlorine atom of the molecule with its twelve nearest neighbours. This "twelve-fold disordered" model was refined by the least-squares method. The results of the refinement are summarized in Table 1 and the absolute values of the structure factors calculated from this model are shown in Fig. 2. The agreement of this diagram with the one in Fig. 1 is well within the experimental error.

Table 1. STRUCTURAL DATA OF DEUTERIUM CHLORIDE AT  $111.5^\circ \text{K}$

Edge length of the cubic unit cell: $a = 5.47 \pm 0.01 \text{\AA}$ .			
Space group: $Fm\bar{3}m$ ; number of molecules in the unit cell: 4.			
Fractional positional parameters of the atoms:			
	$x$	$y$	$z$
Chlorine (in 4-fold position):	0	0	0
Deuterium (1/12 atom in 48-fold position):	$0.151 \pm 0.005$	$0.151 \pm 0.005$	0
Anisotropic temperature factors of the atoms ( $B_{ik}$ in $\text{\AA}^2$ ):			
Chlorine	$B_{11} = B_{22} = B_{33} = 3.5 \pm 0.3$	$B_{12} = B_{13} = B_{23} = 0$ .	
Deuterium	$B_{11} = B_{22} = 7.5 \pm 1.5$	$B_{33} = 15.7 \pm 4.8$	
	$B_{13} = -3.6 \pm 1.8$	$B_{12} = B_{23} = 0$ .	

Final  $R$  factor after six cycles of anisotropic refinement:  $R_1 = 1.9$  per cent.

D-Cl bond length calculated from the positional parameters of the atoms:  $1.17 \pm 0.04 \text{\AA}$ .

This value is considerably less than the one reported previously<sup>1</sup> for orthorhombic DCl. This bond shortening, however, is only apparent; it is a consequence of the large molecular librations in the cubic phase.

The proposed "twelve-fold disordered" model is compatible with the results of the specific heat, infra-red and Raman spectra, dielectric constant and nuclear magnetic resonance investigations referred to in the previous communication<sup>1</sup>. It is also compatible with the results of the latest infra-red investigations<sup>2</sup>. All these investigations indicate that the cubic phase of solid deuterium chloride is disordered but none of them gives a clue to the exact nature of the disorder. Nevertheless, there is a consensus of opinion in favour of an orientational disorder which is different from the entirely random orientation of the molecules, such as one would expect if the molecules were freely rotating.

It had also been suggested<sup>3</sup>, however, that the molecules may rotate freely in the cubic phase of solid hydrogen chloride and the idea of entirely random molecular orientations for both hydrogen and deuterium chlorides has recently been revived in a modified form<sup>4</sup> which does not involve free molecular rotation. The model of randomly oriented molecules has therefore been investigated in detail. In this model the deuterium atoms were distributed over spherical shells about the chlorine atoms with the radius and the thickness of the shell as variable parameters.

It was found that this model could only be refined to a final  $R_1$  value of 4.1 per cent, thus giving a significantly

worse overall agreement with the observed neutron diffraction pattern than the "twelve-fold disordered" model. Furthermore, the model of randomly oriented molecules failed to reproduce the two observed reversals in the graph of  $|F|$  against  $\theta$ . This is shown in Fig. 3 where the vertical bars represent  $|F|_{\text{calc}}$  values giving the best overall agreement with the observed neutron diffraction pattern for the model of randomly oriented molecules. The monotonous decrease of  $|F|_{\text{calc}}$  with increasing  $\theta$  is a basic feature of this model and is independent of the values of the variable parameters.

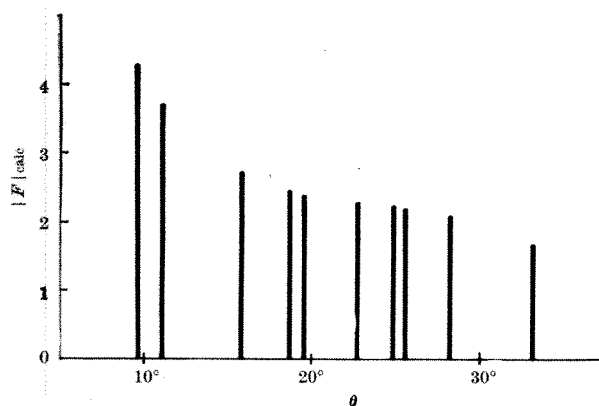


Fig. 3. Randomly oriented model.

Disordered models with a smaller number of molecular orientations than the "twelve-fold disordered" model have also been investigated, in particular a "six-fold disordered" model in which the deuterium atoms lie along the crystallographic axes. This model led to even more unacceptable structure factors than the model of randomly oriented molecules.

Although the proposed "twelve-fold disordered" model differs significantly from the model of randomly oriented molecules, such as would result from free molecular rotation, it still allows considerable freedom in molecular re-orientation. The existence of twelve equilibrium orientations for each molecule combined with the large temperature factors of the deuterium atoms suggest that the deuterium chloride molecules carry out librations of large angular amplitudes about their equilibrium orientations accompanied by random "flipping-over" from one equilibrium orientation to another. Each "flipping over" represents a restricted re-orientation of the molecule in which a hydrogen bond to a nearest neighbour chlorine atom is broken, followed by the formation of a new hydrogen bond with another nearest neighbour chlorine atom.

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## X-ray and Neutron Diffraction Studies of a Tetragonal Hydrogen Bronze $\text{H}_{(0.25)}\text{WO}_3$

THE unstable blue compounds obtained by the reduction of tungstic oxide with active hydrogen were classified as "bronzes" by Glemser<sup>1,2</sup> and shown by X-ray powder photography to exhibit structural variations with composition similar to those observed for the alkali-metal tungsten bronzes. In this note we report a new tetragonal hydrogen bronze phase with the same crystal structure as  $\text{Na}_{(0.1)}\text{WO}_3$  (ref. 3), and with  $0.21 < x < 0.25$ .

$\text{H}_{(0.23 \pm 0.02)}\text{WO}_3$  was prepared by passing suspensions of tungstic oxide (B.D.H., reagent grade) in 2 normal hydrochloric acid through a specially constructed Jones reductor. Both the strength of the acid and the length of the column were used to control the composition of the product, which was determined by quantitative oxidation with standard potassium dichromate solution. The deuterium analogue was prepared by an identical procedure carried out in the complete absence of air and water vapour. The products were precipitated in a centrifuge and washed repeatedly with water (or heavy water), pumped dry and transferred to evacuated sealed tubes.

A Philips P.W. 1010 generator, P.W. 1050 goniometer and P.W. 4231 counter were used to obtain accurate angles and intensities over the range  $2\theta = 0-90^\circ$  ( $\text{CuK}\alpha$ ) by step scanning at intervals of  $0.05^\circ$  and counting for 10 sec. Samples milled with silicone grease (under nitrogen) and embedded in aluminium trays maintained constant composition for 8 h. In addition a large number of powder photographs of samples, sealed under vacuum in 'Pyrex' tubes were obtained using a Debye-Scherrer camera. Neutron diffraction experiments were carried out on an AERE (Harwell) neutron diffractometer at liquid helium temperatures on samples sealed in aluminium tubes.

The X-ray diffraction data at room temperature showed that the compounds have the tetragonal lattice symmetry  $P4/nmm-D_{2h}^{19}$  (the only systematic absences are  $hk0$  with  $h+k$  odd). The lattice constants of the unit cell as obtained from powder photographs are

$$a_0 = 5.2285 \pm 0.0005 \\ c_0 = 3.881 \pm 0.001$$

with a cell content of two formula units of  $\text{H}_{(0.23)}\text{WO}_3$ . The structure factor calculations were based on the following atomic parameters

	B(A) <sup>2</sup>	
2W in 2(c)	0.70	$0\frac{1}{2}z, \frac{1}{2}0z$
20 in 2(c)	0.20	$0\frac{1}{2}(z + \frac{1}{2}), 0\frac{1}{2}(z - \frac{1}{2})$
40 in 4(e)	0.20	$\frac{1}{4}\frac{1}{4}z, \frac{3}{4}\frac{1}{4}z, \frac{1}{4}\frac{3}{4}z, \frac{3}{4}\frac{1}{4}z$

The coefficient for tungsten in the isotropic temperature factor  $B(A)^2$  was derived from intensity data obtained for a cubic hydrogen bronze  $\text{H}_{(0.48)}\text{WO}_3$ , a reasonable value for oxygen being assumed. A value for  $z$  was determined from the reflexions with  $h+k$  odd, which are most sensitive to  $z$  and approach zero intensity as  $z$  approaches  $\frac{1}{2}$ . Calculated and observed intensities are given in the table.

The positions of the hydrogen atoms in the lattice were determined from the neutron diffraction data by a comparison of the relative intensities of a hydrogen and deuterium bronze of the same composition ( $\text{H}_{(0.25 \pm 0.01)}\text{WO}_3$ ). Although line broadening prohibited a complete interpretation of the absolute intensities, it was shown that the light atoms occupy the interstitial (special) positions in the distorted perovskite lattice with the atomic parameters

$$0.5 \text{ H in } 2(a)$$

$$000, \frac{1}{2}\frac{1}{2}0$$

Table 1. X-RAY DATA FOR  $H_{(0.23)}WO_3$ 

<i>hkl</i>	$\sin^2\theta_{obs}$	$\sin^2\theta_{calc}$	$I_{obs}$	$I_{calc}$
001	0.03925	0.03945	34	45
110	0.04340	0.04350	100	91
101	0.06100	0.06120	10	11
111	0.08280	0.08295	55	45
200	0.08695	0.08695	20	25
201	0.12640	0.12640	18	23
211	0.14800	0.14815	5.5	6.7
002	0.15775	0.15780	3.0	3.2
220	0.17395	0.17390	16	15
102	0.17960	0.17955	10	11
112	0.20120	0.20115	8	10
221	0.21320	0.21335	14	15
310	0.21750	0.21740	2.0	18
301	0.23505	0.23510	2.0	1.9
202	0.24475	0.24475	3.2	3.3
311	0.25685	0.25685	15	15
212	0.26640	0.26650	13	14
321	0.32205	0.32205	2.1	2.1
222	0.33185	0.33175	3.0	5.8
400	0.34795	0.34785	2.2	4.5
302	0.35340	0.35345	2.1	3.0
312	0.37525	0.37520	7.5	6.4
401	0.38710	0.38730	4.2	5.2

(Only those reflexions suitable for intensity measurement have been included.)

Although no evidence of ordering of the hydrogen atoms was found, systematic discrepancies between observed and calculated structure factors indicate that the oxygen positions may differ slightly from those given here. It is hoped that additional experimental data will enable a more precise assignment of atomic co-ordinates to be made.

The structure of  $H_{(0.23)}WO_3$  is thus identical with that of  $Na_{(0.1)}WO_3$  (ref. 3) with hydrogen atoms occupying the alkali metal positions. The former compound has also been prepared in this laboratory by the bombardment of tungstic oxide at room temperature with gas phase hydrogen atoms produced in a microwave discharge.

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## CHEMISTRY

### Metastability of Rotationally Hot Dihelium at 77° K

WE report here the detection by kinetic absorption spectroscopy of  $He_2$  ( $a^3\Sigma_u^+(\sigma=0)$ ) produced in the gas phase at 77° K with an intense microwave pulse discharge. Although vibrationally cold, at this temperature the molecules exhibit an extremely non-equilibrium distribution in the high rotational states, which show only a minor degree of relaxation during their lifetime of  $10^{-4}$  sec with total helium pressures up to 100 mm. The observations appear to constitute a unique example, as yet, in which the absorption spectrum of a rotationally hot molecule yields relative reaction rates into individual rotational states. It is suggested that the observed rotational distributions of  $He_2$  ( $a^3\Sigma_u^+$ ) may reveal the states of  $He_2^+$  ( $X^2\Sigma_u^+$ ) from which it is formed.

The single pulse microwave generator incorporated an English Electric M 578 magnetron, the pulse was switched from a 1.5  $\mu$ F condenser, with two English Electric CX 1140 thyratrons, and the output power was transmitted to a waveguide section via a "door-knob" and "double-plunger tuner". The power was coupled to the

gas at low  $Q$  (to prevent mismatch following breakdown) by positioning the quartz reaction vessel (length 1 m) inside a standard waveguide section preceded by a right-angle bend. Power is efficiently absorbed by the gas, with this arrangement. The spectroscopic light-pulse was directed through the reaction vessel onto a lens located inside the waveguide, and was extracted via a 1.5 cm hole in the broad face of the bend. For experiments at 77° K, the waveguide was filled with liquid nitrogen. Spectra were recorded with a Hilger Littrow spectrograph, and the helium was purified by cataphoresis.

In these experiments, the magnetron was pulsed at 23 kV for 3.5  $\mu$ sec with an output power of 400 kW, corresponding to a total energy of 1.4 J/pulse. The formation and decay of rotationally hot  $He_2$  ( $a^3\Sigma_u^+$ ) at 77° K are shown in Fig. 1. The total concentration reaches a maximum about 15  $\mu$ sec after termination of the pulse. The distribution shows a sharp minimum for states  $K=5, 7$  and 9, with a maximum population in levels 17 and 19. There is an abrupt cut-off of higher states, with the  $K=21$  population, one half of that in  $K=19$ ; the level  $K=23$  was just detectable. At 77° K, the plates indicate a slow but definite drift with time to lower  $K$ , though the distribution still showed extreme departure from equilibrium at the longest delay time of about 150  $\mu$ sec, at which  $He_2$  ( $a^3\Sigma_u^+$ ) could still be observed.

Similar experiments were conducted at 150° and 295° K. At 295° K, a linear variation of the log of the ratio of the plate density changes to  $2K+1$ , with the energy of the  $K^{th}$  state, was observed at all time delays corresponding to a rotational temperature of  $380 \pm 80^\circ$  K, and a "Beer-Lambert factor" of about unity. At 150° K, the initial rotational distribution was similar to that observed at 77° K, showing a double maximum, with states  $K=1$  to 5 tending to a Boltzmann distribution. The initial distribution is weighted more into the lower  $K$  states, however, with a third in states below  $K=7$ , compared with a fifth at the lower temperature. At 150° K, the rotational distribution shifts fairly rapidly with time, towards an equilibrium distribution. Relative populations at the shortest delay times are listed in Table 1.

Table 1. RELATIVE POPULATION OF ROTATIONAL LEVELS OF  $He_2$  ( $a^3\Sigma_u^+$ ) IMMEDIATELY FOLLOWING THE MICROWAVE PULSE

Temp. °K	$K=1$	3	5	7	9	11	13
295	0.10	0.27	0.33	0.16	0.08	0.04	0.005
150	0.09	0.133	0.07	0.048	0.040	0.081	0.120
77	0.073	0.068	0.039	0.034	0.038	0.087	0.10
Temp. °K	$K=15$	17	19	21	23	25	
295	—	—	—	—	—	—	—
150	0.133	0.120	0.10	0.048	0.024	—	—
77	0.13	0.15	0.15	0.087	0.039	—	—

The derivation of the relaxation times for the individual quantum states will require an analysis of all processes which simultaneously contribute to the formation and decay of  $He_2$  ( $a^3\Sigma_u^+$ ). At 77° K and 35 mm pressure, the relaxation time in the levels 15–19 appears to be approximately 50  $\mu$ sec, corresponding to rotational relaxation once in about  $10^4$  collisions. The theoretical vibrational relaxation time at this temperature and pressure is  $10^{-6}$  sec ( $Z=300$ ) assuming a minimum in the interaction potential with a characteristic temperature of 77° K and an intermolecular force constant corresponding to 0.18 Å. Although the standard theory seems to exaggerate relaxation rates in systems of small reduced mass, the detection of  $He_2$  ( $a^3\Sigma_u^+$ ) only in  $\sigma=0$  cannot exclude the possibility that the dihelium is produced in excited vibrational states. K. Takayanagi, of Colorado University, tells us that he has estimated that there is a slightly greater vibrational relaxation rate at 298° K, and the temperature coefficient for both vibrational and rotational relaxation should be quite small. Rotational relaxation of  $He_2$  ( $a^3\Sigma_u^+$ ) in the high  $K$  states is apparently slower than vibrational relaxation, and the metastability of the rotationally hot molecules must imply that in collision



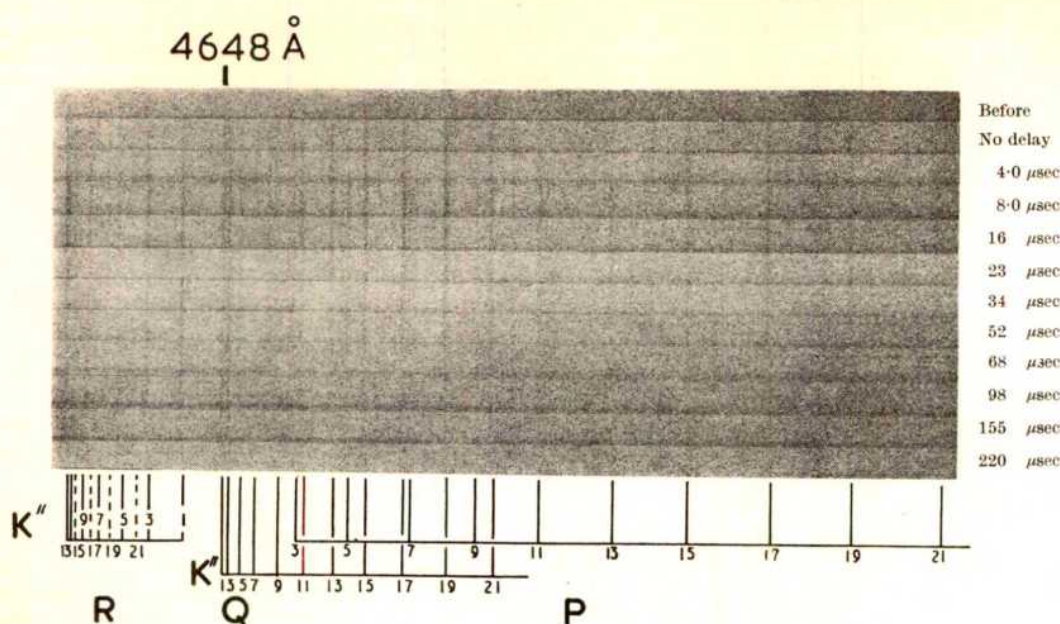


Fig. 1. The formation of  $\text{He}_2$  ( $a^3\Sigma_u^+$ ) at  $77^\circ\text{K}$ . Helium pressure 35 mm.

with atomic helium the interaction equipotential surfaces are very nearly spherical about the centre of the diatomic molecule.

It is now established that the chief reaction causing molecular emission in the early after glow of a helium discharge is collisional combination of molecular ions with electrons<sup>1-4</sup>



Departure from a Boltzmann distribution has been detected in emission at ambient temperature<sup>5,6</sup>. Collisional combination and subsequent cascade radiation should occur without changing substantially either the nuclear separation or the rotational energy. Because rotational relaxation of  $\text{He}_2$  ( $a^3\Sigma_u^+(\sigma=0)$ ) is slow at  $77^\circ\text{K}$ , the observed distribution must correspond closely to that of the molecular ion. Considering such a scheme in further detail, there are several features which are difficult to interpret and will require further study. Following the theory of Stabler<sup>6</sup>, the rotational excitation probably results from multiple non-combinative encounters of  $\text{He}_2^+$  ( $\sigma=0$ ) with electrons during the discharge, the cut-off above  $K=23$  may reflect the instability of the rotationally hot molecular ion in the environment. The principal difficulties concern the slow rate of formation of  $\text{He}_2$  ( $a^3\Sigma_u^+$ ) following the pulse (a similar problem has been discussed in relation to molecular emission following a pulsed discharge<sup>7,8</sup>) and also the invariance of the distribution with time at  $150^\circ\text{K}$ , immediately following the pulse.

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## Decomposition of Nickel Oxide

DURING recent studies of photo-adsorption on high area nickel oxide, decomposition of the oxide was observed at surprisingly low temperatures. Teichner<sup>1</sup> first prepared this nickel oxide by decomposing  $\text{Ni}(\text{OH})_2$  at  $200^\circ\text{C}$  for 2 h at a pressure of less than  $10^{-5}$  mm mercury. It is yellow-green, with a surface area of approximately  $100\text{ m}^2\text{g}^{-1}$  and has a composition which is almost stoichiometric<sup>2</sup>. Both composition and area are little changed by further annealing under the same conditions, but on exposing the oxide to higher pressures of oxygen, it blackens instantly.

If oxygen (at a  $p_{\text{O}_2}$  of 0.06 mm) has been adsorbed to completion on the oxide in the dark at  $120^\circ$  and  $200^\circ\text{C}$ , and the surface is illuminated with water filtered, white light from a Philips 12 V 100 W tungsten lamp, oxygen begins to be rapidly evolved at both temperatures so that after 30 min several times as much oxygen is evolved as has been adsorbed in the dark. The oxide decomposes and metallic nickel is easily detected in the darkened patches of the oxide. Apart from two precautionary comments about thermal decomposition of this oxide at  $400^\circ\text{C}$ <sup>1,2</sup>, and a study by Imoto *et al.*<sup>3</sup> on an unspecified oxide between  $660^\circ$  and  $780^\circ\text{C}$ , no other reports of decomposition of NiO at low temperatures have appeared. Furthermore, the thermodynamic data give  $K_p^{400^\circ\text{C}} = p_{\text{O}_2}^{1/2} = 10^{-14}\text{ atm.}^{1/2}$ , that is, the equilibrium oxygen pressure at this temperature is  $10^{-28}$  mm of mercury.

More controlled experiments without prior dark adsorption showed rapid decomposition (8 per cent in 1 min) of the freshly prepared oxide at  $200^\circ\text{C}$ . After 30 min, 25.65 per cent of the oxide had reacted, although the rate had now slowed to 0.1 per cent decomposition  $\text{min}^{-1}$ . At lower temperatures decomposition was slower, until at room temperature it was much smaller (0.55 per cent in 25 min) but still quite definite. When annealed oxide samples (10 h at  $200^\circ\text{C}$ ) were used, however, the decomposition at each temperature of illumination was reduced to about 10 per cent of that for the freshly prepared samples.

The thermal decomposition of this form of nickel oxide was investigated by raising the temperature slowly in the dark above  $200^\circ\text{C}$  at  $10^{-6}$  mm of mercury. At  $340^\circ\text{C}$  the first oxygen appeared and 2.84 per cent decomposition occurred in 5 min, followed by a further 8.5 per cent in

5 min when the temperature was raised to 370° C. Thus if the decomposition of the illuminated surface is caused by thermal heating of the sample, rather than some truly photoprocess, the hot spots on the illuminated surface must exceed 350° C. A rise of only 2° C was detected, however, on a thermocouple immersed in the sample.

The dark and illuminated decompositions indicate that this oxide has very different thermodynamic properties from low area and bulk NiO. Furthermore, the stability of the fresh oxide differs from that of the annealed oxide, even though these oxides otherwise seem to have similar surfaces and properties.

Several models have been considered to account for these observations. The first model implies that the oxide has a very defective lattice structure with clustered vacancies which might lead to decomposition; but X-ray and electron diffraction studies of the oxide give no encouragement to this model. A second model is based on stored energy for the tiny crystallites may incorporate unrelaxed cubic-to-rhombohedral structures and stacking faults. Attempts to analyse the diffraction line shape for such features were unsuccessful because of the superimposed effect of the particle size. Furthermore, for very similar systems, this type of excess energy has never exceeded 1–3 kcal mole<sup>-1</sup> (ref. 4), whereas a value of at least ten times this amount is required for decomposition of the NiO at 500° K ( $\Delta G^\circ = 10$  kcal mole<sup>-1</sup> compared with  $\Delta G^\circ$  (bulk oxide) = 47 kcal mole<sup>-1</sup>).

The third and most likely model is based simply on the size of the crystallites and the surface arrangements at these low temperatures of formation. For the average crystallite size observed in the oxide, at least 10 per cent of the ions are surface ions, the co-ordination of which depends on the particular packing.

The lattice energy of an ionic crystal is large compared with the enthalpy, and thus the free energy, of formation and small changes in the former can cause large changes in the latter.

Preliminary calculations on model ionic crystallites bounded by (111) and (100) planes respectively suggest that there are variations in lattice binding energy which could account for the observed phenomena. The effect of the annealing would then be to cause simple intracrystalline rearrangements of the surface atoms by increasing their co-ordination and their stability without changing the surface area.

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### Desorption of 3-Phenylpropionaldehyde preceding Polarographic Reduction

In the interpretation of polarographic measurements, it is usually assumed that organic compounds are only reduced when they are adsorbed onto the surface of the mercury electrode<sup>1,2</sup>. Unfortunately, the term "adsorption" is used for a broad range of interactions between the solid and liquid phase.

Two of the simplest electrochemical methods which are assumed to indicate the potential range over which

adsorption occurs, are the change in capacity current on d.c. polarographic curves<sup>3</sup> and the presence of tensametric peaks<sup>4</sup> in a.c. polarography. In many previously reported cases, there have been changes in the double layer, associated with adsorption/desorption effects, at potentials so near to the potential corresponding to a faradaic process that it was difficult to separate the components affecting the a.c. polarographic curves.

3-Phenylpropionaldehyde exhibits good resolution between the adsorption/desorption phenomena and the faradaic reduction process.

The d.c. polarogram comparing the capacity current of the pure buffer and the buffer in the presence of aldehyde (Fig. 1) shows a well defined region between -0.2 V (Fig. 1, potential A) and -1.4 V (potential D) where the capacity current in the presence of the aldehyde is below that of the pure buffer. The reduction wave occurs at a more negative potential (-1.64 V) than the desorption potential (-1.4 V).

The a.c. polarographic curves of the same solutions (Fig. 2) show a decrease of the a.c. current below the value of the pure supporting electrolyte between -0.1 V and -1.42 V (potential D, Fig. 2). The reduction peak (R) at -1.6 V is therefore preceded by a potential region in which the curves of the pure buffer and aldehydic solution overlap. The nature of the indistinct peaks of -0.8 V and -1.2 V is being investigated.

Thus both methods suggest that 3-phenylpropionaldehyde is adsorbed at about -0.1 V and is desorbed at about -1.4 V. The reduction at -1.65 V must therefore occur at a potential at which the compound is usually considered to be desorbed. Such behaviour might be more common than is usually assumed—for example, benzophenone in 60 per cent ethanol also shows no decrease in the a.c. polarographic curve before the reduction peak (Fig. 2b in ref. 5).

3-Phenylpropionaldehyde is therefore a suitable model for the study of adsorption/desorption phenomena.

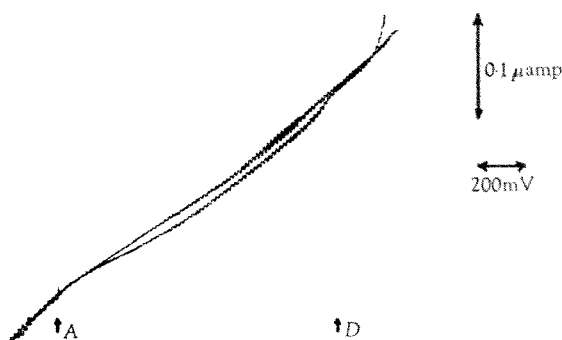


Fig. 1. D.C. polarographic curves of 3-phenylpropionaldehyde. (1) Borate buffer, pH 9.2; (2)  $1.5 \times 10^{-4}$  molar 3-phenylpropionaldehyde in borate buffer, pH 9.2. Curves start at 0.0 V.

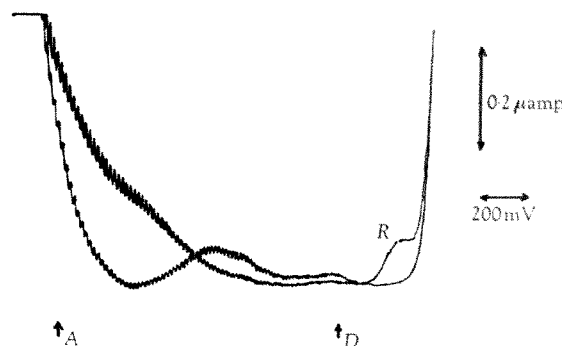


Fig. 2. A.C. polarographic curves of 3-phenylpropionaldehyde. (1) Borate buffer, pH 9.2; (2)  $1.5 \times 10^{-4}$  molar 3-phenylpropionaldehyde in borate buffer, pH 9.2. Curves start at 0.0 V.



because it is a relatively simple molecule and is sufficiently soluble, and particularly because it shows a clear separation of the capacity and faradaic processes.

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## BIOLOGY

### Seasonal Variation of Caesium-137 from Fallout in a Clam, *Rangia cuneata* Gray

In the northern temperate zones, fallout of long lived radionuclides, including caesium-137, varies seasonally, with a maximum during May–June and a minimum during November–December<sup>1</sup>. The concentration of caesium-137 in caribou flesh and in Alaskan Eskimos shows a similar seasonality<sup>2</sup>. Because of its half life of 30 yr and the huge stratospheric reservoir of radioactive debris, the amount of caesium-137 on the surface of the Earth is still increasing, despite the moratorium on atmospheric testing<sup>3</sup>. Although caesium is not a biologically important trace element, chemically it resembles potassium, which is the principal cation of cytoplasm. The bioaccumulation of caesium has therefore aroused considerable interest<sup>4,5</sup>. Whereas the concentration of caesium in marine organisms

is usually about three to thirty times higher than in the surrounding water, the concentration for similar organisms in fresh water may be higher by a factor of 100–1,000 (ref. 5). In laboratory experiments with isopods and snails from brackish water, Bryan<sup>6</sup> showed that the biological concentrations of caesium and potassium were directly related and that the concentration factors of both elements were inversely related to the salinity of the water. In the field study reported here, a bivalve mollusc, *Rangia cuneata* Gray, was collected from six stations in the Trent–Neuse estuary in eastern North Carolina (Fig. 1), and the amount of caesium-137 in the soft tissues was measured periodically for 18 months.

The Neuse River drains some 6,000 square miles of the central Piedmont region and coastal plain of North Carolina and receives radioactive pollution only from worldwide fallout. The Trent, a principal tributary, enters the Neuse at New Bern. *Rangia* is collected on a limited scale for human consumption in some estuaries of North Carolina; it is found fairly abundantly on sandy bottoms throughout the sampling area in salinities ranging from <0.1 to >15 parts per thousand (Fig. 1). *Rangia* were collected at the stations once every 4–6 weeks, the shells were scrubbed and rinsed at the laboratory, and the clams were opened by steaming in a stainless steel bucket on a hotplate. Soft tissues were ashed at 450° C and subjected to gamma-ray spectrometry for analysis of radioactive content<sup>7</sup>. Caesium-137 from fallout and naturally occurring potassium-40 were determined as the sums of the counts comprising their respective photopeaks at 0.662 and 1.46 meV (five 20-keV channels) for a 200-min analysis after correction for background and after spectral stripping of other gamma emitting radionuclides<sup>8</sup>.

The ratio of caesium-137 to potassium-40 in *Rangia* at the four upstream stations exhibited a pronounced seasonal cycle which was not so apparent in clams from Pinecliff or Cedar Point. The salinity of the water, which was measured coincidentally with the collection of each sample, also varied seasonally, and reflected an inverse

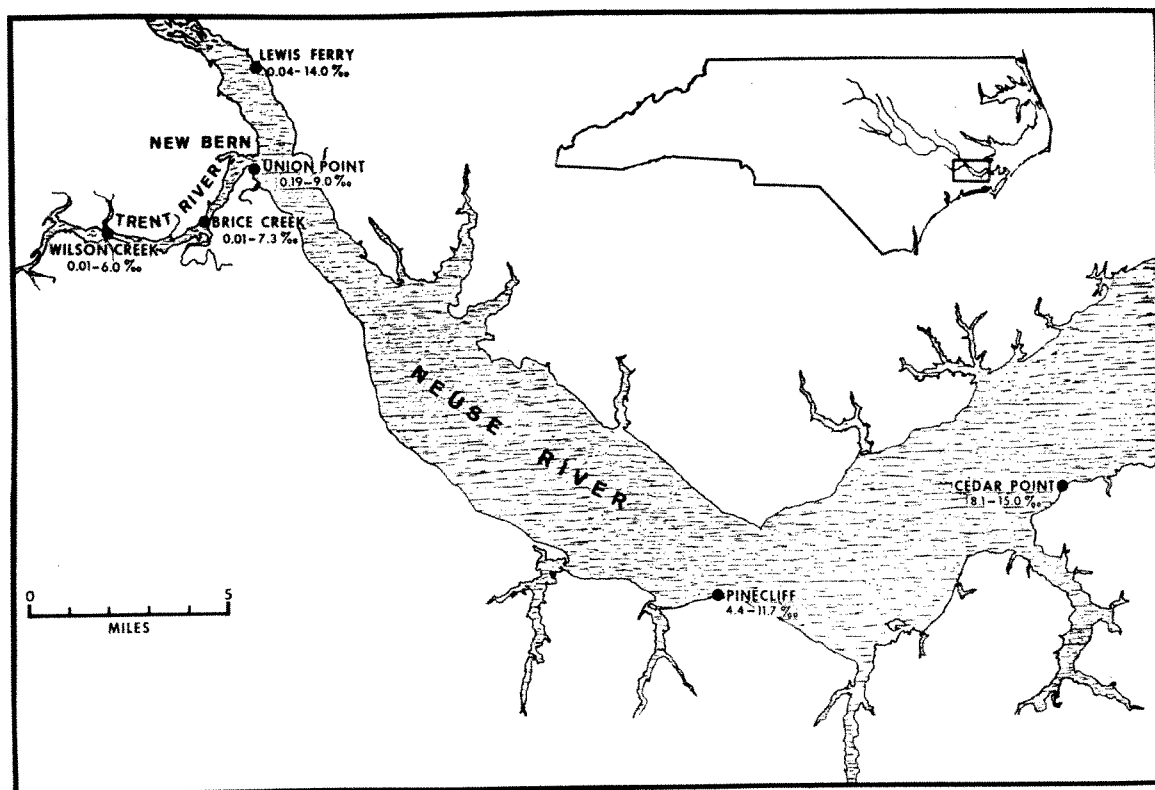


Fig. 1. Location of sampling stations for *Rangia cuneata* in the lower Trent and Neuse Rivers. Measured extremes of salinity are shown for each station.

relationship to the caesium-137/potassium-40 ratio in *Rangia* (Fig. 2). Although the salinity, and therefore the potassium content, of the water was quite different at the six stations (usually <1 part per thousand at Wilson and Brice Creeks and 5–15 parts per thousand at Pinecliff and Cedar Point) the concentration of potassium-40 in the tissues of *Rangia* increased only two-fold with salinity (Fig. 3). In Bryan's<sup>6</sup> laboratory experiments, the potassium content of brackish water isopods (*Sphaeroma*) was also stable over a wide range of salinity, and the variability of potassium content at any given salinity was similar to that for *Rangia* (Fig. 3). Because concentrations of potassium are essentially constant, the variation in the ratio of caesium-137 to potassium-40 chiefly represents changes in the concentration of caesium-137 in the clam. In late spring, when the ratio of caesium-137 to potassium-40 attained its highest value at each station, the concentration of caesium-137 in *Rangia* decreased from station to station in a downstream order (Wilson Creek  $\approx$  Brice Creek > Lewis Ferry > Union Point > Pinecliff  $\approx$  Cedar Point). The greatest difference in caesium-137 content between clams from upstream and downstream stations was six- to seven-fold. The absolute activities of caesium-137 in *Rangia* ranged between 0.93 pc. (Cedar Point, February 9, 1966) and 8.33 pc. (Wilson Creek, May 18, 1966)/100 g wet weight, and averaged for all samples  $2.79 \pm 1.55$  pc./100 g wet weight<sup>8</sup>.

The seasonal fluctuations of caesium-137 content of *Rangia* may be promoted simultaneously by two phenomena, both of which are correlated with rainfall: (a) seasonal variation in the quantities of radioactive debris deposited in the biosphere; and (b) seasonal variation in the salinity (potassium content) of the river water at the sampling stations. The variation of caesium-137 in *Rangia* from upstream corresponded closely to the five-fold range reported for seasonal variation of atmospheric

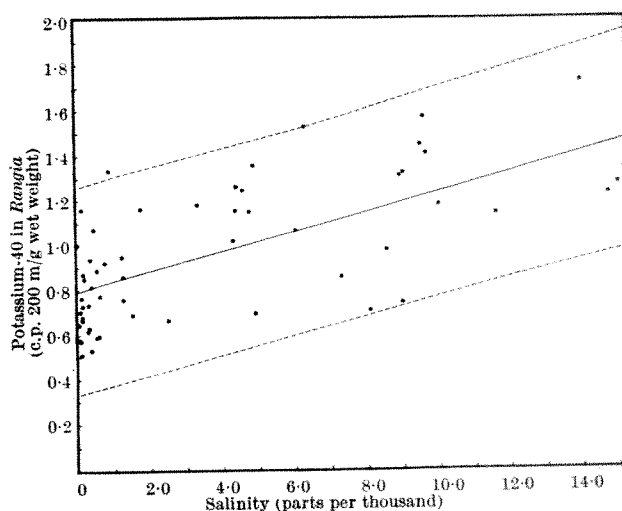


Fig. 3. Least squares regression for the relation of potassium-40 in *Rangia cuneata* to salinity of the water where the clams were collected. Dashed lines are the 95 per cent confidence limits for the regression line.

fallout<sup>1</sup>. The five-fold range of caesium-137 concentration in *Rangia* for a salinity change of more than eighty-fold (from <0.1 to about 8 parts per thousand at Brice and Wilson Creeks) is not consistent with published results of laboratory experiments. Bryan<sup>6</sup> showed that the concentration of caesium-134 in the crab *Carcinus maenas* is inversely proportional to the concentration of potassium of the water. A similar relationship exists for the concentration of caesium-137 by snails and isopods from various dilutions of sea water<sup>5</sup>. At the nearly freshwater stations in the lower Trent and Neuse Rivers therefore the seasonal variation of caesium-137 in *Rangia* is probably caused principally by fluctuations in the deposition of fallout.

If the concentration of caesium-137 was the same in the water at all stations, the concentrations of caesium-137 in *Rangia* at Wilson Creek might be expected to be much higher in relation to those at Cedar Point than were observed. I therefore surmise that the concentration of caesium-137 is much lower in the fresh water of the Trent River than in the estuarine waters of the Neuse, and that this difference accounts for the lack of inverse proportionality between salinity and caesium-137 in *Rangia* at any one sampling time. Because the bioaccumulation of caesium-137 is influenced by the content of potassium in the water, however, estimates of potential concentrations of radiocaesium in estuarine organisms should allow for seasonal and perhaps even tidal variations in salinity.

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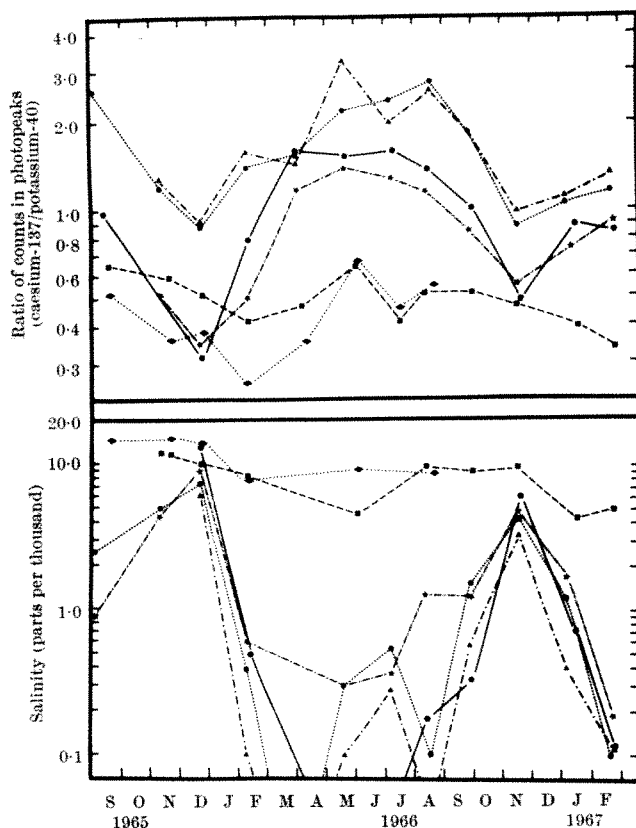


Fig. 2. Variation during 1965–67 of the caesium-137/potassium-40 ratio in *Rangia cuneata* (top) and salinity (bottom) at the stations shown in Fig. 1. Sampling at Cedar Point was discontinued in August 1966 because clams there were relatively scarce. —●—, Brice Creek; —○—, Lewis Ferry; —●—, Union Point; —■—, Pinecliff; —◇—, Cedar Point.



## Variation of Strontium Content within Shells of Recent *Nautilus* and *Sepia*

OUR survey of the strontium content of aragonite shells from living and fossil cephalopods<sup>1</sup> has prompted us to make a more detailed study of the variations of strontium concentration within individual shells of two familiar species now extant—*Sepia officinalis* and *Nautilus pompilius*. Preliminary analysis revealed marked difference in the strontium content of the dorsal and ventral components of the aragonite sepium or cuttlebone of *Sepia*, and so we have investigated in detail samples of these specimens from different parts of the two components of the shells. Most of this communication is, however, concerned with the analyses of samples from two specimens of *Nautilus*. Altogether we have made 350 determinations of strontium, using an X-ray fluorescence technique<sup>1</sup>. All samples were analysed in duplicate and the precision error ranged from  $\pm 1.8$  to  $\pm 2.5$  per cent.

The ventral component of the *Sepia* cuttlebone is the highly porous chambered phragmocone, which is covered dorsally by a thin aragonitic periostracum. Material from the phragmocone was mostly obtained by taking 2 cm<sup>2</sup> of aragonite from various parts of the surface, penetrating the cuttlebone to a depth of 1 cm. In two specimens, some material was obtained from the interior of the phragmocone. Samples from the periostracum were obtained by choosing an area of approximately 1 cm<sup>2</sup> at various positions along the length. All material was ground to pass 200 mesh and analysed for strontium. Subsequently they were treated with a 5 per cent sodium hypochlorite solution to remove organic matter (compare ref. 2) and re-analysed for strontium.

The data for *Sepia* are presented in Table 1. The first figure for each sample gives the value before treatment for the removal of organic matter; the second figure, in parentheses, gives that after such treatment.

Table 1. VARIATION WITHIN *Sepia* OF STRONTIUM

Specimen		Anterior	Anterior/central	Central	Posterior/central	Posterior
1	Periostracum	—	—	2,540 (2,700)	—	—
	Phragmocone	Interior 3,060 (3,230)	—	3,100 (3,260)	—	3,810 (3,840)
2	Periostracum	Exterior 3,550 (3,740)	—	3,290 (3,360)	—	3,900 (4,100)
	Phragmocone	Interior 2,520 (2,800)	—	2,530 (2,900)	—	—
3	Periostracum	3,020 (3,260)	—	3,180 (3,560)	—	—
	Phragmocone	Interior 2,360 (2,580)	2,390 (2,600)	—	2,360 (2,380)	2,370 (2,550)
		Exterior 2,930 (3,280)	2,760 (3,240)	—	2,900 (3,440)	3,050 (3,960)

Values are given in p.p.m.

The values for the periostracum show only slight variations, both within individual specimens and before and after hypochlorite treatment.

Values for the phragmocone are consistently larger, ranging up to 4,100 p.p.m. in treated material, and there is appreciably more variability. The variation within each specimen must relate to differences in strontium deposition during the growth of the animal, allowing for the fact that the chambers lie oblique to the length of the cuttlebone. The data for treated specimens 1 and 3 indicate a decline in strontium values, followed by an increase, between the earliest- (posterior) and latest-formed parts of the phragmocone.

For the principal part of our investigation we used two mature, sagittally sectioned specimens of *Nautilus* which we labelled A and B, 16.1 and 15.9 cm maximum diameter, respectively. Samples of septa were obtained by drilling midway between the septa necks and the venter. Because of the small size of the early septa the whole area between these two positions had to be used for analysis. Venter material was taken at positions adorally 140° from particular septa, thus producing a number of septum/venter pairs, secreted at about the same time. Although most septa were sampled, septum/venter pairs of only the outer whorl were obtained, because it is

difficult to obtain venter material where it is bounded by the preceding whorl. All samples were ground to pass 200 mesh and treated with sodium hypochlorite before strontium was determined.

The data for *Nautilus* are presented graphically in Figs. 1 and 2. In Fig. 1 the strontium values for different positions on the venter are plotted directly above those for the corresponding septa marking approximately the same ontogenetic stage, the scale being displaced slightly to avoid confusing overlap. The graphs indicate the overall similarity of the two specimens. Considering only those septal data for which there are corresponding venter data, mean values of the latter are slightly larger in both specimens (that is, about 1,950 p.p.m. compared with 1,850 p.p.m.). We first thought this was probably related to the difference in aragonitic shell layers. Whereas these consist, in the outer wall, of nacreous, spherulitic-prismatic and semi-prismatic layers in similar proportions, the nacreous layer is dominant in the septa<sup>3</sup>. The analyses of venter samples given in Table 2, however, show that strontium values are greater in the nacreous layer than in the outer two layers, at two positions of the outer whorl of *Nautilus B*. There must be therefore independent biochemical control of strontium precipitation in the septal and ventral nacreous layers.

Considering first the septal data, both specimens show a sharp decline from high to low strontium values from the earliest formed to about the eleventh or twelfth septa, after which a phase of comparative stability sets in, followed by a comparatively gentle rise at about the twenty-fifth septa and a final decline in the last two or three septa. Variation in the venter seems in both cases to be largely independent of the septa, with even a tendency towards an inverse relationship, so that peaks in the one group correspond to some extent with troughs in the other, and vice versa. The venter data, especially as shown in Fig. 2, give a clear indication of a slight secular

decline with age of strontium content, on which is superimposed a pronounced cyclicity. Fig. 2 also shows the striking similarity between the two specimens, the only notable exception being a minor peak in *Nautilus A*.

Some limited deductions and tentative suggestions can be made from our results. The variation of strontium content within morphologically different but mineralogically similar shell layers of the same organism, as found in both *Sepia* and *Nautilus*, is of considerable interest. Whereas it has been known for some time that trace element contents may vary within calcareous shells, this has usually been related to the variable development of calcite and aragonite, that is, it is mineralogically determined. Our data, concerned with only one mineral species, indicate that in addition there must be an independent biochemical control, and that crystal parameters only determine the general level of the strontium content. Biochemical determinants are also required to account

Table 2. VARIATION OF STRONTIUM (P.P.M.) IN VARIOUS CARBONATE LAYERS FROM THE VENTER OF *Nautilus B*

	<i>Nautilus B</i> Position 1 Strontium (p.p.m.)	<i>Nautilus B</i> Position 2 Strontium (p.p.m.)
Spherulitic and semi-prismatic layers	1,790 $\pm$ 30	1,870 $\pm$ 40
Nacreous layer	2,040 $\pm$ 40	1,990 $\pm$ 40

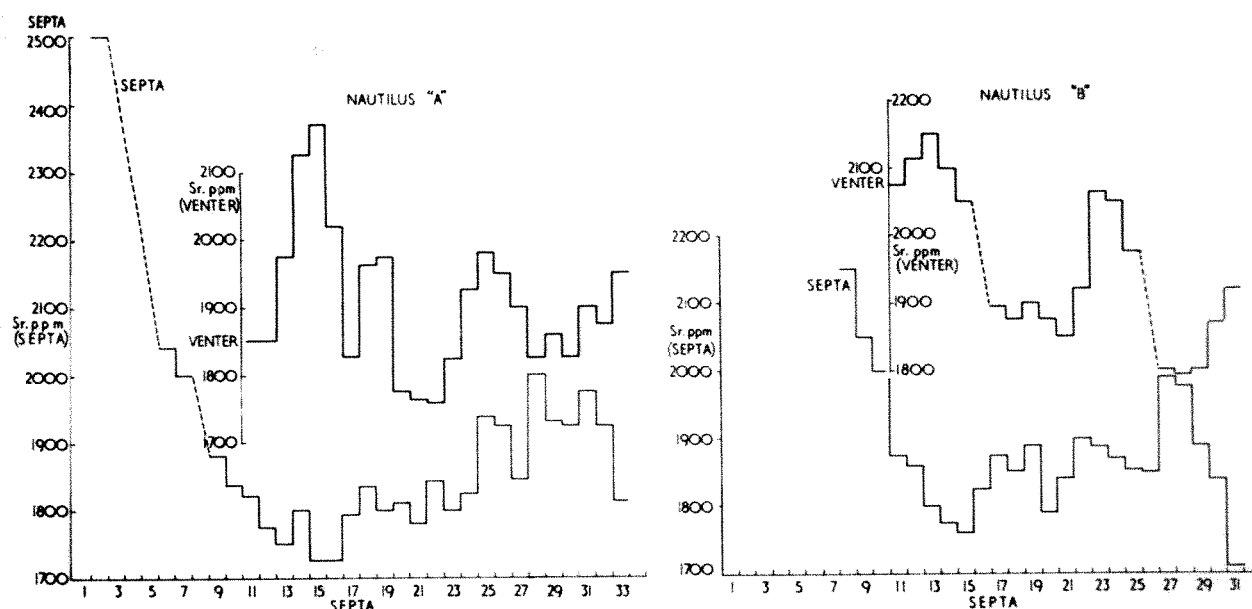


Fig. 1. Variation in p.p.m. of strontium in septa/venter increments in *Nautilus A* and *B*.

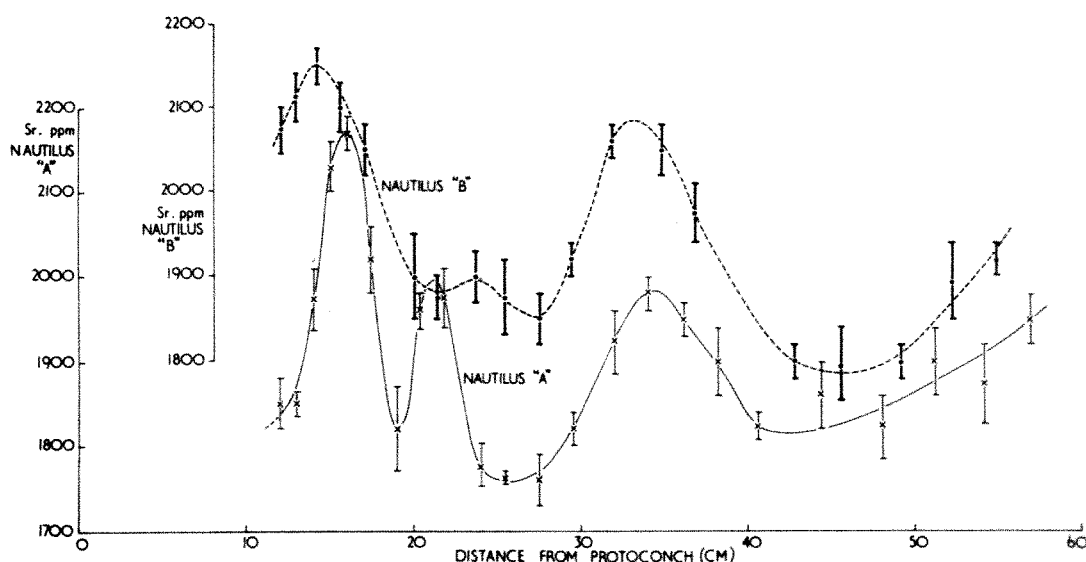


Fig. 2. Strontium variation in venter samples from *Nautilus A* and *B* plotted as distance from protoconch. Vertical lines indicate the deviation of replicate analyses and include errors due to sample preparation and instrument instability.

for the variations within particular shell layers. These variations are essentially of two kinds. Cyclicity is shown in *Nautilus*. The obvious interpretation, which cannot be proved for the specimens in question, is that these cyclic variations in strontium content correlate with seasonal changes. The only published research known to us, with which our own is directly comparable, is that on *Mytilus* by Dodd<sup>4</sup>, who found that the strontium content of the aragonitic nacreous layer varied inversely with temperature in different seasons. This would suggest that the peaks of Fig. 2 should be correlated with winter seasons, but the fact that septal and venter peaks tend not to coincide renders the problem more complex. Furthermore, Denton and Gilpin-Brown<sup>5</sup> have recently argued, on the basis of varying gas pressures within the chambers of *Nautilus*, that maturity is reached within 1 yr, with septa being secreted at fortnightly intervals, a conclusion which implies a remarkably rapid rate of growth, with weight doubling about every 40 days, and would argue against a seasonal interpretation.

The secular decline of strontium values that is also observable in *Nautilus* could indicate migration during the first few years of life from cooler to warmer waters. Alternatively, it might relate more directly to biochemical changes during ontogeny and have nothing to do with the external environment. A recent paper on oxygen and carbon isotopes in the shell of *Nautilus* is important in this connexion. Eichler and Ristedt<sup>6</sup> found a sharp change in  $\delta^{18}\text{O}$  content of both septa and outer shell. This change, between the seventh and eighth septa, was correlated with temperature change, but with the animal passing the early stages of its life in warmer water. A further point of comparison between our work and that of Eichler and Ristedt is that  $\delta^{18}\text{O}$  values in the septa following the sharp change referred to are more constant than those of the outer shell at a corresponding period of growth. Without more information one should not dismiss the possibility that endogenous factors have played at least some part. This possibility becomes intriguing when it is borne in mind that certain fossil ancestors of *Nautilus* had appre-

ciably higher strontium contents, and that this appears to be a biochemically more primitive condition<sup>7-9</sup>.

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### Population Control by Territorial Behaviour in Red Grouse

THERE is uncertainty about the importance of social behaviour in regulating the size of animal populations, as shown by widely different explanations put forward in recent years<sup>1-3</sup>. One difficulty is that firm conclusions on the subject cannot be drawn from correlations derived from simple field observations; and even with birds few experiments have been done in the wild, although the social behaviour of birds is better understood than of most other wild animals. The results of field work are therefore usually open to different interpretations.

The idea that breeding stocks of birds might be limited by their territorial behaviour has been mentioned by so many authors (for review see ref. 2) that it is frequently accepted as an established fact instead of a largely untested idea. Hinde<sup>4</sup>, in his review of the literature on territory in birds, could find no unequivocal support for this idea from field observations. Furthermore, two pioneering experiments<sup>5,6</sup>—still the only important experiments yet published in this field—did not really test this conception. The authors attempted during June–July to eliminate the passerine birds in 16 hectares of forest, and succeeded in reducing them to about 20 per cent of the original level, after which the reduced numbers were maintained by the continued influx of new birds until finally two to three times as many males had been shot as had initially been present. This suggested that the newcomers were surplus birds which had previously been prevented from settling by the presence of the original residents. There was no proof, however, that they were not migrants or territorial birds attracted from somewhere else which might have bred elsewhere if there had been no shooting. In fact, most of the newcomers belonged to one migratory species, and there may have been extra inducement to settle because of abundant food from an outbreak of spruce budworm (*Choristoneura fumiferana*).

To demonstrate that territorial behaviour really limits breeding stocks, it is necessary to show: *a*, that a substantial part of the population consists of surplus non-territorial birds which do not breed; *b*, that these are prevented from holding territory and breeding by the established territory-holders; and *c*, that they are able to take territories and breed, if they are given the chance by the removal of the established birds.

A series of thirteen experiments from 1960 to 1966 has recently been completed, in which red grouse (*Lagopus lagopus scoticus*) were removed from study areas on heather (*Calluna vulgaris*) moorland in north-east Scotland. A useful feature was that the grouse lived on these moorland areas all the year round and did not migrate;

even local movements beyond 5 km were rare<sup>7,8</sup>. In the first three experiments, individual territorial cocks were taken temporarily into captivity and later released. In later experiments, all young or old cocks on certain areas were removed by shooting, and in some experiments the entire stocks of both sexes were shot on areas of up to 52 hectares.

The experiments were preceded by a study of the behaviour of all birds on experimental and control areas, and on ground within 150 m round about. This was backed up by accurate counts<sup>7</sup> of total population size. Red grouse take up their territories in autumn and hold them until the following summer. On each area the grouse population contained both territorial and non-territorial birds, the latter making up about half the autumn population<sup>8</sup>. Territorial birds included cocks which showed territorial behaviour<sup>9</sup>—that is, each cock consistently dominated intruding cocks and kept them out of its individual plot of ground on the heather moor, where it also courted hen grouse—and hens paired up with territorial cocks. Non-territorial cocks and hens did not court, pair up, show territorial behaviour or subsequently breed, and were frequently driven out of the territories by established territorial birds. Because most grouse on and around the study areas were marked with plastic back-tabs for individual recognition, the previous status of most newcomers which appeared on vacant ground was known—a crucial fact for proper interpretation of the experimental results.

The null hypothesis was that there would be no changes in numbers, at least until the breeding season, on control areas and on areas depopulated by shooting between autumn and spring, and that non-territorial birds on or around these areas would also remain as such over the same period. (Effectively, this meant finding whether territorial behaviour limited the size of the breeding stocks.) Neither result was obtained on the experimental areas, while the *status quo* was maintained on the control areas.

Compared with a total of 119 experimental vacancies for territorial birds on five areas between August and June, 111 new birds colonized to take territories by mid-summer. During most of the experiments, most vacancies were filled within 2–3 days and all within 1 week. There were exceptions after experiments in November–December when 2–4 weeks were needed, and in February–March when no replacement occurred until late April. In two experiments in March and one in June, involving nine vacancies in all, there were no replacements until the following autumn. Twelve of the 111 newcomers were of previously unknown status. Fifty-one were juveniles when the August–September experiments were performed (that is, before the time in October when juveniles first took territories in undisturbed populations). Because on average more than half the autumn population later becomes non-territorial and because the mortality rate of first year grouse is the same as in older grouse<sup>7</sup>, more than half these juveniles would probably have become non-territorial if there had been no experiments. Twenty newcomers during experiments after November were previously non-territorial young grouse reared in the previous summer, and four were non-territorial old birds which had lost their former territories before the experiment. Twenty-four newcomers were birds which were occupying territories elsewhere at the time of the experiment and which moved up to 1 km to take territories on the vacant ground.

Two control areas were used, which were roughly the same size as the experimental areas. Out of an initial total of 269 territorial grouse on the control areas before the experiments, there were only three individual changes during the period of removal and colonization on the experimental areas. Two that disappeared were replaced and the third bird moved to colonize vacant ground on the experimental area.

Territorial cocks, the neighbours of which were shot, invariably enlarged their territories before they themselves were shot, which implies that there was some previous pressure against this. Grouse which were previously non-territorial and which took territories on the experimentally vacant ground subsequently bred there, which shows that non-territorial birds are capable of taking territories and breeding, given the chance. On each of the five different experimental areas the breeding stock in the spring after an experimental shooting usually rose to about the same level as before the shooting, irrespective of whether some or all the previous birds were shot, and even though the numbers of grouse on different adjacent areas varied greatly in the same year, and in different years on the same area. Consequently, the ceilings to population size on the different areas were not related to the numbers of birds available on these areas or on adjacent areas. Presumably, these different ceilings were adjusted to some fairly constant environmental feature of each area.

These results show experimentally that territorial behaviour in red grouse sets a limit to the size of the territorial population during the winter months and of the subsequent breeding stock.

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### **In vitro Cultivation of the Tapeworm *Hymenolepis nana* from Larva to Adult**

BERNTZEN<sup>1</sup> described an apparatus for the cultivation of *Hymenolepis nana* in continuously circulating medium. His average recovery of live worms from two specified media was 7 per cent and 11 per cent, and this increased to 17 per cent when the temperature of cultivation was raised from 37° to 39° C. In an unspecified number of these worms, fertile eggs were produced by day 12. The advantages and disadvantages of Berntzen's apparatus and media have been discussed by Hopkins<sup>2</sup>.

We were unable to reproduce Berntzen's results and decided to use the medium used by McCaig and Hopkins<sup>3</sup> for the cultivation of *Schistocephalus solidus*. When this medium was dispensed in roller tubes and gassed with 95 per cent nitrogen and 5 per cent carbon dioxide, it supported growth of *H. nana* to 3 mm in length and early strobilation, but no genitalia developed. Supplementation of the medium with various extracts including autoclaved rat liver extracts failed to improve growth significantly, but a cold liver extract prepared using Stoll's technique<sup>4,5</sup> greatly enhanced development.

In a medium of Hanks balanced salt solution, horse serum, 'Oxoid' yeast extract, glucose and rat liver extract, 37 per cent of the cysticercoids grew to sexually mature adults and 50 per cent of these mature worms had fully formed, visibly normal eggs in some of the proglottids. In later experiments, it was found that lamb liver extract was as good as rat liver extract and because lamb liver is more easily available in quantity it was used for all subsequent work. The method of preparation of liver

extract, excystation of the cysticercoid and cultivation was as follows.

Lamb liver was brought from the abattoir in an iced container, chopped into pieces of about 20 g and stored at -15° C. When required it was thawed at 4° C for 48 h. One part by weight of liver was homogenized with four parts by volume of de-ionized water at 4° C keeping the flask surrounded by crushed ice. The brei was adjusted to pH 4.0 with 1 normal hydrochloric acid (approx. 2.5 ml./100 ml. brei), squeezed through muslin and centrifuged at 5,420g for 1 h at 4° C. The supernatant was collected, filtered through a 0.45µ 'Millipore' filter and sterilized by passing through a 0.22µ filter. The sterile extract was stored in aliquots of 5 and 10 ml. at -15° C.

One hundred ml. of medium was prepared by mixing the following solutions in the order stated: (a) 40 ml. Hanks saline; (b) 10,000 units of sodium penicillin G and 10 mg of streptomycin sulphate; (c) 5 ml. of autoclaved 6.5 per cent glucose solution; (d) 10 ml. of a 5 per cent 'Oxoid' yeast extract, filter sterilized; (e) 10 ml. of liver extract; and (f) 30 ml. of horse serum (No. 2, Burroughs Wellcome, London). The pH of the mixture was adjusted to 7.6 by addition of 1.8 ml. of 0.2 normal sodium hydroxide and 3-4 ml. of 1.4 per cent sodium bicarbonate. After mixing, the medium was dispensed in 5 ml. quantities in 125 × 25 mm roller tubes and gassed for 40 sec with 95 per cent nitrogen and 5 per cent carbon dioxide. The culture tubes were immediately closed with 'Esco' RWH grade rubber bungs, placed in a roller drum incubator and rotated (0.15 r.p.m.) for at least 2 h before inoculation with freshly excysted worms.

Infected beetles (*Tribolium confusum*) were dissected in Hanks balanced salt solution (BSS). The freed cysticercoids were washed twice in sterile BSS and thereafter all procedures were carried out at 37° C using sterile solutions containing 100 u of penicillin and 100 µg of streptomycin ('Crystamycin', Glaxo)/ml. The cysticercoids were placed in 1 per cent pepsin (activity 1:2,500; supplied by B.D.H.) in BSS at pH 1.7 (pH adjusted with 0.2 normal hydrochloric acid) for 12-15 min. They were next washed three times with BSS and transferred to trypsin-bile salt solution (0.5 per cent trypsin (83:1, B.D.H.) and 0.3 per cent sodium tauroglycocholate (B.D.H.) in BSS at pH 7.2, pH adjusted with 0.2 normal sodium hydroxide). About 90 per cent of the worms excysted within 8-10 min; these were washed three times in BSS and collected in a Petri dish of 5 cm diameter. From this dish groups of 16-20 excysted worms were transferred to small glass containers, examined under ×12 magnification and pipetted into a culture tube. The tube was gassed a second time with 95 per cent nitrogen and 5 per cent carbon dioxide and returned to the incubator. The pH of the medium 2-3 h after setting up was 7.2 ± 0.2. The medium was changed on days 3, 6, 9, 11 and 13.

The principal results were as follows. Cysticercoids, during 14 days of cultivation, grew to strobilated worms 7-30 mm long, 60-70 per cent had mature genitalia with active sperm and 20-30 per cent produced eggs in some proglottids. Discharged eggs were found in the medium from the tenth day onwards and these eggs and those from proglottids have been fed to the intermediate host where they developed into normal infective cysticercoids.

Work has started to determine the conditions under which the liver extract is active. It is known to lose its activity gradually after storage for 4 weeks at 4° C, but it retains full activity for at least 4 months at -15° C. Heating the liver extract for 6 min at 53° C does not inactivate it (compare Sayre *et al.*<sup>6</sup>). A precipitate forms within 2 weeks of storage at 4° C which must be resuspended; if this is separated by centrifugation the supernatant is inactive. When the complete medium is prepared a precipitate forms at the time of mixing (associated with changing the pH of the liver extract from about 4 to neutral) or shortly after rotating in the roller tubes. The formation of this encrusting precipitate is important



and is in some way associated with the ability of the worms to grow and mature (compare Smyth *et al.*<sup>7</sup>).

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### Infertility in Mice and Guinea-pigs induced by Feeding with a Fungal Pathogen isolated from Leaves of *Romulea rosea* (Linn.) Eckl.

IN areas of low soil fertility, in the State of Victoria, *Romulea rosea*, known locally as Onion Grass, is a fairly common constituent of pastures. The seeds and corms germinate after the first autumn rains; and in some circumstances these plants provide valuable autumn feed for stock.

Evidence has accumulated during the past 20 yr to show, however, that in certain seasons a disease of sheep is associated with the consumption of pasture containing a high percentage of *R. rosea*. Gorrie<sup>1</sup> clearly defined the symptoms of this disease syndrome which includes infertility, abortion and paralysis; and he named the disease "romulosis".

During the late autumn and early winter of 1965 "romulosis" was reported from several areas within a radius of about 100 miles of Melbourne. All toxic pastures inspected were *Romulea* dominant; furthermore, a leaf spot condition was a conspicuous feature of these plants.

Initially, discrete Chestnut brown<sup>2</sup> spots, about 1-2 mm in diameter, developed in the leaf tissue; later individual spots coalesced and finally complete necrosis occurred, usually extending from the tip downwards.

*Helminthosporium biseptatum* Sacc. and Roum, was isolated from leaves of *R. rosea* collected from very toxic pasture, near Seymour, about 60 miles north-east of Melbourne.

Pathogenicity of the fungus was established by inoculation of healthy seedling plants of *R. rosea* with a pure culture of the isolant.

The inoculation technique, which was carried out in a heated glasshouse, involved atomizing leaves with an aqueous suspension of conidia. Incipient spotting of the leaves appeared after 10-11 days and typical lesions from which *H. biseptatum* was re-isolated developed after 21 days.

Infertility in sheep is an important symptom in the "romulosis" syndrome, and so preliminary feeding trials were conducted to determine whether the *Romulea* leaf-spot fungus had an effect on the breeding efficiency of laboratory animals, such as mice and guinea-pigs.

Free feeding methods were used, and it was essential that the culture medium should provide food acceptable to the animals, particularly the guinea-pigs. Oatmeal was selected after testing the growth of the fungus on several natural plant media. The medium was prepared by mixing 40 g of oatmeal + 50 ml. of water in a 250 ml. Erlenmeyer flask and autoclaving on 3 successive days, for 20 min at 15 lb. pressure.

The fungus was incubated at 21°C until the oatmeal was thoroughly permeated by the mycelium—11 to 12 weeks.

The oatmeal fungal culture was then incorporated in the standard mash used for feeding to laboratory animals (experimental diet), and an equivalent amount of oatmeal medium was added to the standard mash to provide the control diet.

The experimental diet for mice contained 5 per cent fungal culture; guinea-pigs, however, rejected this diet and the fungal additive was reduced to 2.5 per cent.

The feeding trials were designed to comprise four groups: (1) males and females fed experimental diet; (2) males fed experimental diet and females fed control diet; (3) males fed control diet and females fed experimental diet; (4) males and females fed control diet.

In the mouse trial, there were twenty-four females in each group and one male was mated to two females. Mating commenced on day 35 of feeding and was allowed to continue for 16 days. At the end of the mating period the males were killed and examined.

In the guinea-pig trial there were eight females in each group and they were mated at the rate of one male to four females. Mating was commenced on day 21 of feeding and was continued for 21 days after which the males were killed and examined.

Each feeding trial was terminated when all pregnant females had produced their litters. All females and litters were then killed and examined.

The results of these feeding trials are shown in Table 1.

Animal	Group	Control diet	Exper. diet	Pregnant	Not pregnant	No. females in group
Mouse	1		♂ ♀	12	12	24
	2	♀	♂	19	5	24
	3	♂	♀	12	12	24
	4	♂ ♀		18	6	24
Guinea-pig	1		♂ ♀	2	6	8
	2	♀	♂	5	3	8
	3	♂	♀	7	1	8
	4	♂ ♀		8	0	8

Statistical analysis of the number of pregnancies in each group indicates that the fungus caused a reduction in the breeding efficiency of the female mice, significant at 2 per cent and almost at the 1 per cent level.

With guinea-pigs, on the other hand, females were not affected but ingestion of the fungus caused a reduction in the breeding efficiency of the males which was significant at the 1 per cent level.

Post-mortem examination of the experimental animals did not disclose any macroscopic differences between feeding groups; and the results of histological examinations of selected organs have yet to be evaluated.

In certain conditions the *Romulea* leaf spot fungus seems to adversely affect animal reproduction, but the nature of its action has yet to be investigated. The possibility that an oestrogen or related compound may be involved should not be overlooked in view of the finding<sup>3</sup> that two foliar pathogens (*Pseudopeziza medicaginis* and *Leptosphaerulina briosiana*) significantly increased the coumestrol content of alfalfa.

We thank Mr R. Jardine, of the Victorian Department of Agriculture, for the statistical analysis of the feeding trials. We also thank Mrs Brush, of the Veterinary Research Institute, Melbourne, for technical assistance, and Dr Ellis, of the Commonwealth Mycological Institute, Kew, for identifying the fungus.

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### Effect of Carbohydrates on the Symbiotic Growth of Planktonic Blue-Green Algae with Bacteria

PHOSPHORUS and nitrogen have been suggested to be limiting agents in the unwanted growth of Cyanophyta. Removal of phosphorus from sewage effluents is being considered as the preferred method to control eutrophication. The significance of organic matter in lake water has been largely disregarded, although carbonaceous material is always found in productive lakes, for example, from 22 mg to 99 mg/l. of dissolved organic matter in the Great Lakes<sup>1</sup>. Massive growths of blue-green algae have been documented before the use of industrial phosphates, and they probably occurred always after a heavy influx of organic matter. Maximum growth of phytoplankton does not require high concentrations of phosphorus. As little as 0.02 mg of phosphorus/l. in natural lake water may sustain it<sup>2</sup>. Additional phosphorus alone does not increase growth because algal growth will depend on the presence and availability of not less than fifteen essential elements<sup>3</sup>.

Planktonic Cyanophyta are always associated with bacteria, and I have found that abundant algal growth results from a symbiotic relationship within their systems. In particular, the results reported here suggest that the bacteria assimilate added carbonaceous material and produce carbon dioxide which accelerates algal photosynthesis. The addition of a source of carbon also apparently delays the bacterial assimilation of organic chelating agents which are necessary if nutritional elements are to remain accessible at a high pH. Reports<sup>4-10</sup> are consistent with various findings and, as a whole, support my conclusions.

I have grown the algae in Zehnder and Gorham's medium No. 11 (ref. 11) which contains, among other minerals, 6.9 mg of phosphorus/l., 81.7 mg of nitrogen/l. and only 6.7 mg of carbon/l. Half of the heavily inoculated growth medium was mixed with an aqueous solution of sucrose, the other half (the control) received an identical volume of distilled water. Quantities of 50 ml. of the two cultures were placed in 250 ml. Erlenmeyer flasks which were loosely capped with aluminium foil. The flasks were exposed to fluorescent illumination of 50 ft. candles (540 lux) in a 16-8 h cycle of light and dark at 22° C. The

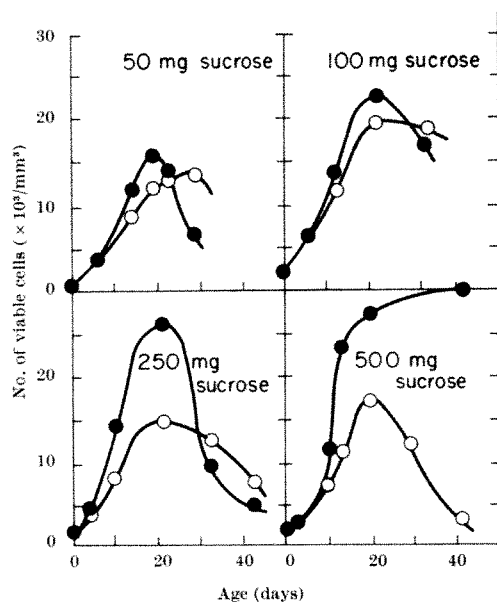


Fig. 1. Effect of added sucrose (mg/l.) on *Microcystis aeruginosa* Kütz. Wisc. 1036 cultures containing bacteria. Starting concentrations were: 6.2 mg of phosphorus/l. as orthophosphate, 73.5 mg of nitrogen/l. as nitrate. Light was 50 ft.-candles (540 lux) at 22° C. Numbers of viable cells plotted versus age of cultures; ●—● sucrose cultures, ○—○ sucrose free control cultures.

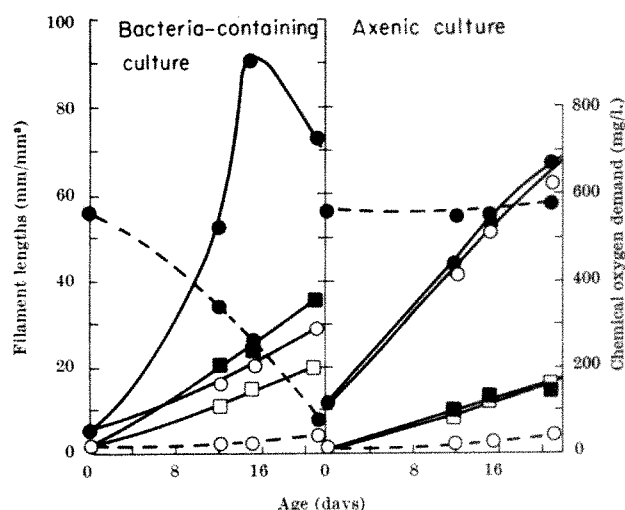


Fig. 2. Effect of added sucrose on cultures containing bacteria, EAWAG 18, Clone 51 (left) and on pure cultures of *Oscillatoria rubescens* DC, EAWAG 19, Clone 51 R (right). Starting concentrations: 5.3 mg of phosphorus/l., 80.6 mg of nitrogen/l., and 500 mg of sucrose/l. Light was 50 ft.-candles (540 lux) at 22° C. Total lengths of algal filaments and chemical oxygen demand of membrane filtrates and of biomass plotted versus age of cultures.

Legend:  
 Filament lengths: ●—● (sucrose-containing), ○—○ (sucrose free control)  
 Chemical oxygen demand of filtrates: ●—● (sucrose-containing), ○—○ (sucrose free control)  
 Chemical oxygen demand of biomass: ■—■ (sucrose-containing), □—□ (sucrose free control)

pH of the cultures ranged from 8.9 at inoculation to 10.5 when growth was at a maximum, and it decreased when bacterial growth and cell lysis prevailed. For analysis, cells appearing viable on the basis of colour and refraction were counted in a haemocytometer, or the lengths of filaments were measured. Chemical oxygen demand was determined on the total culture and on the membrane filtrate; the difference was taken as the biomass.

The unialgal cultures of Cyanophyta containing bacteria which were used are shown in Fig. 1 and Table 3. Professor O. Jaag and Miss B. Egli of the Swiss Federal Institute of Technology provided axenic cultures<sup>12</sup> and bacteria-containing cultures of *Oscillatoria rubescens* (Fig. 2).

In four separate experiments with bacteria-containing *Microcystis aeruginosa* systems at different concentrations of sucrose (Fig. 1), the cultures containing sucrose showed greater growth than the controls. Maximum growth was often achieved within 3 weeks, and then the number of viable cells declined simultaneously with their discoloration. This decline seemed to coincide with the disappearance of the added carbonaceous matter and often was more rapid in the cultures fed sucrose.

The following observations suggest a protective function of the added source of carbon against bacterial degradation of the metal chelates. The membrane filtrate of declining control cultures still contained 1.0 mg of phosphorus/l. and 37 mg of nitrogen/l. The cultures could only be revived by the addition of a solution of ferric citrate, the minor elements, ethylenediamine tetraacetic acid (EDTA), and citric acid in the original concentrations, increasing the carbon content in the final culture by only 2.2 mg of carbon/l. The addition of the chelated metals slowly restored the blue-green colour of the algae, and growth resumed. Deterioration of the cultures soon started again, apparently as a result of bacterial assimilation of the insignificant amount of added organic chelating agents (Table 1). The large number of bacterial cells in deteriorating cultures fed sucrose prevented a revival because the added chelating agents were assimilated too rapidly. The blue-green colour was restored, but growth did not resume and the cultures started to deteriorate further 2 days later.

The effect of an added source of carbon on algal growth was duplicated by raising the concentration of carbon dioxide in the atmosphere (Table 2). Table 3 gives the

Table 1. EFFECT OF THE ADDITION OF EQUAL VOLUMES OF DISTILLED WATER, POTASSIUM PHOSPHATE SOLUTIONS OR SOLUTIONS OF ORGANIC IRON-TRACE METAL COMPLEXES ON A DECLINING CONTROL CULTURE OF *Microcystis aeruginosa*

Equal volumes of solutions added	No. of viable cells ( $\times 10^3/\text{mm}^3$ )		
	0	4	7
Distilled water	7.3	6.4	4.9
6.9 mg of phosphorus/l.	7.3	3.8	2.3
Iron-trace metal complexes	7.3	12.3	9.1

Table 2. EFFECT OF AN INCREASED CONCENTRATION OF CARBON DIOXIDE IN THE ATMOSPHERE UPON THE GROWTH OF *Microcystis aeruginosa*

	Cell No. ( $\times 10^3$ / $\text{mm}^3$ )	pH	Chemical oxygen demand (mg/l.)		
			Total	Filtrate	Biomass
Starting culture	6.4	9.2	116	38	77
Control in normal air, after 7 days	10.0	9.1	206	45	161
Air with added 0.5% $\text{CO}_2$ , after 7 days	22.9	8.8	403	55	348
Normal air, but addition of 250 mg of sucrose/l. to the culture, after 7 days	23.8	9.2			

Table 3. COMPARISON OF CELL COUNTS ( $\times 10^3/\text{mm}^3$ ) AS THE GROWTH RESPONSE OF BLUE-GREEN ALGAE CONTAINING BACTERIA TO ADDED SUCROSE (500 mg/l.)

Species		Days							
<i>Nostoc muscorum</i> Kütz. Wisc	Days	0	2	4	7	11	14	45	
		0.5	0.5	0.8	1.6	6.0	9.4	13.0	
		0.5	0.7	1.1	2.2	6.9	13.0	27.0	
<i>Anabaena circinalis</i> Rab. Wisc. 1038	Days	0	19	26	34				
		0.1	0.3	1.2	3.8				
		0.1	2.6	5.2	10.8				
<i>Phormidium faveolarum</i> Gomont IU 427	Days	0	26	40					
		0.6	12.7	24.6					
		0.6	32.2	74.6					
<i>Lyngbya</i> sp. IU 487	Days	0	13	21	26				
		5.3	29.4	67.0	94.2				
		5.3	52.4	145.7	191.8				
pH		9.4	10.3	10.2	10.4				
		9.2	10.1	9.9	10.9				
Chemical oxygen demand (mg/l.)									
Total		15	121	271	385				
		580	721	765	911				
Filtrate		14	19	49	69				
		576	475	119	151				
Biomass		1	102	222	316				
		4	246	646	760				

The upper value is the sucrose free control, the lower that of the sucrose culture.

results for other alga-bacteria systems fed 500 mg of sucrose/l. in similar conditions.

Axenic cultures and cultures of *Oscillatoria rubescens* containing bacteria, a strict photolithoautotroph<sup>12</sup>, were grown in the presence or absence of sucrose. Sucrose was of no value to the axenic alga, but it stimulated the growth of the association of bacteria and alga (Fig. 2).

Work in progress indicates that many sugars and polyhydric alcohols, glucuronate, citrate and starch give results similar to sucrose. Also, using closed containers at decreasing ratios of enclosed air and algal culture volumes with proper illumination, *Microcystis* and its associated bacteria may grow in low concentrations of oxygen without an atmospheric source of carbon dioxide if enough organic matter is available.

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## Action Potentials in the Reproductive System of Plants

DURING the process of sex differentiation, male and female metabolic systems develop in the flower. Their unification at pollination creates a single system with a metabolism of extremely high intensity<sup>1-3</sup>. Electrophysiology may show when the pollen starts to affect the pistil tissue and give information about the nature of the primary reactions induced.

We used *Incarvillea grandiflora* and *I. delavayi* species of an entomophilous genus of the Bignoniaceae. The bilobate stigmas are sensitive to mechanical pressure or irrigation. *Lilium martagon*, a plant with an insensitive stigma, was used as the control.

Fig. 1 shows the scheme for attaching measuring devices to the pistil of *Incarvillea*. A microelectrode of a d.c. amplifier (DCA<sub>1</sub>) was inserted into the top of the stigma lobe, and the electrode of the second amplifier (DCA<sub>2</sub>) was inserted into the junction of the lobes, and the electrode of the third d.c. amplifier (DCA<sub>3</sub>) into the tissue of the style. Microthermistors of the Karmanov type<sup>4</sup> were applied to the tissues of the lower part of the lobe or to the style to record the temperature and possible flow of water. An electronic amplifier and a potentiometer were used to record potentials. The diameter of the micropipette end of the glass electrodes was less than 1–2  $\mu$ . The internal resistance of electrodes was measured before and after the experiments. Pipettes were made of 'Pyrex' and filled with 3.0 molar potassium chloride. Calomel electrodes were used as pickups. Ovaries were placed into a chamber of organic glass and the pickup of oxygen was recorded by an electrochemical method<sup>5,6</sup>.

Electrophysiological investigations of stigmas of *Incarvillea* and simultaneous recording of their movements with a kymograph showed that action potential (AP<sub>1</sub>) appeared in response to mechanical irritation of the top of the lobe with a soft brush in about 0.2 sec (Fig. 2a). AP<sub>1</sub> spread to the base of the stigma with a velocity of 1.8 cm/sec, and in 0.8–1.0 sec it reached the point where they joined, that is, the tissues which give rise to the motor reaction of stigma. The lobes started to move 0.1 sec after this and the stigma closed in 6–10 sec.

If mechanical irritation was not followed by pollination the stigma began to open some minutes after closing and was fully open and ready for the next irritation in 17–22 min. It is interesting that the action potential of mechanical irritation (AP<sub>1</sub>) reached only as far as the junction of stigma lobes and could not penetrate into the style because of the physiological non-conductivity block in it.

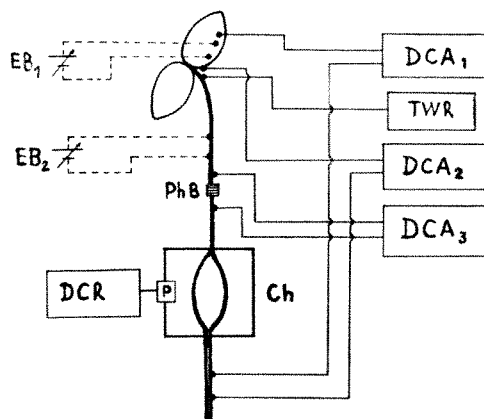


Fig. 1. Scheme for measuring potentials and intensity of respiration in the flowers of *Incarvillea*. DCA<sub>1</sub>–DCA<sub>3</sub>, d.c. amplifiers; TWR, recorder registering temperature and water flow along the tissues; P, electrochemical pickup (Clark microelectrode) for registration of partial oxygen pressure; DCR, d.c. recorder; Ch, chamber for measuring of respiration intensity; EB<sub>1</sub>, EB<sub>2</sub>, electrotonic blocks; PhB, physiological block.

If pollen was placed on the stigma lobes with caution and there was no mechanical irritation,  $AP_1$  was not aroused and the stigma did not close in the first seconds; closing started only 3–17 min after application of pollen. It was preceded by polarization of rest potentials in the column by 30–40 mV (Fig. 2b), after which the second action potential ( $AP_2$ ) with the amplitude of 80–90 mV appeared in the stigma (Fig. 2c) and caused it to close.

A negative rest potential caused the non-conductivity block in the column to disappear and  $AP_2$  spread down the style at a rate of about 2.9 cm/sec. It was recorded by the electrodes of the third d.c. amplifier (DCA<sub>3</sub>). Primary and reversible stimulation of the oxygen consumption up to 5–11 per cent was recorded in ovary tissue 60–90 sec after  $AP_2$  reached them (Fig. 2d).

In 4–16 min, respiration intensity returned to the initial level and then increased slowly and sometimes irregularly and reached a maximum in 10–36 min, depending on the maturity of the stigma. In the case of simultaneous mechanical irritation and pollination, reciprocal reactions appeared in succession. Generation of action potential ( $AP_1$ ) and motor reaction of stigmas were observed first and they were followed by negatization of the rest potential, and generation and spreading of  $AP_2$  down the column. Finally, stimulation of oxygen consumption by the ovary was observed. Amplitude, velocity of spreading and the forms of  $AP_1$  and  $AP_2$  were analogous to those of plants which have motor reactions<sup>7–11</sup>.

Spreading of  $AP_1$  and  $AP_2$  can be stopped by blocking their path with electronic and physiological (solution of 2,4-dinitrophenol) "blocks". Then the primary reciprocal reaction expressed as motion of the stigma and stimulation of respiration at the ovary are not observed when pollen is applied to the stigma lobes. These processes recommence after removal of the electrotonic blocks.

The stigma of *Lilium martagon* has no motor reaction and does not generate  $AP_1$  (Fig. 2a<sub>1</sub>). The rest potential

generation and spreading of  $AP_2$  as well as stimulation of the respiration of the ovary become negative after pollination (Figs. 2b<sub>1</sub>–2d<sub>1</sub>). Thus the electrophysiological response of pistils to pollen seems to be common for *Incarvillea*, *Lilium* and *Zea mays*<sup>12</sup>. Differences are concerned only with the specific stigma sensitivity of *Incarvillea*.

Thus there is a relation with time of the generation of spreading potentials  $AP_1$  and  $AP_2$  with the reciprocal physiological reaction some distance from the place of irritation and also with the absence of such a reaction when spreading potentials are blocked. This supports the function of  $AP_1$  and  $AP_2$  as a signal.

$AP_1$  appears as a result of pressure or mechanical irritation of the sensory cells of the stigma and activates motor cells at the junction of the stigma lobes which cause the stigma to close.  $AP_1$  is localized in the stigma.

$AP_2$  appears in the stigma first as a result of chemical action of the pollen secretion (it is interesting that rest potentials became negative and  $AP_2$  developed when solutions of 2,4-D and IAA were applied to the stigma).  $AP_2$  spreads down the column and signals to the ovary that pollen is on the stigma. The ovary responds to this signal by changing its metabolism, which is probably the beginning of the preparation of the female reproductive area for fertilization. This is usually expressed in an increase of respiration. In other investigations intensification of the flow of phosphorus-32 to the ovaries has been found soon after pollination<sup>1,3</sup> and restoration of Feulgen reaction in the egg cells before pollen tubes reach the ovaries has also been found<sup>13</sup>. The physiological significance of such reactions is most clearly expressed in some orchids and trees in which the development of the embryo sac and the ovules begins only after pollination and goes on for many weeks and months simultaneously with the growth of pollen tubes in conductive tissues of the pistil.

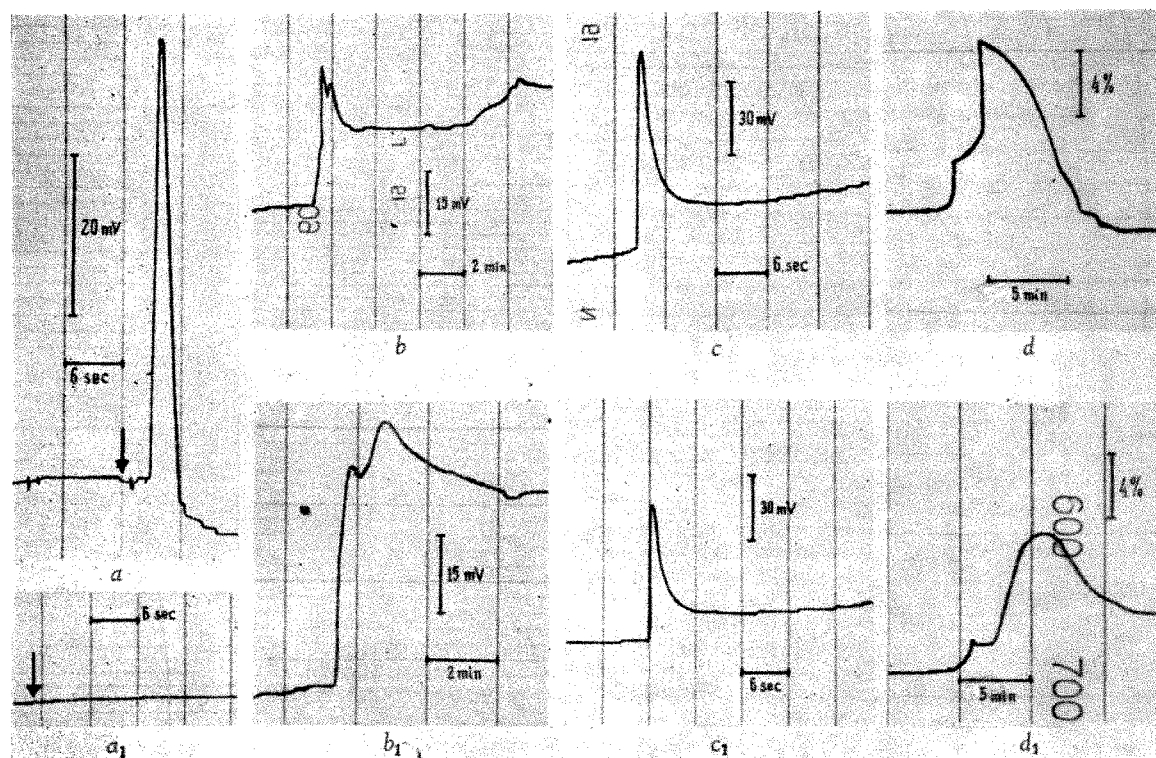


Fig. 2. Potentiograms of potential and oxygen consumption by pistils. Upper row: pistils of *Incarvillea*: (a)  $AP_1$  action potential in stigma in response to mechanical irritation; (b) negatization of rest potential in style; (c)  $AP_2$  action potential in response to application of pollen; (d) primary reversible stimulation of oxygen consumption under the influence of  $AP_2$ . Lower row: pistils of *Lilium*: (a<sub>1</sub>) absence of  $AP_1$  in response to mechanical irritation; (b<sub>1</sub>) negatization of rest potential; (c<sub>1</sub>)  $AP_2$  action potential in response to pollen application; (d<sub>1</sub>) primary reversible stimulation of oxygen consumption under the influence of  $AP_2$ . Arrows: the beginning of mechanical irritation.



Our data may explain the observations<sup>14</sup> that the stigmas of sensitive plants may close because of the action of an electric current (which is analogous to our AP<sub>1</sub> action) or that the cutting and maceration of the unpollinated style have no immediate effect on the closing of the stigma (in our opinion, because of its non-conductivity).

The ability of tissues of the column of *Incarvillea* to conduct one action potential and to block the other shows how useful reciprocal reactions during pollination have evolved. Ovaries are protected from the necessity to respond to an incidental mechanical irritation of stigmas, including irritation caused by insects bringing no pollen.

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### Simple Immunological Method for the Diagnosis of Pregnancy in Mares

THE diagnosis of pregnancy in women has been routinely effected by utilizing the reaction in which human chorionic gonadotrophin (HCG) inhibits the agglutination of HGC-coated red blood cells<sup>1,2</sup> or latex particles<sup>3</sup>. The same method was then used for the assay of pregnant mare serum gonadotrophin (PMSG)<sup>4</sup> and it was shown that PMSG in the sera of pregnant mares was detectable after about day 44 of pregnancy, increased to a maximum around the seventieth day as reported earlier<sup>5</sup> and had decreased to small concentrations by day 120. Similar experiments done by other workers seem to have been less successful<sup>6-8</sup> chiefly because mare sera cause variable degrees of non-specific agglutination even after extraction with acetone or ethanol.

It has also been shown that there is in PMS during the relevant period of gestation an  $\alpha_2$ -globulin soluble in perchloric acid which precipitates with rabbit serum anti-PMSG that has been absorbed with non-pregnant mare serum (NMS)<sup>7-9</sup>. This could provide a method for pregnancy diagnosis, but King<sup>10</sup> had difficulty in producing rabbit antisera to PMSG. He found that some antisera failed to precipitate with his preparations of PMSG after absorption with NMS; others precipitated with PMS but the amount of precipitate did not correlate with the PMSG as measured by its biological activity, and the precipitin reactions were too feeble to be used for pregnancy diagnosis.

It seemed reasonable to suppose that hormones such as PMSG would not be very antigenic in other mammalian species and, because all the existing preparations contained other proteins, it would not be easy to make the antisera specific for the hormone by absorbing them with NMS. On the other hand, injection of PMSG into birds

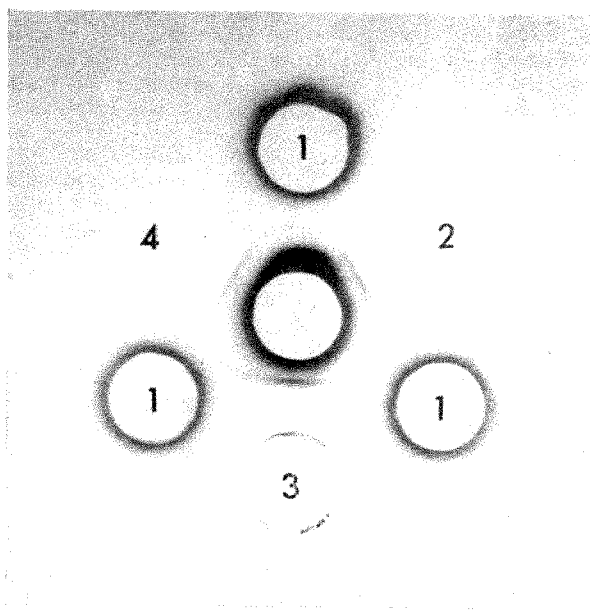


Fig. 1. Reactions in agar gel of turkey serum anti-PMSG (absorbed with an equal volume of NMS) with (1) PMS (day 63 of pregnancy), (2) PMSG (1,000 IU/ml., 'Organon'), (3) Endometrial cup secretion and (4) PMSG (1,000 IU/ml., Leo Pharmaceuticals). 1.5 per cent agar containing 1.5 molar sodium chloride, 0.04 molar phosphate buffer pH 7.0, 0.01 molar sodium azide.

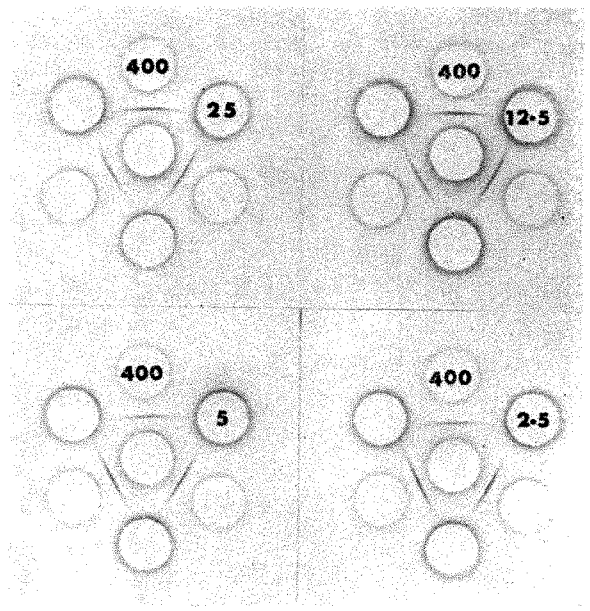


Fig. 2. Reactions of absorbed turkey serum anti-PMSG with NMS to which has been added PMSG to the concentrations (in IU/ml.) shown. This polygon-plate method permits detection of PMSG at concentrations as low as 2.5 IU/ml.

might be expected to give antisera in which the ratio of anti-PMSG to antibodies to impurity antigens would be much greater than was found with mammals.

Accordingly, commercially available PMSG and PMSG derived from endometrial cup secretion (DEAE-2 and upper phase fractions prepared by Dr W. R. Butt<sup>11</sup>) were mixed with Freund's complete adjuvant and injected, subcutaneously into turkeys. After 2 months their sera contained useful amounts of precipitating antibodies and the antisera have improved several hundred-fold in the intervening 18 months. The commercial PMSG (presumably prepared from PMS) was more satisfactory than that derived from endometrial cup secretion in that the latter

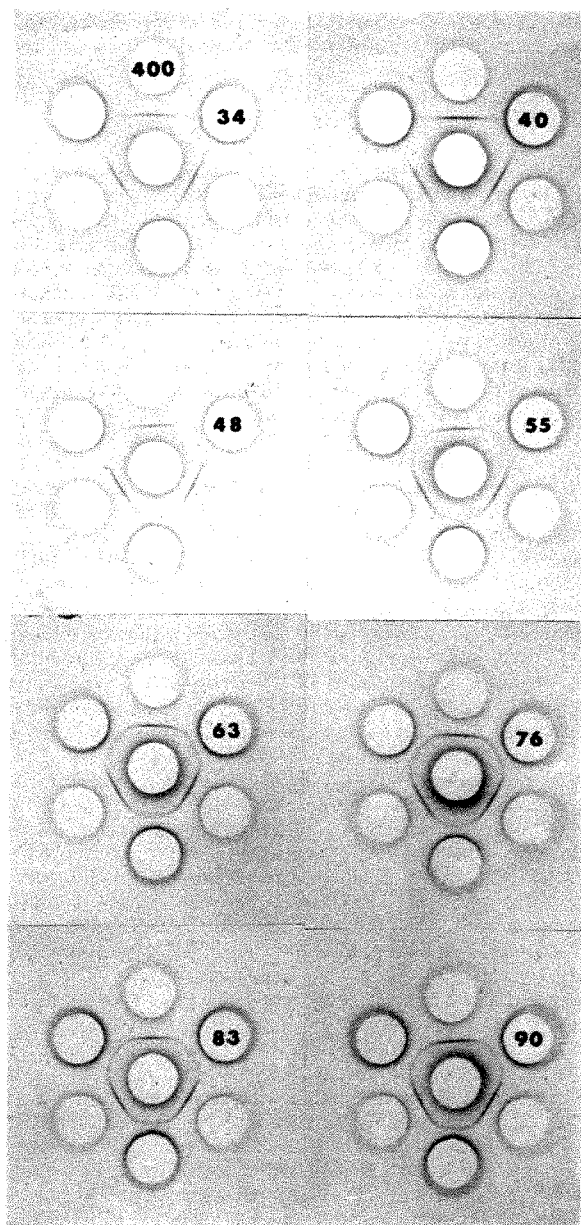


Fig. 3. Pregnancy diagnosis using the polygon-plate method. In each set of wells alternate peripheral wells contained sera obtained from a pregnant mare on days 34, 40, 48, 55, 63, 76, 83, and 90. PMSG was clearly present on day 48 and later.

caused the production of a greater proportion of antibodies to impurity antigens.

Absorption of the antisera (twenty volumes) with NMS (one volume) removed antibodies to all but two components of PMS. Absorption using equal volumes of antisera and NMS was necessary to remove antibodies to the second component. Fig. 1 shows that only one precipitation line is formed with PMS, commercial PMSG and endometrial cup secretion, the reaction being one of complete identity throughout.

At very low concentrations of the antigen the intensity of the precipitation line in Ouchterlony plates is too low for it to be detected either before or after staining with amido schwarz. It is, however, possible to detect as little as 2.5 IU of PMSG/ml. of solution by the use of the polygon-plate method of analysis<sup>12</sup>. Fig. 2 shows an experiment in which alternate peripheral wells have been filled with NMS to which PMSG has been added to give a final concentration of 400 IU of PMSG/ml. These give rise to strong

precipitation lines with the absorbed antiserum in the central wells. The remaining peripheral wells have been filled with NMS having 25, 12.5, 5 or 2.5 IU PMSG/ml. Clearly the presence of antigen at a concentration of 25 IU/ml. in the intervening wells has caused a marked deviation of the principal lines. At 2.5 IU/ml. there is almost no deviation of the lines, but there is just sufficient to be detectable.

This technique has been applied to the detection of PMSG in mare sera for the purposes of pregnancy diagnosis. Fig. 3 shows the results obtained with sera from a typical pregnant mare. In this instance the presence of PMSG is very evident at day 49 of pregnancy and its concentration is maximal between 63 and 76 days. In one case PMSG could just be detected as early as day 39. Of fifteen mares in which pregnancy was later confirmed the presence of PMSG in the serum was detected in all before day 50. When using the fully absorbed antiserum no false positives have so far been obtained with sera from stallions and non-pregnant mares.

This method is extremely simple in practice. The preparation of the agar gel-plates was similar to one described elsewhere<sup>13</sup> except that the solvent contained 1.5 molar sodium chloride instead of the usual 0.15 molar sodium chloride. This is necessary because turkey antisera precipitate maximally with the antigens at the higher salt concentration.

In general the detection of antigen by the method of inhibition of haemagglutination (HI test) is more sensitive than the precipitin test and an attempt was made to apply it in this case in the hope of detecting pregnancy a few days earlier. But at present the HI test is slightly less sensitive than the precipitin test and much less reliable. Probably very little can be gained by increasing the sensitivity of the test below the level of 2.5 IU PMSG/ml. of serum from the point of view of pregnancy diagnosis, but it would be useful for fundamental research on the production of PMSG by the endometrial cup.

Subsequently turkeys have been used for the production of antisera to a number of other mammalian hormones with much success. Samples of the absorbed and freeze-dried turkey serum anti-PMSG are available on application.

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## GENETICS

### Induction of Genetically Recombinant Chromosomes in the Absence of Induced Mutation

GENETICALLY recombinant chromosomes can be induced (at a low frequency) in the *Drosophila melanogaster* male, although crossing-over does not usually occur in the male. The agents which have been shown to induce crossing-over are all mutagens (for example, formaldehyde<sup>1-3</sup>, diethyl sulphate<sup>4</sup>, nitrogen mustard<sup>5</sup>, dihydroxy-

dimethylperoxide<sup>6</sup>, X-rays<sup>7</sup> and  $\gamma$ -rays<sup>8</sup>). It is therefore difficult in general to exclude the loss of a coupled genetic marker by mutation or by chromosomal deletion over one (or more) of the genetic markers. Usually, the latter event is operationally defined by lethality of the homozygote for the apparently recombinant chromosome, although the simultaneous presence of an independent recessive lethal mutation cannot necessarily be ruled out.

A study of induced crossing-over should thus ideally be carried out with an agent which is either not a mutagen, or else a mutagen which is not mutagenic under readily definable conditions: the latter situation is fulfilled only for the case where formaldehyde is administered by the larval feeding method in *Drosophila* larvae. In fact, formaldehyde is the only mutagen for which a direct attempt has been made to study the mechanism of action of a mutagen in a multicellular organism, and for which there is direct experimental evidence for the mode of action.

The present paper reports on the induction of genetical recombination by formaldehyde in the *Drosophila* male under conditions where it is mutagenic, and under conditions where it is not mutagenic.

Mutations can be induced in *Drosophila melanogaster* by administering formaldehyde by two different methods: injection of formaldehyde into the abdominal cavity of the adult male, or the addition of formaldehyde to the culture medium on which larvae are allowed to develop. Results obtained by these two methods of administration suggest that formaldehyde is capable of producing mutations by two qualitatively different mechanisms.

Following adult injection, it has been suggested that formaldehyde exerts its mutagenic effect by the formation *in vivo* of an organic peroxide, dihydroxydimethylperoxide<sup>9</sup>: when injected into the adult male, this peroxide behaves as a mutagen with much the same behaviour as formaldehyde in preferentially affecting mature and almost mature spermatozoa, and spermatogonial germ cell stages<sup>9</sup>.

By the larval feeding method, it has been shown that formaldehyde exhibits no mutagenic activity towards *Drosophila melanogaster* larvae unless a source of adenylic acid (or adenosine) is present in the treatment medium<sup>10</sup>. Adenylic acid may be present either as the free monoribonucleotide (-3' or -5' isomers) or bound in the (yeast) RNA polynucleotide<sup>11</sup>, but its presence in the treatment medium is essential for the mediation of the mutagenic activity of formaldehyde (as measured by the frequency of sex-linked and second chromosome recessive lethal mutations). Because the chemical binding of formaldehyde by RNA and its individual constituent nucleotides appears to be a specific function of the amino groups of the bases, reaction(s) *in vitro* (in the treatment medium) at the extranuclear 6-amino group of adenylic acid (or adenosine) has been postulated as forming an essential metabolite(s) concerned in the mediation of the mutagenic activity of formaldehyde<sup>10</sup>. This hypothesis is supported by the observation<sup>12</sup> that the replacement of the 6-amino-group of adenylic acid (or adenosine) by a hydroxyl group, as in inosinic acid (or inosine), completely removes the ability of these compounds to mediate the mutagenic activity of formaldehyde, despite the apparently similar utilization of both these adenine and hypoxanthine derivatives by *Drosophila* larvae. Reaction of different concentrations of formaldehyde with a standard concentration of adenylic acid (adenosine-5'-phosphate) before addition to the treatment medium has shown that the monohydroxymethylation of adenosine-5'-phosphate by formaldehyde produces an effective mutagenic derivative, whereas the dihydroxymethylated derivative of adenosine-5'-phosphate does not appear to be mutagenic<sup>13</sup>. Similar experiments where yeast RNA was reacted with formaldehyde, and dialysed free of unbound formaldehyde, have shown that here, too, the monohydroxymethylation reaction of formaldehyde with RNA produces an effective mutagenic derivative(s)<sup>13</sup>.

Because free formaldehyde is not present in the treatment medium in the formaldehyde-treated RNA experiments, it would appear that the monohydroxymethylation reaction of formaldehyde with RNA (and adenylic acid) is in itself both a necessary and sufficient condition for the mediation of the mutagenic activity of formaldehyde. Recent experiments, as yet unpublished, have further shown that whereas adenosine-5'-phosphate is a mediator of formaldehyde-induced mutagenesis, its deoxyribose derivative (deoxyadenosine-5'-phosphate) is not effective: this result suggests that formaldehyde-induced mutagenesis is mediated by way of RNA synthesis. Observations on the patterns of mutational response of larval germ cells (primary spermatocytes, and spermatogonia) indicate that the sensitive period for the activity of formaldehyde is restricted to the auxocyte stage in primary spermatocyte development<sup>14</sup>, that is, the protracted growth stage which precedes meiosis in the larval testis: spermatogonia are not affected, unlike the situation in the adult testis when free formaldehyde is injected into the adult male. Free formaldehyde is not found to be mutagenic when injected into *Drosophila* larvae<sup>15</sup>.

On the basis of these observations, I decided to use the *Drosophila* larval feeding method to test previously reported findings<sup>2,3</sup> (using this method), that formaldehyde is capable of inducing genetically recombinant chromosomes in the *Drosophila* male. Because it is known that the presence of a source of adenylic acid in the larval treatment medium is essential for the mediation of the mutagenic activity of formaldehyde, experiments on the induction of recombinant chromosomes from the *Drosophila* male were carried out under conditions where formaldehyde is mutagenic (adenylic acid present), and under conditions where formaldehyde is not mutagenic (adenylic acid replaced by inosinic acid); the frequencies of sex linked recessive lethal mutations were determined to verify that the required conditions were attained. Larvae which were heterozygous for the coupled second chromosome markers, dumpy wing (*dp*), cinnabar eyes

(*cn*), and brown eyes (*bw*) ( $\frac{+dp+cn+bw}{dp\ cn\ bw}$ ) were treated

for the entire larval life, at a density of 100 larvae/25 ml. of medium, on a formaldehyde-containing ( $1.4 \times 10^{-2}$  molar) chemically defined and axenic medium<sup>16</sup> which contained no source of purines or pyrimidines other than the ones indicated. Because the *Drosophila* Oregon-K wild type stock used to construct the hybrid has a partial dietary requirement for cytidine, however, 0.025 per cent cytidine-3'-phosphate was always present in the treatment media. Males hatching from each treatment medium (and the control) were divided into two batches; males from one batch were individually mated to two dumpy, cinnabar, brown females for 3 days for the detection of recombinant chromosomes, and males from the other batch were individually mated to two Muller-5 females for 3 days for the estimation of sex-linked recessive lethal mutations<sup>17</sup>.

Table 1 illustrates the results obtained when larvae were cultured on the chemically defined medium (plus adenylic acid, or inosinic acid) in the absence of formaldehyde (data combined for the control), on the medium containing formaldehyde and adenylic acid (0.1 per cent), and on the medium containing formaldehyde and inosinic acid (0.1 per cent). Recombinant chromosomes are seen to be induced by formaldehyde both under conditions where formaldehyde is mutagenic (formaldehyde + adenylic acid) and under conditions where formaldehyde is not mutagenic (formaldehyde + inosinic acid): no recombinant chromosomes occur in the progenies of the control males. All tested recombinants were found to be viable when homozygous. Some evidence for a differential effect on one region of the chromosome is suggested by the greater frequency of the cinnabar, brown recombinants compared with the dumpy, cinnabar re-

		ADENYLIC ACID		Total offspring (n) and recombinants (r)		Percent
Treatment	Non-lethal/lethal chromosomes	Per cent lethals	n	r	r	
Control + 0.1 per cent rA5'-P or 0.1 per cent rH5'-P	1,790/2 (derived from 182 males)	0.10	8,064 (progeny from 324 individual males)	—	—	0.0
Formaldehyde + 0.1 per cent rA5'-P	540/26 (derived from 130 males)	4.80	5,590 (progeny from 1,128 individual males)	bw dp cn cn bw cn bw	—	0.07
Formaldehyde + 0.1 per cent rH5'-P	1,260/2 (derived from 146 males)	0.16	17,575 (progeny from 1,125 individual males)	bw bw dp cn bw cn bw cn bw cn bw * cn bw cn bw * cn bw cn bw * cn bw cn bw dp cn dp cn	—	0.07

Per cent lethals, percentage of sex-linked recessive lethal mutations. rA5'-P, Adenine ribonucleoside-5'-phosphate (adenylic acid). rH5'-P, Hypoxanthine ribonucleoside-5'-phosphate (inosinic acid).

The present technique of sampling one 3-day brood from males treated with formaldehyde from the beginning of larval life recovers larval germ cells which were present predominantly as primary spermatocytes at the time of treatment; the primary spermatocyte has been shown to be the germ cell stage to which the mutagenic activity of

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BIOCHEMICAL studies have shown that aryl sulphatase is concentrated in lysosomal fractions of liver<sup>1,2</sup>. This can now be confirmed cytochemically because hydrolytic enzymes other than acid phosphatase can be identified at the ultrastructural level. Histochemical methods of producing electron-dense enzyme reaction product at the sites of localization of *E*.600-resistant esterase<sup>3</sup>, and aryl sulphatase<sup>3</sup>, will provide important new information on the rapidly enlarging concept of lysosomes<sup>4,5</sup>. de Duve<sup>6</sup> stressed the importance of developing these cytochemical



methods for a wide spectrum of lysosomal enzymes. It is to be hoped that these may to some extent augment acid phosphatase as lysosomal markers.

Utilizing *p*-nitrocatechol sulphate as a substrate<sup>7</sup> buffered at pH 5.2, and barium chloride as a capture reagent as suggested by Hugon and Borgers<sup>8</sup>, the ultrastructural localization of aryl sulphatase in Swiss albino mouse kidney and liver has been investigated. Frozen sections (50 $\mu$ ) from tissue blocks fixed in 3 per cent buffered glutaraldehyde were incubated at 37° C for periods of up to 1 h. After fixation in buffered osmium<sup>9</sup> the sections were dehydrated and embedded in 'Araldite'. Thin sections were examined electron microscopically, initially without any contrast enhancement. After identification of the sites of barium sulphate localization, the thin sections were stained with lead citrate to enhance the photographic image. Correlated light microscope 15 $\mu$  sections were examined after converting the reaction product to the sulphide as in the Gomori methods for acid phosphatase<sup>10</sup>.

A moderate concentration of enzyme reaction product was observed overlying organelles with the morphological characteristics of lysosomes<sup>11</sup> (Fig. 1). There is good correspondence between the form of organelles stained by methods for acid phosphatase<sup>12</sup> and those observed in this study. The enzyme reaction product which in this case appears to represent sites of aryl sulphatase type B (ref. 13) has a similar lysosome-specific localization, in liver sections after 25–30 min incubation. There was no artefactual staining of nuclei as is often the case with acid phosphatase methods utilizing beta-glycerophosphate as a substrate.

The distribution of aryl sulphatase seems to be heterogeneous in nature. The reaction product does not appear over all the organelles which correspond to the accepted morphological appearance of subcellular bodies belonging to the lysosome family. Serial sections were examined to ensure that the reaction product was not concentrated in areas of the organelles which did not appear in any particular section. It is not possible to state at present whether this heterogeneity of distribution indicates a true differential localization of lysosomal enzymes or is caused by specific inhibition of particular enzymes. The differential distribution of small quantities of enzymes is of course difficult to detect by these insensitive histochemical methods. Hayashi<sup>15</sup> has, however, demonstrated

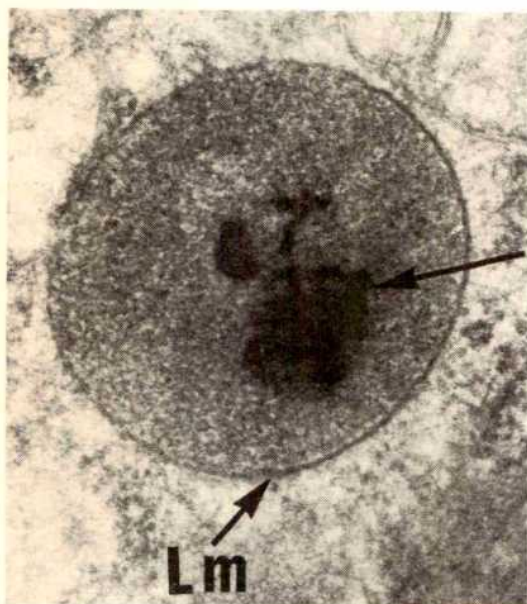


Fig. 1. Electron micrograph of a lysosome in mouse kidney proximal tubule cell.  $\leftarrow$  indicates enzyme reaction product of aryl sulphatase technique after incubation for 30 min. Lm, lysosomal membrane. ( $\times 63,000$ .)

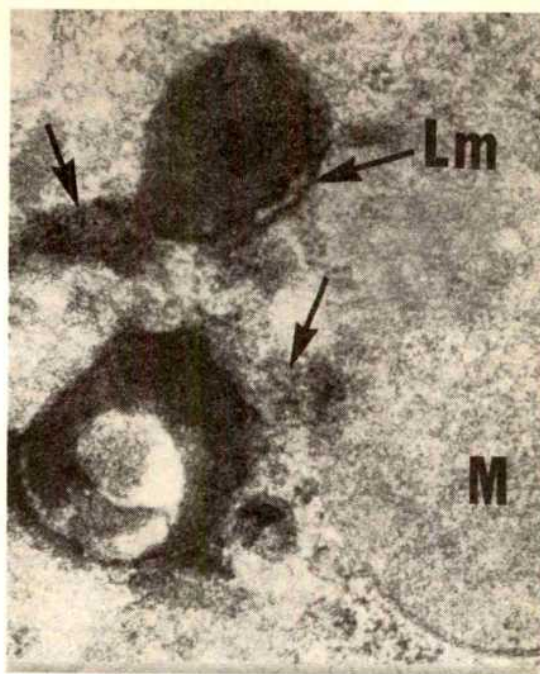


Fig. 2. Kidney lysosomes after 45 min incubation. Barium sulphate is concentrated over the bodies of the organelles.  $\leftarrow$ , released enzyme reaction product. M, mitochondrion; Lm, lysosomal membrane. ( $\times 52,500$ .)

qualitatively different distributions of beta-glucuronidase, N-acetyl-beta-glucosaminidase and acid phosphatase activity in various tissues of the rat. Interpretation of the results suggests that a functional differentiation among lysosomes in various tissues may exist.

The restricting nature of the single membrane of lysosomal bodies is observed in sections that are incubated for periods of 1 h. The enzyme reaction product was observed in the cytoplasm surrounding the ruptured organelles (Fig. 2). Incubation in the acid medium at 37° C eventually disrupts the membrane, thus releasing the enzymes and producing the artefactual staining. The reaction product often appears to be concentrated near the lysosomal membrane in such over-incubated sections. Control sections incubated in medium free of substrate showed no reaction product even after incubation for 90 min. Further investigations are required regarding the heterogeneity of distribution of the various lysosomal enzymes as well as the nature of substrates on which the enzymes act *in vivo*.

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## MICROBIOLOGY

Aggregation in a Thermophilic *Oscillatoria*

*Oscillatoria terebriformis* (Ag.) Gom., isolated from Hunter's Hot Springs, Lakeview, Oregon, shows both gliding and flexional movements of individual trichomes. Laboratory clone populations of trichomes, when dispersed by agitation, rapidly aggregate in still liquid medium. If the population density is high enough aggregation produces a single dense ball or clump. The clump may be redispersed by swirling or shaking. Fig. 1 demonstrates the rapidity of the aggregation phenomenon at an optimal temperature of 47° C. Growth in an unagitated flask takes place with essentially all of the trichomes in a single ball. At Hunter's Hot Springs and other Oregon hot springs this species forms dark brown mats up to about 2 mm in thickness from 53° C to about 47° C, which corresponds closely to the optimal growth temperature range in the laboratory. Growth medium consisted of the following (1 l. of distilled water): 0.1 g of nitrilotriacetic acid; 1.0 ml. of trace element solution (1 l. of water, 0.5 ml. of concentrated sulphuric acid, 2.28 g of manganese sulphate, 0.5 g of zinc sulphate, 0.5 g of boric acid, 0.025 g of copper sulphate, 0.025 g of sodium molybdate, 0.045 g of cobalt chloride); 0.2 mg of ferric chloride; 0.06 g of calcium sulphate; 0.1 g of magnesium sulphate; 0.008 g of sodium chloride, 0.103 g of potassium nitrate, 0.689 g of sodium nitrate; and 0.111 g of sodium phosphate. The pH was adjusted to 8.2 with sodium hydroxide, and was about 7.5 after autoclaving. Field material, when removed by suction and dispersed in a vessel, also aggregates rapidly, as in the laboratory cultures.

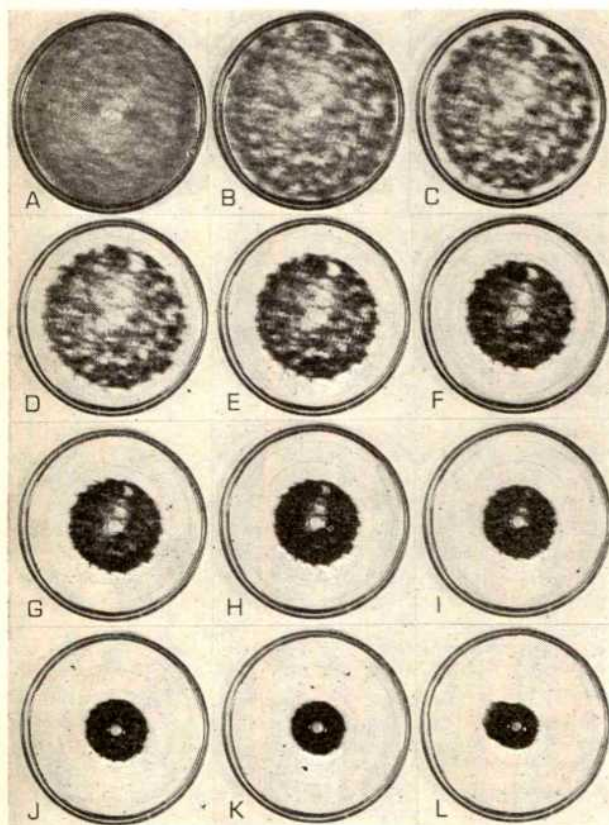


Fig. 1. The aggregation of *O. terebriformis* at 47° C and 1,000 lumens/ft.<sup>2</sup> in a glass Petri plate with a radius of 4.5 cm, containing 20 ml. of culture medium. (A) Immediately after dispersal; (B) 10 sec after dispersal; (C) 20 sec; (D) 30 sec; (E) 40 sec; (F) 50 sec; (G) 60 sec; (H) 70 sec; (I) 90 sec; (J) 130 sec; (K) 4 min; and (L) 30 min. In the last frame streamers are beginning to radiate from the central mass.

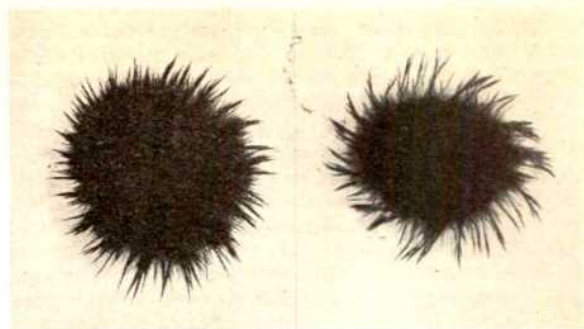


Fig. 2. Two aggregated masses of *O. terebriformis* in unagitated liquid culture medium with typical streamers of roped trichomes extending from the peripheries. The diameter of each mass is approximately 2 cm.

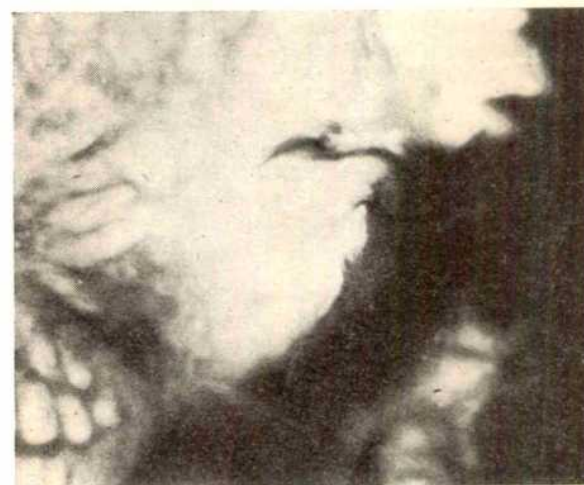


Fig. 3. A mat of *O. terebriformis* overlying and bordering a mat of a species of *Synechococcus* in Hunter's Hot Springs, Lakeview, Oregon. Tufts and streamers of trichomes may be seen extending from the edge of the dark *Oscillatoria* mat. The length of the field pictured is approximately 6 cm.

The mechanism of aggregation is now being investigated, but is still not well understood. It appears to be a result, first, of strong flexional movements of the trichomes, already reported for this species<sup>1</sup>. When trichomes contact they glide against each other and coil. The rope like strands formed continue to flex and undergo contortions and apparent contractions. In the case of high trichome density, the movements result within a few seconds in a single interwoven network which contracts evenly into a single clump. With a lower density of dispersed trichomes the network formed is irregular. At very low densities numerous small clumps form. Reducing the length of the trichome with a blender also results in numerous small clumps. After the aggregation of trichomes of normal length has been completed, the dense mass gradually "sends out" streamers of roped trichomes around the periphery (Fig. 2). Such streamers are also quite apparent at the edges of the *Oscillatoria* mat in the hot springs (Fig. 3).

The aggregation phenomenon seems to be a result chiefly of undirected flexional and contractional or torsional movements<sup>2</sup>, and there is no evidence of chemotactic attractions. There is more than microscopical evidence to show that the motility of individual trichomes is required for aggregation. *Oscillatoria* can be killed by various methods which do not cause immediate cell lysis, but which do stop individual motility and aggregation. A weak IKI solution or 4 per cent formalin was sufficient. Momentary freezing or drying and subsequent rewetting were also effective. A reversible inhibition of motility and aggregation was caused by solutions of sodium

chloride of more than 0.3 per cent and by pH below about 6.5. The aggregation rates were normal for a time in distilled water, but declined in medium of increased viscosity. Nevertheless, the process still occurred slowly in a methyl cellulose solution of 6.7 g/l. A 0.2 per cent solution of the detergent 'Tween 80' had no apparent effect on aggregation or redispersal.

Preliminary experiments have shown that the addition of chelating agents (for example, 0.5 mmolar EGTA) stops gliding and flexional movements, as well as aggregation. Complete activity was restored by the addition of calcium ions, but not by magnesium or sodium ions. These results suggest that a protein contractility phenomenon may be involved in all types of *Oscillatoria* movements<sup>3</sup>.

The rate of aggregation was temperature dependent below about 37°C and was saturated to about 52°C, above which it declined steeply to the lethal temperature of 54–55°C. Exponential phase cultures grown in the light aggregated in both light and darkness above about 27°C, but cultures which had been starved of light for 24 h or more at 45°C showed no movement in the dark. A subsequent exposure to light resulted in an aggregative response within a few minutes. The dark aggregation of normal light grown cultures was stopped almost immediately by  $2 \times 10^{-4}$  molar 2,4-dinitrophenol (DNP), but the rate in the light was still substantial. It appears that adenosine triphosphate is available as an energy source for motility from both photo- and oxidative-phosphorylations.

Recently, a mutant lacking flexional and gliding motility has been recovered from the normal clone culture. This material shows no ability to aggregate.

The freedom of trichomes to disperse with agitation depends on the presence of one or more species of bacteria during the growth of the culture. In axenic cultures, a large quantity of excreted gel like material accumulates in the centre of the growing clump; this tends to bind at least portions of most of the trichomes. This material is apparently metabolized as rapidly as it is released in bacterized cultures and in nature.

In this species of filamentous blue-green alga the ability to aggregate seems to have a clear adaptive significance. In hot spring drainways most of the blue-green algae hold their position in the flowing water by forming mats in which the trichomes or cells are bound together by gel like substances (unpublished work of T. D. Brock). In the case of thermophilic *Oscillatoria terebriformis* the integrity of the dense mat is maintained principally by the coiled and interwoven disposition of the trichomes. The photosynthetic thermophiles occupy only the few surface millimetres and are underlain by gelatinous layers of filamentous heterotrophic organisms. *O. terebriformis* completely dominates the surface of this undermat in its optimal growth temperature range, and so it is believed that the non-mucilaginous nature of the *Oscillatoria* layer and the movements of the trichomes provide a very poor substrate for the colonization and growth of other thermophilic species which could potentially occupy the same temperature range<sup>4</sup>.

Field observations also indicate an adaptive value for gliding movements in this *Oscillatoria*. When flow patterns of the thermal stream shift, resulting in temperature changes at various points in the drainway, *Oscillatoria* is able to rapidly colonize the gelatinous mat composed of other more sedentary species if the 53°–54°C boundary is extended away from its edge. On the other hand, if the temperature at the edge of the mat is gradually increased, the mat seems to be able to retract towards lower temperature without the mass mortality that would result from extended exposures to temperatures over 54°C. When patches of mat in the optimal temperature range were cut out and removed from the underlying sand-gravel substrate, re-invasions by migrating *Oscillatoria* from the edges occurred at the rate of up to 1 cm/h.

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### Significance of the Characteristic Chemical Pattern of Gram Positive and Gram Negative Bacterial Cell Walls

GRAM positive and Gram negative bacteria are known to have different and characteristic patterns of chemical composition of their cell walls. Gram positives usually have a relatively small number of amino-acids and Gram negatives have a much larger number<sup>1</sup>. Some Gram positive genera are characterized by the possession of a specific sugar, whereas Gram negatives are not<sup>2</sup>, and they also have fewer amino-sugars and more lipids than Gram positive walls<sup>1</sup>.

These facts have been widely interpreted as implying that there is a phylogenetic significance in these compositions, but it is common experience that single strains of bacteria may have variants of both staining types. In this laboratory, it has been observed that Gram positive variants of *Haemophilus* have the Gram positive pattern of cell wall composition, whereas the original strains are Gram negative in both composition and staining reaction. Similarly, it has been pointed out that *Neisseria* species have the Gram negative cell wall<sup>3</sup>, whereas they are usually regarded as related to other cocci, most of which are Gram positive, and may themselves be variable.

Thus the conclusion that this taxonomy provides conclusive evidence of bacterial affiliations is badly at variance with other available evidence, and we suggest two explanations to resolve this anomaly. The first is that the Gram negative pattern may be caused in part by blocked synthesis of the Gram positive pattern. The second is that the differences may be accentuated by experimental procedures.

In a recent investigation, one Gram positive strain of *Corynebacterium cervicis* was found to contain 6-deoxytalose, rhamnose, mannose, glucose and galactose in its cell walls, whereas a Gram negative variant had mannose, glucose and galactose only; but the glucose was in significantly greater quantity. This is explicable as a blockage of rhamnose synthesis at glucose<sup>4,5</sup>. It has also been shown<sup>6</sup> that 6-deoxytalose is a hydrolysis product of rhamnose, and presumably the same blockage caused the loss of this also. Thus the original pattern, which is significantly like that of *Actinomyces bovis*, is lost in the variant. Similarly, lysine and DAP rarely occur in comparable quantities together in Gram positives<sup>2</sup>, but frequently do so (in our experience) in Gram negatives. DAP has been shown to be a precursor of lysine<sup>7,8</sup>, so that this difference may also be explained by blockage of synthesis. Further similar illustrations could be adduced from work inside and outside this laboratory, especially where loss of Gram positivity and blockage of synthesis are simultaneously produced by the presence of penicillin. For example, glucosamine-6-phosphate accumulated in a penicillin-induced variant of *Haemophilus influenzae*, replacing glucosamine that was present in the original strain, the former being a precursor of the latter<sup>9</sup>.

The second explanation is that where analyses are conducted by chromatography, adjustment of the technique to detect the larger quantities of a smaller range of



amino-acids in Gram positives may result in the smaller quantities of other, supposedly atypical, amino-acids failing to register. Where we compared the results of thin-layer chromatography with those given on the same extracts by a Technicon auto-analyser, it was found that only four major and six minor amino-acid components could be detected by chromatography in the cell wall of a group *D Streptococcus*, whereas twenty-two minor components were found by the autoanalyser, and these were those supposedly characteristic of Gram negatives, and absent from Gram positives. In this case, very careful control of the purity of the cell wall preparations, before hydrolysis, was made by the electron microscope, to avoid the risk of contamination by residual cytoplasm.

Thus the chief difference between Gram positive and Gram negative cell wall composition may prove to be the balance between the precursors and their synthetic products; the former being present in smaller proportions, even in the Gram positive wall.

It also seems that the theory of degenerative evolution of some Gram negative bacteria, from Gram positive ancestors, advanced by Bisset<sup>10</sup>, is not necessarily contradicted (as has been suggested<sup>3</sup>) by the evidence of cell wall analysis.

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### Purification and Concentration of Influenza Virus by Auto-aggregation

THE suggestion that influenza virus tends to aggregate was based on the assumption that the increase in titre of suspensions treated with trypsin is due to disaggregation<sup>1</sup>. Precipitation of partially purified influenza virus by dilution with distilled water has previously been observed<sup>2</sup>.

During the dialysis of allantoic fluid containing influenza A virus we noticed a precipitate. After separation the haemagglutinin was confined to the precipitate while the supernatant was almost free from haemagglutinin. To investigate this phenomenon further a standard test was developed. All the virus suspensions used were first centrifuged at 1,100*g* for 10 min and then dialysed against 50 volumes of tap or distilled water at 37° C. Separate specimens were collected at hourly intervals and again centrifuged at 1,100*g* for 10 min. Supernatants and deposits were then assayed for virus activity, protein content and electrical conductivity. The results of the standard test with fresh allantoic fluid suspensions of influenza B are shown in Table 1.

An increase in virus activity in the deposit with loss of protein and electrolytes in the supernatant is evident. The protein purification factor is significant and compares favourably with results from other methods<sup>3-5</sup>. Similar results were obtained with influenza A allantoic fluids, influenza C amniotic fluids and calf kidney tissue culture fluids of influenza B. In a standard test, Newcastle disease virus (Herts strain), parainfluenza 1 (laboratory strain), bovine parainfluenza 3 (Frant strain), adenovirus 11, echovirus 7 and vaccinia (Jenner) showed no increase of haemagglutinin in the deposit.

Haemagglutinin tests were performed by the Takátsy microtitration method<sup>6</sup> with red blood cells appropriate to the virus tested. Infectivity titres of influenza virus were determined by haemadsorption on calf kidney cells.

The relatively low centrifugal force used to concentrate the influenza virus suggested aggregation, and this was confirmed by electron microscopy. Negatively stained specimens from deposits showed aggregates of closely packed particles with influenza morphology<sup>7</sup> up to several microns in size.

Aggregation under the conditions of the standard test was not prevented by specific antiserum, receptor destroying enzyme, inactivation by 1:10,000 formalin or by heating at 56° C for 30 min. Because of the unspecific character of the phenomenon the term "auto-aggregation" is suggested.

The amount of aggregation is inversely proportional to the molarity of the solutions against which dialysis is performed. This was established with caesium chloride, potassium chloride, potassium dihydrogen phosphate and sodium hydrogen phosphate. For example, in experiments where the molarities of sodium chloride and sucrose used for dialysis were varied by two-fold steps from 0.4 to 0.025, the haemagglutinin titre of the supernatant at the end of dialysis fell from 2,560 to 160 and that of the deposit rose from 320 to 1,280.

The pH of the solutions had no apparent effect over the range pH 5–pH 9, because when the virus suspension was dialysed against M/15 Sørensen phosphate buffers over this range it gave the same haemagglutinin titres in the deposits. The temperature at which dialysis is performed is not critical because the same titres were obtained at 37° C, room temperature and 4° C.

Some aggregation takes place when influenza allantoic fluid is allowed to stand at room temperature for 4 h (Table 1). Addition of salts to a final concentration of 0.2 molar or greater prevents this aggregation. This effect has been used in this laboratory for stabilization of influenza virus suspensions.

The aggregates formed after dialysis are not easily dispersed even by vigorous shaking in saline. A four-fold increase in titre was found after treatment of the re-suspended material with 2 mg/ml. crystalline trypsin at pH 7.6 for 30 min at 37° C or with 1 per cent Difco trypsin.

The aggregation of influenza virus seems to be a property of the virus envelope for the soluble antigen which is a common factor of the whole influenza sub-group of myxoviruses did not aggregate under the conditions of the standard test. On the other hand, after disaggregation the infectivity seems to be preserved and corresponds to the haemagglutinin titre.

Table 1. ALLANTOIC FLUID INFECTED WITH INFLUENZA VIRUS B DIALYSED AGAINST 50 VOLUMES OF WATER

Time of dialysis at 37° C (h)	Haemagglutinin unit/ml.	Supernatant		Conductivity ( $\mu$ Mho/cm)	Haemagglutinin unit/ml.	Deposit Protein* (mg/ml.)	Unit/mg † protein
		Protein* (mg/ml.)	Units/mg † protein				
0	5,120	4.39	1,166	98 × 10 <sup>3</sup>	320	0.135	2,370
1	1,280	3.94	325	14 × 10 <sup>3</sup>	1,280	0.165	7,757
2	1,280	3.95	324	10.5 × 10 <sup>3</sup>	2,560	0.222	11,531
3	1,280	3.90	328	8.4 × 10 <sup>3</sup>	5,120	0.289	17,716
4	640	3.74	171	7.3 × 10 <sup>3</sup>	5,120	0.319	16,500
Control (4 h at 37° C not dialysed)	2,560	4.35	588	96 × 10 <sup>3</sup>	320	0.106	3,020

\* Protein determinations by the Folin phenol method<sup>4</sup>.

† Units of haemagglutinin/mg of protein (purification factor).



The phenomenon of auto-aggregation is eminently suited to be a simple and economical method of partial purification and concentration of influenza virus for vaccine production or an intermediate step in more demanding purification procedures. Auto-aggregation, because it is limited to the influenza sub-group, can also be used as a criterion in the classification of myxoviruses.

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## BIOCHEMISTRY

### Albumin Mexico, a New Variant of Serum Albumin

AN inherited variant of albumin (albumin Naskapi) has recently been described which has an electrophoretic mobility greater than that of common albumin (albumin A)<sup>1</sup>. This variant is relatively common in many North American Indian tribes, but it has not been found in the United States white and negro sera so far tested, nor in many European sera. We describe here a further variant which was discovered while examining a group of sera from Mexico. This variant moved slower than common albumin, but faster than the variant albumin B which has been described in several European and American families<sup>2</sup> (Fig. 1).

Starch gel electrophoresis was carried out with whole serum with Ashton and Braden's discontinuous buffer system at pH 8.6 (ref. 3). The gels were prepared from hydrolysed starch (Connaught) at a concentration 25 per cent greater than that recommended by the manufacturer. The electrophoresis was performed in a vertical

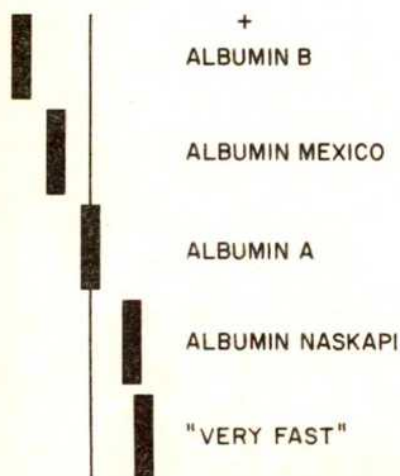


Fig. 1. Relative electrophoretic mobilities of albumin variants.

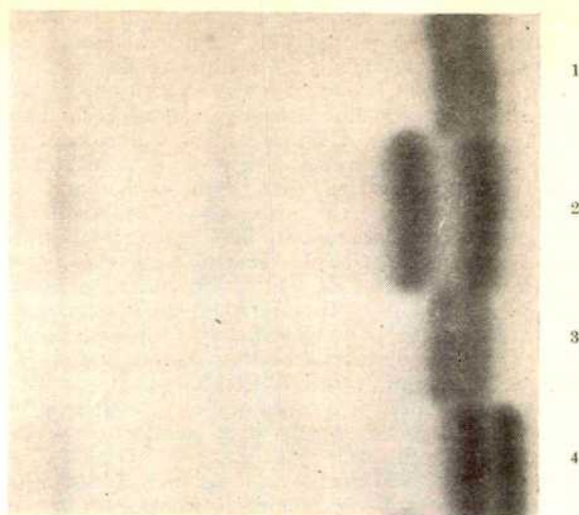


Fig. 2. Albumin variants, starch gel electrophoresis in Ashton and Braden's buffer. 1, A/Mexico; 2, A/B; 3, A/Mexico; 4, A/Naskapi.

system with a constant voltage of 9 V/cm and the run was continued until the "borate-line" had migrated 12–15 cm from the origin.

A total of 281 sera from Mexican mestizo communities were studied. In addition, 20 Mazatec (Huautla de Jimenez), 123 Zapotec (Guelatao de Jurez) and 263 Maya (Yucatan) Indians were studied, as well as other sera (Table 1). The populations are described elsewhere<sup>1,4,6</sup>. With few exceptions, the individuals sampled were not closely related. Sera from the family of an individual from Mexico City with the Mexican albumin variant were also studied.

Table 1. DISTRIBUTION OF ALBUMIN A/A (COMMON ALBUMIN) AND A/Me (COMMON ALBUMIN AND ALBUMIN MEXICO) IN MESTIZO AND MEXICAN INDIAN GROUPS

Population	Location	Total	A/Me
Maya	Yucatan	263	0
Mazatec	Huautla de Jimenez	20	0
Zapotec	Guelatao	123	1
Mestizo/Zapotec	Pochutla	36	1
Mestizo/Mixtec	San Pedro Mixtepec	20	0
Mestizo/Mixtec	Ometepec	20	0
Mestizo/Mixtec	Cuajinicuilapa	20	0
Mestizo	Tlaxcala and Hidalgo States and Mexico, D.F.	185	10

Albumin Mexico was not found in any of the following American Indian and Eskimo sera: Naskapi, Canada, 151; Montagnais, Canada, 112; Sioux, US, 160; Athabaskan, Alaska and Canada, 20; Tlingit, Alaska, 100; Haida, Canada, 365; Quechua, Peru, 100; Cashinahua, Peru, 92; Eskimos, Alaska, 443 (ref. 1).

Blood was collected by venipuncture and the serum separated by centrifugation. The Mexican sera had been stored at  $-25^{\circ}\text{C}$  for about 5 yr–1 yr when the electrophoresis experiments were carried out.

Twelve of the sera (excluding the family sera) contained a variant (in addition to the common albumin) the mobility of which was slower than common albumin. The variant will tentatively be referred to as albumin Mexico. One albumin Mexico variant was found among the Zapotec Indians from Guelatao and another from Pochutla where the Indian component of the population studied is also Zapotec. Ten of the albumin Mexico variants were found in the mestizo communities. None of the other sera tested had a variant with the same mobility as albumin Mexico. The albumin Mexico is shown in Fig. 2 where it is compared with albumin A (common albumin), albumin B and albumin Naskapi.

One parent and three of the sibs of the albumin Mexico propositus had albumin Mexico in addition to albumin A (Fig. 3). This segregation is consistent with simple autosomal co-dominant inheritance. The same mode of inheritance has been reported for the other albumin variants.

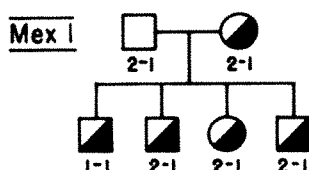


Fig. 3. Segregation of albumin Mexico in a Mexican family. A/Me heterozygotes are indicated by the half darkened circles (females) or squares (males) and A/A homozygotes by the empty box. The Gc phenotypes (1-1 or 2-1) of the family members are also shown. The propositus is the first offspring.

The number of apparent heterozygotes among the offspring is rather more than expected, but this could be the result of chance.

There is a diagram in Fig. 1 which shows the relative mobilities of the albumin variants which have been compared in this laboratory. Albumin A is the common form of albumin. Albumin B is the slow moving variant referred to as "very slow" by Weitkamp *et al.* (unpublished work). This apparently is the slow moving variant found rarely in Europeans and described originally as "bis-albumin". Albumin Naskapi<sup>1</sup> has a mobility faster than albumin A. We have compared an albumin variant found in the serum of a Navajo Indian by Dr J. L. Robbins of Salt Lake City and found it to be the same as albumin Naskapi. From this, it appears that the samples designated as "faster" by Weitkamp *et al.*<sup>2</sup> have the same mobility as albumin Naskapi. The albumin described by Wieme has the fastest moving mobility of any of the albumins tested. Quantitative comparisons of these and other variants will be given elsewhere (Melartin, in preparation).

Additional family studies will be necessary to confirm the apparent inheritance of this trait. If this variant is inherited, however, as most of the others seem to be<sup>4</sup>, then these Mexican populations would be particularly interesting to study. It may be possible to identify family segregating for three (or more) variants to determine if they segregate at one or more loci. Furthermore, using data from families segregating for albumin B<sup>2</sup>, and albumin Naskapi<sup>1</sup>, data have been obtained which suggest close linkage between the albumin and Gc loci. These populations could be used for more detailed studies of this linkage.

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## Effect of Inhibitors of Protein Synthesis on the Plastic Deformation and Growth of Plant Tissues

MANY workers have confirmed that auxin increases the irreversible deformability ("plasticity") of plant tissues, for example, coleoptiles or storage tissue<sup>1-3</sup>. A primary effect of auxin in growth may therefore be to promote extensibility of the cell wall, a change which precedes cell expansion. The mechanism of action of auxin in causing this change is not known, although it has been suggested that the substance affects the linkage of pectin chains to each other.

In the past few years it has become clear that nucleic acid and protein synthesis are required for auxin-induced growth. This is suggested partly by the finding that various inhibitors of the synthesis of these macromolecules prevent the growth-promoting action of auxin. As yet it is not clear which part of the growth process in plant cells is controlled by auxin-induced proteins, although Noodén and Thimann<sup>4</sup> have suggested that proteins affecting the condition of the wall might be particularly important.

In this note we describe experiments performed to test the possibility that auxin-induced plasticity is dependent on protein synthesis. This has been studied by examining the effects of inhibitors of protein synthesis on the irreversible deformability of plant tissues. A similar approach has been taken by Cleland<sup>5</sup> and Morré<sup>6</sup> using the inhibitor of RNA synthesis, actinomycin D, but these workers rather differed from each other in their conclusions.

In the present experiments, we have used three methods for measuring the irreversible deformation under static external load. The first method (Fig. 1C), employing wheat coleoptile sections, is identical to that described by Tagawa and Bonner<sup>2</sup>. In the second method (Fig. 1B), coleoptile sections were stretched by a 10 g weight. The

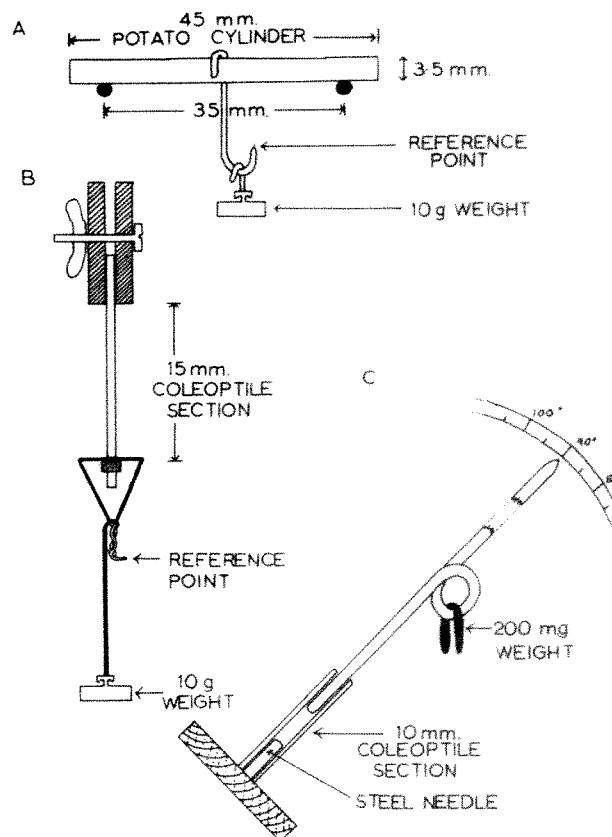


Fig. 1. Methods used to measure irreversible tissue deformation.

increase in length and the recovery when the weight was removed were measured with a travelling microscope. In the third method, cylinders of potato tuber tissue were arranged as shown in Fig. 1 and the bending and recovery were again measured with a travelling microscope. In each experiment the cylinders were cut from a single large tuber, ensuring highly uniform material. All experiments were carried out in glass containers saturated with water vapour, at room temperature (approximately 22° C) in the light. The techniques were carefully checked to establish that changes in material other than the plant tissues, for example that used for attaching the weights, did not contribute to the measured deformations.

The coleoptile sections and potato cylinders were placed in water for 1 h or in solutions of the inhibitors of protein synthesis, actidione (cycloheximide) or D-chloramphenicol. They were then transferred to solutions of indoleacetic acid (IAA) for 90 min, a time sufficient to cause the maximum increase in plastic deformation. Except for the auxin control the IAA solutions also contained one of the inhibitors. Other control tissues were kept in water throughout. After these treatments, measurements of deformation were made for 30–50 min. Fig. 2 shows that the tissues behaved in the expected manner towards IAA, with respect to their plasticity. Fig. 2 also shows the result of treatment with IAA in the presence of actidione (10 µg/ml.). This substance strongly reduced the plastic component of the IAA-induced deformation,

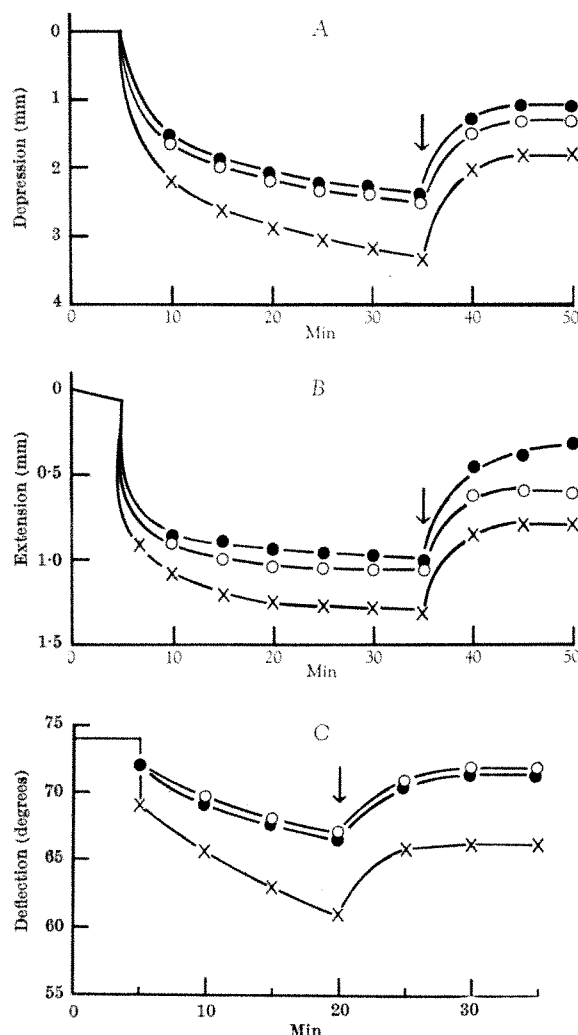


Fig. 2. Time course of tissue deformation and recovery. A, Potato cylinders; B, wheat coleoptiles, extension method; C, wheat coleoptiles, bending method. The arrows show the time at which the load was removed. ○, Treatment with water; ×, treatment with IAA (10 µg/ml.); ●, treatment with IAA + actidione (10 µg/ml.).

Table 1. EFFECTS OF ACTIDIONE AND CHLORAMPHENICOL ON RESIDUAL AND AUXIN-INDUCED PLASTICITY AND ON WHEAT COLEOPTILE GROWTH

Treatment	Plasticity Index			Per cent increase in coleoptile length
	Potato	Coleoptile (B)	Coleoptile (C)	
Water	100	100	100	31
IAA (10 µg/ml.)	150	120	124	59
IAA + actidione (1 µg/ml.)	100	—	106	—
IAA + actidione (10 µg/ml.)	93	60	110	6
IAA + chloramphenicol (2 mg/ml.)	100	63	117	44
Actidione (1 µg/ml.)	100	—	100	—
Actidione (10 µg/ml.)	90	3	89	4
Chloramphenicol (2 mg/ml.)	93	31	106	30

in two cases (A and B) to a level even lower than the water control. Similar effects were obtained with other concentrations of actidione and with chloramphenicol (Table 1). In this table the percentage plasticity in the water controls was taken as 100 and all the others were related to this (the plasticity index). Other coleoptile sections were treated with inhibitors and auxin in the same manner but afterwards were transferred to water for 24 h when their lengths were determined (Table 1). The effects of the inhibitors on auxin-inducible deformation were clearly paralleled by effects on the growth of the sections.

These results indicate that protein synthesis seems to be an essential event in the mechanism of auxin-induced plasticity. Auxin possibly controls the synthesis of enzymes which "soften" the cell wall before expansion of the cell by turgor forces. It is interesting to note that the residual plasticity present in the water controls was severely reduced by treatment with the inhibitors. This could mean that the protein responsible for promoting plastic changes has a rapid turnover time, that is, a short life. Cleland<sup>5</sup> found that auxin increased plasticity even though 90 per cent of the RNA synthesis was arrested by actinomycin D. This finding considered with the others presented here might suggest that the "message" for the proteins controlling plasticity has a rather long life and that in these systems auxin might not act on RNA synthesis but at a subsequent stage in protein synthesis. Masuda *et al.*<sup>7</sup> have reached a similar conclusion on the basis of their studies on RNA metabolism in growing coleoptiles.

Note added in proof. Results have recently been published<sup>8</sup> which show that actidione markedly reduces auxin-inducible plasticity in soybean hypocotyls.

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### Protein and RNA Synthesis in Trisomic Down's Syndrome Leucocytes

IN Down's syndrome (mongolism) increased activities have been reported in the leucocyte enzymes alkaline<sup>1,2</sup> and acid<sup>3</sup> phosphatase, glucose-6-phosphate dehydrogenase (G-6-PD) (ref. 3), galactose-1-phosphate uridyl transferase<sup>4</sup> and 5-nucleotidase<sup>5</sup>. It has been suggested that such increases may be due to the siting of structural genes for the relevant enzyme on the trisomic "G"



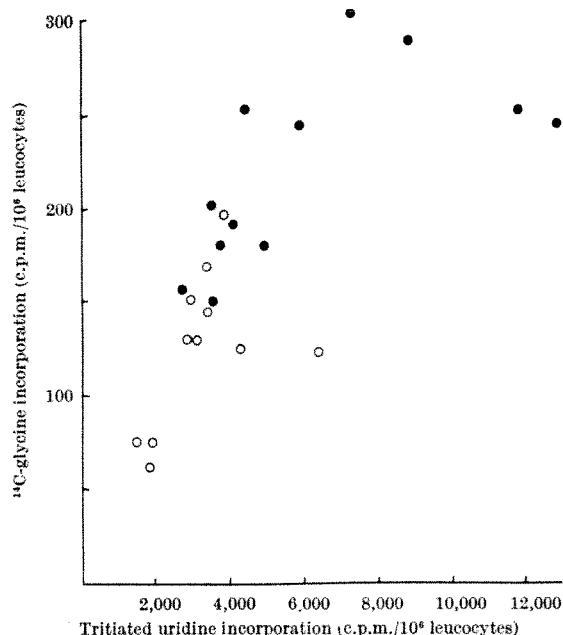


Fig. 1. Rates of incorporation of <sup>14</sup>C-glycine and tritiated uridine. ●, Down's syndrome; ○, control.

group autosome<sup>1</sup>. This seems unlikely, however, at any rate in the case of G-6-PD, for there is good evidence that the structural gene for this enzyme is carried on the X chromosome<sup>3,4</sup>. An alternative possibility is that the trisomic autosome may carry genetic material responsible for regulation of protein synthesis.

Increase in enzyme activity may be due to either enzyme activation or enhancement of *de novo* synthesis of enzyme protein. This article presents evidence that, in primary trisomic Down's syndrome, the rates of leucocyte RNA and protein synthesis are increased.

Leucocytes were obtained from children with primary trisomic Down's syndrome and mentally subnormal controls matched for age, sex and domicile. All subjects appeared to be free from infection at the time of the investigation. 10 ml. of heparinized blood were collected, and leucocytes isolated under sterile conditions by sedimentation in dextran<sup>5</sup>. Total and differential cell counts were performed after thorough mixing with a mechanical mixer. In accordance with the experience of others<sup>3</sup>, this method of leucocyte isolation yielded differential counts which were similar in subjects with Down's syndrome and controls.

Approximately  $10 \times 10^6$  leucocytes were collected by gentle centrifugation (80g for 15 min at 4° C) and resuspended in either 2 ml. of autologous plasma with 6-<sup>14</sup>C-urotic acid (0.4 µc/ml.) or in 2 ml. of culture medium (Difco T.C. 199 containing 30 per cent pooled human serum without antibiotics) containing U-<sup>14</sup>C-glycine (3 µc/ml.) and 5-tritiated-uridine (3 µc/ml.). After incubation at 37° C for 4 h the leucocytes were washed once with 0.85 per cent saline. Protein and RNA were extracted, using a modification of the Schmidt-Tann-

Table 2. INCORPORATION OF <sup>14</sup>C-GLYCINE INTO LEUCOCYTE PROTEIN

	Incorporation of <sup>14</sup> C-glycine into protein (c.p.m./10 <sup>6</sup> leucocytes)	
	Control	Down's syndrome
Mean	122.9	171.0
S.D.	42.6	63.8
No. studied	38	38
Average age (yr)	11.4	11.0
P*	< 0.01	

\* Calculated from *t* test for paired observations.

hauser technique<sup>6</sup>, but alkaline hydrolysis was omitted when tritiated uridine was the RNA precursor<sup>6</sup>.

The results are summarized in Tables 1 and 2. Incorporation rates of <sup>14</sup>C-urotic acid or tritiated uridine into leucocyte RNA were higher in Down's syndrome than in controls ( $P < 0.01$  and  $< 0.02$  respectively) (Table 1). The rate of incorporation of <sup>14</sup>C-glycine into leucocyte protein was also higher in Down's syndrome leucocytes than in controls ( $P < 0.01$ ) (Table 2). No relationship was observed between either age or sex. In individual subjects, a positive relation was apparent between the rates of incorporation of <sup>14</sup>C-glycine and tritiated uridine (Fig. 1).

These results indicate that rates of protein and RNA synthesis are increased in primary trisomic Down's syndrome leucocytes during short term culture.

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### Relative Susceptibilities of Isotopically Labeled and Unlabeled Nuclei to Deoxyribonuclease I

THE use of tritiated thymidine as a specific precursor of DNA is well established in the study of DNA synthesis and the kinetics of cell population. The usefulness of the method depends on the conservation of labelled DNA and on the rapid catabolism of non-utilized nucleoside. During experiments in which mammalian nuclei were treated with deoxyribonuclease (DNase I) I found that labelled nucleic acid was more labile to the enzyme than DNA which carried no isotope—an observation which may have a bearing on the use of radioactive precursors.

Murine lymphoma cells of strain L 5178Y in culture were labelled during exponential growth by the addition of 0.25 µc. of <sup>14</sup>C-formate/ml. of Fischer culture medium<sup>1</sup>. Initial cell numbers were such that after 70 h of growth in the isotope between  $2$  and  $5 \times 10^5$  cells/ml. were collected. The cells were washed with unlabelled medium, and the nuclei isolated by seventy to eighty manual reciprocations in a 'Teflon' glass homogenizer, size A. The nuclei were treated with DNase I at a concentration of 160 µg/ml. suspension at room temperature. The suspension consisted of  $10^7$  nuclei/ml. of Creasey Stocken medium C, pH 7.2, augmented with 0.02 molar magnesium ions<sup>2</sup>. Samples of the suspension were withdrawn in duplicate at various times, and the enzyme action was stopped by the addition of ice-cold 0.2 molar perchloric

Table 1. INCORPORATION OF <sup>14</sup>C-OROTIC ACID OR TRITIATED URIDINE INTO LEUCOCYTE RNA

		Incorporation into RNA			
		<sup>14</sup> C-urotic acid		Tritiated uridine	
		Control	Down's syndrome	Control	Down's syndrome
C.p.m./10 <sup>6</sup> leucocytes	Mean	2.79	5.78	3.230	6.160
	S.D.	1.49	3.11	1.300	3.460
Number studied		20	20	12	12
Average age (yr)		9.2	9.0	11.7	10.5
*P		< 0.01		< 0.02	

\* Calculated from *t* test for paired observations.



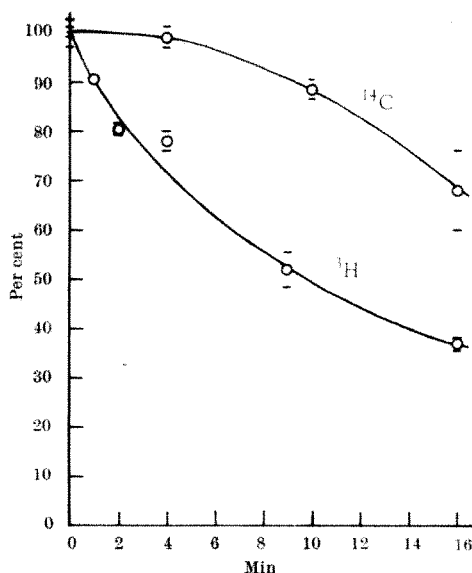


Fig. 1. Percentage of specific activity remaining in labelled nuclei after digestion with 160 µg/ml. DNase I, as a function of time.

acid. DNA was estimated by spectrophotometry after extraction by a modified Schmidt-Tannhauser procedure<sup>3</sup>. Radioactivity was estimated in a 'Tracerlab' LSC 30 liquid scintillation counter. Similar experiments were also performed on nuclei which had been labelled with tritiated thymidine by the addition of the isotope in eight fractions of 0.01 µc./ml. spread over the 70 h period of growth. A fractionated treatment was used because there is evidence that cell suspensions degrade thymidine<sup>4</sup>. The specific activities of the DNA were expressed as a function of the control (untreated nuclei), and plotted against time of enzyme action (Fig. 1).

It is apparent that the enzyme preferentially removes labelled DNA, particularly in the case of DNA labelled with tritium. A high concentration of enzyme was used here to emphasize the effect, but a noticeable decrease in specific activity was also obtained after the first few minutes with concentrations of 30 µg/ml. Some preliminary results along similar lines have been obtained using kidney nuclei from rats treated with folate<sup>3</sup> which were flash-labelled with tritiated thymidine 1 h before they were killed. The fall in specific activity after treatment with DNase was much less than with the cultured cells exposed to label for 70 h, but still appreciable, and this effect is being further investigated.

The explanation of this effect is unknown at present, but two possibilities may be considered. The nuclear DNA may be so damaged by the radiation from the isotope label that the active regions are more intrinsically labile to enzyme attack. Alternatively, the damaged DNA is undergoing repair and the action of the DNase I is to hydrolyse already excised material. The greater effect of the tritium label as compared with the carbon-14 label is probably due to the greater energy absorption from the tritium  $\beta$  particle, although in murine lymphoma cell cultures the number of bases labelled is about ten times higher in the case of carbon-14. It has recently been reported elsewhere that loss of tritium activity from acid-precipitable material after labelling with tritiated thymidine can occur over the course of some hours<sup>5</sup>. This was attributed to the localized absorption of energy from tritium disintegration, because it did not occur after labelling with carbon-14.

The use of carbon-14 or tritium precursors can thus produce cells in which the chromosomal material is changed such that a proportion of the DNA is more labile to enzyme attack. Whereas this effect may have implications in the use of labelling techniques, it is not yet certain what the biological consequences of such lability might be.

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### Formation of Blue Oxidation Product from Psilocybin

Gilmour and O'Brien<sup>1</sup> have reported that a blue colour develops when psilocybin (4-phosphoryl-*N,N*-dimethyl-tryptamine) is incubated with a preparation of rat brain mitochondria. The colour is apparently not dependent on oxygen for its formation, is associated only with the particulate matter and cannot be brought into solution by treatment with various organic solvents, detergents, acid or alkali. The reaction is inhibited (80 per cent) by EDTA.

Experiments carried out in this laboratory and elsewhere provide a reasonable explanation for this phenomenon. Psilocin, the dephosphorylated derivative of psilocybin, forms a deep blue colour on incubation with ceruloplasmin—the copper oxidase of mammalian serum—or with a copper oxidase obtained from the gill plates of *Mytilus edulis*. The reaction is accompanied by the uptake of oxygen<sup>2,3</sup>. The colour has a spectral maximum at about 620–625 m $\mu$  and a smaller peak at 400 m $\mu$ . Although 5, 6 and 7-hydroxyindole derivatives are also oxidized in the presence of both enzymes, only psilocin yields the blue colour. The blue material is a fairly stable free radical (Blumberg, W. E., and Peisach, J., personal communication), while the free radicals formed from other ceruloplasmin substrates are rather short lived<sup>4</sup>. The reaction catalysed by ceruloplasmin is unaffected by EDTA. This is in contrast to the reactions with other substrates such as *p*-phenylenediamine, which are inhibited by about 50 per cent by EDTA (refs. 5 and 6).

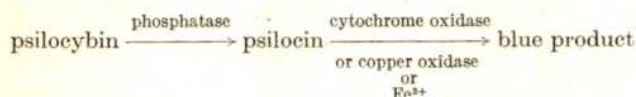
During the formation of blue material in the presence of the *Mytilus* enzyme the colour becomes associated principally with particulate matter and resists extraction with various non-polar solvents. Much of the colour can, however, be brought into solution with molar hydrochloric acid. If psilocin is oxidized in a system with very little protein the blue oxidation product appears to remain soluble.

The oxidative formation of blue colour can also be elicited without enzyme in the presence of ferric ion, which acts as an electron acceptor. As would be expected, EDTA and other chelating agents block this non-enzymatic reaction. When treated with alkali, the oxidation product of psilocin turns pale green, and can readily be extracted into butanol, where the deep blue colour reappears. The blue product can be re-extracted into 0.1 molar hydrochloric acid. The hydrochloric acid solution at this point contains no detectable iron, and the blue colour cannot therefore be due to a metal complex.

With psilocybin neither formation of blue colour nor uptake of oxygen occurs during incubation with ceruloplasmin or with ferric ions. Thus a free, non-esterified phenolic hydroxyl group is required for reactivity. These reactions could be related to those described by Gilmour and O'Brien in two possible ways. First, their psilocybin may have been contaminated with a small amount of psilocin. No evidence for purity was presented. The colour is so intense that even a slight contamination would produce visible colour in their system. Second, because prolonged incubation was required for the colour to

form (16–20 h at 5° C) it seems likely that during this period of time a phosphatase could remove the phosphate and thus expose the free phenolic hydroxyl to oxidative attack either by a copper enzyme or by a metal ion such as the ferric ion. Horita and Weber<sup>7,8</sup> described the dephosphorylation of psilocybin by homogenates of various mammalian tissues, as well as by purified alkaline phosphatase. They found that the psilocin produced in this reaction is transformed into a deep blue colour. The latter reaction may be catalysed by cytochrome oxidase which readily oxidizes psilocin to the blue product at physiological pH (7.4), although the optimum pH for this reaction is 9 (ref. 9). Because Gilmour and O'Brien found EDTA inhibited the formation of blue colour, the reaction probably involves some iron which is either free or loosely bound so as to be accessible to chelating agents. This would also explain why the reaction could take place without oxygen.

I therefore propose that the formation of blue colour from psilocybin in the presence of the brain fractions described by Gilmour and O'Brien be attributed to the following reaction.



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### Primary Structure Heterogeneity in Ribosomal Proteins from *Escherichia coli*

FUNCTIONAL heterogeneity of ribosomal proteins from *Escherichia coli* is extensive and *in vitro* polypeptide synthesis requires at least six different protein fractions<sup>1</sup>. The structural heterogeneity of ribosomal proteins is even more impressive; on gel electrophoresis of 70S ribosomal protein from *E. coli* about thirty protein bands separate and opinion favours the presence of at least as many distinct proteins<sup>2–10</sup>. Little is known, however, about the extent to which the primary structure of these proteins is related. In this communication a comparison between the amino-acid composition and tryptic fingerprints of eight simplified protein fractions, isolated from 70S ribosomal proteins of *E. coli*, will be made. In addition the purification and some properties of three single ribosomal proteins, one acidic and two basics, will be presented. Fig. 1 gives the results of applying 1 g 70S ribosomal protein (prepared using glacial acetic acid<sup>2,10</sup>) to a column of CM cellulose. Four different peaks were reproducibly resolved. The electrophoresis patterns on polyacrylamide gels (15 per cent) of selected fractions are shown in Fig. 2. As Table 1 shows, the amino-acid compositions of these fractions, determined on a Technicon autoanalyser, are quite distinct. Each fraction contains

at least one amino-acid, the percentage of which is substantially greater or less than the percentage of that amino-acid in any other fraction. The relative difference in the contents of basic over acidic amino-acid residues increases regularly from a value of –7 mole per cent to 12 mole per cent. Tryptic fingerprints confirm the presence of extensive differences in primary structure between each of these fractions; with the exception of fractions 1a and 1b, each fraction shows a different tryptic fingerprint with strong spots uniquely missing or present on comparison with any of the other fingerprints (Fig. 3).

The chief component in peak 1 (compare Fig. 1), called ribosomal A-protein, was purified on DEAE cellulose and hydroxyl apatite until this protein moved as a single band in polyacrylamide gel electrophoresis. From the

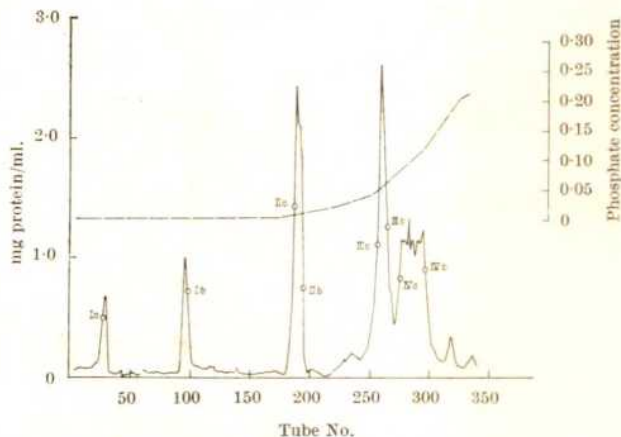


Fig. 1. Chromatography of *E. coli* ribosomal protein on CM cellulose. The column (43 cm x 3.2 cm) at 14° C was loaded in two portions: 400 mg of ribosomal protein, dissolved in 20 ml. of starting buffer, 0.005 molar ammonium phosphate, 8.0 molar urea, pH 8.0, was applied to the column and the acidic protein portion was eluted with the same buffer. At tube 60, the second portion, 550 mg of ribosomal protein was applied in a similar manner. The gradient, 600 ml. each of 0.005 molar, 0.10 molar and 0.30 molar ammonium phosphate buffer, all 8.0 molar urea, pH 8.0, was started at tube 140. Protein concentrations were determined by the Lowry method<sup>11</sup>. Circles refer to the tubes (8 ml.) selected for further characterization. Recovery from the column was 88 per cent of the input. Pooled fractions, after dialysis and freeze drying, were recovered in 94 per cent yield. Peak I, 8.7 per cent, Peak II, 14.8 per cent, Peak III, 23.7 per cent, Peak IV, 26.2 per cent, Peak V, 4.1 per cent, Peak VI, 1.0 per cent.

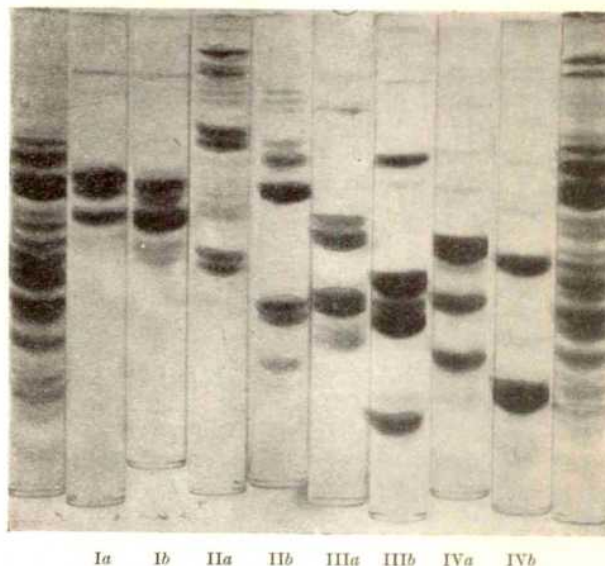


Fig. 2. Electrophoresis on polyacrylamide gels of the CM cellulose fractions indicated by the circles in Fig. 1. The cathode is at the bottom and the origin is indicated by a faint line near the top. Solvent was 8.0 molar urea, 0.3 molar formic acid and 0.06 molar potassium formate, pH 3.5 (ref. 9). Gels of unfractionated ribosomal proteins appear on either end as a control. There is similarity between the band patterns of fractions 1a and 1b.



analysis of the amino-acids of this acidic protein and the number of tryptic peptides on a fingerprint the minimum chemical molecular weight was estimated to be 15,000. The physical molecular weight for ribosomal A-protein was 21,000 (ref. 10), a value greater than found chemically. No terminal amino group could be detected by the dansyl method<sup>13</sup>. Analysis of the amino-acids of the spots eluted from the tryptic fingerprints yielded fifteen authentic peptides. The presence of this acidic protein, ribosomal A-protein, to the extent of a few molecules for each 70S ribosome<sup>6,9</sup> is interesting, and so is the fact that neither washing with 0.5-2 molar ammonium chloride in the presence or absence of EDTA nor centrifugation in concentrated caesium chloride solution releases this protein preferentially from the ribosomes into the supernatant.

Two basic proteins were purified by CM cellulose and hydroxyl apatite chromatography to the level of a single

Table 1. AMINO-ACID COMPOSITION OF VARIOUS FRACTIONS OF *E. coli* RIBOSOMAL PROTEINS ISOLATED BY CHROMATOGRAPHY ON CM CELLULOSE

Amino-acid	Ib	IIa	IIb	IIIa	IIIb	IVa	IVb
Aspartic acid	8.1	9.3	8.3	7.5	8.9	5.0	7.0
Threonine	3.3	4.6	5.3	4.9	3.9	5.4	3.6
Serine	4.2	3.7	5.3	3.6	4.1	5.0	4.2
Glutamic acid	14.2	9.7	9.5	11.7	9.9	6.7	9.0
Proline	2.6	3.6	4.3	2.8	3.8	5.0	4.8
Glycine	7.6	6.8	10.2	7.8	9.0	14.4	7.0
Alanine	21.6	14.8	12.0	14.1	12.3	10.5	15.7
Valine	8.2	7.1	9.7	8.9	9.6	6.4	5.1
Methionine	2.2	1.7	1.2	1.1	0.4	0.9	1.7
Isoleucine	4.4	5.3	5.2	4.7	5.9	5.3	5.2
Leucine	5.8	8.1	6.5	6.6	6.6	7.4	6.0
Tyrosine	0.9	1.2	1.3	1.4	0.9	0.8	0.9
Phenylalanine	1.8	2.8	2.3	2.5	2.3	2.6	2.3
Lysine	9.2	10.1	11.3	12.4	12.9	12.6	16.0
Histidine	1.9	1.5	2.0	2.6	1.7	2.1	2.4
Arginine	3.9	9.8	5.4	7.7	7.8	9.7	9.3
Excess basic	—	2.4	0.9	3.5	3.6	12.7	11.7
Excess acidic	7.3	—	—	—	—	—	—

Results are expressed in moles/100 moles of amino-acid recovered. The fractions selected are indicated in the chromatogram of Fig. 1. Amino-acid analyses were performed on 22 h hydrolysates. Values for the contents of cysteine and tryptophane are not included in total.

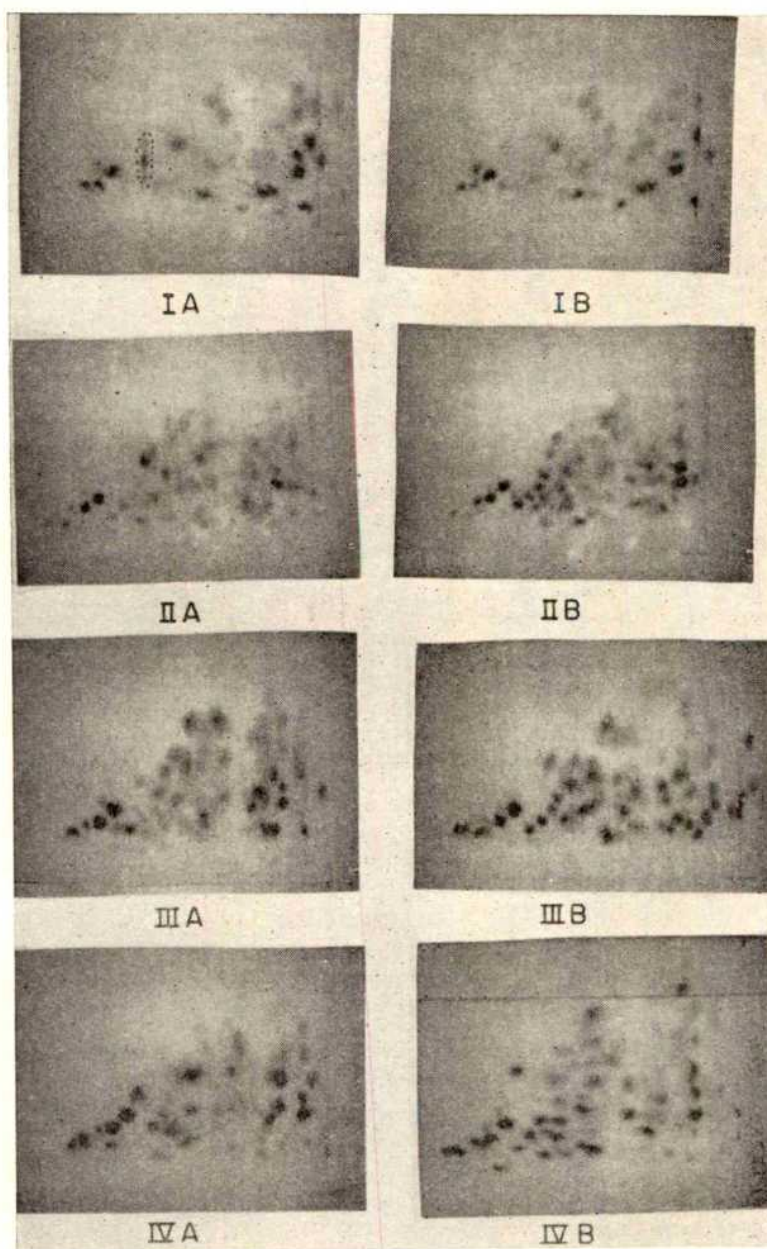


Fig. 3. Tryptic fingerprints of the protein fractions Ia to IVb, indicated by circles in Fig. 1. Each print represents 2 mg of digested chains. The three spots enclosed by a dotted line in the fingerprint of Ia were not reproducible and coincided with the top rod of the rack. The fingerprint technique used has been described before<sup>14</sup>.



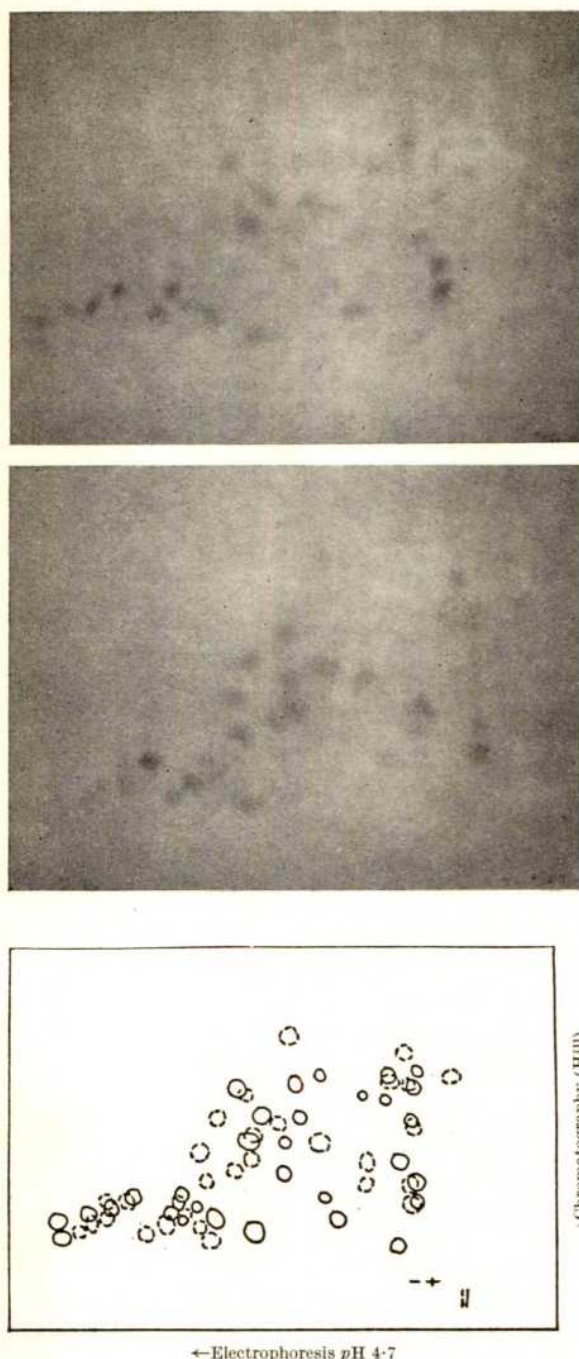


Fig. 4. Fingerprint of tryptic digest of two basic ribosomal proteins, 28B (top) and 28D (middle). Superposition (bottom), full circles, 28B; dotted circles, 28D.

band on polyacrylamide gel electrophoresis. Each protein yielded about thirty tryptic peptides, of which a maximum of ten spots overlapped (Fig. 4). On comparison, the tryptic digest of the acidic protein has at the most six spots in common with those of the two basic proteins. The amino-acid compositions of the two basic proteins were distinctly different. Furthermore, the predominant amino terminal was found to be aspartic acid for 28B and alanine for 28D.

From the results of the combined experiments we conclude that (a) 70S ribosomal proteins from *E. coli* contain at least seven proteins of different primary structure, and (b) three single bands, separated by gel electrophoresis of 70S ribosomal protein, originate from proteins of different primary structure.

Because the total number of bands is about thirty, there are probably at least as many distinct proteins. In that case each protein, on the average, is present in the ribosomes in an amount not exceeding a few per cent of the total ribosomal protein at the most, a prediction so far fulfilled for three purified proteins (ribosomal A-protein 3.5 per cent, 28B protein 2.5 per cent; 28D protein 1.4 per cent).

The distribution of these proteins among individual ribosomes is completely unknown. Peptidyl transferase<sup>14,15</sup> is probably contained by all 70S ribosomes. On the other hand, a number of ribosomal proteins of related enzyme activities—for example, co-enzymes, specific for positioning amino acyl sRNA onto the messenger template—but different structures, may not be distributed uniformly throughout all particles<sup>16</sup>. Thus the large degree of structural heterogeneity of ribosomal proteins could be consistent with what seems to be a self-contained biological unit. It is hard to visualize otherwise how so many distinct proteins in a size range of 10,000–40,000 molecular weight<sup>9</sup> could be assembled in a 70S ribosome which accommodates maximally in the order of thirty protein molecules of an average molecular weight of 25,000 (ref. 16).

After completion of this work we received information from R. R. Traut<sup>17</sup>, P. B. Moore, H. Delius, H. Noller and A. Tissières, who have come to similar conclusions with regard to differences in primary structure of ribosomal proteins from *E. coli*. We thank Dr M. Naughton for his help. This work was supported by grants from the US National Institutes of Health.

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## Glucose Tolerance after Kwashiorkor

ALTHOUGH permanent organic defects can probably result from prolonged chronic malnutrition, no lesion has so far been proved to follow treated kwashiorkor in man<sup>1</sup>. Persistence of low lactase activity in the jejunal mucosa is a possible exception<sup>2</sup>.

Fourteen children have been studied who had been patients at the MRC Infantile Malnutrition Unit, Kampala, and who had suffered from kwashiorkor between 6.2 and 12.3 (mean 9.5) yr before. Their ages when they were treated for kwashiorkor ranged from 1.0 to 4.4 (mean 1.9) yr. Twelve had had severe and two moderately severe



kwashiorkor<sup>3</sup>. Twenty children whose parents denied a history suggesting kwashiorkor and who came from highly similar environments were studied in parallel. All the children were clinically well nourished; there was no evidence in the children who had had kwashiorkor of their illness, and no evidence of any previous vitamin deficiency. The group who had had kwashiorkor consisted of eight males and six females; ten from the Ganda, three from the Hutu and one from another Bantu tribe. The control group consisted of thirteen males and seven females; nine Ganda, nine Hutu and two from other Bantu tribes. Three (21 per cent) of the post-kwashiorkor children and five (25 per cent) of the control group had enlarged parotids, while two (14 per cent) and four (20 per cent) had slight hepatomegaly, and five (36 per cent) and seven (35 per cent) had splenomegaly. Their mean weights were 29.8 (23.3–38.5) and 25.2 (18.6–39.2) kg and their mean heights 133.8 (119.4–148.6) and 127.9 (113.0–149.9) cm. The mean haemoglobin was 13.1 (7.1–17.6) and 12.9 (8.0–14.6), total plasma-protein 7.7 (6.6–9.4) and 7.3 (6.6–8.4) g/100 ml. (normal range > 7.0 g/100 ml.), and amino-acid ratio<sup>4</sup> 2.1 (1.2–3.2) and 2.7 (1.5–3.7) (normal range < 3.0) in the two groups respectively. There was no statistical difference between any of these figures for the two groups. For the post-kwashiorkor group, body weights were 68.8–98.4 (mean 81.5) per cent, and heights 87.1–100.4 (mean 93.7) per cent of those for a group of well nourished, upper class Ganda schoolgirls of similar ages<sup>5</sup>. Needle biopsies of the liver were performed on nine of the children who had had kwashiorkor. None of them showed any disturbance of architecture but one had slight stellate-fibrosis radiating from the portal areas<sup>6</sup>.

All the children were admitted to the Mulago Hospital, Kampala for the present study, and all received a ward diet containing about 500–600 g carbohydrate (expressed as monosaccharide) daily for 3 days before the tests. All tests were performed between 8.00 and 11.00 a.m. After a 10 h fast a 40 per cent solution (w/v) of glucose was injected intravenously (0.5 g glucose/kg body weight) over 2 min. Capillary blood-glucose was estimated before the injection and at 10, 20, 30, 40, 50 and 60 min after the mid-point of the injection, by a glucose-oxidase method<sup>7</sup>. The concentrations of glucose in the blood (mg/100 ml.) were plotted on semi-logarithmic paper.

The rate of fall of glucose (per cent/min), ( $K_G$ ), was calculated from a straight line drawn visually through the initial linear part of the curve, usually consisting of three or four points, beginning with the 10 min point. Fig. 1 summarizes the mean blood-glucose values at the various

times after injection of glucose. The mean  $K_G$  (calculated from the individual curves) was very significantly lower in those who had had kwashiorkor than in the others ( $t=4.71$ ;  $d.f.=32$ ;  $p<0.001$ ). The mean concentrations of glucose in the blood at 30, 40 and 50 min were also significantly different ( $p<0.02$ ,  $<0.01$  and  $<0.02$  respectively).

In addition to their hospital diet two of the children who had had kwashiorkor were given 150 g glucose daily for 3 days before the test; both had very low  $K_G$  values. Intravenous glucose tolerance shows a decrease at puberty<sup>8</sup>; only two of the children, however, had any evidence of incipient puberty. There was no significant correlation between  $K_G$  and body weight or height in either of the groups.

The cause of the relative impairment in glucose tolerance in the children who had been treated for kwashiorkor requires further study. In old age, decreased intravenous glucose tolerance has been associated with a decrease in the serum insulin response to intravenous glucose<sup>9</sup>. The pancreas is severely damaged in kwashiorkor<sup>10,11</sup>, and malnutrition has been incriminated in the pathogenesis of the chronic pancreatitis which is common in Uganda and other tropical countries<sup>12,13</sup>. Permanent disease of the exocrine pancreas after recovery from treated kwashiorkor, however, has previously been thought to be unlikely<sup>14,15</sup>.

I thank Mr Y. M. Semindi for tracing the children. The haemoglobins were estimated by Dr R. A. Tozer, and the plasma proteins and amino-acid ratios by Mr G. R. Howells. I also thank Professor R. A. McCance for discussion.

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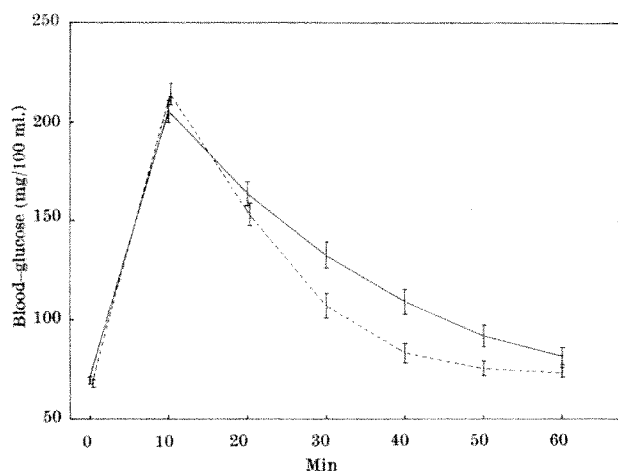


Fig. 1. Comparison of the blood-glucose response to intravenous glucose (0.5 g/kg body weight) in the children after kwashiorkor with the control group. The mean ( $\pm$  S.D. of the mean) for each group is shown at each time interval after the injection. —, After kwashiorkor,  $K_G=2.15 \pm 0.17$  per cent/min; ---, controls,  $K_G=3.79 \pm 0.27$  per cent/min.

## Potential Toxicity of Solutions of Dimethyl Sulphoxide

THE recent popularity of neat or aqueous dimethyl sulphoxide (DMSO) as a solvent for biological work—for example, in tissue culture studies of substances causing enucleation of fibroblasts<sup>1</sup> and of the rate of virus penetration of HeLa cells<sup>2</sup>—has encouraged us to make the following report.

Solutions in DMSO-water (30 : 70 v/v) of aromatic hydrazo- and azo-compounds or carcinogenic amines of the benzidine class readily penetrate certain types of rubber and surgical gloves.

Similar solutions of nitrobenzene and amyl nitrite are rapidly absorbed through the skin to give the characteristic unpleasant symptoms, but negligible penetration or side effects occur when solutions in ethanol, methyl cellosolve or dioxan are used.

Aqueous or neat DMSO is known to be readily absorbed dermally and rapidly distributed throughout the body<sup>3</sup>

and also when used in relatively high concentrations in the solvent, to facilitate the penetration of certain pharmaceuticals and other compounds<sup>4,5</sup>, but our observations (which were noted incidentally to other studies) emphasize the additional caution necessary when toxic, carcinogenic, or perhaps even compounds which are usually regarded as innocuous are handled in solution in this medium. Similar precautions may be necessary for other dipolar aprotic solvents because dimethyl formamide has been shown to be even more active than DMSO in promoting skin penetration by *d*-tubocurarine chloride<sup>6</sup>.

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### D-Xylose: Active Intestinal Transport in a Sodium Ion Substituted Lithium Medium

It is becoming increasingly evident that the active transport of a wide variety of substances by the small intestine requires sodium ions<sup>1-4</sup>. The mechanism of active sugar transport, postulated by Crane<sup>5,6</sup>, accounts for the movement of a sugar against its own concentration gradient by virtue of an opposing sodium ion gradient. D-xylose is the only pentose which has been demonstrated to be actively transported by the small intestine<sup>7</sup> and it has been suggested that its mechanism of transport is similar to that of the hexoses<sup>8</sup>. A criterion used to arrive at this conclusion was the apparent dependency of active d-xylose transport on sodium ions.

In 1962, Faust<sup>9</sup> reported that the rates of absorption (disappearance from the mucosal medium) of various pentoses, including D-xylose, by the rat jejunum *in vitro* were not inhibited when lithium was substituted for sodium in the incubation medium. Consequently, it became of interest to investigate whether D-xylose is transported against its own concentration gradient into intestinal mucosa from medium in which sodium is replaced by lithium. It also was of interest to determine the effects of potassium on D-xylose transport.

Fed male rats of the Sprague-Dawley strain, weighing between 200 and 260 g, were used. Cannulated everted sacs of jejunum were filled and incubated at 37° C for 1 h in 100 ml. of gassed (oxygen : carbon dioxide, 95 : 5) sodium, lithium or potassium bicarbonate saline solution, at pH 7.4, containing 6.66 mmoles of either D-xylose, D-ribose, L-arabinose or D-arabinose. When sodium was replaced, equal molarities of either lithium or potassium were used. Lithium or potassium chloride was substituted for sodium chloride, and lithium carbonate or potassium bicarbonate was substituted for sodium bicarbonate. In some cases, an inhibitor was added to both the mucosal and serosal fluids. After incubation the everted sacs were gently wiped with absorbent tissue, then cut open and the tissue placed mucosal side up on strips of Whatman No. 1 filter paper. The tissue was blotted with filter paper and the mucosa was scraped off with a glass microscope slide. The wet mucosa was weighed, lyophilized, and reweighed to determine its tissue water. The dry mucosal tissue was then pulverized in a glass mortar with a glass pestle and mixed with Ba(OH)<sub>2</sub> and Zn(SO<sub>4</sub>)·7H<sub>2</sub>O. After centrifugation, pentoses were determined in the supernatant by the method of Roe and Rice<sup>10</sup> and tissue

pentose concentrations were calculated. No interference with this assay method was observed with control tissue which had been incubated in the absence of pentose.

Table 1 shows that, by using this method, the final concentration of D-xylose within the mucosa in the presence of sodium is almost twice as great as the initial concentration in the incubation medium. It is also obvious that in this sodium bicarbonate saline the other pentoses, D-ribose, L-arabinose and D-arabinose, have a final mucosal tissue concentration which is similar to the initial medium concentration of 6.66 mmoles. A tissue to medium concentration ratio (T/M) greater than 1.00 further exemplifies that D-xylose is the only pentose within this group which has been transported from the bathing medium to the mucosal tissue against its own concentration gradient. In a lithium bicarbonate saline medium, as shown in Table 1, D-xylose also is actively transported. There is, however, a reduction in this active transport, as indicated by its T/M ratio, which is less than that observed in the sodium medium ( $P > 0.02$ ). No change was seen with the other pentoses under this condition.

The active transport of D-xylose is not evident when potassium is substituted for sodium, the final concentration being  $5.01 \pm 0.32$  mmoles (seven animals) within the mucosal tissue. A T/M ratio of  $0.76 \pm 0.05$  further illustrates that the presence of a high potassium concentration in the incubation medium is inhibitory to the movement of D-xylose into the mucosa toward an equilibrium.

Table 1. PENTOSE TRANSPORT INTO RAT MUCOSAL TISSUE FROM A SODIUM AND A SODIUM SUBSTITUTED LITHIUM MEDIUM

Pentose	Na <sup>+</sup> bicarbonate saline medium		*T/M
	Number of animals	Final pentose conc. in mucosal tissue (mmolar)	
D-Xylose	12	12.64 ± 0.57	1.90 ± 0.09
D-Ribose	4	6.72 ± 0.40	1.01 ± 0.06
L-Arabinose	4	7.04 ± 0.15	1.06 ± 0.02
D-Arabinose	4	6.78 ± 0.70	1.02 ± 0.14
Pentose	Li <sup>+</sup> bicarbonate saline medium		*T/M
	Number of animals	Final pentose conc. in mucosal tissue (mmolar)	
D-Xylose	8	10.20 ± 0.71	1.53 ± 0.11
D-Ribose	4	6.67 ± 0.30	1.00 ± 0.05
L-Arabinose	4	7.02 ± 0.17	1.05 ± 0.03
D-Arabinose	4	7.16 ± 0.40	1.07 ± 0.06

In Tables 1 and 2, experiments were conducted over a period of 1 h at 37° C. The initial pentose concentration on both sides of the tissue was 6.66 mmolar. Volume of mucosal tissue water was not corrected for extracellular fluid volume. The S.E. of the mean is indicated for each result.

\* Ratio of final pentose concentration in mucosal tissue water to final pentose concentration in the mucosal incubation medium.

In the experiments involving sodium substitution, the question arose as to whether or not there is any sodium in the incubation media. Flame photometric analysis indicated that the sodium concentration after incubation within the mucosal and serosal fluids of the lithium bicarbonate saline were  $0.63 \pm 0.05$  and  $3.34 \pm 0.17$  mequiv./l. (four animals) respectively, and for the potassium bicarbonate saline,  $0.59 \pm 0.04$  and  $4.35 \pm 0.32$  mequiv./l. (four animals). Although the incubation media are not absolutely free of sodium, it seems unlikely, at the present time, that the extremely low concentrations of sodium present in the lithium medium after incubation play a part in the active transport of D-xylose.

Additional experiments were performed in order to compare the active transport of D-xylose in sodium and lithium bicarbonate saline. The data in Table 2 illustrate the effect of various inhibitors on this phenomenon. Active D-xylose transport in both the sodium and lithium media is inhibited by phlorizin, a competitive inhibitor of active intestinal sugar transport<sup>11-12</sup>, iodoacetic acid and sodium fluoride, inhibitors of glycolysis<sup>13</sup>; and 2,4-dinitrophenol, an uncoupler of oxidative phosphorylation<sup>14</sup>. It is interesting to note that oligomycin, an inhibitor of oxidative phosphorylation<sup>14</sup>, is very effective in inhibiting active D-xylose transport in the presence of sodium, but is not nearly as effective when sodium is replaced by lithium. This may be a reflexion on the mechanism of action of oligomycin in a lithium medium, because 2,4-dinitrophenol

Table 2. EFFECT OF VARIOUS INHIBITORS ON ACTIVE D-XYLOSE TRANSPORT BY RAT MUCOSAL TISSUE IN A SODIUM AND A SODIUM SUBSTITUTED LITHIUM MEDIUM

Inhibitor	Na <sup>+</sup> bicarbonate saline medium			*T M
	Conc. of inhibitor (mmolar)	Number of animals	Final D-xylose conc. in mucosal tissue (mmolar)	
None	—	12	12.64 ± 0.57	1.90 ± 0.09
Phlorizin	0.1	4	6.96 ± 0.17	1.04 ± 0.03
Iodoacetic acid	12.0	5	7.45 ± 0.16	1.12 ± 0.03
Sodium fluoride	12.0	6	7.13 ± 0.32	1.07 ± 0.05
2,4-Dinitrophenol	0.5	4	7.13 ± 0.32	1.07 ± 0.05
Oligomycin	0.026	8	7.43 ± 0.30	1.12 ± 0.05
Inhibitor	Li <sup>+</sup> bicarbonate saline medium			*T M
	Conc. of inhibitor (mmolar)	Number of animals	Final D-xylose conc. in mucosal tissue (mmolar)	
None	—	8	10.20 ± 0.71	1.53 ± 0.11
Phlorizin	0.1	4	6.23 ± 0.37	0.94 ± 0.06
Iodoacetic acid	12.0	4	6.80 ± 0.28	1.02 ± 0.04
Lithium fluoride	12.0	4	6.80 ± 0.12	1.02 ± 0.02
2,4-Dinitrophenol	0.5	4	6.43 ± 0.25	0.97 ± 0.04
Oligomycin	0.026	11	8.67 ± 0.14	1.30 ± 0.02

The experimental conditions were the same as those for Table 1. D-xylose and inhibitors were placed on both sides of the tissue. The initial D-xylose concentration was 6.66 mmolar. The S.E. of the mean is indicated for each result.

\* Ratio of final D-xylose concentration in mucosal tissue water to final D-xylose concentration in the mucosal incubation medium.

under this condition completely inhibits active D-xylose transport.

These experiments illustrate that the mechanism of active D-xylose transport by mucosa from rat jejunum is similar in either sodium or lithium bicarbonate saline. In addition, the energy requirements for this process are similar to those observed for the active intestinal transport of other sugars<sup>15,16</sup>. The fact that lithium can replace sodium in the incubation medium is the only apparent difference in the mechanism of active transport of this pentose and other sugars.

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### Identification of Prostaglandin E<sub>2</sub> as the Principal Vasodepressor Lipid of Rabbit Renal Medulla

INVESTIGATORS in several laboratories have reported on the isolation of vasoactive lipids from renal medulla of several species<sup>1-7</sup>. Muirhead *et al.*<sup>1</sup> have separated these lipids into two classes: the antihypertensive lipids which prevent canine renoprival hypertension<sup>2</sup> and lower canine renal hypertension<sup>3</sup>; and the more acidic vasodepressor

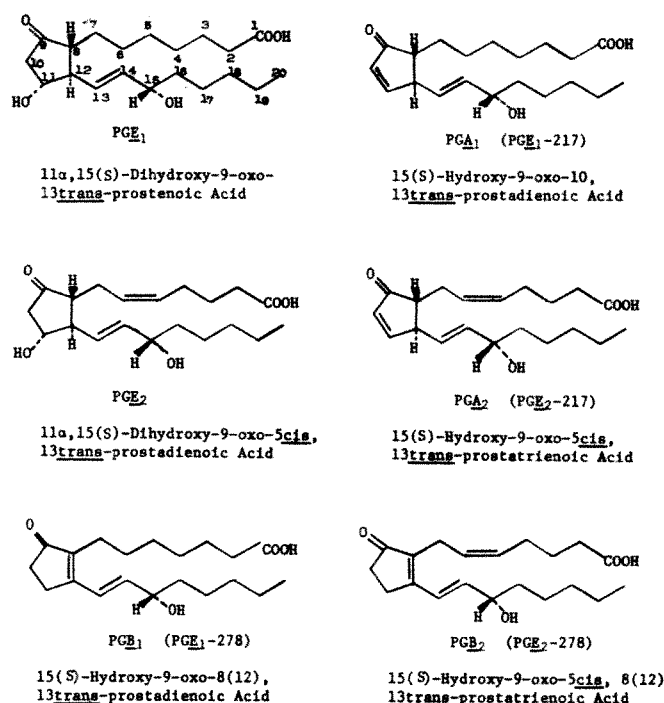


Fig. 1.

lipids which acutely lower the blood pressure of the anaesthetized, vagotomized dog or rat treated with pentolinium<sup>4</sup>.

Hickler *et al.*<sup>5</sup> suggested that the acidic depressor lipids in extracts of renal medulla are prostaglandins<sup>6</sup>. In a similar study Lee *et al.*<sup>6</sup> separated two depressor lipids, one tentatively identified as PGE<sub>1</sub> (Fig. 1). The other depressor lipid differed chromatographically and was unable to stimulate non-vascular smooth muscle and was named "medullin". Subsequently, this material was identified<sup>7</sup> as PGE<sub>2</sub>-217 (now termed PGA<sub>2</sub> (ref. 10)). Strong *et al.*<sup>7</sup> also isolated vasodepressor lipids from rabbit renal medulla and interpreted their findings to indicate that their "purified VDL" was PGE<sub>1</sub>. They pointed out the possibility of the formation of "medullin" from PGE<sub>1</sub> by acid treatment and the similarity in the properties of "medullin" and PGE<sub>1</sub>-217 (PGA<sub>1</sub>) (ref. 11).

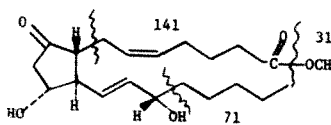
This communication concerns the identification of the acidic depressor lipids from rabbit renal medulla using less equivocal methods. Five kilograms of rabbit renal medulla were homogenized for 5 min in a Waring blender with an equal volume of water (pH adjusted to 7.0). After the addition of three volumes of acetone, the mixture was stirred and then allowed to settle overnight at 4° C. The insoluble residue was removed by filtration and the filtrate (75 per cent acetone) was concentrated *in vacuo* to an aqueous extract containing 10–20 per cent acetone. The solution, still at pH 7, was extracted twice with equal volumes of petroleum ether (boiling point 30°–60° C). The aqueous phase was adjusted to pH 3.0 with hydrochloric acid and extracted three times with 1/4 volume of methylene chloride. These three phases were combined and concentrated to approximately 1 l. The combined methylene chloride solution was extracted four times with 1/4 volume of 0.2 molar phosphate buffer, pH 8.0. After evaporation to dryness the methylene chloride layer provided the "neutral" fraction which was reserved for other studies. The aqueous buffer phase was acidified to pH 3.0 with hydrochloric acid and extracted three times with 1/3 volume of methylene chloride. After evaporation of the solvent, 0.35 g of acidic lipid fraction was obtained. All the activity detectable by the acute vasodepressor assay<sup>4</sup> was found in this acidic fraction.

Silicic acid chromatography<sup>7</sup> of pooled acidic lipids (984 mg) provided only two depressor fractions which were predominantly *E* (103 mg) and *A* (13 mg) prostaglandins. Thin-layer chromatography of the  $\text{PGA}_4$  fraction and subsequent elution with methanol from the silica gel<sup>12</sup> impregnated with silver nitrate plates indicated that  $\text{PGE}_2$  was the principal component of this fraction. The behaviour of this sample on thin-layer chromatography compared with an authentic sample and the conversion by alkali degradation to  $\text{PGB}_2$  (Fig. 1) were the signs used to identify this material.

Preparative thin-layer chromatography of 50 mg of the  $\text{PGE}$  fraction from the silicic acid column on a silica gel plate (20 × 20 cm) impregnated with silver nitrate 1 mm thick, developed with chloroform : methanol : acetic acid (CMA) (90 : 5 : 5), yielded 31 mg from the  $\text{PGE}_2$  zone on elution with methanol.  $\text{PGE}_1$ , if present at all, constituted less than 10 per cent of the  $\text{PGE}$  content of the renal medulla.

A portion of the  $\text{PGE}_2$  eluted from the chromatographic plate was esterified with ethereal diazomethane. The resulting methyl ester was shown by thin-layer chromatography and mass spectrometry to be identical to an authentic sample of  $\text{PGE}_2$  methyl ester (Systems MI, MII, MIII (ref. 13) (Table 1)). In still further confirmation of its identity another portion of the  $\text{PGE}_2$  was treated with alkali and converted to  $\text{PGB}_2$  which exhibits strong ultra-violet absorption at 278 m $\mu$  and has thin-layer chromatographic mobility distinguishable from that of  $\text{PGB}_1$ .

Table 1. MASS SPECTRAL DATA FOR  $\text{PGE}_2$  METHYL ESTER

						
<i>m/e</i>	<i>M</i> <sup>*</sup>	<i>M</i> -18	<i>M</i> -(2 × 18)	<i>M</i> -(18 + 31)	<i>M</i> -71	<i>M</i> -(18 + 71)
Per cent of base peak	366	348	330	317	295	277
	1.0	21.0	11.6	6.7	10.9	21.0
			<i>M</i> -(18 + 140*)	<i>M</i> -(36 + 140*)		
<i>m/e</i>			208	190		
Per cent of base peak			56.7	44.7		

\* Fragmentation involves hydrogen transfer.

In view of these findings it would appear that there is little doubt that the principal vasodepressor lipid of rabbit renal medulla is  $\text{PGE}_2$ . This is consistent with reports that the  $\text{PGE}_2$  precursor, arachidonic acid, is present in much larger quantities than the  $\text{PGE}_1$  precursor, dihomog- $\gamma$ -linolenic acid, in renal medulla<sup>14</sup>.

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## IMMUNOLOGY

### Interferon and the Interaction of Allogeneic Normal and Immune Lymphocytes with L-cells

It has been supposed<sup>1-3</sup> that the effect of immune lymphocytes is connected with the transmission between the lymphocytes and target cells of the informative active parts. The destruction of homologous cells induced by lymphocytes is similar to the primary cytopathic effect in virus diseases. Nuclei of immune lymphocytes proved to exert the same destructive effect on target cells as that of intact immune lymphocytes<sup>2,3</sup>.

Our histochemical and autoradiographic investigations have demonstrated that cytoplasmic connexions ("bridges") containing DNA and RNA are formed between lymphocytes and target cells, and that H<sup>3</sup>-uridine-labelled RNA of lymphocytes and numerous granules containing DNA appear in the cytoplasm of L-cells. These processes are more marked with immune lymphocytes than with normal allogeneic lymphocytes of BALB/c mice<sup>4</sup>.

We consider it possible that interferon is produced in the course of immune lymphocyte target cell interaction; and that the interferon induced by virus should suppress that action of immune lymphocytes.

In experiments carried out with Dr. Inessa A. Svet-Moldavskaya, we attempted to detect interferon production in the course of interaction of immune lymphocytes BALB/c with L-cells. Interferon was tested by means of variola virus plaque formation in L-cells culture. All these experiments produced negative results. Investigation of virus interferon, however, revealed an unexpected result.

Continuous L-fibroblasts were used as target cells to which immune or normal lymphocytes from BALB/c mice were added (Rosenau and Moon's system<sup>5</sup>). BALB/c mice were immunized twice intraperitoneally with  $2.5 \times 10^6$  L-cells at an interval of one month, and one month later the mice were inoculated into the spleen with  $2.5 \times 10^6$  L-cells in 0.1 ml. Four days after inoculation the spleens were removed aseptically and made into a suspension in medium 199 by means of Potter's homogenizer. In the same way suspensions of cells were prepared from the spleens of non-immunized BALB/c. The cells were filtered and the numbers of live lymphoid cells were counted.

Interferon was obtained according to Hare and Morgan<sup>6</sup>. The chick embryo allantoic fluid containing Newcastle disease virus was irradiated with ultra-violet light until the complete loss of infectivity to chick embryos. The allantoic fluid of normal chick embryos was irradiated in the same way. Each of these fluids was diluted 1:20 with medium 199 and was then added to monolayer L-cell cultures. Cultured fluids from L-cells treated both



with irradiated fluid containing virus and irradiated normal allantoic fluid were collected separately after 48 h and acidified with 1 normal hydrochloric acid to pH 2. They were then kept at 4° C for 18 h and were neutralized with 1 normal sodium hydroxide to pH 7.5. The interferon preparation and the control fluid were treated similarly and then stored at 4° until use.

Our samples of interferon in the dilution 1:10 completely protect *L*-cells from the > 100 TCID<sub>50</sub> of encephalomyocarditis virus.

Interferon and the control fluid were added to the *L*-cells together with lymphocytes or 1 h after the incubation of lymphocytes with *L*-cells. In one experiment (No. 40), interferon was added to *L*-cells 1 h before contact with lymphocytes. In all the experiments, there were control tubes in which interferon and control fluid were added to the *L*-cell without any lymphocytes. The results of experiments were obtained by counting the number of live *L*-cells on the second or third day of incubation.

It is evident from Table 1 that the interferon did not suppress or destroy the *L*-cells. In the presence of interferon, however, normal lymphocytes affected *L*-cells in the same way as the immune cells, and thus the number of live *L*-cells was significantly reduced in the tubes to which normal lymphocytes *BALB/c* plus interferon were added (see Table 1).

Interferon did not enhance the sticking of lymphocytes to *L*-cells.

Holm *et al.*<sup>7</sup> and Möller and Möller<sup>8</sup> reported that non-immune allogeneic lymphocytes produced destruction of target cells when added to substances which agglutinated lymphocytes, in particular, with phytohaemagglutinin.

We studied the effect of phytohaemagglutinin in the system of *L*-cells plus lymphocytes from normal *BALB/c* and *C<sub>3</sub>H/f* mice.

Table 1. EFFECT OF INTERFERON IN THE SYSTEM OF *L*-CELL PLUS IMMUNE AND NON-IMMUNE ALLOGENEIC LYMPHOCYTES (*BALB/c*)

Experiment No.	Interferon with lymphocytes*		Control fluid in the presence of:		Interferon without lymphocytes	Control
	Immune	Non-immune	Immune	Non-immune		
51	7.5 ± 1.5	20 ± 3	19 ± 8	118 ± 8	141 ± 5.5	110 ± 31
40	104 ± 7	108 ± 14	102 ± 14	139 ± 14	—	—
59	nt	73 ± 10	nt	135 ± 13	238 ± 11.5	151 ± 10

\* In experiments No. 51 and No. 59 15 × 10<sup>6</sup> lymphocytes/tube were added; in experiment No. 40 2 × 10<sup>6</sup> lymphocytes/tube were added.

The agglutinating activity of phytohaemagglutinin proved not to be associated with its ability to destroy *L*-cells on contact with normal lymphocytes. The freshly diluted phytohaemagglutinin caused marked sticking of lymphocytes from normal *BALB/c* and *C<sub>3</sub>H/f* mice to *L*-cells and in both instances destruction of target cells was observed. The same phytohaemagglutinin diluted and stored for 2 months at 4°–8° C retained its haemagglutinating capacity but completely lost its capacity to cause death of *L*-cells when even large amounts of *BALB/c* or *C<sub>3</sub>H/f* lymphocytes (10 million/ml.) were added (see Table 2).

It is possible that the effect of lymphocytes on allogeneic cells in the presence of phytohaemagglutinin depends on the interferon formation. The induction of interferon by means of phytohaemagglutinin was described by Wheelock<sup>9</sup>.

The mechanism of the action of interferon in the system of normal and lymphocytes *L*-cells is obscure. Initially we made the assumption that normal lymphocytes trans-

mit some informative moieties to the other cells. Any impairment of information exchange between the lymphocyte and the cell leads to dysfunction and destruction.

It was shown recently in our laboratory that addition of methotrexate to the system normal *BALB/c* lymphocytes *L*-cells in a dose which was not toxic for these cells resulted in the destruction of *L*-cells by lymphocytes, as in the presence of interferon. Methotrexate did not increase the sticking power of lymphocytes to *L*-cells. It is possible that any agents which damage or denature lymphocyte DNA will act in the same way.

The cytological and cytochemical pattern of methotrexate and interferon in the system of normal lymphocytes target cells is similar to that with immune lymphocytes.

Blastoid transformation of lymphocytes appears to be close in its mechanism to the interaction of lymphocyte-target cell. It is possible that in blastoid transformation a portion of lymphocytes functions as donors and a portion of lymphoid cells as recipients of DNA. Transfer of the excess syngeneic DNA results in blastoid differentiation of cells. In other words, in both instances the same principal mechanism is hybridization but the final effects are different: cell differentiation or lethal morphogenesis resulting in death.

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### Suppression of Viral and Chemical Carcinogenesis by means of Artificial Heterogenization

WE have demonstrated the possibility of artificial induction *in vivo* of viral antigens of the transplantation type in tumour cells. The antigens are induced by a chemical carcinogen, after intravenous or intracardiac inoculation of polyoma viruses, vacuolating virus *SV<sub>40</sub>* and adenovirus type 16 (refs. 1–3).

Administration of 7,12-dibenz-( $\alpha$ )-anthracene simultaneously with *SV<sub>40</sub>* or adenovirus, type 16, gave rise to tumours containing transplantation antigens of these viruses. Simultaneous inoculation of a chemical carcinogen and a mixture of two DNA viruses, polyoma and adenovirus 16, resulted in induction of tumours of which the cells contained transplantation antigens of both viruses<sup>4</sup>.

Based on these facts, the following two experiments to suppress chemical and viral carcinogenesis by artificial heterogenization were developed. (a) Newborn hamsters were inoculated with carcinogen and at the same time with *SV<sub>40</sub>* or adenovirus. When adult, these animals

Table 2. EFFECT OF ACTIVE AND INACTIVE PHYTOHAEMAGGLUTININ IN THE SYSTEM OF *L*-CELLS PLUS SYNGENEIC (*C<sub>3</sub>H/f*) AND ALLOGENEIC (*BALB/c*) LYMPHOCYTES

Experiment No.	PHA	The number of lymphocytes in 1 ml.	The average number (± S.E.) of living <i>L</i> -cells × 1,000 after incubation in the presence of:				PHA*	Control
			PHA* with lymphocytes	PHA* with lymphocytes	PHA* with lymphocytes	PHA* with lymphocytes		
			<i>BALB/c</i>	<i>C<sub>3</sub>H/f</i>	<i>BALB/c</i>	<i>C<sub>3</sub>H/f</i>		
69	Fresh diluted	5 × 10 <sup>6</sup>	24 ± 2	42 ± 2	82 ± 5.5	104 ± 7	87 ± 9	124 ± 15
64	Was kept in diluted condition for 2 months	10 × 10 <sup>6</sup>	73 ± 9	64 ± 6	96 ± 9	75 ± 5	80 ± 6	124 ± 17

\* PHA was added in dilution 1:100.

were immunized with the corresponding virus. (b) New-born hamsters were inoculated with  $SV_{40}$  virus and adenovirus type 16. Adult animals were immunized with one of these viruses.

Vacuolating virus  $SV_{40}$  (strain A-426) was received from the Museum of Oncogenic Viruses in our institute. The virus was carried through three passages in green monkey kidney cell cultures. The titre of the virus was  $10^7$  TCID<sub>50</sub>/0.1 ml. Adenovirus type 16 was supplied by Dr R. S. Dreizin. Its titre was  $10^3$  TCID<sub>50</sub>/0.1 ml. when assayed in HeLa cell cultures at 7–8 days.

In experiments concerned with viral carcinogenesis, 0.1 ml. of virus was inoculated subcutaneously into the back of newborn hamsters (no older than 24 h). Another group of animals received 0.2 ml. of a mixture of  $SV_{40}$  and adenovirus 16 consisting of equal volumes of fluids containing viruses. In experiments concerned with the suppression of chemical carcinogenesis 7,12-dibenz-( $\alpha$ )-anthracene was dissolved in sterile apricot-kernel oil in a concentration of 1 mg/ml. The carcinogen was administered subcutaneously into the back of newborn hamsters in a dose of 60  $\gamma$ /0.06 ml. The first group received carcinogen alone; the second group received carcinogen and immediately after it  $SV_{40}$  virus in the same site ( $10^7$  TCID<sub>50</sub> in 0.1 ml.); the third group received carcinogen and immediately afterwards adenovirus in the same site ( $10^3$  TCID<sub>50</sub> in 0.1 ml.).

At the age of 1 month each litter in all groups of experimental animals, in experiments with both chemical and viral carcinogenesis, were divided into three subgroups. One subgroup was not immunized and the other two were immunized with adenovirus or  $SV_{40}$  virus, respectively. Immunization was given twice, on the thirtieth and forty-fifth day after birth, by intraperitoneal inoculation of 0.5 ml. of fluid containing the virus. Every 10 days the hamsters were palpated. The developing tumours were recorded and their percentage incidence in the surviving animals was calculated. Animals dying of other causes were excluded from the calculation. The data were then treated statistically.

Table 1. CARCINOGENESIS INDUCED BY  $SV_{40}$  VIRUS AND SUPPRESSED BY ARTIFICIAL HETEROGENIZATION WITH ADENOVIRUS

Subgroup	Inoculation	Immunization	Development of tumours				Total*	%
			Months					
Control	1 <i>SV</i> <sub>40</sub>	None	4	5	6	7	18/30	60
	2 <i>SV</i> <sub>40</sub>	Adeno 16	1	6	3	3	13/19	68
	Total		2	10	10	9	31/49	63
	3 <i>SV</i> <sub>40</sub> + adeno 16	None	6	11	3	5	25/38	66
Experiment	4 <i>SV</i> <sub>40</sub> + adeno 16	Adeno 16	2	6	3	3	14/35	40
	5 <i>SV</i> <sub>40</sub> + adeno 16	<i>SV</i> <sub>40</sub>	2	1	1		4/37	11

\* Numerator: number of animals developing tumours; denominator: total number of animals inoculated.

Table 1 shows that viral carcinogenesis caused by administration of a mixture of two viruses,  $SV_{40}$  and adenovirus 16 was suppressed by vaccination both with  $SV_{40}$  alone and adenovirus alone, that is, carcinogenesis induced by  $SV_{40}$  may be suppressed in such circumstances by adenovirus type 16 which is practically non-oncogenic for hamsters. The difference between figures is statistically significant. Vaccination with adenovirus of hamsters inoculated at birth with  $SV_{40}$  alone produced no effect on the rate and frequency of tumour development. Tumours in animals inoculated with a mixture of  $SV_{40}$  and adenovirus 16 began to appear by 116–126 days. Immunization of these animals with  $SV_{40}$  or adenovirus 16 did not in any way influence the time of appearance of tumours, although the number of developing tumours was definitely reduced.

Table 2 shows that there was suppression of chemical carcinogenesis by artificial heterogenization. Immunization with adenovirus of hamsters inoculated at birth with carcinogen plus adenovirus resulted in a significant reduction of the number of tumours in comparison with those in the immunized subgroup. In this experiment, immunization also influenced the rate of tumour development. First tumours in non-immunized animals developed at 58 days, while the first tumour in animals immunized with adenovirus appeared much later on day 114.

In the next experiment, newborn hamsters were inoculated simultaneously with carcinogen and  $SV_{40}$  (Table 3). There was no difference in the time and rate of tumour development in groups of animals which were not immunized or in those immunized with  $SV_{40}$  and adenovirus. Animals receiving simultaneously carcinogen and  $SV_{40}$  showed marked stimulation of carcinogenesis in comparison with the animals receiving carcinogen only. The difference was not only in the incidence of developing tumours but also in the time of their appearance: first tumours induced by carcinogen plus  $SV_{40}$  appeared as early as 28 days after inoculation, while 7,12-dibenz-( $\alpha$ )-anthracene alone induced them only after 72 days. A stimulatory effect was manifest only in simultaneous administration of carcinogen and  $SV_{40}$ . Table 3 also shows that subsequent inoculation of  $SV_{40}$  and adenovirus to hamsters which at birth had received carcinogen alone did not influence the time of appearance and incidence of tumours.

Induction of viral antigens of the transplantation type during chemical carcinogenesis raises the question of whether individually specific antigens of tumours induced by chemical carcinogens are actually antigens of occasional passenger viruses.

Suppression of viral and chemical carcinogenesis by means of artificial heterogenization creates various poss-

Table 2. CARCINOGENESIS INDUCED BY 7,12-DIBENZ-( $\alpha$ )-ANTHRACENE AND SUPPRESSED BY ARTIFICIAL HETEROGENIZATION

Table 2. CARCINOGENESIS INDUCED BY 1,12-DIBENZ (a, h) ANTHRAcene AND SUBSEQUENT TUMOUR DEVELOPMENT											
Subgroup		Inoculation	Immunization	Development of tumours					Total*	%	
				Months							
Control	6	Carcinogen	None	2	3	4	5	6	7	14/27	52
	7	Carcinogen	Adeno 16		5	1	3	4		14/19	73
	8	Carcinogen	SV <sub>40</sub>		3	4	5	2		16/24	66
			Total		11	9	17	6	1	44/70	63
	9	Carcinogen + adeno 16	None	2		3	3	2	4	14/24	58
Experimental	10	Carcinogen + adeno 16	SV <sub>40</sub>		1	1	1	2	1	6/14	43
			Total	2	1	4	4	4	5	20/38	53
	11	Carcinogen + adeno 16	Adeno 16			1	4			5/23	21

\* See footnote to Table 1.

Table 3. TUMOURS IN HAMSTERS INOCULATED AT BIRTH WITH CARCINOGEN PLUS  $SV_{40}$  AND IMMUNIZED WITH  $SV_{40}$  OR ADENOVIRUS

Subgroup			Inoculation	Immunization	Development of tumours				%	
					Months			Total*		
Control	12	Carcinogen	Total	None	1	2	3	4	6/27	22
	13	Carcinogen		SV <sub>40</sub>		5	1		7/21	33
	14	Carcinogen		Adeno 16		3	4		7/27	26
						11	9		20/65	26
Experimental	15	Carcinogen + SV <sub>40</sub>	Total	None	1	5	11	4	21/21	100
	16	Carcinogen + SV <sub>40</sub>		SV <sub>40</sub>	1	7	10		18/21	31
	17	Carcinogen + SV <sub>40</sub>		Adeno 16	3	9	12		24/27	89
					5	21	33	4	63/69	91

\* See footnote to Table 1.

ibilities for using this method to control malignant growth.

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## PATHOLOGY

### Bleeding Time and *in vivo* Platelet Adhesiveness in von Willebrand's Disease: Effect of Iontophoresis of Adenosine Diphosphate

MEASUREMENT of the adhesiveness of platelets *in vitro* has yielded variable results in von Willebrand's disease<sup>1-6</sup>. Hopes of increasing the discrimination between von Willebrand's disease and normal subjects using adenosine diphosphate (ADP) in an *in vitro* system have not been fulfilled<sup>2,3,6</sup>.

In patients with von Willebrand's disease, the *in vivo* platelet adhesiveness of Borchgrevink<sup>7</sup> has been measured in areas of the skin treated with ADP by iontophoresis<sup>8</sup>. A current of 2 m.amp/in.<sup>2</sup> at 90 V was passed for 5 min through a blotting paper pad, applied to the forearm, carrying 0.02M ADP in phosphate buffer pH 6.8. Contralateral control areas were iontophoresed with buffer only. After rinsing the site with buffer, a cuff at 40 mm of mercury was applied to the upper arm and three stab wounds 1 mm long and 3 mm deep were made in quick succession in each treated area. The wounds were lightly blotted every 15 sec to obtain the bleeding time, but this process was interrupted after the first two blottings to allow the outflowing blood to be sampled with a polythene automatic micropipette for platelet counts<sup>9</sup>. Blotting was resumed at 90 sec and continued until bleeding ceased, the end point being calculated from the mean of the three readings. Punctures apparently entering veins were rejected. Platelet counts<sup>10</sup> (about 200 cells) were made "blind", under phase contrast, in duplicate, in a random order and ignoring clumps because these were regarded as part of the haemostatic response. (Clumps were not seen in known samples of venous blood, which was always collected from an arm vein on the control side.) Platelet adhesiveness was given by the ratio (venous count - skin count)/venous count<sup>7</sup>. Factor VIII activities were measured on venous blood collected before iontophoresis, to avoid an adrenaline response to the procedure<sup>11</sup>, by a single stage assay based on the partial thromboplastin time<sup>12</sup>. Patients were diagnosed as suffering from von Willebrand's disease when they had: a history of haemostatic failure; close relations of both sexes who were also affected; a bleeding time >10 min; and a factor VIII level <45 per cent on at least one occasion. Results are shown in Table 1.

In normal subjects ADP increased the platelet adhesion and decreased the bleeding time. In von Willebrand patients the platelet adhesiveness was decreased, but the effects on the bleeding time were more difficult to interpret as six of the ten patients tested had bleeding times longer than 15 min and no end point was obtained. In those von

Table 1. EFFECT OF ADP IONTOPHORESIS ON THE BLEEDING TIME, PLATELET COUNTS AND PLATELET ADHESIVENESS *in vivo*

	38 Normals			10 von Willebrand's disease		
	Venous blood	Buffer site	ADP site	Venous blood	Buffer site	ADP site
Mean platelet count (10 <sup>9</sup> /cmm)	229	162	125	201	148	171
Pooled S.E. mean		9.0			17.6	
Mean platelet adhesiveness (per cent) (calculated from above)	—	28.4	44.9	—	28.2	17.9
Mean difference (ADP-buffer)	—	+17 per cent (P < 0.01)		—	-11 per cent (P < 0.01)	
Bleeding times*						
Range of readings	—	2.9-7.8	2.5-6.8	—	4.7-12.0	8.0-15.0
No. of subjects	—	0	0	—	6	6

\* For normal subjects, the mean for the buffer site = 4.97 min and for the ADP site, mean = 4.37 min; mean difference (ADP-buffer) = 0.60 min (P < 0.01).

Willebrand patients with recordable bleeding times, however, three out of four showed lengthening of the bleeding time by ADP. Von Willebrand's patients could be distinguished from normal subjects irrespective of their factor VIII level at the time of testing. These results are shown in Fig. 1.

The arrest of bleeding from small wounds may usually depend on the interaction of ADP and thrombin<sup>13</sup>. It is likely, however, that ADP introduced into the skin would be broken down to AMP and adenosine<sup>14</sup>, and also other substances may be produced<sup>15</sup> which inhibit platelet aggregation<sup>16,17</sup>. If therefore there were a delay in thrombin generation, as might occur in von Willebrand's disease, a proportion of the iontophoresed ADP might already have degraded to inhibitory products. These might further impair the haemostatic response, accounting for the effects described. Preliminary experiments suggest that iontophoresis of partially degraded ADP will produce a von Willebrand like response in normal subjects.

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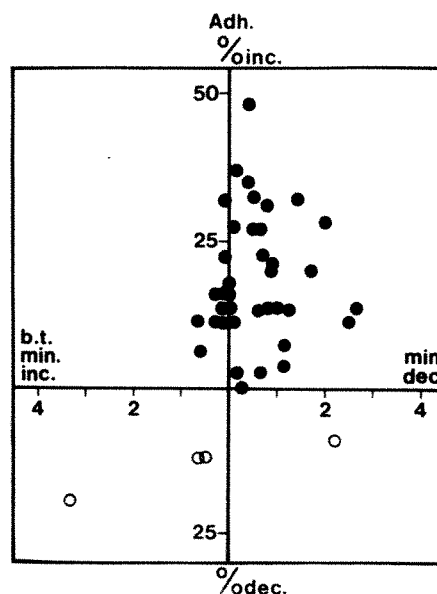


Fig. 1. The effects of iontophoresis of ADP on the bleeding times and *in vivo* platelet adhesiveness of thirty-eight normal subjects and four patients with von Willebrand's disease. The differences between control and treated areas are plotted against each other; differences in *in vivo* adhesiveness on the ordinate; differences in bleeding time on the abscissa. ●, Normal subjects; ○, von Willebrand's disease (patients with bleeding times <15 min). Factor VIII levels on the four von Willebrand patients, measured at the time of testing and reading from right to left, were: 28 per cent, 131 per cent, and 55 per cent. The fourth patient (extreme left) had a factor VIII level of 13 per cent measured on a previous occasion, and this measurement was not repeated at the time of testing with ADP. The remaining six patients were not plotted as their bleeding times were all >15 min and end points were not recorded.

Royal Free Hospital for making available some of her patients with von Willebrand's disease.

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### Some Serum Protein Nitrogen Mustard Complexes with High Chemotherapeutic Selectivity


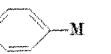
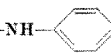
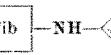
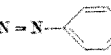
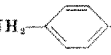
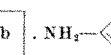

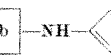

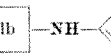
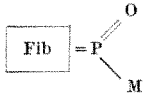

THE use of proteins as carriers of cytotoxic groups has been put forward on several occasions, based on indications that some specificity of uptake is thereby achieved. Busch and Greene<sup>1</sup> have shown that administration of radioactive albumin leads to the selective incorporation of radioactivity into tumours compared with other host tissues. Experimental evidence has also been advanced to show that radioactivity is localized selectively in tumours after administration of anti-fibrin antibody labelled with iodine-131 (ref. 2) and of similarly labelled fibrinogen<sup>3</sup>. This effect was more marked in the case of transplantable tumours than for spontaneous tumours but the results obtained provide a sound experimental basis for the use of this protein as a vehicle for the preferential introduction of cytotoxic agents.

Several years ago, we reported some preliminary experiments on the use of albumin as a carrier of latent cytotoxic groups<sup>4</sup>. No anti-tumour activity was apparent in the assessment of this compound, but dose levels used were far below toxic. It was considered at that time that macromolecules containing a more reactive cytotoxic group would not be very likely to be effective because of intramolecular alkylation with consequent loss of activity. Discussions with Dr B. Larsen (Aarhus) in connexion with his experiments with albumin and melphalan, some results of which have been reported recently<sup>5</sup>, indicated that the use of relatively highly reactive cytotoxic groups might be feasible. Our own experience with polyamino-acid carriers has confirmed this (unpublished work).

We wish to report preliminary data on the use of fibrinogen and two other proteins as carriers of reactive and latent cytotoxic groups. Bovine fibrinogen (Armour) was treated in buffer (pH 8.0) with di-2-chloroethyl-phosphoramidic dichloride to introduce latent cytotoxic groups in which the nitrogen mustard is deactivated by virtue of being linked by a phosphoramidate bond. As a more reactive species, *N,N*-(di-2-chloroethyl)phenylenediamine was coupled to the protein using a water soluble carbodi-imide [1-cyclohexyl-3-(2-morpholinoethyl)carbodi-imide metho-toluene-p-sulphonate].

As control compounds, protein was stirred in buffer with *N,N*-di-(2-chloroethyl)-phenylenediamine in the absence of carbodi-imide to see whether the nitrogen mustard base

Table 1

CB No.	Compound	ADJ-PC64		
		LD <sub>50</sub>	ID <sub>50</sub>	Therapeutic index
1128	HCl.NH <sub>2</sub> -  -M*	6.0	0.36	16
1134	AcNH-  -M	27	1.9	13.5
3494	Fib-  -M	1,900	96	20
3496	Ala-  -M	1,200	10	120
3497	Fib-N=N-  -M	1,700	20	85
3525	Fib-NH <sub>2</sub> -  -M	560	12.8	44
3524	Ala-  -M	1,700	17	100
3528	Glob-NH-  -M	1,200	< 15.6	> 77
3527	Ala-  -M	> 2,400	< 37.5	> 64
3529	Alb-NH-  -M	1,400	< 15.6	> 90
3530	Ala-  -M	1,400	< 15.6	> 90
3212	MPOCl <sub>2</sub>	35	> 20	< 1.7
3498/B	MPOCl <sub>2</sub> Blank†	560	90	6
3492	Fib- 	2,000	430	4.6
3495	Ala- 	1,700	270	6

\* M = -N(CH<sub>2</sub>CH<sub>2</sub>Cl)<sub>2</sub>.

† Supernate fraction after stirring CB 3212 24 h, in buffer.

could be conjugated to protein without the actual formation of a covalent bond. The modified protein was in each case dialysed against 0.2 per cent sodium citrate solution before freeze drying. Coupling was also effected by the use of diazotized *N,N*-(di-2-chloroethyl)phenylenediamine (ref. 6). In this case the product was precipitated from the buffer solution almost immediately and was separated by centrifugation. In addition to fibrinogen itself, an alanylated product was also modified in the hope of obtaining products of greater water solubility<sup>7</sup>. Alanylation was carried out by treating fibrinogen in phosphate buffer, pH 7, with a solution of *N*-carboxyanhydro-DL-alanine in dioxan. After dialysis to remove polyalanine, the product was freeze dried. Analysis showed that about 65 per cent of the lysine ε-amino groups had been modified with DL-alanyl side chains of average length nineteen residues.

Compounds were analysed for amino-acid content, chlorine and alkylating power as measured by the Epstein technique<sup>8</sup> suitably modified for this type of compound. It was found that values for nitrogen mustard content



calculated from the latter two parameters agreed within the limits of experimental error, indicating that the chlorine present in the substance was still covalently bound and was not present as an associated hydrolysis product.

The compounds were tested for inhibitory potency on the *ADJ/PC6A* plasma cell tumour<sup>9</sup> in mice using an assay system similar to that previously described for the *ADJ/PC5* tumour<sup>10,11</sup>. Female *BALB/C* mice were implanted subcutaneously by trocar, and after 10 days a suspension of the protein derivative in oil was injected intraperitoneally at a wide range of dose levels. After a further 10 days the tumours were dissected out and weighed. The  $ID_{90}$  (90 per cent inhibitory dose) was the dose required to reduce tumour growth to 10 per cent of that of the controls, and the ratio  $LD_{50}/ID_{90}$  was the therapeutic index.

Considering first the phosphoramidate mustards (*CB* Nos. 3212, 3498/B, 4392 and 3495), it would appear that coupling with fibrinogen or its alanylated derivative produced no increase in effectiveness. The derivatives of the chemically more reactive *N,N*-di-2-chloroethylphenylenediamine, however, gave results which were of considerably greater interest. The derivative of fibrinogen itself (*CB* 3494) was of the same order of effectiveness (as measured by therapeutic index) as the acetyl derivative (*CB* 1134) of the base, but if alanylated fibrinogen was used (as in *CB* 3496) the index rose by a factor of 6–120.

Somewhat surprisingly, the two derivatives in which the base was not covalently bound to protein (*CB* 3524 and 3525) also showed a high degree of anti-tumour activity being several times more effective than the free compound (*CB* 1128). An Epstein determination showed that the mustard had lost none of its alkylating ability during the protein treatment in buffer and so apparently had neither alkylated the protein nor been hydrolysed. The potentialities of this particular mode of protecting chemically highly reactive compounds and the mechanism whereby it is achieved have yet to be fully elucidated.

Modifications were also carried out in which human albumin and human gamma globulin were alanylated and coupled using carbodi-imide. The resulting compounds (*CB* 3527–3530) have not yet been examined to the limit of their inhibitory potencies, but the data obtained indicate a high degree of anti-tumour effect.

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## PHYSIOLOGY

### Individual Differences in a Physiological Effect of Water Restriction in Rats

WHEN their water intake is restricted, rats will learn to run a maze or press a bar to obtain water. Individual rats learn these tasks with varying speeds, and there are many factors which can contribute to speed of learning and hence to differences between individuals. Among these is the possibility that the same amount of time spent without water does not arouse the same "need" for water in different animals. A "need" for water arises at least in part from the dehydration of body tissues and this is in turn reflected by a fall in body weight which is larger than can be accounted for by the concurrent fall in food intake<sup>1</sup>. There is little information on changes in body weight consequent on water restriction schedules in different animals. The experiments reported here were concerned with estimating the effect of a standard schedule of water restriction on the body weight of rats varying in age, sex or strain.

Rats (Glaxo hooded and Sprague-Dawley) were fed powdered food *ad lib*. (Wyatt GR/R/3: crude protein, 18.8 per cent; oil, 2.1 per cent; crude fibre, 3.1 per cent) and were housed in pairs at 24°–26° C. Body weight, food and water intake were recorded daily at about 10.30 a.m. During periods of water restriction, water bottles were available only between 4.00 p.m. and 5.30 p.m. In addition to water intake during this time the animals obtained about 1.5 ml. water, as 0.1 ml. rewards, in a bar pressing apparatus.

Fig. 1 shows that adult rats who had reached a stable body weight and young growing rats lost the same absolute amount of weight (about 18 g) when placed on a 22.5 h water restriction schedule. The young rats continued to grow during the restriction period. When restriction ended, both groups put on the same amount of weight as they had lost at the start. Changes in body weight followed the same pattern on subsequent short water restrictions and the amounts of water lost and regained were the same as on the first occasion.

Because adult and young rats lost the same absolute amount of weight it follows that the young rats, being lighter initially, lost more as a percentage of initial weight.

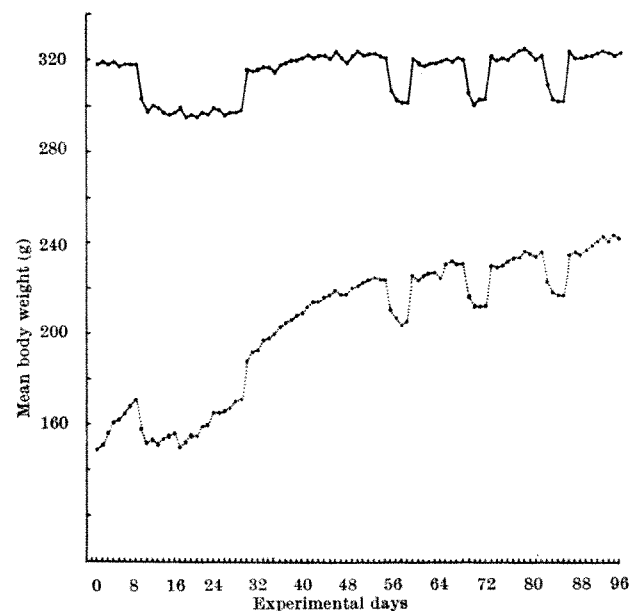


Fig. 1. Mean body weight of male rats submitted to a schedule of water restriction at different ages. Adult rats (●—●) were first water restricted at 245 days of age and young rats (●---●) at 59 days of age. Water was available for 1.5 h each day. Both groups lost about 18 g each time water restriction was imposed and regained this weight when restriction ended.  $n = 4$  in each group.

Table 1. BODY WEIGHT OF MALE RATS FOLLOWING WATER RESTRICTION  
Results are expressed as a percentage of initial weight

Sprague-Dawley				Hooded (young)				Hooded (adult)			
Age (days)	Initial	Body weight %	S.D.	Age (days)	Initial	Body weight %	S.D.	Age (days)	Initial	Body weight %	S.D.
83	235.25	91.09	1.76	83	171.50	87.13	2.37	245	318.25	92.41	1.10
111	389.13	94.21	0.38	111	224.88	91.12	0.55	273	321.75	93.54	0.66
125	405.38	95.05	0.61	125	231.50	91.98	1.39	287	321.88	94.08	0.59
139	426.38	94.97	0.81	139	237.13	91.90	0.60	301	323.25	93.68	0.88
				167	244.63	92.19	0.89	329	324.50	93.30	1.35

Table 1 shows body weight changes of groups of male hooded and Sprague-Dawley rats on successive water restrictions expressed as a percentage of initial body weight. The two strains grow at different rates, Sprague-Dawley growing faster. At the same initial body weight (though necessarily different age) changes in body weight were similar for the two strains. At the same age, however, hooded rats, being smaller, fell to a lower percentage of their initial body weight.

Comparisons can also be made between sexes (Tables 1 and 2): at an initial body weight of about 231–235 g male rats lost more weight (in terms of percentage loss) than Sprague-Dawley females whether they were older (male hooded) or younger (male Sprague-Dawley). The corresponding figure for female hooded rats agrees with these results. The variability was usually higher for the female animals which suggests that the effect of water restriction may be reflected differently, on the body weight measure, in females at different stages of the oestrous cycle.

Table 2. BODY WEIGHT OF FEMALE RATS FOLLOWING WATER RESTRICTION  
Results are expressed as a percentage of initial weight.

Sprague-Dawley				Hooded (young)			
Age (days)	Initial	Body weight %	S.D.	Age (days)	Initial	Body weight %	S.D.
83	168.63	91.69	1.81	89	157.0	91.40	1.22
111	231.13	95.70	1.48				
125	245.25	94.18	3.47				
139	252.63	95.52	4.43				
167	258.88	94.51	1.14				

The percentage figures shown in Tables 1 and 2 were obtained by taking the lowest body weight occurring during the restriction period and expressing this as a percentage of the body weight on the morning preceding

water restriction ("initial" body weight). Similar results were obtained when body weights on the 3 days following water restriction were expressed as a percentage of the three preceding days.

Groups of hooded rats first placed on the restriction schedule at different stages of growth were compared with a group of unrestricted controls of the same age (Fig. 2). The group of rats restricted during the period of faster growth, from 59 to 78 days, grew faster during water restriction than the rats restricted later, from 83 to 103 days. At the age of 174 days and after a total of five periods of restriction, there was no difference in body weight between these groups. Comparison with the unrestricted control group shows that water restriction does not interfere with the rate of growth. The apparently greater body weight of the restricted groups at 174 days is of no significance because the control rats, which had been matched for body weight at 50 days, developed into smaller animals. Such a difference in growth rate is not unexpected<sup>2</sup>. The restricted animals had simply maintained at 174 days the body weight advantage they had over the controls at an earlier age.

Thus the physiological consequences of the same water restriction schedule differ in individual animals. Age, sex and strain of animals of the same species may all influence the extent of loss of body weight following the same water restriction schedule. Our results, as well as considerations of diet composition<sup>3</sup>, may help to interpret discrepant results obtained from different types of rats in experiments on motivational factors<sup>4,5</sup>.

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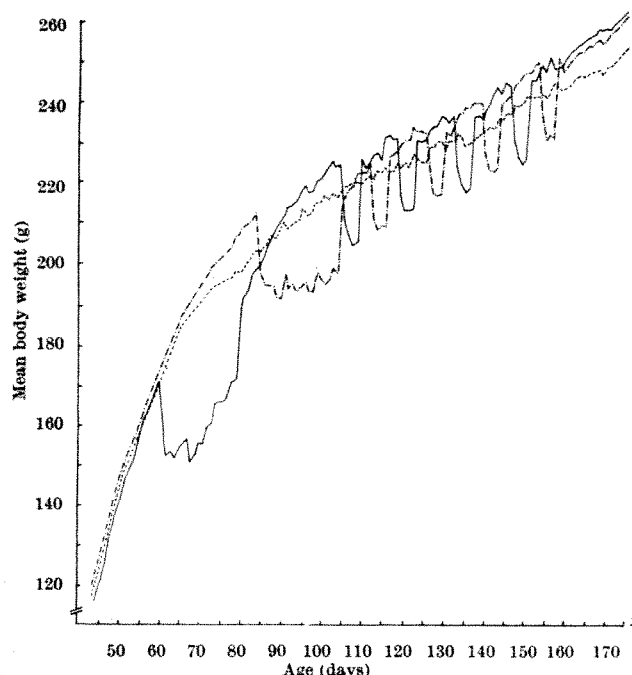


Fig. 2. Mean body weight of young, growing male rats submitted to water restriction at different stages of growth, or unrestricted. Restricted at 59 days old —; restricted at 83 days old - - -; unrestricted . . . . . Water was available for 1.5 h each day. Both groups of restricted rats continued to grow at the same rate as the unrestricted controls.  $n = 4$  in each group.

## Potentials measured from Glycerinated Cardiac Muscle

SZENT-GYÖRGYI<sup>1</sup> has shown that muscle extracted in glycerol serves as a model for the actinomyosin contractile system. He found that molecular orientation is well preserved and that the contractile process can be investigated in the presumed absence of a functioning cell membrane. The contractions of glycerinated fibres from skeletal, cardiac and smooth muscle induced by ATP have since been studied by several workers. Chichibu<sup>2</sup> showed that the resting membrane potential of the extensor muscle of the American freshwater crayfish is decreased by glycerol. 2 molar glycerol reduced the membrane potential from  $-80$  mV to less than  $-20$  mV within 3 h, but fibres kept in glycerol for more than a few hours were not studied.

Nayler and Merrillees<sup>3</sup> stated that a transmembrane resting potential persisted in the glycerinated ventricular muscle of the toad, and ranged from  $-37$  mV to  $-44$  mV in muscle extracted in 50 per cent glycerol for 3 weeks, and from  $-30$  mV to  $35$  mV in muscle extracted for 6 weeks. They suggested that, if these results were interpreted in accordance with the ionic hypothesis of cellular electrical activity<sup>4</sup>, some functional activity must persist in the plasma membrane of glycerinated cardiac muscle. This would make tenuous previous work using muscle extracted in glycerol as a contractile protein model.

We made the investigation reported here to determine whether the potentials described by Nayler and Merrillees represented true transmembrane resting potentials.

The potential difference between modified Tyrode solution<sup>5</sup> and glycerol-Tyrode solutions containing glycerol in concentrations from 0.25 to 50 per cent was measured (Fig. 1). The two solutions were connected through a Tyrode-agar bridge and the difference between the potentials measured through glass capillary microelectrodes filled with 3 molar potassium chloride. Electrodes *D* and *E* in Fig. 1 were first placed in the modified Tyrode solution (*B*), and differences in tip potential eliminated. Then electrode *D* was transferred to the glycerol-Tyrode solution (*A*), and the potential difference between the electrodes measured. In this apparatus four junctions were measured: Tyrode-glycerol; potassium chloride-glycerol; potassium chloride-Tyrode; and Tyrode-Tyrode. In measuring potentials from a preparation of glycerinated fibre (Fig. 2), three junctions are measured when one microelectrode is placed within the fibre and another in the surrounding bath: Tyrode-glycerol; potassium chloride-glycerol; and potassium chloride-Tyrode. Thus the measuring system in Fig. 1 duplicates the junction potentials measured in the preparation of glycerinated fibre except that it contains a Tyrode-Tyrode junction, which should be insignificant, and that it lacks the junctions between the glass capillary microelectrodes filled with 3 molar potassium chloride and the colloid substances contained in the glycerinated fibre. No significant junction potentials were found between the Tyrode and glycerol solutions.

Glycerinated strips of dog ventricular muscle were prepared and stored in 50 per cent glycerol at  $-20^{\circ}\text{C}$  for 1-7 months. Benson and Hallaway<sup>6</sup> noted no change in the contractile properties of similarly prepared muscle bundles stored for 6 months. The resting potential of fresh cardiac muscle is  $-90$  mV, and we recorded potentials in the range of  $-32$  to  $-56$  mV from the glycerinated cardiac fibres, with an average of  $-46$  mV. There was no significant change in the recorded potentials with time. The resistance of the microelectrode invariably increased two to three times when the tissue was impaled.

No change occurred in the measured potential when Tyrode solution containing 25 mmolar or 45 mmolar potassium chloride was substituted for control Tyrode (potassium = 2.7 mmoles/l.). Substitution of Tyrode solution containing 1.35 mmolar calcium chloride or no

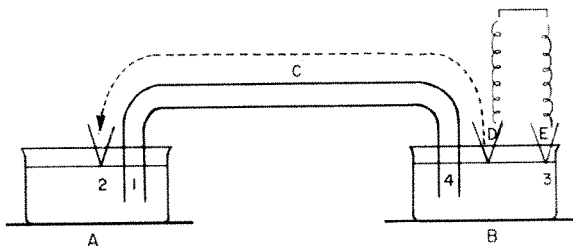


Fig. 1. Petri dish *B* contained the modified Tyrode solution. Petri dish *A* contained varying concentrations of glycerol in Tyrode solution. *C* represents the Tyrode-agar bridge and *D* and *E* microelectrodes. (1) Represents a Tyrode-glycerol junction, (2) a potassium chloride-glycerol junction, (3) a potassium chloride-Tyrode junction and (4) a Tyrode-Tyrode junction.

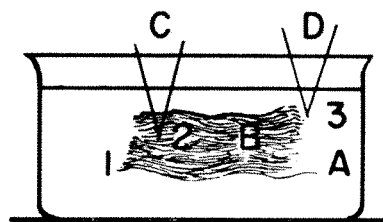


Fig. 2. *A* represents the bathing modified Tyrode solution, *B* the fibre containing glycerol and *C* and *D* microelectrodes. (1) Represents a Tyrode-glycerol junction, (2) a potassium chloride-glycerol junction and (3) a potassium chloride-Tyrode junction.

calcium chloride for the control Tyrode (calcium = 2.7 mmoles/l.) failed to affect the measured potential, as did the addition of epinephrine (0.01-0.06 mg/ml.).

Stimulation of the glycerinated fibres extracted for 1 month, while a potential of  $-50$  mV was being recorded, using rectangular pulses of up to 30 V which lasted up to 100 msec, produced no change in the potential.

Addition of ATP to the concentration of  $4 \times 10^{-3}$  moles/l. caused those glycerinated fibres which had been stored for 7 months to contract, but did not affect the measured potential of  $-48$  to  $-52$  mV.

The surface of glycerinated cardiac muscle which had been stored for 3 months was scraped with the blade of a scalpel. The average potential measured from these abraded areas was  $-28$  mV. Recordings from pulverized cat papillary muscle stored for 3 months were in the range of  $-24$  mV.

A difference in potential similar to that described by Nayler and Merrillees was found between glycerinated cardiac fibres and the extracellular bathing solution, and it was shown that a significant junction potential does not exist between glycerol and Tyrode solution. Bergen and Terroux<sup>7</sup> showed that in mammalian atria an increase in extracellular potassium ions results in depolarization, but increasing the extracellular concentration of potassium chloride did not affect the potential measured from glycerinated cardiac muscle. Hoffman and Suckling<sup>8</sup> showed that severe depletion of extracellular calcium causes a marked decrease in the resting potential of Purkinje fibres. The potential difference measured from glycerinated cardiac muscle did not change even when calcium was removed from the extracellular solution; nor did addition of epinephrine change the potential obtained from glycerinated cardiac fibres. Furthermore, procedures that would surely damage and destroy a cell membrane, such as scraping the surface of a fibre or pulverizing a muscle bundle, did not abolish the potential recorded from the glycerinated muscle. Thus the potential measured from the glycerinated cardiac muscle does not behave like a true transmembrane resting potential. Our observations show that contraction of glycerinated cardiac fibres is induced by ATP independently of any effect on the potential recorded from the tissue. There was therefore no demonstrable action of ATP at the membrane site analogous to excitation. The relatively free access of ATP to the interior of the cell strongly suggests that there is no significant differential permeability of the remnant of plasma membrane with respect to the small ions such as potassium, sodium and chloride. Such differential permeability is a prerequisite for the existence of the membrane potential<sup>9</sup>.

The nature of the potential measured is uncertain. The invariable increase in electrode resistance suggests an interaction at the electrode tip between the colloid components of the glycerinated tissue and the glass or 3 molar potassium chloride. The absence of a significant junction potential in the system of Tyrode solution and glycerol in water suggests that some component of tissue is necessary.

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## Sodium Extrusion by Giant Muscle Fibres from the Barnacle

THE neuromuscular physiology of giant muscle fibres from the barnacle has been described in some detail by Hoyle and Smyth<sup>1</sup>. Single striated muscle fibres up to about 2 mm in diameter have been found. Such fibres have cross-striations similar to those of vertebrate skeletal muscle cells and maintain an internal electrical negativity of from -74 to -80 mV.

In our laboratory, we have found the average intracellular content of sodium of freshly isolated single giant muscle fibres from the barnacle, *Balanus nubilus*, to be 15 mmoles/kg fibre (Table 1), which agrees with the measurements of Brinley<sup>2</sup>. Also measured and presented in Table 1 are the contents of potassium and water of isolated single fibres. If the intracellular sodium is uniformly distributed in the cell water, the internal concentration must be about 20 mmoles/l. The external sodium concentration is about 470 mmoles/l., so the electrochemical theory for passive ionic fluxes<sup>3</sup> predicts that the influx of sodium is about 500 times greater than its efflux. To maintain a steady state with the low internal concentration of sodium, it is evident that, like vertebrate skeletal muscle fibres, the fibres of the giant barnacle must actively transport sodium out of the cells by a process coupled to cell metabolism. The finding<sup>2</sup> of a strophanthidin sensitive and saturating component of sodium efflux is consistent with this conclusion.

Table 1. CATION AND WATER CONTENT IN FRESH BARNACLE FIBRES

Sodium mequiv./kg fibre	Potassium mequiv./kg fibre	Water per cent of wet weight
13	109	75
10	111	77
7	117	76
20	103	—
19	120	—
17	103	69
16	106	67
19	100	67
16	100	67
Mean 15	108	71
± S.E. 1.5	2.4	1.7

The purpose of the work presented in this report was to provide a direct demonstration that giant muscle fibres from the barnacle can produce a net extrusion of sodium ions against an electrochemical gradient. It is well known that vertebrate skeletal muscle fibres can extrude sodium in the presence of external potassium after they have been enriched with sodium<sup>4</sup>. In these conditions, the extruded sodium is replaced with potassium.

We applied methods similar to those used to demonstrate extrusion of sodium in skeletal muscle fibres. Small bundles of barnacle giant fibres were carefully isolated

and stored for 16 h at 4° C in a potassium-free medium of the following composition: Na<sup>+</sup>, 465 mmolar; Ca<sup>++</sup>, 25 mmolar; Mg<sup>++</sup>, 8 mmolar; Cl<sup>-</sup>, 533 mmolar; SO<sub>4</sub><sup>=</sup>, 4 mmolar; *tris* buffer, 5 mmolar (pH = 7.6). At the end of the period of sodium enrichment, single fibres were carefully isolated from the bundle for analysis for sodium and potassium. The remaining bundles of fibres were then transferred to a medium containing potassium ions at a concentration of 10 mmolar; the other ions were present at the concentrations given previously. These bundles were kept in the solution containing potassium for 6 h at 20° C. At the end of this period, single fibres were again isolated and prepared for sodium and potassium analyses. The cation analyses were performed by flame photometry after ashing the fibres in platinum crucibles at 550° C for 10 h. Before ashing, all fibres were rinsed for 10 min in a solution with the sodium and potassium removed and replaced with an osmotic equivalent of sucrose, to wash away any extracellular sodium and potassium adhering to the fibres.

The results, which are given in Table 2, show that, on the average, the fibres recovering in the medium which contained potassium have extruded 12 mequiv. of sodium/kg of muscle and gained 15 mequiv. of potassium/kg of fibre. The net outward extrusion of sodium has obviously taken place against an electrochemical gradient because the outside concentration of sodium is more than ten times the magnitude of the intrafibrillar concentration and the fibres maintain an internal electrical negativity.

The process which actively transports sodium in vertebrate skeletal muscle cells is known to be inhibited by the drug strophanthidin<sup>5</sup> and Brinley<sup>2</sup> has observed a strophanthidin sensitive portion of sodium efflux in barnacle giant muscle fibres. It should therefore be possible to abolish the net sodium extrusion which takes place in isolated barnacle fibres by application of strophanthidin. The procedure followed was identical to that used to demonstrate extrusion except that the recovery medium containing potassium also contained 10<sup>-6</sup> molar strophanthidin. The results are shown in Table 3. It is clear that strophanthidin has prevented the sodium extrusion and potassium gain which takes place in its absence. Fibres in the

Table 2. RECOVERY OF FIBRES ENRICHED WITH SODIUM IN SOLUTION CONTAINING 10 MMOLES/L. OF POTASSIUM

Before recovery			After recovery		
Sodium mequiv./kg fibre	Potassium mequiv./kg fibre	Water per cent of wet weight	Sodium mequiv./kg fibre	Potassium mequiv./kg fibre	Water per cent of wet weight
25	85	73	18	103	72
20	91	72	17	97	71
24	97	71	13	106	69
31	86	73	29	95	72
28	93	72	13	108	70
27	93	71	15	103	70
53	92	70	21	125	69
22	109	69	11	125	67
24	113	67	29	120	68
41	104	67	20	125	69
30	109	67	17	121	67
29	106	67	9	125	67
Mean 30*	98†	70	18*	113†	69
± S.E. 2.6	2.8	0.7	1.7	3.4	0.6

\*  $P < 0.001$ .

†  $P < 0.005$ .

Table 3. ACTION OF 10<sup>-6</sup> MOLAR STROPHANTHIDIN ON THE RECOVERY OF FIBRES ENRICHED WITH SODIUM IN SOLUTION CONTAINING 10 MMOLES/L. OF POTASSIUM

Before recovery			After recovery		
Sodium mequiv./kg fibre	Potassium mequiv./kg fibre	Water per cent of wet weight	Sodium mequiv./kg fibre	Potassium mequiv./kg fibre	Water per cent of wet weight
35	79	70	45	72	71
18	105	69	29	88	70
22	88	70	33	84	72
18	94	70	34	84	73
23	90	71	29	95	71
32	100	70	28	96	70
28	94	71	27	85	77
34	84	72	28	84	70
28	91	71	—	—	—
Mean 26*	92†	70	32*	86†	72
± S.E. 2.2	2.6	0.3	2.1	2.7	0.8

\*  $P > 0.05$ .

†  $P > 0.1$ .



recovery medium containing strophanthidin continued to gain some sodium and lose some potassium. All the results reported are statistically significant at the stated levels.

It is interesting that strophanthidin prevented the gain of potassium as well as the extrusion of sodium. The inward movement of potassium ions to replace the extruded sodium ions must be in some way linked with the mechanism for transporting sodium ions outwardly. Keynes *et al.*<sup>6</sup> have shown that, in vertebrate skeletal muscle, the inward potassium movement during recovery may be in the direction of or against the electrochemical gradient for potassium ions, depending on the conditions. The nature of the inward potassium movement during recovery of barnacle giant muscle fibres from the sodium-rich state is now being investigated in this laboratory.

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## GENERAL

### Biological Entropy Pump

THE recent exchange between Woolhouse<sup>1</sup> and Popper<sup>2</sup> presents the enigma of the low entropy content of living systems. The means whereby their rapid decay into the inert state of "equilibrium" is avoided are serious problems to the theoretical biologist. It is, of course, to Schrödinger<sup>3</sup> that we owe the classic analysis of biological entropy. It was the source of negentropy which Schrödinger was unable to discover, as the recent discussion indicates. That from one generation to the next, a complex coding and decoding can occur without net loss of negentropy is problem enough, but in the large sequence of generations through geological time, we see that, if anything, the informational system becomes fuller and the organization of many species more improbable. It is my purpose here to show what I believe to be the answer to this seeming paradox and to describe the "entropy pump" whereby the species of living matter not only prevent a drop into a position of greater positive negentropy at each generation, but may in fact acquire more negentropy as their reproduction continues. This will involve only principles widely known and easily defended.

From the second law of thermodynamics, we learn that at temperatures above absolute, chemical change always proceeds, in a statistical system, from states of lower to higher entropy. Put in a biological reference we might express it thus: if a female codfish lays a million eggs, these eggs (considered en masse) will show the expected degradation and the egg genomes will have an average entropy content higher than the original egg genome of the parent. Very few generations of such deterioration can be envisaged as compatible even with the continuance of life. Yet we know that such a succession of generations of nearly infinite extent precedes our hypothetical drama. The ace in the hole, so to speak, is that in such a system as the million eggs from one parent, the entropy content

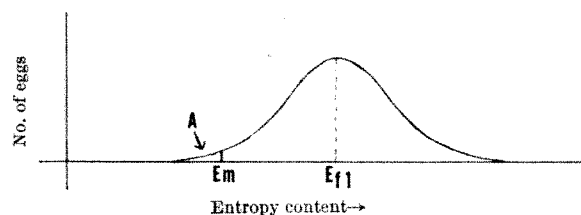


Fig. 1. The "entropy pump" as illustrated by the hypothetical distribution of entropy content of the genomes of 1,000,000 eggs produced by one codfish. The entropy content of the maternal genome is indicated by  $E_m$ . The mean entropy content of the genomes of the eggs is denoted by dotted line  $E_f$ . A marks area where egg genomes have entropy lower than or equal to that of the parent.

of the individual genomes may be expected with certainty to distribute in a simple fashion about a mean value which by the dictates of the second law must be of a higher value for entropy than that of the original egg genome. In such a set we might suppose that, in perfect consistency with the second law, one tenth of 1 per cent or some such low proportion of the eggs actually has a genome with an entropy content equal to or less than that of the parental egg (Fig. 1). These remarkable ones are most likely to grow up and repeat the reproductive process. It will be seen that this is but a re-statement of the mechanism of natural selection, fully consistent with the meaning of the term as used by Darwin and as still used by our contemporaries.

This simplification does clarify one point which has perhaps not been sufficiently stressed in statements of evolutionary mechanisms. For this I suggest the term "chemical selection" to denote the very intense selection which goes on in such a clutch of eggs long before the "survival of the fittest" exerts its statistical leverage. Many of the eggs would not hatch because, as they are at the high end of the entropy distribution, they have such serious defects as the lack of some essential enzyme which crosses them out of the survival race at the starting gate. When we consider that mammalian sperm frequently show 10 per cent morphological abnormalities, the reality of this chemical selection may be appreciated. Selection at this molecular level has been discussed previously by Lima-de-Faria<sup>4</sup>, although in a context apart from the present one. It has also been appreciated by Whyte<sup>5</sup> as "internal selection".

One might be tempted to the view that such an "entropy pump" would be a characteristic of living processes and might indeed furnish a definition to distinguish the living from the non-living world. Since the advent of subcellular biology and of the molecular approach to life processes, it would be gratifying to differentiate the world of living things from that of the non-living with such a succinct criterion. The note of Spiegelman *et al.*<sup>6</sup> extends the process of chemical selection down to the adaptation of enzymes, thus raising the question of the propriety of defining life only with reference to cellular processes. There seems to be little objection to the hypothesis that life preceded the evolution of cellular structure and that subcellular mechanisms may show replicative or reproductive processes. The differentiation of the living from the non-living may perhaps be made on the basis of such an "entropy pump".

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<sup>1</sup> Woolhouse, H. W., *Nature*, **213**, 952 (1967).

<sup>2</sup> Popper, K. R., *Nature*, **214**, 322 (1967).

<sup>3</sup> Schrödinger, E., in *What is Life?* (Cambridge University Press, 1944).

<sup>4</sup> Lima-de-Faria, A., *J. Theoret. Biol.*, **2**, 7 (1962).

<sup>5</sup> Whyte, L. L., in *Internal Factors in Evolution* (Tavistock Pub., London, 1965).

<sup>6</sup> Spiegelman, S., Mills, D. R., and Peterson, R. L., *Science*, **156**, 542 (1967).

## BOOK REVIEWS

### PROBLEMS WITHOUT INSIGHT

#### Major Problems in Developmental Biology

Edited by Michael Locke. (25th Symposium of the Society for Developmental Biology.) Pp. x+408. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1966.) 128s.

THIS anniversary volume celebrates publication of the twenty-fifth symposium of the Society for Developmental Biology. What has developed in developmental biology during this period? For one thing, a new view has appeared, and seems now to generally prevail, to wit: "Differentiation (is) the outward sign of selective gene action . . . We shall make use of . . . the increasingly plausible concept . . . that superimposed on the basic template principle . . . there are control mechanisms." (James Ebert and M. E. Kaighn, second chapter of the present volume.) That development constitutes the orderly, sequential turning off and on of the right genes in the right places at the right times is a proposition which few would contest today. The central questions of developmental biology are: How are genes repressed? How are they turned off and on? and How does the programming of gene expression work? These questions, which lie at the heart of developmental biology, are not dealt with in the present volume. *Major Problems in Developmental Biology* deals rather with the new descriptive embryology, the fact that different specialized cells have different enzymes, respond to different media with different growth patterns, are differently affected by hormones, and so on. It may well be that hidden in this wealth of descriptive material of different developmental systems is the system which, by its study in depth, will reveal a new central principle or principles about how development works. One cannot today point to that system.

*Major Problems in Developmental Biology* is none the less not to be sneezed at as a book to read. The introductory chapter by Jane Oppenheimer, a "what were they doing 25 years ago?" kind of chapter, is wonderful. Holtfreter was studying the development of pieces of gastrulae; Harrison, induction by the neural crest; and Brachet, the number and location of sulphhydryl groups in developing amphibian embryos. Change was, however, just around the corner. Beadle, Ephrussi and Tatum were shortly to establish biochemical genetics and hence molecular biology, and then Ebert's and Kaighn's present formulation of developmental biology. The second chapter, by James Ebert and M. E. Kaighn, is a thorough review of modern work of development on the cellular level, and even to some extent on the molecular level. "Are there cases of differential DNA replication other than that which has to do with the nuclear organizer?" they ask—a good question and one that deserves to be settled. Great emphasis is placed, too, on the numerous cases of which we now know in which DNA replication is the prerequisite to change of gene state from repressed to derepressed, or vice versa. A third chapter by E. Hadorn reviews his work on determination as studied with fragments of imaginal disks of insect larvae, which can grow apparently for ever as cell cultures in the abdo-

men of adults, but which will differentiate briskly if transplanted to a larva. C. H. Waddington discusses fields and gradients, a subject obviously not to his taste, while J. P. Trinkhaus reviews morphogenetic cell movements, coming to the conclusion that there are many unsolved problems in this field. So far as the aggregation of similar cells out of a mixture of dissimilar cells, Trinkhaus votes for selective adhesion of like cells. H. Ursprung in "Patterns of Development" comes to the conclusion that it may well be that all patterns flow from the amino-acid sequences of proteins, and thus directly from nucleotide sequence in the gene. Maybe so—but it's a long step from nucleotide sequence to patterns in butterfly wings or banding of feathers. D. E. Koshland and M. E. Kirtley bring a new discipline, protein chemistry, to the Society for Developmental Biology. They develop a new general theory of control of gene and cell activity. On the level of transcription, Koshland and Kirtley propose that a polynucleotide, presumably the product of the specific DNA sequence involved, is bound covalently to a protein, possibly also the product of that gene, which in addition contains an appropriate binding site for a small effector molecule. They do not specify how such an RNA protein molecule by binding specifically to the gene in question would cause that gene to be repressed so far as transcription is concerned.

Anton Lang next takes up the new knowledge of the action of plant hormones. Thus, it is now clearly established that for gibberellic acid to exert its effect by increasing the rate of plant cell elongation, DNA synthesis must first occur. Induction of cell elongation by indole-acetic acid, a second plant hormone, does not require this intermediate step. Nature is clearly telling us something here, but we do not yet understand her message. Interestingly enough, the influence of gibberellic acid on the aleurone layer of the barley endosperm, an influence which causes the *de novo* production of  $\alpha$ -amylase and other hydrolytic enzymes, does not appear to require DNA synthesis. J. W. Saunders and J. F. Fallon review cell death, and point out that many cells die in the course of and as a part of normal development. Cell death in early developmental stages is therefore apparently genetically pre-programmed. H. Rubin discusses in detail contact inhibition and the loss of contact inhibition in the transformation of tissue culture cells to cancer cells *in vitro*. Rubin believes that contact inhibition is caused because the cultured cells prefer to stick to the culture dish rather than to each other, and that loss of contact inhibition is caused by loss on the part of the cells of their affinity for the culture dish. He further suggests that transformation is caused by some sort of self-perpetuating change in the affinity of cell membranes for things like the bottoms of culture dishes. His suggestion should be a testable one.

Finally, and this is a new direction for the Society for Developmental Biology, Marcus Jacobson, in a long and interesting chapter, discusses "starting points for research in the ontogeny of behavior". This important new direction is certainly one still appropriate for study by descriptive methodologies.

*Major Problems in Developmental Biology* does not therefore really state or encompass the central principal problems of the subject. It does none the less provide an evening of pleasant reading. JAMES BONNER

### WHAT SCIENCE EXPLAINS

#### Completeness in Science

By Richard Schlegel. Pp. xi+280. (New York: Appleton-Century-Crofts, a Division of Meredith Publishing Company, 1967.) \$7.50.

As the tide of protest against scientism slowly gathers momentum it is very important that a number of funda-

mental questions about science should be asked in the spirit which is commonly claimed by, and often manifested in, scientists themselves—dedication, caution, and humility. Professor Schlegel's approach admirably fulfils this first condition. These questions are about science and so they are philosophical questions—or if you prefer, metascientific; but not, if you please, "the science of science", a phrase that any mediaeval schoolman would have pounced on as failing to employ a term univocally. But the questions are about science; and because science, as commonly understood, is involved in action, only actors in the drama are likely to possess the insight necessary for an accurate appraisal of the content of scientific knowledge. It is thus to scientists themselves, standing back in philosophic detachment, that we must look for answers to these questions. Unfortunately, the last "scientist" appears to have been Aristotle; today we have (on the most optimistic view) only "physicists", "chemists" (said to be approaching extinction) and "biologists". Further progress in fundamental physics seems likely to turn as much on philosophical insight as on particle accelerators, thus it is not surprising that the majority of scientists approaching these philosophical problems are physicists. "Nothing is here for tears", providing that "physics" is not tacitly (less often blatantly) regarded as coextensive with "science". Professor Schlegel (whose exposition of the physical aspects of the questions conveys to me a sense of deep understanding but whose accuracy in detail I am not competent to assess) explicitly repudiates (page 5) any such claim; a slight relapse (page 249) does not seem to have coloured his final judgment. A mere outline of Schlegel's book would be mainly a recitation of the obvious problems that arise in relation to the central question, hence such exposition as is necessary may be combined with critical comment. This procedure is all the more necessary in view of my suspicion that an error of strategy has been committed that to some extent reduces the impact of an otherwise very timely and accomplished piece of work.

I have referred at the beginning of this review to a "number of questions" whereas the author appears to ask only "How Completely Science may Describe the Universe", which is his sub-title. But the first chapter is headed "The Problem of Completeness in Science", and the first paragraph ends: "In a sentence our problem is: What do we mean by completeness in science, and to what degree is this completeness achieved or achievable?" Although this question is highly relevant to the first it is not the same question. That I have not merely misunderstood the author's intention of asking the same question in different words is I think shown by the inclusion of the fifth chapter on "The Gödel Incompleteness Theorem". This, it will be recalled, is concerned with formally undecidable sentences in formalized systems. Until "science", as distinct from (possibly) parts of physics, has been completely formalized—a consummation even the outlines of which are as yet hardly conceivable—the Gödel theorem cannot strictly apply. Schlegel is of course aware of this, but claims its relevance on the grounds that a "complete" enumeration of the sentences of a "descriptive science" would include (page 80) undecidable sentences. But is such a "complete" inventory relevant to the function of science? And even if it were, ought not the author to make it quite clear that the "incompleteness" inherent in formal undecidability is hardly if at all related to the incompleteness expressed in his own words: "the explicit structure of science must be regarded as a statement of a few abstracted aspects of the totality of nature; aspects that have universality and are of importance to us, but do not constitute the entirety of experience. In a word science is a very, very partial account"?

There are thus two distinct questions—completeness in science and completeness of the scientific account of—

account of what? Here again, although providing many illuminating insights towards answering the question—the tenth chapter "The Observer in Quantum Physics", and the section of the second chapter in which he applies Margenau's epistemological schema of "constructs", are at the very heart of the problem—Schlegel never makes explicit whether he is talking about "nature" as a convenient symbol for the totality of human experience ("including ourselves", page v), or what Eddington called "Nature", designated by Schlegel as "her" (page v). But, leaving aside the question of our "knowledge" of sensible objects, once we pass beyond this, "interaction" becomes virtually a postulate: knowledge of, say, an electron is restricted to the "interaction" between some arbitrarily applied "stimulus" and some component of nature—there is no need to complicate the issue by introducing an "observer". To the unsensed "continuant" (Whitehead's "historic route of occasions") it is convenient to give the name "electron". Our knowledge of it is thus determined by what we compel it to do: of electrons "in a state of nature" we have and can have no knowledge, any more than of the "smell" of nitric oxide. Although Dingle may be accredited with administering the necessary shock, it is, I believe, the "fishing net" analogy suggested by Eddington and the more abstract approach of von Weizsäcker and Cassirer that put the matter in a manner avoiding solipsism or extreme idealism. Yet despite Schlegel's admission that nuclear ejects are probably "made" by "interaction" with artificially accelerated particles (page 227), the general reader is given no hint of the fundamental importance of "interaction" until a fairly late stage in the study of "quantization"; and its general relevance to the problem of "complete description of nature" is nowhere worked out.

For somewhat similar reasons I am suspicious of the relevance of the otherwise fascinating application of the theory of transfinite cardinals and infinite sets to the problem of the "number of objects in the universe". Schlegel's distinction between the "infinite" as an "operator" ("make the finite cardinal as large as you please"), and the transfinite aleph zero as an actual cardinal, having defined (or postulated) properties based on one to one correspondence, with counting explicitly excluded, is well taken. But the application of transfinite correspondence to attaining "a measure of understanding and information with respect to infinite [countable] quantities of any kind: atoms, stars, parsecs, or years" seems to me open to the objection that the transfinite are arbitrarily constructed objects to enable mathematicians to deal with such arbitrary objects as the "points" in the continuum. As long as you follow the rules of correspondence you can deduce relationships otherwise inaccessible; but the essence of "quantization" in nature is the recognition of the non-existence of infinitely divisible objects. The spatio-temporal continuum has held out so far, but has been under fire from many quarters.

One further aspect of these questions calls for critical comment—the language of science. Dedicated scientists will not be much perturbed by the question of "completeness of description"; but ordinary folk, including ordinary school children, are showing signs of disquiet. The time may come when ordinary tax-payers begin to question the increasing annual sacrifice to the sacred cow. If the risk of a retreat from science into anti-science is to be avoided, scientists must heed Professor Denys Wilkinson's warning and refrain from expressing the relations of abstract systems in terms trailing clouds of confusion from long association with the familiar concrete. I have already referred to the "existence" of mesons "in" the nucleus. But "expanding universe", "curved space", "infinite distance" (which Carnap long ago replaced by "straight line having no greatest co-ordinate"), even "position and velocity at an instant" are likewise replete with possibilities of confusion and internal

contradiction, although they refer to concepts that can be given a precise connotation within a theory. Schlegel seems to accept this in principle (page 151) but fails to fasten the reader's attention to its importance.

This review has been almost entirely critical: I hope Schlegel would regard it as a response to his para-Gödelian sentence, "I would have known that I had failed if I had come to a final answer". My comments are intended to supply a small contribution towards the strengthening of what appear to me to be a few weaknesses in the philosophical foundations in a book that has made me think and think hard. I hope that it will engage the thoughts of a wide circle of readers. It is admirably produced: only two trivial typographical slips caught my eye.

WILLIAM P. D. WIGHTMAN

## ODDMENTS OF OPTIMIZATION

### Topics in Optimization

Edited by George Leitmann. (A Series of Monographs and Textbooks on Mathematics in Science and Engineering, Vol. 31.) Pp. xv+469. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1967.) \$18.00.

THIS book is a collection of ten papers dealing with various aspects of optimal control theory. The stated purpose of the collection is to record in permanent format worthwhile contributions which are too long for inclusion in technical journals. Some material in the longest contribution, "On the Geometry of Optimal Processes", by Blaquiére and Leitman, however, has already appeared in book form, while several other contributions are short enough to appear in journals. Indeed, as in other similar collections, it is unlikely that more than one or two of the contributions will be of particular interest to a reader. This book is, however, distinguished by a most interesting and important contribution by Halkin, "Mathematical Foundations of System Optimisation". In this chapter Halkin outlines a geometric proof of the maximum principle, which he developed several years ago, and whose exposition he has refined in successive papers. By means of a new theorem he is now able to develop the proof "without introducing measurable control functions which are mathematical artifacts, completely out of place in control theory". This simplification makes the proof understandable by a relatively wide audience, and will be much appreciated. This chapter is, without doubt, the most important in the book. Another useful and interesting contribution is the chapter on singular extremals by Kelley, Kopp and Moyer. The maximum principle does not provide an adequate test for optimality on singular subarcs; this results in the failure of normal gradient methods of optimization. Singular extremals occur in problems of practical interest, and so the development of suitable numerical procedures is an important task. This is not done in this contribution, but a useful derivation of the conditions of minimality of singular arcs over short intervals of time is presented. The chapter by Blaquiére and Leitman presents (in 107 pages) a geometric approach to optimal processes which is at first sight very appealing, but which leads to considerable complexity. Halkin's proof of the maximum principle is undoubtedly preferable. The remaining contributions are either actually or potentially short enough for publication in journals, and deal with: bang-bang principle for non-linear systems; the relationship between classical calculus of variations, functional analysis and optimal control theory; inequalities and discontinuities in variational problems; determination of optimal control of a boosting vehicle; optimal problems in the theory of magnetohydrodynamics; numerical methods for the determination of minimum time control.

D. Q. MAYNE

## INFRARED SPECTRA

### Interpreted Infrared Spectra

Vol. 3. Including a Cumulative Index. By Herman A. Szymanski. Pp. ix+275. (New York: Plenum Press Data Division, 1967.) \$12.50.

THE third volume of Szymanski's *Interpreted Infrared Spectra* is devoted to phenols, ethers and peroxides. For each class, spectra with vibrational assignments are presented for typical compounds. This is followed by a short discussion of correlations and a table of appropriate group frequencies. Then the spectra of some fifty or sixty compounds in each class are reproduced, attention being drawn to the various group frequencies and any special points in each case. The aim is to provide instruction in spectral interpretation by showing how the significant bands may be identified and recognized in the spectra of related compounds, even where interfering bands may be encountered in some instances.

A short literature survey on phenols covers hydrogen bonding, and the effects of *ortho*- and *para*- substituents. Ethers are dealt with at some length in two separate sections, non-cyclic and cyclic ethers. Group frequencies for peroxides and hydroperoxides are discussed in relation to ethers and alcohols respectively. Some tentative —O—O— stretching frequencies are assigned but this band has only a limited diagnostic value.

The reasons underlying the choice of spectra are not always clear. Thus for the first member of the ethers, dimethyl ether assignments reported in 1945 and 1957 are given but more recent work is not included. Consequently, the book has limited value to those interested in detailed assignments. It will be of most value to those who wish to make use of group frequencies and know the class of compound they are working with. Interestingly enough, the author confesses to the view that where it is a question of identifying an unknown from an infrared spectrum he is convinced the only method is an exact matching of each band using a computer sort.

A cumulative index covering not only phenols, ethers and peroxides, but also alcohols and alkynes, is provided. The book is splendidly produced, tables well set out, and spectra clearly reproduced.

D. J. MILLEN

## LINEAR ALGEBRA

### Linear Algebra

By Walter Nef. Translated from the German by J. C. Ault. (European Mathematics Series.) Pp. x+305. London: McGraw-Hill Publishing Company, Ltd., (1967.) 80s.

THERE are many books on linear algebra. What is different about this one? The special feature is the blending of pure and applied aspects of the subject. This book is not a practical primer on the matrix calculus, such as might be useful to those engaged in computation; and it is not devoted exclusively to the theory of vector spaces and their linear mappings. Instead it lays the foundation for the theory of linear algebra and then proceeds to an introduction to linear programming and the theory of games. As a result, the book will be of special value to those students who will be concerned with applications but who wish to understand the fundamental theory at the back of what would otherwise be little more than a set of rules and recipes.

The book opens with a short chapter on sets and mappings and then proceeds to the initial treatment of vector spaces, including the theory of cosets (linear manifolds) and quotient spaces. The next chapter is concerned with bases of a vector space, especially a space of finite dimension. It includes a discussion of the exchange method of numerical calculation. The following chapter, on determinants, starts with a treatment of permutations



and then defines the determinant, of order  $n$ , as a scalar function of  $n$  vectors (each with  $n$  components) which satisfies certain conditions. The reader will notice that, so far, matrices have not been introduced. But the next chapter, which introduces linear mappings of a vector space, presents the matrix as a representation of such a mapping. Numerical inversion of matrices (of low order) is discussed. Linear functionals are introduced in the following chapter and are used in a discussion of the important concept of duality in a finite dimensional vector space. Then there is a chapter on systems of linear equations and systems of linear inequalities, the latter occurring in the subject of linear programming, which is treated in the following chapter. The treatment is not, of course, exhaustive but it includes a discussion of free variables and the duality law. There is, in the next chapter, an account of Chebyshev approximations and this is of particular value because the topic is usually omitted in books on linear algebra. A short chapter on game theory, which proceeds as far as the evaluation of games by the simplex method, is followed by chapters on quadratic and Hermitian forms and on Euclidean and unitary vector spaces. In this second chapter Hilbert space is introduced and thus contact is made with the subject of linear analysis. The book closes with two chapters covering eigenvalues, eigenvectors and canonical forms.

It is likely that some students will not find the book an easy one. The exposition is actually very clear, but it presupposes an introduction to abstract algebra at the matriculation and first year level, which many students may not have had. This highlights again the need for overall revision of syllabuses at the school level. On the other hand, it must be admitted that not all those interested in the use of mathematics will be capable of much abstract thought. For these, as far as linear algebra is concerned, the present book is not the text to follow.

L. S. GODDARD

## MACHINES COME TO LIBRARIES

### Library Planning for Automation

Edited by Allen Kent. (Based on the proceedings of a conference held at the University of Pittsburgh, June 2-3, 1964.) Pp. ix+195. (Washington, D.C.: Spartan Books, Inc.; London: Macmillan and Co., Ltd., 1965.) 52s. net.

THIS volume begins with a visionary description by Dr Stafford L. Warren of a proposed national science library system for the United States, consisting of a network of terminals linked electronically to a central store, by means of which on-line access could be had to the whole text (not simply to the titles) of scientific periodical literature. This would be an extension of the system developed by the National Library of Medicine, which is still the most sophisticated development of automated bibliography. There follows a chapter by Dr Andrew D. Osborn of the University of Pittsburgh, who introduces the professional librarian's realism and practical expertise.

The book illustrates the lack of *rapprochement* which can result from the failure to distinguish between the development of a purely scientific information system primarily relying on periodical literature, and the possible role of automation in a general library. Dr Warren writes with the first of these in mind, while Dr Osborn criticizes him from the second standpoint and issues some salutary caveats. A well indexed book is as good a form of random access as has yet been devised. It is important that lack of sympathy for the information officer's approach should not blind librarians to all that automation can achieve in a general library (up-dating of catalogues, output printing, control of accessions and borrowing). The overall plans produced by Dr Warren are stimulating and ought to shape much thinking in this country. Dr Warren is a

special assistant to the President of the United States. The expenditure he advocates is on a very large scale. When, in this country, shall we see advocacy on this level of such far-reaching and imaginative schemes?

ROBERT SHACKLETON

## FURTHER DEVELOPMENT

### The Biochemistry of Animal Development

Edited by Rudolf Weber. Vol. 2: Biochemical Control Mechanisms and Adaptations in Development. Pp. xiv+481. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1967.) 168s.

THE biochemistry of development is not a subject of which all aspects can be discussed fully in two volumes of review articles, especially if development is used in its widest sense to include regeneration, metamorphosis, growth control and so on. For this reason the editor has selected eighteen aspects of the subject for detailed review (ten in the first volume and eight in the second), and these books are to be regarded as collections of essays, between which overlap has been largely avoided. The second volume deals mainly with the control of developmental processes and contains articles on primary embryonic induction (by Tiedemann), metabolic regulation of growth and differentiation (by Papaconstantinou), nucleo-cytoplasmic interactions (by Chen), metamorphosis (by Weber), and regeneration (by Flickinger); the last three chapters, by Williams, Villet and Fisher, concern respectively yolk utilization, the mammalian placenta, and nitrogen metabolism in embryos. The main emphasis has been on vertebrate embryos and is justified by the distribution of current biochemical knowledge of embryos and differentiating cells.

Each article in the book constitutes a detailed and comprehensive review of the topic concerned, and this is where the main merit of the work lies. At a time when embryologists are required to have some familiarity with work classified as genetics, cytology, molecular and cell biology, there is a real danger that old, or even recent, as opposed to current, literature will be overlooked. Lengthy review articles of the kind included in this volume constitute most valuable sources of information and references, and speculation which is not extensive is clearly distinguished from facts. As might be expected, however, this treatise does not constitute light reading, and serves more as a source of results and facts than of ideas. Thus several contributors have chosen, as in the first volume, to divide the work they discuss into categories corresponding to the taxonomic groups of animals concerned. This kind of treatment tends to result in uncohesive lists of achievements, but is of value in subjects, like the biochemistry of development, where a new finding may well turn out to link up a number of otherwise isolated pieces of information the significance of which was previously little, if at all, understood.

This volume is devoted to the control of developmental processes. At our present level of knowledge this usually involves showing a relationship between a developmental event and an altered biochemical pattern or pathway. As several contributors have pointed out, this does not show whether the relationship is causal or consequential. Until questions of this kind can be answered, ideally by creating known biochemical alterations in living cells, progress requires the accumulation of as much information as possible about the biochemical events associated with a given developmental process. Much of this information has been conveniently collected in these two volumes.

The limitations of these books are largely unavoidable and not serious. First, there are necessarily some aspects of the biochemistry of development, which are not fully covered, but these are surprisingly few and of these most are at least mentioned by one or other contributor.

Second, most articles appear to have been completed in 1965, about two years before publication. This is not a disadvantage as long as the contributions are recognized as digests of the relevant literature up to 1964/65. The price of the book is high, but it is well produced and the illustrations appear good.

The first and second volumes of this treatise constitute a most useful contribution to the study of development. They will be valuable as reference books for several years to come, especially for graduate students and research workers. It is perhaps unfortunate that two other books composed of collections of essays on the biochemistry of development should also have been published very recently: *Current Topics in Developmental Biology*, Vol. 1, and *Comprehensive Biochemistry*, Vol. 28, edited by Florkin and Stotz. The latter together include review articles by no less than five of the same authors as have contributed to *The Biochemistry of Animal Development*. In at least one case the two contributions of the same author are not easily distinguishable.

J. B. GURDON

## EXCITABLE CELLS

### The Molecular Properties and Evolution of Excitable Cells

Vol. 35. By C. J. Duncan. (International Series of Monographs in Pure and Applied Biology: Division of Zoology.) Pp. xi + 233. (Oxford, London and New York: Pergamon Press, Ltd., 1967.) 70s. net.

THE object of this monograph is to present a concrete hypothesis linking the evolution and the molecular properties of excitable cells. This is a formidable task, made all the more so by the multi-disciplinary approach entailed in any attempt to formulate a comprehensive theory. The author takes as his starting point the findings in two neurophysiological monographs published in 1964 on axonal and synaptic transmission by the two distinguished authorities, namely A. L. Hodgkin and Sir Jack Eccles. In the presentation of his theory concerning the phenomena underlying these findings, he adduces supporting evidence which is largely that available in the relevant reviews in histochemistry, electronmicroscopy, biochemistry, molecular biology, pharmacology and physiology.

Obviously, such a theory can only represent the views of one man, Dr. Duncan, and attempts to collate findings in many fields call for critical selection and the discarding of data from the plethora of those available. Selection of data, together with the interpretations placed thereon, carries the personal imprint of Dr. Duncan and, of course, can hardly be expected to receive universal approbation but, nevertheless, this brave attempt can hardly fail to stimulate its readers.

Dr Duncan points out that the primitive form, amoeba, reacts to mechanical deformation of its surface membrane by a locomotor action combining receptor and effector in one cell. Development interpolates a conducting cell or neurone which provides a specialized mechanism for excitation by stimuli, propagation of the excitation and its delivery to an effector cell. This development of rapid transmission of excitation by the axon requires little modification of inherent membrane properties.

In this volume Dr Duncan discusses the mechanoreceptors and the evidence for an enzyme transducer mechanism in sensory cells and attempts to relate ATPases with cation-permeability. He turns then to the mechanisms by which chemical, thermal and light stimuli are converted into nerve impulses. Dr Duncan shows that transduction of these four forms of stimuli could arise from a common underlying mechanism, a further example of supreme exploitation by Nature on the lines of the utmost economy. He goes on to consider cholinergic synapses and the relation of acetylcholine

and cholinesterases to substances which modify synaptic function. The roles of adrenaline, noradrenaline,  $\gamma$ -aminobutyric acid (GABA) and of inhibitory synapses are considered and then sodium permeability and the release of synaptic chemical transmitters are examined. He touches on RNA and Nissl substance and on the fascinating hypothesis of the incorporation of a memory trace within the RNA molecule.

In the final chapter, Dr Duncan summarizes the main points of his theory and provides a master diagram to illustrate the main components and the points at which it is vulnerable to chemical agents. He relies strongly on pharmacological findings but some may feel that his speculations based thereon ask, in some instances, more from the evidence than is justified.

While the book would be unsuitable for medical, dental and ophthalmic optical students, it could prove of special value to senior B.Sc. students and graduates in the biological sciences because of its trans-disciplinary approach and wealth of information together with references to review sources which might not otherwise come to the attention of its readers.

It should be read, however, as a scholarly attempt to correlate knowledge from many different sources and to provide intellectual stimulation to critical assessment of the evidence and of the author's speculations. Such books are invaluable as catalysts of new ideas and new research and should be found a place in every biological library.

J. P. QUILLIAM

## YEASTS ALIVE

### The Life of Yeasts

Their Nature, Activity, Ecology, and Relation to Mankind. By H. J. Phaff, M. W. Miller and E. M. Mrak. (Harvard Books in Biology, No. 5.) Pp. ix + 186. (Cambridge, Mass.: Harvard University Press; London: Oxford University Press, 1966.) 44s. net.

THIS small volume makes no pretence to being comprehensive. Thus it is practically devoid of chemical formulae and makes little more than passing reference to well-known uses of yeasts on the large-scale such as in baking, brewing and wine-making. This latter remark is, however, in no way an adverse comment. It is rather intended to show how welcome is this simple but wide-ranging account of a field which for various reasons has hitherto mostly been described only in a few specialized texts or in many respects only piecemeal in original publications.

For instance, "yeast life" here basically embraces yeast morphology and life cycles. Topics such as yeast cytology, genetics, nutrition and ecology comprise ancillary fields while yeast spoilage and industrial uses of yeasts are treated more in an illustrative than in a fully descriptive way. The result is an essay which is not only interesting but well balanced and scholarly. Most botanists and microbiologists will appreciate this book as a more coherent account within its limitations of space than has hitherto been available. The individual specialist in, for example, yeast genetics or biochemical transformations will of course be aware of important developments which the authors have not included but will be agreeably surprised by the succinct way in which so much has been co-ordinated. Most readers including specialists will be surprised to learn or be reminded how recent are many of the findings in this field. It is easy to forget that the very concept of yeasts as distinct from other unicellular organisms is only about a century old, that the early faltering attempts at classification go back only about seventy years, that the first really systematic scheme of Stelling-Decker dates from 1931 and that even the present taxonomic schemes of Lodder-Kreger van Rij (1952) and Kudriavtzev (1954) together with technical methods (for

example, of Wickerham in 1951) remain subjects of debate. Likewise it will surprise many to have it recalled that the recognition of the alternation of generations in yeasts goes back only to 1935, that the concept of "sexual" mating types followed little more than twenty years ago and that the comparatively little that is known of yeast genetics is even more recent.

With its wealth of newly co-ordinated information the few quite unimportant errors can be disregarded. They are more than compensated by a number of appendices including a useful listing of genera and diagnoses. It is slightly regrettable that no original papers are cited though a list of probably all the major monographs of the field will to a large extent meet this need indirectly.

A. H. COOK

## LICHENS FOR STUDENTS

### The Biology of Lichens

(Contemporary Biology.) By Mason E. Hale, Jun. Pp. viii + 176. (London: Edward Arnold (Publishers), Ltd., 1967.) 42s. net boards; 21s. net paper.

THIS is a good book. It is an eminently successful account of lichens for the student. Although it does not aim to be an encyclopaedic work of scholarship, its coverage of the literature is remarkably good, and the clarity with which it is written is outstanding. Its scope is wide: it deals with the fundamental aspects of the morphology, reproduction, ecology and classification of lichens, and lays particular emphasis on the subjects of symbiosis, physiology and chemical taxonomy.

The most valuable quality of this book is the excellent way it deals with those aspects of the biology of lichens which are almost always misunderstood by students. The wealth of fascinating problems concerning reproduction of lichens is presented very clearly. In discussing concepts of symbiosis, Dr Hale gives very lucid explanations of what various authors have had in mind, but stresses our continuing ignorance of the nature of the lichen symbiosis. A section on the physiological relationships of the symbionts begins with the excellent statement: "Dialectic arguments about symbiosis are no substitute for experimental proof". At first glance, the chapter on the chemistry of lichens may seem to require more knowledge of organic chemistry than should be expected of a biology student. It is written, however, with superb clarity, and is an essential precursor to the succeeding chapter on chemical taxonomy, the best in the whole book.

As in any book, there are some small errors. I am embarrassed to be credited on page 61 with a comparison of rates of  $^{14}\text{CO}_2$  fixation between *Trebouxia* and *Chlorella*: this work was actually carried out by Cecil Fox. Drew's studies of *Peltigera polydactyla* mentioned on page 59 showed that glucose is converted to mannitol in the algal layer, not the medulla, during photosynthesis. Some of the plates are poor, and 1D, 1E and 6B look more like objects from outer space than algal cells and soredia. In the next edition, the printers really must try to make the fifteen labels on plate twelve more legible.

I found the chapter on ecology the least stimulating. I am most sorry that Ried's (1963) work on zonation of lichens was omitted, because I believe this to be the best and most thorough piece of physiological ecology carried out on lichens, and I feel it deserves a whole section in a book of this kind. In dealing with atmospheric pollution, it is a pity to find no mention of Gilbert's work on the Newcastle area, because this provides better direct evidence than most other ecological studies of the effects of sulphur dioxide on lichen distribution.

But despite such criticisms, which are the sort that one specialist can always make about another, this book is to be highly recommended.

D. C. SMITH

## USING THE LIGHT

### Primary Productivity in Aquatic Environments

Edited by Charles R. Goldman. (Proceedings of an I.B.P. PF Symposium, Pallanza, Italy, April 26-May 1, 1965.) Pp. 464. (Berkeley and Los Angeles: University of California Press; London: Cambridge University Press, 1966.) \$7; 56s. net.

THIS volume is neither a recipe-book methodology nor a simple collection of research papers. Most of the contributions have a strong emphasis on the methods employed in production research and their potential sources of error, or on their underlying philosophy. Presumably the intention is to give examples of how freshwater productivity data can be obtained so that participants in the International Biological Programme can follow, modify or develop these examples.

Three examples from the twenty-seven contributions indicate the sort of topics and their treatment. Jorgensen and Steemann-Nielsen discuss the ways in which planktonic algae adapt themselves to factors such as temperature and light intensity, primarily by varying the concentrations of photosynthetic pigments and various enzymes. This is obviously important to anyone wishing to extrapolate from experimental data to the field situation.

Fogg and Watt discuss the release of glycolic acid as an extracellular product of photosynthesis by phytoplankton. The amounts released can be quite significant, more so in oligotrophic than eutrophic waters, and especially in surface waters at high light intensities. This is a potential source of error in the use of the radiocarbon method for estimating photosynthesis.

Margalef contributes a stimulating paper on the relationship between primary production and community structure in phytoplankton communities. This emphasizes the importance of (and therefore the need to measure) community structure either as some index of species composition, or as a simple pigment ratio.

Other important topics discussed include mechanisms of photosynthesis, the factors limiting primary production in natural phytoplankton populations, the importance of planktonic bacteria, production by higher plants in aquatic environments, the value of standing crop data in estimating primary production and the comparison of seasonal and spatial variation in phytoplankton populations and production rates in a temperate and a tropical lake.

Each paper has an abstract and a reference list and the whole volume is well edited and produced. The instigation and speedy publication of well-designed conferences will clearly be an important function of IBP and it has got off to a good start.

P. J. NEWBOULD

## POTTED MAMMALS

### Recent Mammals of the World

A Synopsis of Families. Edited by Sydney Anderson and J. Knox Jones, jun. Pp. viii + 453. (New York: The Ronald Press Company, 1967.) \$12.50.

THIS book is the culmination of a series of check lists or bench books compiled for their own use by a succession of graduate students and staff members of the University of Kansas. It began as long ago as 1953 when the contributors prepared an outline of each order and family of living mammals; this proved so useful that it was improved and extended in 1959, and then further revised and expanded with the assistance of mammalogists in other institutions to form the present volume. As the editors say, the final result reflects a commendable degree of teamwork on the part of all contributors in writing to agreed standards of objectivity and conciseness.

The introductory chapter summarizes the characters of mammals, the zoogeographical regions—with a table showing the distribution and diversity of recent mammals—and explains the methods of presentation of the information in the main body of the work. The second chapter gives a synopsis of the fossil history of the families of recent mammals, and packs much information into a small space. Simpson's classification is followed for the greater part, but the cetaceans are treated as two orders, and the pinipedes are ordinarily separated from the fissipede carnivores. The writing throughout is telegraphic in style for the sake of brevity.

The synopsis of each family is arranged under a set of standard headings: diagnosis, general characters, habits, habitat, recent distribution, a list of recent genera with the number of species, geological range, major fossil groups, and remarks. The orders are treated similarly *mutatis mutandis*, and a distribution map is provided for nearly every family. The information given is well documented, and there is an extensive bibliography and a full index. The similarity of organization throughout the volume makes it easy to find and compare information in the accounts of the different families. In a compilation from so many sources, by so many contributors, there are bound to be some mistakes, but a set of test questions failed to reveal any errors.

The book is intended to provide a ready source of information about the living mammals not only for mammalogists but also for other people interested in mammals who work in other disciplines on which mammals impinge such as botany, entomology, ecology or geography. In addition to giving an enormous amount of information in small compass, it is an excellent source book for more detailed studies. It is one of the most useful books about mammals that has appeared since Flower and Lydekker's *Introduction to the Study of Mammals* was published in 1891, and all those to whom it is addressed will find it indispensable and will be accordingly grateful to the team of authors for their enterprise and skill.

L. HARRISON MATTHEWS

## SMALL WORLD

### An Illustrated Catalogue of the Rothschild Collection of Fleas (Siphonaptera) in the British Museum (Natural History)

By G. H. E. Hopkins and Miriam Rothschild. Vol. 4: Hystriehopsyllidae (Ctenophthalminae, Dinopsyllinae, Doratopsyllinae, and Listropsyllinae). Pp. viii + 549 + 12 plates. (London: British Museum (Natural History), 1966.) n.p.

THE publication of a volume of the *Illustrated Catalogue of the Rothschild Collection of Fleas* is a real event for anyone interested in siphonapterology. This so-called catalogue is actually nothing less than a handbook for the identification of all the known species and sub-species of fleas. It not only catalogues the numerous specimens present in the Rothschild and British Museum (Natural History) collections of fleas but also gives keys for identification and short descriptions of all taxa.

The work is of great interest because of its contents, and the way in which the great task of compiling it has been carried out deserves much admiration. The keys and descriptions are lucid; the latter are not long-winded; they have been reduced to essential characters. The numerous excellent figures (926 in this volume) of structures of taxonomic importance are supplemented by twelve plates with photographs of whole fleas or of particular taxonomic features. It is impossible to appreciate here all the excellent details of this work which must rank among the best publications in the field of systematic entomology.

The present volume contains the greater part of the large family Hystriehopsyllidae, including the large genus

*Ctenophthalmus kolenati*. The classification of this genus follows modern ideas, especially those of Smit, and the genus is subdivided into thirteen sub-genera. A "list of taxa not in volumes I to III inclusive", in the appendix, gives references to the taxa belonging to families treated in previous volumes of the catalogue and which have been described since.

The two authors and the trustees of the British Museum (Natural History) are to be congratulated for this admirable book and to be thanked for the painstaking work involved in its completion. I trust that it will be widely used and distributed. It should not be missing from any entomological library, because it should serve, apart from its special subject matter, as a model for papers on taxonomy.

E. HAESELBARTH

## BACKWARDNESS IN READING

### The Disabled Reader

Education of the Dyslexic Child. Edited by John Money. Pp. xiii + 421. (Baltimore: The Johns Hopkins Press; London: Oxford University Press, 1966.) 68s. net.

THIS is a collection of articles—some new, others reprinted—concerned with various aspects of the problem of selective backwardness in reading and spelling. It is "inter-disciplinary" in the modern fashionable sense, with the authors drawn from child psychiatry, paediatrics, experimental psychology, linguistics and remedial education. (Oddly, there is only one neurologist, and he is half psychologist.) While predominantly American in tone, British workers are by no means unrepresented.

The book is divided into two main parts, the first concerned with reading and the analysis of reading backwardness, and the second with teaching methods and the organization of classes for the backward reader. Then come five clinical case reports and an account of some special disabilities in perception and orientation encountered in backward readers. There is a short concluding section, a glossary and a bibliography.

Although the nature and origin of severe backwardness in reading are still imperfectly understood, it now seems clear that at least two major varieties of the disability exist. The first appears in the context of overall language retardation which, in some cases at least, may have a genetical basis. The second appears along with more widespread defects in visual orientation and spatial judgment, and is not infrequently linked with early, minimal brain damage.

The nature of the disability, too, appears to differ somewhat in these two main groups, being largely a difficulty in linking visual with phonetic elements in the first, and of visual orientation and recognition in the second. It should not be impossible to devise remedial methods specifically adapted to these particular forms of disability.

As regards the remedial methods described in this book, there is no doubt that they are largely *ad hoc*, or at all events based on a distinctly insecure rationale. It would be an immense step forward if this book should lead psychologists, doctors and remedial teachers to design genuinely inter-disciplinary research on methods of remedial treatment and to test their efficacy by controlled experiment.

Dr John Money has done much in recent years to focus interest on backwardness in reading and to stress the need for more adequate scientific study of its causes and treatment. In spite of the scrappiness inseparable from compilations of this kind, his book should be warmly welcomed by all concerned with the very real problems of the dyslexic child and those who teach him.

O. L. ZANGWILL



## OBITUARIES

### Professor Gregory Goodwin Pincus

GREGORY PINCUS, who died on August 22, was born in Woodbine, New Jersey, on April 9, 1903. He received his B.S. from Cornell University in 1924 and both his M.S. and Sc.D. degrees from Harvard in 1927. After graduation he served as a Fellow of the National Research Council for three years. In 1931 he became assistant professor at Harvard in the department of general physiology of Professor W. J. Crozier, and in 1938 he went to Clark University as a visiting professor of experimental zoology, holding a Guggenheim Fellowship from 1939 to 1941.

In 1944 he and I established, at Clark University, the entirely independent Worcester Foundation for Experimental Biology, and we have served as its co-directors since its foundation. This institute has become a distinguished centre of biomedical research and postdoctoral training.

Pincus' work in the field of endocrinology has been very notable. He was responsible for new methods of steroid analysis. He and his collaborators conducted many studies of stress in relation to adrenal cortical functions; studies of the role of steroids in cancer of the breast and of the reproductive system; studies of steroid hormones in relation to cardiovascular disorders and also in relation to problems of human ageing. During the mid-1930's his work on mammalian reproduction produced the first parthenogenetic mammals—rabbits that had mothers but no fathers. He was a pioneer in producing multiple ovulation in animals and in transplanting animal ova from females producing the ova to females that carried and developed the embryos to term. Since 1950 his work on the control of fertility has had widespread repercussions with regard to the "population explosion". He was the principal investigator in the development of the first successful oral contraceptive and he and his group continued to investigate a number of steroid contraceptive agents.

Pincus was chairman or a member of many important national committees and of international conferences and congresses. He was a Fellow of the American Academy of Arts and Sciences and a member of the National Academy of Sciences. He was a member or honorary member of physiological and endocrinological societies throughout the world. He was the recipient of numerous prizes, including the Oliver Bird Prize for 1957, the Albert D. Lasker Award in Planned Parenthood, 1960, the Sixth Annual Julius A. Koch Award, 1962, the Modern Medicine Award for Distinguished Achievement, and the City of Hope National Medical Center Award, both in 1964, the Cameron Prize in Practical Therapeutics (University of Edinburgh) in 1966, the Barren Foundation Medal also in 1966, and the American Medical Association Scientific Achievement Award in 1967. He had also been made an honorary professor at the San Marcos University in Lima, Peru.

His books include *The Eggs of Mammals*, published in 1936, and *The Control of Fertility*, published in 1965. He was the author of many scientific papers and served on editorial boards of *Experimental Biology Monographs* and the *American Journal of Physiology*, and was editor or co-editor of books such as *Hormones and the Aging Process* and *Hormones and Atherosclerosis*. He was co-editor of five volumes entitled *The Hormones* and he was consulting editor to *Life Sciences*.

Dr D. Ewen Cameron, past president of both the American Psychiatric Association and the World Psychiatric Association, wrote, in the *New York Times*, concerning the contributions of Pincus in the field of fertility control: "few contributions to medical knowledge have done so much to bring to women everywhere a sense of worth and dignity".

HUDSON HOAGLAND

### Appointments

PROFESSOR B. KATZ, professor and head of the Department of Biophysics at University College, London, has been appointed a member of the Agricultural Research Council on the retirement of Professor T. A. Bennet-Clark.

### Announcements

DR L. H. TURNER of the Leptospirosis Reference Laboratory of the London School of Hygiene and Tropical Medicine, Keppel Street, London, WC1, is acting as convenor of a group of 40 people concerned with the taxonomy and nomenclature of the order Spirochaetales. One possibility is that the group may eventually become a taxonomic sub-committee under the aegis of the International Committee on the Nomenclature of Bacteria. Persons wishing to be associated with the project should write to Dr Turner.

NOMINATIONS are invited for the Beilby Medal and Prize which is awarded annually to a British scientist in recognition of his work in such fields as chemical engineering, fuel technology and metallurgy. Further information can be obtained from the Convenor of the Administrators, Sir George Beilby Memorial Fund, The Royal Institute of Chemistry, 30 Russell Square, London, WC1.

NOMINATIONS are invited for the award of the Meldola Medal which is made annually to the British chemist under the age of 30 who shows most promise in his or her published chemical work. Further information can be obtained from the President, The Royal Institute of Chemistry, 30 Russell Square, London, WC1.

THE opening meeting of the 61st session of the Society of Tropical Medicine and Hygiene will be held at Manson House, 26 Portland Place, London, W1, on October 19 at 7.30 p.m. Further information can be obtained from the Royal Society of Tropical Medicine and Hygiene at the above address.

### Meetings

PEAT and Organic Soils, September 25–27, Southport (Mr H. T. Davies, North of England Soils Discussion Group, Department of Soil Science, National Agricultural Advisory Service, Government Buildings, Lawnswood, Leeds).

GRANITES and Basement of North-Eastern Brazil and their Comparison with those of West Africa, October 1–14, Recife, Brazil (M. J. Lombard, Secrétaire Général de l'Association des Services Géologiques Africains, 12 rue de Bourgogne, Paris 7e).

ULTRASONICS, October 4–6, Vancouver (R. L. Guthrie, Boeing Scientific Research Laboratories, P.O. Box 3981, Seattle, Washington).

SINGLE-CELL Protein, October 9–11, Massachusetts Institute of Technology (Single-cell Protein Conference, Room 16–325, Massachusetts Institute of Technology, Cambridge, Massachusetts).

ANALYTICAL Chemistry in Nuclear Technology, October 10–12, Gatlingburg, Tennessee (C. K. Tipton, Oak Ridge National Laboratory, P.O. Box X, Oak Ridge, Tennessee).

PHYSICS of Selenium and Tellurium, October 12–13, Montreal (Selenium-Tellurium Development Association, Inc., 11 Broadway, New York, NY).

DENTAL Medicine, October 12–14, High Tatras Mountains, Czechoslovakia (Secretary of the Congress, Dr J. Ležovič, Bezručova 5, Bratislava, ČSSR).

APPLICATION of Automation in the Process Industries, October 16–17, Amsterdam (The Institution of Chemical Engineers, 16 Belgrave Square, London, SW1).

## FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

## Tuesday, September 19

SOCIETY OF CHEMICAL INDUSTRY, HEAVY ORGANIC CHEMICALS GROUP (at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Dr A. R. Graham: "Resorcinol from M-Diisopropylbenzene".

## Wednesday, September 20

SOCIETY OF INSTRUMENT TECHNOLOGY (at the A.E.I. Cinema, 33 Grosvenor Place, London, S.W.1), at 5.30 p.m.—Inaugural Meeting followed by "Practical Data Logging—Possibilities, Pitfalls and Progress" (A 'Manufacturer and User' Forum).

OIL AND COLOUR CHEMISTS' ASSOCIATION, LONDON SECTION (in the New Engineering Block, University College, Gower Street, London, W.C.1), at 6.30 p.m.—Mr N. R. Wheeler: "Art Forms from Newer Materials".

## Wednesday, September 20—Friday, September 22

BRITISH CAST IRON RESEARCH ASSOCIATION (at the University of Technology, Loughborough)—Conference on "Electric Melting and Holding Furnaces in the Ironfounding Industry".

INSTITUTE OF PHYSICS and the PHYSICAL SOCIETY (at University College London, Gower Street, London, W.C.1)—Conference on "Stress Analysis in Bio-Engineering".

SOCIETY OF INSTRUMENT TECHNOLOGY (at the University, St. Andrews, Fife)—Conference—"On-Line Measurement and Inspection—Their Impact on Quality".

## Thursday, September 21

INSTITUTION OF MINING AND METALLURGY (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Dr A. J. Ellis, Dr J. S. Tooms, Prof J. S. Webb and Mrs J. V. Bicknell: "Application of Solution Experiments in Geochemical Prospecting"; Mr T. R. Groundwater: "Role of Discounted Cash Flow Methods in the Appraisal of Capital Projects".

INSTITUTION OF MECHANICAL ENGINEERS, MANUFACTURE AND MANAGEMENT GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion Meeting on "Getting the Best Out of People".

## Thursday, September 21—Friday, September 22

CHALLENGER SOCIETY and REPRESENTATIVES from the MARINE LABORATORIES (Natural Environment Research Council Scheme) (at the National Institute of Oceanography, Wormley, near Godalming, Surrey), at 10 a.m. daily—Papers on Physical Oceanography and Biological Oceanography.

INSTITUTE OF PHYSICS and the PHYSICAL SOCIETY (at the Central Electricity Research Laboratories, Leatherhead, Surrey)—Conference on "Electroless Breakdown of Gases, including Laser Produced Breakdown".

ROYAL ENTOMOLOGICAL SOCIETY OF LONDON (at the Imperial College of Science and Technology, London, S.W.7)—Symposium on "Insect Abundance".

SOCIETY FOR WATER TREATMENT AND EXAMINATION (in the Physics Lecture Theatre, The University, Newcastle-upon-Tyne)—Autumn Meeting. Mr R. Briggs and Mr K. Melbourne: "Recent Advances in Water Quality Monitoring"; Mr D. G. Hager and Mr R. D. Fulker: "Adsorption and Filtration with Granulated Activated Carbon"; Mr K. D. B. Johnson: "Desalination in Britain"; Mr E. E. Finney and Mr N. J. Nicolson: "The Evaluation of Analytical Methods".

## Friday, September 22

BRITISH MYCOLOGICAL SOCIETY (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 10.45 a.m.—Symposium on "Some Aspects of the Fungal Hypha".

## Friday, September 22—Saturday, September 23

SOCIETY FOR ANALYTICAL CHEMISTRY, MICROCHEMICAL METHODS GROUP and SPECIAL TECHNIQUES GROUP (at the University of East Anglia, Norwich), at 9.45 a.m. daily—Meeting on "Molecular Spectroscopy in Microanalysis".

## Monday, September 25—Wednesday, September 27

INSTITUTE OF CANCER RESEARCH: ROYAL CANCER HOSPITAL (at Imperial College of Science and Technology, London, S.W.7)—International Nuclear Medicine Symposium on "Radioactive Isotopes in the Localization of Tumours".

## Monday, September 25—Friday, September 29

INSTITUTION OF MECHANICAL ENGINEERS (at Church House, Westminster, London, S.W.1)—Conference on "Lubrication and Wear—Fundamentals and Application to Design".

## APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

LECTURER (with high academic qualifications and preferably special experience in certain aspects of industrial metallurgy) in METALLURGY—The Principal, Lanchester College of Technology, Priory Street, Coventry (September 19).

SENIOR LECTURER (with substantial research and teaching experience and eligible to apply for recognition as a teacher of London University) to take charge of COMPARATIVE PHYSIOLOGY—The Secretary, Sir John Cass College, Jewry Street, London, E.C.3 (September 20).

SENIOR SCIENTIFIC ASSISTANT (with a certificate in microbiology at H.N.C. level or its equivalent, or with equivalent experience) in the BACTERIOLOGY DEPARTMENT, for duties which include general laboratory organization and training and supervision of scientific assistants—The Secretary, Hannah Dairy Research Institute, Ayr, Scotland (September 20).

PRINCIPAL LECTURER (honours graduate in pharmacy with a higher degree or equivalent research experience) in PHARMACEUTICS—The Secretary, Robert Gordon's Institute of Technology, Aberdeen, Scotland (September 25).

RESEARCH FELLOW or ASSISTANT (graduate in mathematics, physics or electrical engineering, and preferably experience in the communications field or in programming a digital computer) in ENGINEERING for studies in the synthesis of lumped linear electrical networks—The Registrar, The University, Leicester (September 25).

RESEARCH FELLOW (with research experience of electronics and high vacuum techniques) in the DEPARTMENT OF PHYSICS to work on a hydrogen beam maser project under the direction of Dr D. C. Lainé—The Registrar, The University, Keele, Staffordshire (September 26).

ASSISTANT EXPERIMENTAL OFFICER (with a degree, H.N.C., or equivalent qualification) for electron microscopy of various biological materials—The Secretary, Rothamsted Experimental Station, Harpenden, Herts, quoting Ref. 1052/102 (September 29).

LECTURER or ASSISTANT LECTURER (with a science degree with a major in physics) in PHYSICS at the University of Botswana, Lesotho and Swaziland—The Inter-University Council, 33 Bedford Place, London, W.C.1 (September 29).

EXPERIMENTAL OFFICER (with experience of instrument design or construction involving electronics or optics, and preferably some experience in spectroscopy) in the DEPARTMENT OF CHEMISTRY—The Registrar, The University of Manchester, Manchester, 13, quoting Ref. 144/67 (September 30).

PROFESSOR OF AGRICULTURAL SYSTEMS AND ORGANIZATION in the DEPARTMENT OF AGRICULTURE—The Registrar (Room 39, O.R.B.), The University, Reading (September 30).

PROFESSOR OF CROP PRODUCTION in the DEPARTMENT OF AGRICULTURE—The Registrar (Room 39, O.R.B.), The University, Reading (September 30).

RESEARCH FELLOW (with or expecting to receive a Ph.D. and preferably previous research experience relevant to the project) in the SCHOOL OF PHYSICS, for work on the growth, structure and properties of single crystals of oxides, nitrides and carbides with the aim of developing new composite ceramic materials—The Registrar, University of Warwick, Coventry, Warwickshire (September 30).

COMPUTER MANAGER—The Secretary, University of Stirling, Stirling, Scotland (October 2).

TEACHING FELLOW (with an honours degree in science or equivalent qualifications) in the SCHOOL OF CHEMISTRY, University of New South Wales, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 2).

HEAD (preferably with interest in the development of research projects) of the DEPARTMENT OF PHARMACY—The Principal, College of Technology, Belfast, Northern Ireland (October 7).

LECTURER in BIOCHEMISTRY—The Secretary, The University, Dundee, Scotland (October 7).

DIRECTOR (with postgraduate training in computer science and numerical analysis plus experience in their application) of the UNIVERSITY COMPUTER CENTRE, University of Ibadan, Nigeria—The Inter-University Council, 33 Bedford Place, London, W.C.1 (October 9).

LECTURERS or ASSISTANT LECTURERS in the DEPARTMENT OF SOCIOLOGY, University of Singapore—The Inter-University Council, 33 Bedford Place, London, W.C.1 (October 10).

SENIOR LECTURER or LECTURER (preferably with an interest in taxonomy and plant ecology) in the DEPARTMENT OF BOTANY and PLANT PATHOLOGY, University of the West Indies, Trinidad—The Inter-University Council, 33 Bedford Place, London, W.C.1 (October 10).

LECTURER in the DEPARTMENT OF FUEL TECHNOLOGY and CHEMICAL ENGINEERING—The Registrar, The University, Sheffield (October 16).

DEMONSTRATOR/SENIOR DEMONSTRATOR in the DEPARTMENT OF INORGANIC CHEMISTRY—The Registrar, The University, Newcastle upon Tyne, 2 (October 20).

LECTURER/SENIOR LECTURER in BIOCHEMISTRY at the University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 20).

DIRECTOR of the COMPUTER LABORATORY—The Registrar, The University, Liverpool, quoting Ref. RV/170/N (October 31).

FORBES CHAIR OF ORGANIC CHEMISTRY—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh (October 31).

RESEARCH FELLOW (with a Ph.D. degree or equivalent experience) in the COMPUTER CENTRE, Australian National University, to take part in the work of the Centre and to undertake research in an appropriate branch of computer science—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 31).

SENIOR LECTURER or LECTURER in SOCIAL ANTHROPOLOGY, University of Cape Town—The Secretary-General, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1; and The Registrar, University of Cape Town, Private Bag, Rondebosch, Cape Town, South Africa (October 31).

CHAIR OF OPHTHALMOLOGY and EYE HEALTH at the University of Sydney, Australia—The Secretary-General, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, November 15).

ASSISTANT EXPERIMENTAL OFFICER (experienced in bacteriology and the usual biochemical techniques; in chromatography (paper and column); and in high voltage paper electrophoresis) in the CHEMICAL MICROBIOLOGY DEPARTMENT for research in the enzymology of milk and the behaviour of the fat globule membrane during milk processing, with special reference to cheese making—The Secretary, National Institute for Research in Dairying, Shinfield, Reading, Berkshire, quoting Ref. 67/31.

SENIOR TECHNICIAN (preferably with background experience of histology and cytology) in the SCHOOL OF BIOLOGICAL SCIENCES, Half Moon Lane, S.E.24, for the preparation of material for electron microscopy and to attend to the day-to-day running of the microscope—The Secretary, Ref. 1118, King's College, Strand, London, W.C.2.

SENIOR TECHNICIAN I (with experience in chemical pathology) to work in the RENAL TRANSPLANT UNIT in conjunction with the Medical Unit—The House Governor, St. Mary's Hospital, Paddington, London, W.2.

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## KEEPING OPTIONS OPEN

THE conference on the school curriculum at Cambridge at the weekend (see page 1329) is an interesting and important sign of the times, if only because it shows how the interaction between the schools and the universities is coming to occupy a central place in the interests of those concerned with educational reform. But this does not, of course, imply that the ill effects of the traditional system of specialization in the schools have not previously been recognized. For much of the past decade and for a variety of reasons, there has been a steady stream of argument in favour of reform. What has happened now is that several independent lines of argument have come together so as to make quite irresistible the case for some drastic revision of the procedures by which young people move from school to university. Is it unreasonable to hope that reform will come quickly now that the need of it is obvious?

First of all, it is important to be clear what reforms are necessary. In this connexion it is important that the British educational system is different from almost all others in the way in which young people are required from a tender age to make decisions about their future education and careers. Some schools require students to make a choice, at thirteen or fourteen, between a science course in chemistry perhaps and a course in some foreign language. But the real trouble starts in what is called the sixth form, where students with academic ambitions usually find themselves following courses in three related subjects chosen to bring success in the competition there will later be for vacant places in particular kinds of university departments. Intending scientists—even when they know that that is what they are—often find themselves in cleft sticks, having to choose between a curriculum which includes no biology and one which has no mathematics. And, although more enlightened schools now take considerable care to see that specialized courses are filled out with extras, nobody really takes this seriously, if only because competitive entry into the universities serves powerfully to concentrate attention on those parts of the curriculum which are rigorously examined. It is no joke, as Mr Carter pointed out at Cambridge, that the newer universities find themselves having deliberately to broaden the kind of education provided by the schools.

Why is this bad? It is only fair to say that fifty years ago the system was able to provide a taste of an academic education to great numbers of people for whom a university would have been quite out of the question. Now young people with the same interests (and a great many more as well) need not depend on the schools for an opportunity to study something in depth (which is not to imply that Britain is well provided with university places). Schools must

now cater for a subversive diversity of interest among the students. The result is that a secondary school system which was once a great source of strength has become almost an obstacle to the advancement of society. Within this pattern, it is no longer easy to provide the kind of education that will best serve the interests of young people. Not only are there new things to learn. It has become even more self-destructive for a young person to abandon chemistry, or French, or Shakespeare. But if young people are cramped, so is society as a whole. Talents are not fully developed and used. Square pegs finish up in round holes. Scientists are particularly concerned just now because of the suspicion that disinclination of young people to put their noses to the grindstone of sixth-form science is one of the reasons why universities are hard pressed to recruit all the students they would like (see page 1333).

So what is to be done? Some remedies are obvious. A general pattern of education up to sixteen is now an urgent need, which in itself entails that a number of practical decisions should be taken. For one thing, the study of science below sixteen should not be so obtrusive that students (and sometimes schools) find themselves having to imitate in their own timetables the names given to science departments at universities half a century ago. Plainly, somebody or some institution should undertake to develop a curriculum intended for young people in general. It is also important that British schools should pay more attention than is their present habit to the teaching of French and other foreign languages. Performance is at present patchy. And then it is clear—this was one of the themes running through the Cambridge conference—that British schools need some means of teaching mathematics in such a way as to provide young people, not only potential scientists, with a skill in the kind of mathematics which is likely to be useful in an increasingly interesting environment. Here, too, is a strong case for adventurous curriculum development, and for a pattern of studies in the sixth forms which will include some mathematics for everybody.

But man—even modern man—cannot survive on mathematics alone. What else should the sixth forms teach? The conference last week brought out clearly the dilemma in which all those who would reform the system sooner or later must find themselves. Should the objective be to design a broad curriculum and to see that all young people with similar interests are taught on much the same pattern, possibly by asking that all entrants to the universities should pass examinations in a range of subjects? Or would it be better to aim at a less oppressive load of examinations so that the schools themselves can provide a broad foundation for a modern liberal education? If



there had been a show of hands at Cambridge, broadness would probably have won the day, chiefly because any other pattern may require that students should make firm decisions on future studies two years or so before leaving school. In the circumstances, it is earnestly to be hoped that the Schools Council will not too fiercely hold to the proposals which it is considering for a sixth-form pattern in which the range of externally examined subjects is restricted, not increased. The schools are right to hope that one result would be a diminution of the pressure of examinations. The snag, of course, is that if there is not at the same time a cast iron agreement with the universities, the pressures of the competition for university places could mean more narrow specialization in the schools. Given the inertia of the system, that would be too big a risk to take.

Whatever pattern seems most desirable, however, a closer liaison between the schools and the universities is essential, chiefly (in this connexion) on matters concerned with entry to universities. There is no doubt that the continuing pressure of the selection process gives bite to the pattern of specialization in the schools, and it is also plain that, if within the British system the curriculum is to be made broader, it will be necessary for university departments to be less exacting in what they ask of newcomers. The intake will be more varied. Some new students in, say, chemistry will know less chemistry than they would at present. But this is not very much to ask. Surely it is a great confession of failure when universities confess that they cannot take young people through first degree courses unless they have gone part of the way at school. It is true that doing this might well mean extra trouble, and sometimes extra time as well, but if the schools were properly provided with broadly educative sixth-form courses, very little would be lost and enormous benefits would be won. The treaty between the schools and the universities most to be desired would be one in which both parties would agree to design some sufficiently liberal realistic curriculum for students to be able to compete for places at universities and not, as at present, for places within individual departments. A little consideration of what happens in countries other than Britain would show that such a goal need not be unattainable.

But how will all this happen? In a sense, the initiative must come from the universities, and the most immediate need is a means by which the universities can speak with one voice on admissions and curriculum. Another urgent need is some means by which the opinion and experience of those in the universities who deal day by day with problems of admissions could be brought more fully into the formation of policy. Although the Committee of Vice-Chancellors may lack the power to make decisions on behalf of all universities, it can at least make propaganda on behalf of a cause which has become of central importance to the development of British society—how to educate young people without putting them in blinkers.

## WHERE PLASTIDS COME FROM

EVEN to look at (with an electron microscope) the mitochondria and the chloroplasts found in animal and plant cells seems as if they have an existence of their own. They are, for one thing, totally enclosed within a membrane and they can be used even when extracted from their parent cells to manufacture energetic chemicals such as ATP. The suspicion that they may be semi-autonomous entities—cells within cells—was strengthened by the discovery, a few years ago, that both mitochondria and chloroplasts contain DNA. Genetic evidence for genes outside the chromosomes points in the same direction, and now there is also evidence that chloroplasts and mitochondria contain the enzymes necessary for the replication and transcription of DNA and ribosomes as well as the enzymes and other components of the cellular apparatus necessary for the manufacture of proteins. The isolation and characterization of ribosomes from mitochondria now reported by Küntzel and Noll (see page 1340) is further evidence to support the notion that mitochondria and chloroplasts are potentially autonomous. But how does it come about that ordinary cells contain entities like these?

This is the question which has led in recent years to the suggestion that both mitochondria and chloroplasts may have arisen from bacteria which were at some stage in the evolution of living cells symbiotically associated with more primitive forms. The idea has several obvious attractions. It has a certain elegance. There is also biochemical evidence to suggest that there is very little in common between the nucleotide sequences of DNA taken from mitochondria and from cell nuclei. The processes by which mitochondria and chloroplasts manufacture protein seem to resemble each other in many ways and to differ from the ways in which protein is manufactured in the cytoplasm; antibiotics such as chloramphenicol will interrupt the synthetic activity of mitochondria and of bacteria, but leave protein synthesis in the cytoplasm almost unaffected. It has also been shown that chloroplasts and bacteria both yield *in vitro* systems for synthesizing protein molecules which function most efficiently at similar ionic concentrations. In other words, there does appear to be a case for saying that the systems for synthesizing proteins which are present in mitochondria and chloroplasts have something in common with those found in bacteria.

The evidence which Küntzel and Noll have now provided is another argument of the same kind. Professor Noll and his associates have already shown that the ribosomes obtained from chloroplasts have sedimentation coefficients which are more like those measured with bacterial ribosomes than the sedimentation coefficient, which characterizes the ribosomes of the cell cytoplasm. What they have now done is to show that ribosomes from the mould *Neurospora* also belong to the bacterial class. To have done this at all is a

considerable and an important technical achievement. Kuntzel and Noll also point out that within the ribosomes of a bacterial character there is a gradation in which the ribosomes of chloroplasts and of a photosynthetic bacterium are at one end of the scale, ribosomes from the bacterium *Escherichia coli* are in the middle and in which the ribosomes from mitochondria and from the bacterium *B. licheniformis* are distinguished by the largest sedimentation coefficients. So is it possible, they ask, that there has been a progressive increase in the size of bacterial ribosomes in the course of evolution?

At this stage, of course, this evidence is not a conclusive proof that chloroplasts and mitochondria have an exogenous origin as symbiotic bacteria. Indeed, it is hard to see how it would ever be possible to be sure of the correctness of a hypothesis like that. It is also prudent to remember that there may be all kinds of reasons why a certain degree of autonomy should be delegated to mitochondria or chloroplasts by the nuclei of cells. It will help a great deal when more is known of the details of the process by which the organelles manufacture protein molecules. But even if in the long run it should turn out that the hypothesis of exogenous origin is a March hare, the chase should be stimulating and illuminating.

## SIR JOHN COCKCROFT

EVERYBODY will be sorry that Sir John Cockcroft is dead, and surprised that he was seventy. He had such a flair for being busy that there was a temptation to think that he had stopped ageing just a little short of the usual retiring age. It is only a few weeks since he stopped going to the Atomic Energy Authority once a month. Quickly, he had settled in a splendid gesture of magnanimity for being president of Pugwash. By now he would also have been president of the Liberal Party, with Asquith and Lloyd George as well as Rutherford as heritage. It would have been fascinating to see how he would have shaped as a politician, but underneath his armour of diffidence he was as tough as nails.

It was, of course, a fine notion of his to have designed a machine to produce to order the kinds of nuclear disintegrations which others had only been able to bring about with the help of fast particles from radioactive nuclei. He and T. S. Walton may have been lucky to have done—with what is now called a Cockcroft-Walton generator—something which might have needed a cyclotron instead, but that was no more than they deserved. It was extremely enterprising of them to have taken seriously some hints of Gamov's about the likelihood of a charged particle tunnelling through a potential barrier and to have recognized that half a million volts might be enough to produce interesting results when protons are flung at lithium-7. People from the north of England will say that this is

just what anybody would expect from somebody who was born in Todmorden, in that neutral zone between Lancashire and Yorkshire, but that is not the whole of it.

The truth is that Cockcroft had a flair for aiming shrewdly and with great determination at more distant goals than most people attempt. This, at any rate, is the best explanation for his particular blend of enthusiasm and doggedness. He seems, for example, to have been sure, ever since 1932, that nuclear energy would be practicable, when Rutherford was thoroughly sceptical. By the outbreak of the Second World War he was deeply involved first in building radar defences and then in the organization of the first developments of nuclear energy in Britain under the wing of the Maud Committee. His great success in working out the basis for collaboration between Britain, the United States and Canada during the visit of the Tizard Mission to the United States in 1941 was as much diplomacy as physics. Yet in a curious sense his real triumph was the Harwell Laboratory of the Atomic Energy Authority, where he won great respect for acuity, kindness, lack of side and fierce stubbornness in the face of interference or even the threat of interference from outside. This is probably the reason why his shyness with people was as much a help as a hindrance to his reputation, which has been distinguished for the best part of a generation.

## LUMP IN THE THROAT

THOSE concerned with the success—even the continuation—of the international agreements whereby there are formal exchanges of scientists between Britain and countries in Eastern Europe and China will have been downcast to learn of the melodramatic manner in which Dr V. Kachenko eventually departed from London this week (see page 1322). The danger, of course, is that what seems to have been mismanagement by the diplomats on both sides might have put in hazard the programme of exchanges as a whole. Although everybody concerned—the Foreign Office, the Home Office and the Russian Embassy in London—now seems anxious to ensure that the consequences of the affair shall not be exaggerated, it is presumably too soon to know for certain that no damage will be done. In the circumstances, it is important that the governments of Britain and the Soviet Union should fully appreciate the great value of these programmes for exchanging scientists. In the past few years they have played a crucial part in the progressive integration of science in the east and west of Europe. It is unthinkable that this process should now be interrupted. This is why it would be sensible if the unhappy incident of Dr Kachenko were taken as an opportunity for making the working of the exchanges still more informal—there is still some way to go in that direction.

## NEWS AND VIEWS

### Education in the Air

THE British Government is moving almost imperceptibly towards its aim of setting up a university of the air. The idea first saw the light of day before the 1964 election, and by 1966 had reached the status of a White Paper, *The University of the Air*. Since then it has changed its name to "The Open University", and it was formally set on its way by its most fervent and sometimes its only supporter, Miss Jennie Lee, at a meeting in London this week. It will be a while before the first students can tune in to education, however; there is much to be done, and the open university will not begin until the autumn of 1970.

What Miss Lee has done is to set up a committee to plan the open university. The committee, seventeen strong, will be chaired by Sir Peter Venables, Vice-Chancellor of the University of Birmingham, and includes among its members Sir Eric Ashby, Professor Asa Briggs, Lord Fulton, Dr Brynmor-Jones, Lord Ritchie-Calder, Dr E. W. H. Briault and Lord Goodman.

The open university will be grant-aided directly from the Department of Education and Science and will not come within the University Grants Committee. The cost of setting it up will be less than £1 million, and annual running costs are thought to be about £3-4 million. Television programmes will be broadcast on the British Broadcasting Corporation's second channel, BBC 2. Courses may also be offered on radio, but exactly how this will work is still under discussion between the BBC and the education departments. As well as television and radio, the courses will include correspondence, short residential courses, and the equipping of local centres in libraries, schools and colleges with audio-visual aids. A fee will be charged, and it is expected to be about the same as the present fee for correspondence courses. The degrees of the open university, the department says, will be guaranteed by the universities, either individually or as a consortium, and the courses will be more like the Scottish or American model than the English—credits, for example, will probably be given at the end of each year. Plainly, Sir Peter Venables and his men will have a lot to do before 1970 comes along.

### Russian Salad

FOR much of this week the British newspapers have been filled with a story which has taken readers back to the chilliest days of the cold war. Dr Vladimir Kachenko, a Russian physicist who had been working at the University of Birmingham, decided to go home. He went to the Russian Embassy in London, arriving apparently in the early hours of the morning, and was told to come back later. Later in the day an anonymous passer-by saw Dr Kachenko carried—or bundled—into

a car near the embassy. When the police were told, they raced to Heathrow Airport, surrounded the aeroplane, and took Dr Kachenko into custody. At first the British authorities said that Dr Kachenko had asked for political asylum, but 24 hours later agreed with Russian officials that he was in fact unwell and should be returned home as soon as possible. He flew back to Russia on September 19.

Nobody emerges with credit from this peculiar affair. Trouble would have been avoided if the Russian Embassy had given advance warning of the need to fly Dr Kachenko home, instead of hustling him on to the first flight in a way almost calculated to arouse suspicion. But the British authorities seem to have acted hastily, and in removing a Russian national from a Russian aeroplane may have set an embarrassing precedent. It will be sad if the affair affects the exchange agreement between the Royal Society and the Russian Academy of Sciences. The present agreement, signed in October 1965, provides for exchanges at three levels. Four highly qualified scientists from each side are exchanged each year, for about three weeks, to give lectures and to get to know the scientific institutions of the other country. Three leading scientists are exchanged for periods of up to two months, to visit institutions in the other country and to give lectures. Finally, four scientific workers from each side are exchanged annually to work in institutions in the other country for periods of up to ten months.

Dr Kachenko was one of the third group, and had been working with Professor W. F. Vinen at Birmingham on the stability of vortex lattices in superfluid liquid helium. Technically the Russian Academy of Sciences should have told the Royal Society of Dr Kachenko's departure fifteen days before he left, giving the exact date and time two days in advance, but it is unlikely that the Royal Society will complain of this, as it is eager to extend the agreement. There is no deadline for arranging this, fortunately, so there should be time for the dust to settle. The Royal Society also has formal exchanges with Bulgaria, Hungary, Rumania and China, in addition to its extensive Western European activities. The Chinese agreement seems a bit one-sided; during 1966 thirteen Chinese scientists arrived in Britain, while only two British scientists went to China.

### Advice on a Shoe-string

LAST week the Select Committee on Science and Technology got down to writing its report on the British nuclear power industry. As part of the task of gathering evidence, two sub-committees have been abroad; one, led by the chairman of the select committee, Mr Arthur Palmer, visited the United States, while the other, led by Mr David Price, visited Europe.

The European party went to Germany and Belgium, but not to Italy or France. Italy would have been particularly interesting, since it is one of the few countries to which British companies have been able to export nuclear hardware. (The Nuclear Power Group commissioned a 200 MW station at Latina in 1963.) Some members of the committee are disturbed by the modest scale on which their inquiry has been carried out, and what is regarded as a self-defeating parsimony about the expenses and the costs of employ-

ing qualified staff. Under the present arrangements, the committee can only adopt a quasi-judicial function, listening carefully to what everybody has to say and then deciding who is the most convincing. Without research staff to ferret out the facts and make independent assessments, the committee can only act by balancing assertions from one vested interest against those from another, a process which could in principle go on for ever without establishing the truth. Despite the obvious need, it is probably fair to say that staff are unlikely to be recruited unless Parliament is prepared to vote much larger sums of money to its committees. Salaries of the staff of the House of Commons are not yet adequate to ensure that people of sufficient quality are recruited.

The select committee hopes that the report on the nuclear power industry will be finished in this session of parliament, which ends on October 27. Next session the committee will be examining the defence research establishments, with particular reference to the employment by them of qualified scientific and technical staff. If the committee keeps to its practice of conducting proceedings in open session, this could be a most interesting investigation. Clearly one of the preoccupations of the committee will be whether some of the qualified people in defence research establishments would be better employed elsewhere.

## Uneven Largesse

THE annual report of the Science Research Council for 1966-67 (see *Nature*, **215**, 1215; 1967; and also page 1424, this issue) gave details of the allocation of research grants and awards for postgraduate study for the academic year 1966-67. The figures show actual numbers of studentships and total sums of money involved. Not surprisingly, Cambridge topped the list of research studentships with 333. In London, Imperial College had 248 and the London School of Economics just one. Obviously a comparison on these terms means very little, but when related to the numbers of science students the figures make more sense. All the 3,472 students at Imperial College were scientists, but SEL took only one—a mathematician.

Working from provisional figures for student populations for 1966-67, the oldest and the newest universities seem to have had best results. Oxford heads the list with one research studentship for each eight students. Essex, Warwick, Lancaster, East Anglia and Sussex all have one studentship to about 10 students, followed closely by Durham, Cambridge and Aberdeen with about one to 12. The other older universities have a ratio of one to 40, but the new technological universities come out less well. The City University (London) has only one studentship to 190 students; Brunel, Loughborough, Bradford and Heriot-Watt all lie in the one to 70-90 range and Bath heads the technological league with one to 43.

Research grants can be treated in the same way, but the resulting sums per head of the student population are slightly misleading, for large amounts of capital expenditure distort the picture. Glasgow, for example, was allotted a grant of more than £1 million, 92 per cent of its total grant, for nuclear physics, which works out at £598 per science student. Oxford tops this list with £693 per head, but 40 per cent of the £1.6 million grant was for nuclear and other physics and

once again includes a large amount of capital expenditure. Imperial College, with eggs in every basket, had the highest total grant of £2 million, which worked out at £600 per head. By comparison, £127,000 for Essex seems a small sum, but with only 230 science students this stands high at £555 per head. The medium sized middle-aged universities received sums per head ranging through the hundreds, but the range below £100 was left to the technological universities. Surrey had £105 per head, and the sum falls to £13.5 per head for the City University and £12 per head for Salford.

The SRC has stated its intention of encouraging technology, and regrets the disappearance into industry with only a first degree of many of the best students of engineering and technology. This, of course, explains the small number of postgraduates at the universities of technology. (On the other hand, the SRC is encouraging pure scientists to go into industry.) As well as awarding grants to the most promising research projects, the SRC can encourage work in what it believes to be important fields by discussing with universities the topics that will receive most favourable consideration by the grants committee.

## Soviet Splashdown

IN March 1965, the Soviet Voshkod 2 spacecraft overshoot its target area and landed in a snow covered forest in the Urals. Its crew, cosmonauts Belyev and Leonov, spent two days in the forest before rescuers reached them. Since then it has seemed probable that Russian space scientists would turn their attention towards sea recovery for manned flights, in the American pattern. A recent report in the Soviet Defence Department journal *Red Star* confirms that this is indeed happening; Russian cosmonauts are now undergoing training for sea recovery. The same report reveals that Leonov nearly suffered a fatal accident during training—his parachute failed to open until he was a few hundred feet above the ground.

Landing over water would not have saved the life of Colonel Komarov in the April Soyuz 1 accident. When the parachutes fail, water is no softer than land at great speeds. But sea recovery would offer the Russians several advantages. At present the Russian space flying season is restricted to 5-6 months in the summer, and sea recovery would make it possible to extend the season. It would also prepare the way for the return trip from the Moon, when it is likely to be difficult for the cosmonauts to land within the normal Russian recovery area. The two-thirds of the globe covered by water offers a much larger target, although clearly the pilot will be hoping to land somewhere near a recovery ship.

Such a radical change of technique will not be achieved by pressing a switch. It is possible that the new flatter orbit, at an inclination of 51°, which was flown by Soyuz 1 and by four Cosmos recoverable satellites during the previous six months, was an intermediate stage in the shift. For sea recovery, a fleet equipped with sophisticated radio aids would have to be deployed, but perhaps the United States forces in the Pacific could be relied on to pick up a straying cosmonaut—the new space treaty guarantees the return of space travellers to their country of origin.



## Gentle Persuasion

from a Correspondent

IN an effort to disabuse PhD students of the notion that academic research is the only respectable outcome of a scientific education, the Science Research Council and the Careers Research and Advisory Centre have just organized an eight day summer school at University College, Oxford, in association with the London Business School. The aim was to introduce postgraduate students to the many different types of industrial and administrative problems, and to show that these are stimulating and as worthy of attention as more academic work. The presence of 50 PhDs from industry gave the postgraduate students the opportunity for discussing informally aspects of careers in industry and administration.

Extensive use was made of case studies of actual companies and group exercise methods involving the full participation of the students. The contents of the course were generally completely new to the postgraduate students, and included topics as diverse as operational research, the financial problems of capital project appraisals and systematic management development. Aspects of business not usually looked on favourably by postgraduates—marketing, for example—were made to seem interesting. Demonstrations of modern techniques such as operational research and systems analysis, which help the manager in making decisions, won an attentive audience—although one which frequently asked to what extent these scientific methods are actually applied in British industry.

The highlight of the week for the majority of the students was a business game—an attempt to simulate a dynamic business situation for a number of competing teams made up of students representing the senior executives of the companies concerned. As well as giving the participants a chance to test their management acumen, the game provided an understanding of the workings of a company and an appreciation of the conflicts which can arise in the board room.

The course was not in any sense a careers convention and there was no recruiting by specific firms. It succeeded in providing a valuable insight into the mechanisms of business and a glimpse of the administrative skills required of a successful manager. Many of the PhD students remained sceptical, however. Doubts were expressed whether anyone entering a large company through a research and development department, as the majority of those present would do, could ever rise to a senior executive position. Beliefs like this, which have developed over several years in an academic climate, cannot be altered so easily, and a first impression of the course is that it may have hardened attitudes rather than have changed them.

The organizers are wisely being cautious in judging the success of their venture. A questionnaire designed to discover attitudes to industry was filled in by all the participants before and after the summer school and will be completed again in several months, when the effects of the course will be more readily discernible. Until the results are known it will be too early to draw any definite conclusions.

## Informed Chemists

ALL five hundred chemistry PhD students in their final year at British universities who are being supported

by an SRC grant are to take part in an experiment with the Chemical Society's Research Unit in Information Dissemination and Retrieval at the University of Nottingham. The unit will provide the chemists with relevant references from the literature, which are selected by computer so that the value of the service can be assessed and changes made if necessary. Six liaison officers, who are themselves chemists, from the Chelsea College of Science and Technology, and the universities of Edinburgh, Oxford, London, Warwick and York, have been appointed to provide a link between the students and the unit.

The liaison officers will meet the heads of the university chemistry departments in their areas next month to explain the scheme. They will then meet the students and, using the references the students have found useful, will work out a reference "profile" for each student to be fed into the unit's computer. The computer will then give the student a list of up to date references relevant to his line of research every two weeks. The liaison officers will meet the students six or seven times during the year to make sure that they are getting a suitable service. It is hoped that the service will give the students a good introduction to the use of mechanized information services and that the experience gained will be spread widely when the students take up employment in government, industrial and university laboratories.

The Office for Scientific and Technical Information, which is organizing this project, is also giving a grant of £56,000 to the Chemical Society's Unit to extend its programme of work for the next two years. The research unit, which gave a demonstration at the Chemical Society's autumn meeting in Durham this week, is financed jointly by the Chemical Society and the Office of Scientific and Technical Information. During the past nine months it has regularly provided about 250 people in various government, industrial and university research laboratories with lists of titles of research papers. The titles have been selected by a computer comparing a machine readable statement of their research interests with magnetic tape versions of two Chemical Abstracts Service publications, *Chemical Titles* and *Chemical Biological Activities*.

## Dispensing with Meteorologists

THE Proceedings of the World Meteorological Organization's Conference on Automatic Weather Stations, held in Geneva a year ago, have now been published. In his opening statement, Mr D. A. Davies, secretary-general of WMO, said that studies had shown that while observational satellites undoubtedly marked a turning point in meteorology, the full benefits would only be reaped if a complementary system for observing the atmosphere from the Earth's surface itself was available. The problems of establishing and maintaining an adequate network of observing stations, especially in the uninhabited regions, meant that automatic weather stations would become increasingly important.

In a report on US Weather Bureau Automatic Stations, Mr A. N. Hill said that the US Weather Bureau had a large number of "co-operative observers" who took daily readings, but because of increased personal mobility they were becoming reluctant to give daily readings and for this reason would need to be replaced. A simple data logger was required and

the development of one had begun in 1966 and was expected to take one and a half to two years.

He also outlined plans for a Co-operative Hurricane Reporting Network, CHURN, of about one hundred stations. Small automatic weather stations would record wind speed, wind direction, temperature and pressure, and report them to an area collecting station where the information would be put on teletypewriters as required. Mr Hill said that in the vast forested areas of the US there was a great need for more meteorological data—particularly when there was a danger of forest fires.

At the conference the various forms of power supply for automatic weather stations were reviewed. These included air depolarized primary batteries, wind driven generators supported by either lead-acid or nickel-cadmium batteries, dry batteries, propane heated thermoelectric generators with secondary batteries, nickel-cadmium batteries charged by a propane fuelled generator, and thermoelectric generators heated by radioisotopes. The radioisotope generators were considered rather expensive, but because of their long life it was hoped that their capital cost could be reduced to make them an economic proposition.

The conference also considered the possibility of introducing automatic or semi-automatic equipment for merchant ships. Although there are some 4,500 merchant ships which supply data, of which perhaps 3,000 should be at sea and reporting weather conditions at any one time, a one day count conducted by WMO on July 1, 1963, revealed that only about 800 ships had reported.

## Bargain Journals

THE Institute of Metals and the Institution of Metallurgists set up a joint trust at the beginning of this year, and among its functions is the publication of two journals—the monthly *Metals and Materials* and the bi-monthly *Metal Science Journal*. The former contains topical articles as well as more esoteric reviews of scientific development and will be issued free to all members of the Institution of Metallurgists. The *Metal Science Journal* will be available to members for thirty shillings a year. At the same time, the Institution has made special regulations to allow science teachers without qualifications in metallurgy to take part in its conferences and to receive its magazines. The annual subscription of the Institution is £3 10s. or less, and members will be able to apply for tax relief on this as payments made to a professional society. A year's subscription to the two journals would cost a non-member more than £20, so that members have good reason to congratulate themselves on a bargain.

## Doing Sums Again

A SURVEY carried out among students of adult education classes has revealed some interesting facts about the retention of mathematics learnt at school. The survey was carried out by Mr Harry Frost, an extension staff lecturer in physical sciences at the University of London, in 1962. The results have now been published (*University of London Extra-Mural Studies Occasional Papers*, No. 1, 4s. 6d.). Adult students in various parts

of England and a small control group of staff from Senate House, University of London, were asked to answer a test paper of thirty-seven questions of the type which they would have faced between the junior school and scholarship level. In marking the papers, credit was given for method rather than numerical correctness, although there was plenty of indication that many of the students were beset with the schoolboy worry of "getting their sums right". Slightly more than half of the papers were returned by both student sample and control group. Willingness to return papers seemed to reflect personality rather than ability, but there was some tendency for weak mathematicians to be reluctant.

Men with a residual mathematical age of less than ten—based on the teaching ages assumed to correspond to the questions—were much fewer than women with such a low residual mathematical age. This seems to indicate inferior retention of mathematics by the women students, few of whom are required by their work to think quantitatively. In the control group this difference between the sexes was less pronounced; in this case the women had not recently spent several years in mathematically undemanding home duties.

Retention—the ratio of the age at which mathematical education ended to the residual mathematical age—is inversely related to education: the more there is to forget, the more is forgotten. The number of years between the end of mathematical education and taking the test is not significant. Of the students whose mathematical education ended forty years ago, 78 per cent still retain 70 per cent of their mathematics. The oldest participant finished his mathematics education seventy years ago and his retention was 87 per cent. Regular use of mathematics is, however, more significant; few people retain mathematical ability beyond that of the fourth form of the grammar school unless they regularly use the subject. People clearly retain more mathematics than they say they do, and even students with a minimum of mathematics education who use the subject very little could be expected to have a knowledge above the standard of the 11-plus examination.

Thus most of the students in the sample should have been capable of understanding and appreciating the adult science courses, which have been organized to present scientific ideas without jargon and without mathematics. Within the survey sample, however, most people who joined scientific classes had more than sufficient mathematics, and those without an "excess" of mathematical knowledge stayed away. If the popularization of science is to be successful it will be necessary to convince those people who suppose that they could never appreciate science that they are wrong, and that science is not inextricably bound up with numbers.

## Scientific Conservation

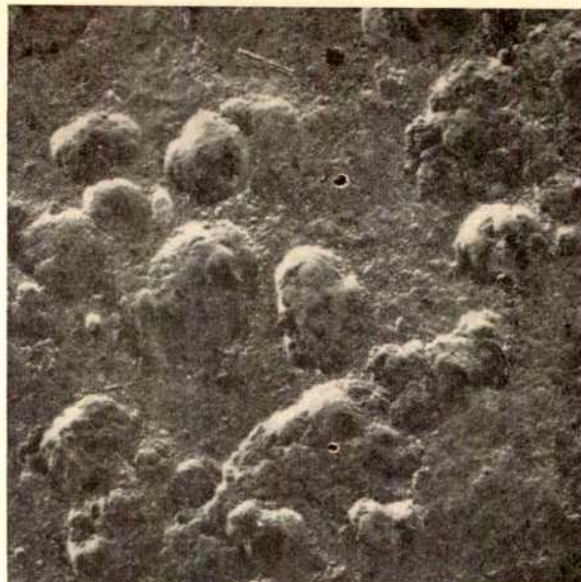
IN 1960, the cave paintings at Lascaux, which had survived since the Stone Age, began to show signs of a green mould. The delicate climate of the cave had been affected by the constant flow of visitors, and by the need to install machinery to control the ventilation. The cave was closed to visitors. Progressively more ruthless attacks on the mould with antibiotics seemed to have solved the problem, but in 1964 a new danger



threatened. The calcite formations on which the paintings are supported began to spread, covering the walls with a white surface. This, it was decided, could only be prevented by the most rigorous control of the temperature, humidity and carbon dioxide content of the cave. It is not yet clear that this will ever be compatible with the desire to re-open the cave to the public.

The problems at Lascaux may be the most extreme that conservators have faced in recent years, but they indicate some general principles. These were discussed at a conference held this week in London by the International Institute for Conservation of Historic and Artistic Works. Added to the simple problems of conservation—which are severe enough to make conservators wonder how anything at all has survived—there are hazards in the increasing tendency for works of art to be transported about the world from exhibition to exhibition. The floods in Florence and Venice are another unpredictable hazard in which conservators are involved.

Mr Thomas H. Carver, who is Assistant Director of the Rose Art Museum in Massachusetts, defined the problem of atmospheric pollutants. Since most large art galleries are in cities, the air is full of impurities. Even within the museum, the air is likely to be contaminated—from visitors, construction projects within the museum—or indeed carried in from outside. Thus all the air within the museum must be properly filtered. Light is another problem. Ultra-violet light, either from the Sun or from fluorescent tubes, can cause irreparable harm to paintings. It is necessary to balance the need for enough light against the danger of damaging pictures by supplying too much—which is why the Leonardo cartoon in the National Gallery in London spends its life in reverential gloom. Variations of humidity, particularly those which happen suddenly,



Green mould on the walls of the cave at Lascaux.

are one of the most destructive forces for works of art; while humans are most comfortable at humidities of 30–35 per cent, paintings need 45–55 per cent. With a scientific approach to these problems, conservators hope that the preservation of works of art can be guaranteed. In the past, more often than not, they have survived by sheer good fortune.

## Disappointing Shower

from an Astronomy Correspondent

It is now clear that the return of the Leonid meteor shower in November last year, eagerly awaited, was a disappointment to most observers in Britain. In the United States, on the other hand, the display seems to have compared well with the Leonid meteor storms of the nineteenth century.

Interest in the Leonids—and in meteors in general—was awakened by the great display over North America on the night of November 12–13, 1833. The frequency of meteors was then estimated to be “about half that of flakes of snow in an average snowstorm”. This spectacle recalled a similar event over South America observed by the explorer Alexander von Humboldt on November 11, 1799, who saw “thousands of meteors and fireballs moving regularly from north to south with no part of the sky so large as twice the Moon’s diameter not filled each instant by meteors”. Records of the shower can be traced back to A.D. 902, brilliant displays occurring at intervals of 33 or 34 years.

As expected, there was a magnificent show in 1866, but the recurrences of 1899 and 1932 were weak, possibly because of perturbations of the stream by close approaches to Saturn and Jupiter in the meantime. In the early 1960s, the zenithal hourly rate of Leonid meteors began to rise from 6 up to 30 in 1964 and 50 in 1965, and it looked as if 1966 would be a good year. However, descriptions of the shower on the morning of November 17 by observers reporting to the American journal, *Sky and Telescope* (33, 4; 1967), exceeded the predictions of the most confident astronomers.



Egyptian cat with severe bronze disease. This is caused by soluble salts in the layers of early bronzes; when exposed to moisture, green spots appear on the surface.

Where sky conditions were favourable, observers in the south-western United States saw too many meteors to make a direct count as the shower reached its climax, at 11.55 universal time. The burst of activity started at about 11.30 and lasted for an hour. A group on Kitt Peak, Arizona, estimated that 40 meteors could be seen in a one-second sweep of the observer's head at the peak of the shower, with more than 1,000 meteors a minute from 11.30 to 12.30 (4.30 to 5.30 a.m. mountain standard time). Similar reports, estimating tens of meteors per second at peak activity, came from California, Colorado, New Mexico and Texas. Accounts from the Atlantic states also indicate heavy activity, but are not quite so impressive since dawn began to interfere while the rate was still increasing.

Meteor showers are believed to be the debris of comets, and the 33 year periodicity of the Leonids is caused by an intense concentration of meteoric particles near the point on the orbit where the parent comet is located. The non-appearance of the display over Britain can be attributed to the comparatively small size of this swarm, but it is evident that the Leonids are still capable of giving a brilliant display.

## Mechanism of Polarity

from our Cell Biology Correspondent

As Imamoto and Yanofsky (*J. Mol. Biol.*, **28**, 1 and 24; 1967) have now shown, polar mutations cause failure in transcription; genetic polarity is not caused exclusively by defects in translation.

Transcription of polycistronic operons, like the tryptophan and lactose operons of *E. coli*, produces a single mRNA molecule containing information for several enzymes. Amber, ochre and frame-shift but not mis-sense mutations in such operons may show polarity; a polar mutation prevents the expression of the cistron in which it occurs and also leads to reduced levels of the enzymes specified by the subsequent, operator-distal, cistrons of the operon. Not all polar mutations in any one cistron show the same degree of polarity; in the *lac* operon, for example, there is a continuous gradient of polarity across the  $\beta$  galactosidase cistron from the very strongly polar mutations at the 5', N terminal end of the cistron to the very weakly polar mutations at the 3', C terminal end.

How do amber and ochre mutations cause polarity? There are three possibilities: (1) by reducing the frequency of the translation of regions of mRNA beyond the nonsense codon; (2) by preventing transcription of mRNA from DNA after the nonsense codon; or (3) by a combination of both these processes. Because suppressor gene transfer RNA prevents chain termination by amber and ochre codons and also relieves polarity, it seemed that polarity might result from defects in translation, and several groups have devised purely translational models for polarity.

Attardi *et al.* (1963), however, found that extremely polar mutants of the *E. coli* operon fail to make *lac*-mRNA; perhaps polar mutants prevent transcription. Using a hybridization technique to detect *tryp*-mRNA, Imamoto and Yanofsky have now shown that strains of *E. coli* with strongly polar mutations in the tryptophan operon do indeed make less *tryp*-mRNA than identically treated wild type cells

or mis-sense mutants. The *tryp*-mRNA molecules of the polar mutants are short; they lack the regions that are normally transcribed from the DNA beyond (operator distal) the site of the polar mutation. Furthermore, the size of the predominant *tryp*-mRNA species in different mutants increases in relation to the genetic distance between the beginning of the first cistron of the operon and the site of the mutation.

Estimates of the relative proportions of complete and short mRNA molecules made by the different mutants correlate fairly well with their previously observed translational polarity; for example, a mutant with 14 per cent translational polarity—it makes only 14 per cent wild type levels of enzymes specified by the cistrons following the mutated cistron—made only 10 per cent complete *tryp*-mRNA molecules and 90 per cent short molecules. Pulse labelling of the *tryp*-mRNA as it is synthesized revealed that the mutants make normal numbers of mRNA molecules and that the short molecules are not produced by degradation of complete molecules. Polarity, therefore, certainly involves and may even be caused entirely by failure in transcription.

How can this finding be reconciled with evidence that polarity is a translational phenomenon? A non-sense codon itself is not a signal to RNA polymerase to stop transcription, and genetic translation and transcription are closely coupled; ribosomes somehow regulate mRNA synthesis (see *Nature*, **214**, 228; 1967). Hence Imamoto and Yanofsky suggest the continued transcription of DNA by RNA polymerase depends on the normal movement of ribosomes translating that part of the mRNA molecule already synthesized. If this ribosome movement is impeded by a nonsense mutation and a subsequent untranslatable region of mRNA, then the greater the distance between the nonsense codon and the initiation site of the next cistron in the DNA being transcribed the lower the probability that RNA polymerase will continue transcription and so the mRNA molecule will be terminated.

## Protean Proteins

from our Molecular Biology Correspondent

It is interesting to speculate whether the native state of a protein represents a single uniquely stable conformation, or whether it is better regarded as one of a number of states occupying potential energy minima, between which transitions can occur in response to environmental adjustments. The proteins trypsin and trypsinogen have recently been subjected to careful scrutiny by several groups working in France, and the results which have been reported have a general bearing on this question.

Lazdunski and Delaage (*Biochim. Biophys. Acta*, **140**, 417; 1967) have studied pH-dependent conformational transitions in both the enzyme and zymogen, and have observed the existence of three reversible equilibria, linking four definable conformational states. The changes are followed on the one hand by difference spectra, which reflect the state of exposure of tyrosine and tryptophan residues, and on the other by optical rotation. Three at least of the observed isomeric states appear not to be denatured forms, in the sense that the protein retains in each case characteristics



of globular structures, undergoes a transition to an unfolded form on heating, and in two of the states is protected against denaturation by specific inhibitors. (That is to say, the active centre remains intact.) There is a broad correspondence between these various states in the enzyme and zymogen, and in both cases phase diagrams have been constructed, which define the stability ranges of the various native and denatured forms with respect to *pH* and temperature.

The same authors (Delaage and Lazdunski, *Biochem. Biophys. Res. Commun.*, **28**, 390; 1967) have also examined the binding of calcium ions—which are known to have an influence on the activation of the zymogen by directing the hydrolysis of the correct peptide bond and suppressing adventitious cleavage. There is evidence that both possess a specific calcium-binding site, which is strong in one of the conformational states, weak in another and absent in the remaining two. The one state (that prevailing at neutral *pH*) is therefore strongly stabilized by calcium, both with respect to transitions to other isomers, and denaturation by heat or urea. The observation that the site is still present after conversion of the zymogen to the enzyme suggests some rather extensive similarities of conformation. A second, and weaker, calcium binding site is present only in the zymogen, and is believed to lie in the peptide which is cleaved on activation.

A further study, of the interaction of trypsin with inhibitors and substrates, comes from D'Albis and Béchet (*Biochim. Biophys. Acta*, **140**, 435; 1967), who find that interaction occurs even at relative extremes of *pH*. This, using the approach of Delaage and Lazdunski, may presumably be seen in terms of the stabilization by the ligands of those isomeric states to which they bind. The curious observation is reported that one tyrosine residue becomes unavailable for titration when an inhibitor is present (in addition to the five already masked), until the protein is unfolded. This suggests a structural change on binding the inhibitor. There are also some changes in optical rotatory dispersion, which may be interpreted in terms of small conformation changes (in accordance with the "induced fit" notion). On the other hand, because the inhibitor contains a phenyl chromophore, which may well become optically active on binding, a new Cotton effect could also explain the result observed.

A further current article (Labouesse and Gervais, *Europ. J. Biochem.*, **2**, 215; 1967) clarifies a rather ambiguous situation, relating to the acetylation of trypsin and its effect on the enzymatic activity. Conditions have been established for the preparation of a derivative, in which all the  $\epsilon$ -amino groups, but not the tyrosines or the  $\alpha$ -amino group, are acetylated. It is confirmed that both the lysines and tyrosines can be modified without destroying the activity, and indeed acetylation of tyrosines is found to enhance the estero-lytic activity. The form in which only the  $\epsilon$ -amino groups are acetylated has normal activity. The possible involvement of the terminal amino group in the active site remains unestablished.

## Beards and Bugs

from our Microbiology Correspondent

A SOMEWHAT UNUSUAL piece of research has been reported recently by a team of microbiologists working

in the Industrial Health and Safety Office at Fort Detrick in Maryland. These workers (Barbeito, Mathews and Taylor, *Appl. Microbiol.*, **15**, 899; 1967) are concerned by the revival of beards among persons working with pathogenic micro-organisms and have investigated the hypothesis that a bearded man provides a risk of infection if his beard becomes contaminated in the laboratory. To test this hypothesis, a group of volunteers had their beards sprayed with cultures of non-infective *Serratia marcescens* and *Bacillus subtilis* var. *niger*, pigmented bacteria which are easily picked out in sampling programmes. With proper regard for their colleagues, Barbeito and co-workers used a bearded mannequin to study the transmission of Newcastle virus disease (*Clostridium botulinum* type A toxin) by beards, with chickens and guinea-pigs as test animals.

Sampling of contaminated beards was made by various means—swabs, membrane filters, agar impressions and saline rinses. In addition, beards were shorn and suspended in nutrient broth. Clean shaven subjects acted as controls and the effect of washing with a disinfectant soap was estimated for both groups. Comparison of the sampling procedures demonstrated that shorn beards blended in broth produced greater numbers of bacteria and that the other methods were likely to underestimate potential infection doses which may result from contact with unwashed beards. Here the investigators emphasize that several diseases are known for which the inhaled human infection dose is about ten microbes. Beards clearly held bacteria more effectively than skin even after soaping and rinsing. It is comforting to note, however, that in order to recover bacteria from unwashed beards it was necessary to apply populations of  $10^4$ – $10^5$ , and laboratory contamination of this magnitude is unlikely to occur without the subject inhaling sufficient organisms to produce an illness. Thus beard-infection of close associates appears feasible only when the wearer has a recognizable accident or is subjected to regular doses of microbial aerosols, as might arise when carrying out a repetitive technique. On the other hand, results of experiments with NDV and the botulinum toxin indicate that contamination within the range of likely accidental beard contamination would create a serious human health hazard.

Interesting and salutary as the results of these trials are, one must keep in mind the fact that very many more people working with pathogens have hair on the top of their heads than on their faces and the issues raised in connexion with beards are surely more widely applicable. Clothing is another potential agency of dissemination and several reports have appeared on the persistence of micro-organisms, especially viruses, on clothing fabrics. To combat this, searches are being made for effective viricidal agents with which to impregnate fabrics. Sidwell, Dixon and McNeil (*Appl. Microbiol.*, **15**, 921; 1967) have illustrated the efficacy of treating fabrics with quaternary ammonium compounds or triazone resin in inactivating or greatly reducing the persistence of vaccinia and polio viruses on a variety of woollen and cotton materials. The treatment of fabrics to render them self-sanitizing is an attractive proposition, particularly when they are used in places liable to contamination by pathogenic micro-organisms.

# Towards a Broader Curriculum

A conference on educational problems concerned with the transition from school to university was held at Gonville and Caius College, Cambridge, on September 15-16 at the initiative of Sir Nevill Mott and with the sponsorship of *Nature*. Several independent lines of argument led the participants to agree that a broader curriculum in the sixth form would be desirable. The report of the proceedings which follows, prepared by the staff of *Nature*, is intended to show how this conclusion was arrived at and to suggest some of the problems which now call for attention.

SIR NEVILL MOTT begins by saying that the conference has its origins in the discussions within the Schools Council of how the sixth form curriculum may be reorganized. The idea, current a year ago, that there may be major and minor courses in the sixth form has now been dropped. Instead, there are discussions about a scheme intended to limit the externally examined subjects studied in the sixth form. But there is no assurance that the result will be a real broadening of the sixth form curriculum—it could be the opposite. And yet the objective, crucial for science and technology, is a broadening of the curriculum. We believe it is an educational must that the age of choice should be delayed for as long as possible. And from that all kinds of other issues follow. Should there be a fourth year at the university for a great many people? How much would this cost?

Professor Allanson says the most serious problem is that decisions about future courses and careers now have to be made at a ridiculously early age. He knows the Schools Council has had this in the forefront of its mind, but doubts whether it has given it a high enough priority. Has it taken account of the complicated patterns in the school curriculum, the larger sixth forms and the wider range of ability and interest in them? And he is afraid we forget that the whole world is not composed of scientists.

Miss Miles thinks that her problems may be easier because girls' schools are more accustomed to a liberal curriculum. She deplores the artificial distinction between things which are examined and things which are not examined. At present, she says, the curriculum is broad until the age of sixteen; after that it becomes specialized, and then we have to make great contortions to bring back the general subjects which have been dropped. Why not, instead, learn and go on learning?

Mr Jennings agrees that the postponement of decisions by pupils is desirable, but thinks the Schools Council has been maligned. What is this talk about compulsory subjects? To Professor Keohane the crucial question is what kind of education should be provided throughout the educational system? Only fear of a shortage of scientists has brought us together, which is a pity. So far as science is concerned it is wrong to have O-levels in separate subjects—physics, chemistry and biology, for example. The chief problem is that people are required to take out options at thirteen or fourteen. He wants a broader education up to sixteen and thinks that it would then be permissible in the sixth form to have broad courses in science such as the Nuffield Foundation's physical science course.

Professor Lauwerys agrees that this is a good starting point. It is a question of when, between sixteen and thirty, options should be introduced. In the sixth form, he thinks that there should be a broader course of studies—something like the old matriculation exam for entrance to universities—mathematics, the mother tongue, a

foreign language and two others. Professor Elton goes one better—there ought to be a general education up to eighteen.

Professor Elvin stands out against this line of argument. A three year degree may be unusual, but the Robbins Report found it an advantage, and in any case some 20 per cent of British undergraduates spend four and not three years on their studies. He wants to encourage this tendency, but would not like to see it become universal overnight. And he would preserve the way in which the sixth forms in most British grammar schools prepare

## PARTICIPANTS

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- C. F. CARTER, *Vice-Chancellor, University of Lancaster.*
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- CHRISTOPHER PRICE, *MP.*
- L. ROSENHEAD, *Professor of Applied Mathematics, University of Liverpool.*
- S. J. TESTER, *Lecturer in Classics, University of Bristol.*
- MRS SHIRLEY WILLIAMS, *MP, Minister of State for Education and Science (for one session).*

people for universities. For him, a three year undergraduate course preceded by three years in the sixth form is the best. It could even be cheaper and could accord with the trend of social fashion to stay longer at school which the Robbins Committee underestimated. (Mr Christopher remarks that in his experience the inclinations of young people are going the other way.)

But are the schools just prep schools for universities? This is what Mr Tester wants to know. He wants a system in which anybody who leaves school at sixteen is as well educated as one who leaves at eighteen. He wants schools to be concerned with the whole education of students. And he thinks it does not matter if schools neglect to prepare students for particular university courses, for he does not believe there is a subject which cannot be taught at university in three years—given a basis in mathematics or linguistics. (Tester is not the only one to single out the chemists for being the laggards.) He emphasizes that there is nothing wrong with specialization as such—indeed, it is virtuous as well as unavoidable. He disputes the view that the logical structure of the sciences makes it possible for people to leave them and impossible to come back—nothing is more logical than the classical languages. And he wants to know why the teaching of science should be dominated by this sacred cow of the laboratory. But Professor Allanson does not believe that he could produce an electrical engineer in three years without some A-level studies in experimental science.

Mr Price says that the real trouble is the belief that schools are really meant as miniature universities—those who teach in them are university lecturers *manqués*. This is why there is specialization—the teachers like it that way. He does not think that things will be right until non-graduates are teaching in the schools in greater numbers.

Dr Nicol argues that the educational system should be looked at as a whole. We are asking how the sixth and seventh years in school are to be restructured. This cannot be done without asking about the structure of further educational training. The great need is to strengthen the tenuous links between schools and universities and to decide what is educationally desirable in both. What, for example, should be the role of postgraduate teaching? And is the honours school worth keeping?

Professor Hoselitz says that schools should not be expected to provide university courses, and people from outside should not go to the schools expecting to give advice which is not really appropriate. The scientists have made a mistake by putting science on a pedestal. It will be better to teach children what the world they live in is really like—that is how to get more scientists in the long run.

Professor Rosenhead pleads that the schools and the education system as a whole must be organized for flexibility. He asks that the goals of the education system should be chosen by trying to assess what society is going to be like twenty years from now. Business men have done better than educationists by making deliberate attempts to find out what life is going to be like for young people in A.D. 2000, and the schools have been separated from the universities, which is bad for the universities and wrong for the schools. Is it not possible to make both parts of the system work together? And Professor Rosenhead emphasizes that though it is desirable to defer choices, it is also necessary for young people to make up their minds on important matters such as their careers before they reach the grave.

Mr Carter reflects that a great many of the new universities are trying to provide students with a broader education. How odd it is that we have an educational system which makes people specialize between fourteen and eighteen and then has to have universities with general courses where people have to go back on their specialization. Should it not, Carter asks, be the other

way around? He is also worried that what the Schools Council is proposing is an organic change in this system which will still leave Britain different from the rest of the world.

He asks for a more thorough examination of the whole question of the transition from school to university. There are all kinds of practical questions to be settled. When, for example, should the transition from schools to universities take place? Should it be eighteen, as now, or seventeen or even twenty? And for that matter, why should there always be either 24 or 36 weeks in the university year? Why should there be three terms in the year? Why even should there be an integral number of years in the course? These are the kinds of question to be asked.

But if the universities influence the schools, they are themselves influenced by the needs of professional bodies. The great variety of careers in engineering, and the need to prepare students for all of them, provides a safeguard. But how can students be selected by universities? Professor Diamond believes that mathematics at A-level is the best indicator of quality—he is not worried if a student lacks an A-level pass in physics, but continues to ask for it because it is the usual thing to do. Mathematics is better suited to the examinations pattern, and is frequently better taught than physics. For this, the dull A-level physics papers must take some of the blame.

But it is not only the numbers of A-levels and the quality of the passes gained that are important. Professor Mitchell believes that it is possible to establish the content of courses by first deciding what students taking careers in science and technology are going to need at the age of twenty-one. This is particularly true in scientific subjects where factual knowledge is important in helping to communicate concepts, and it should be possible to determine what students should know in order to make an effective contribution at that age. The schools are shielded from the demands on the output of the system by the universities, but even universities should not feel obliged to provide all the highly specialized technology which industry needs—industry itself has a part to play in specialist training. If mathematics is to be a compulsory subject for scientific entrants, it must be taught at school as seen in action through engineering, chemistry and physics.

How important is experimental work? Mr Tester denies that he is opposed to learning by doing, but he does not believe that present practical courses are an effective means of providing this. If schoolchildren are properly taught they can go on to read either science or the humanities at university. Mr Elvin disagrees. Science and the humanities employ different modes of discourse. In saying "My love is like a red red rose", it is clear that the context is not botanical. Studies in science and in the humanities develop in different ways. But if this is so, Tester argues, why should children not acquire the language of both in the sixth form?

Mr Morrison suggests that we need a treaty between the schools and the universities to define the responsibilities of each. Morrison believes that this would avoid the depression and boredom which afflict history students faced for the fourth time with study of the Tudors. The older universities have an even heavier responsibility—their scholarship examinations make schools do university work two years before students actually reach university. Mr Jennings agrees that this can lead to disillusion.

Where is the opposition to change? Mr Tester believes that it is in the schools and particularly the grammar schools. What happens when you try to reform the syllabus? The opposition comes from school teachers who fear that their ability to get their pupils through examinations will be lost if they allow the syllabus to change. As Mr McMullen puts it, examinations are not chains round the teachers' necks, but their feather beds.

Mr Jennings feels that students are similarly inclined.

For most of them, the motivation is the desire for a training which will guarantee a comfortable job. Far from welcoming a broad curriculum, pupils prefer the straitjacket of specialization which gives them such good support. And Jennings utters a solemn warning—advances in education can only be bought at the cost of accepting dictation from above.

Then now should school and university education be reorganized in order to take advantage of a wider curriculum? Mr Elvin begins by putting great emphasis on the difference between school and university education, in order to ensure that teachers do what they are best at. Until the age of sixteen, he thinks, a certain number of subjects should be compulsory—or nearly so. These should include mathematics, the mother tongue, one science subject, perhaps one foreign language, and history and geography. After sixteen, young people no longer wish to continue in schools they have known since the age of eleven, and it is here that sixth form colleges can provide a new element in the educational pattern. Some young people may then be happy with a broad curriculum, but others may actually prefer to become specialists, and the structure must incorporate a measure of flexibility to allow for this. Within the large sixth forms or sixth form colleges could be concentrated the best and most scholarly teachers. For most people, however, the sixth form college is not a welcome solution.

Professor Elton argues that if the education of young people is to take an extra year, it should be spent in universities, that this should be at the postgraduate level and that universities should be willing to accept a more restricted first degree.

Mr Morrison has an entirely new suggestion to make. What is needed is an examination in five subjects which should include English, mathematics, physical or biological sciences, one foreign language and history or geography. Certainly it could not be called A-level but might represent a sort of junior *abitur* or *baccalaureat*. The form of the examination should be the basis of a treaty between the schools and the universities in which each party could recognize its responsibility for maintaining a broad curriculum.

Sir Nevill Mott is sure that dons believe in the need for a wider education, and is therefore puzzled to know why in practice they tend to behave as if they do not. What is needed is a structure which would enable universities to make just choices between the specialist and the generalist. Mr Tester is doubtful that the Morrison scheme would achieve this. What matters in selection procedure is not the principles, but the practice. There would be a real danger that new forms of specialization would be grafted on to the Morrison scheme. But Miss Miles is more hopeful. The scheme would be much less dangerous than the Schools Council scheme, or something like it. The Morrison scheme would not necessarily rule out a degree of specialization; children would take all five subjects but could specialize in some of them.

But how to discover what the universities think? Dr Nicol sees a need for a representative body. Professor Allanson believes the body already exists—it is the Standing Conference on University Entrance. Everybody agrees that the difficulties may never entirely be resolved while pressure on university places remains. While nobody denies that a broad curriculum is educationally desirable, Mr Tester doubts whether universities would be prepared to ignore the student who has specialized in the subject he wishes to read at university. Would the Morrison proposals really introduce compulsory breadth into university entrance? Professor Allanson says that perhaps the best solution would be for the universities to adopt the Morrison proposals together with Mr Tester's suggestion that studies beyond the Morrison level should be ignored for the purposes of selection.

But Mr Morrison says that his scheme is really only one attempt to give a little reality to the general view that

options should be kept open as long as possible. He acknowledges that if you keep five subjects instead of three in the sixth form, the standards of entrance will be lower. But schools should offer five subjects and should not expect to teach more specialized courses than those on offer to everybody. To be sure, it would be entirely proper for those who are convinced already that they know what to do at university to drop one of the subjects—but not both—on the side which interests them least. And he says that it is essential to find some way of arranging for a link between schools and universities and some way of negotiating a treaty between them.

This raises all kinds of criticisms, some practical, some concerned with principle. Keohane is not sure that a treaty between the universities and the schools can last. Nicol considers the real need is to reach some kind of agreement at the level of admissions tutors in universities. But there are a thousand of them—what chance is there of keeping them all equally well informed?

Mr Dancy raises a discordant note. He says that if you lay it down that no subject may be studied by anybody beyond a certain level, and if this amounts to simply five teaching periods every week, the best teachers in schools will say their job is not worth doing and will clear out of education altogether. He says that one of the glories of English education is the way in which sixth formers are dealt with in British secondary schools. If he were forced to choose between a broad curriculum of the kind which Morrison has suggested, with five teaching periods a week for the academic subjects, and a system like the one the Schools Council has devised, he would settle for the latter.

To Professor Hoselitz, this is a kind of heresy. It ought to be a challenge to teachers to have to teach academic subjects which at present take seven or eight periods a week in a mere five. There is no reason why this should not be done. After a number of attempts to construct timetables, Sir Nevill Mott says he wants above all an A-level with useful mathematics.

Professor Diamond is uneasy about the assumption that changes in the schools will make necessary a four year university course. As far as he is concerned, what matters most is that people should go into industry as quickly as possible. This does not mean that a broader curriculum in the sixth form is a bad thing, but it is important that it should not impede the transition from university into industry. But we have to have a broad curriculum and a way of postponing options.

Mrs Shirley Williams considers that in planning the British educational system we may not sufficiently be paying attention to the diversity of the sixth form in the modern world, in which a great many have eyes on other things than entry to a university. Not merely are there different needs of pupils to be catered for. How will teachers deal with the problems of teaching children with a variety of backgrounds when they are used to academic courses? She also raises the problem of where the science teachers are coming from and hopes there may be something to be gained from the boys or girls—particularly the girls—who stop short at a general degree at university. There is also the problem of what the sixth form population is asking for at present. Young people seem to want to make a contribution to adult life which it is not possible to ignore. That said, she considers that the pressure on the interaction between schools and universities may be eased because of the tendency for a greater proportion of people at universities to take second degrees. And here, she says, more does not mean worse—more means just more.

On the proposed treaty between the schools and the universities, many people argue that the Standing Conference on University Entrance is already equipped to do this job. It seems agreed that SCUE has considerable achievements to boast about. But Allanson points out that SCUE is only completely successful when



there is agreement. In practice, dissent among a small number of universities could block good proposals. The problem is not so much that university departments fail to delegate authority to their representatives on SCUE, but that they are not at present sufficiently well informed of new proposals coming up for a decision. And so it is considered necessary that SCUE should be more active and help to introduce new ideas for discussion in the universities.

Dr Nicol says that something different is necessary and for him there should be some sort of body or agency capable of superintending the restructuring of the sixth form in the first two years of higher education. He is impressed to see how much has been done on the schools side, where the Schools Council is empowered to deal with the whole problem of the school curriculum. It is different with the universities. People are unfamiliar with the problems. Can we go on like this? He wants to see an organization which can bridge the gap.

Jennings raises an uncomfortable question. One of the characteristics of Britain is that 10,000 people will jump to the defence of academic freedom if they consider this to be in peril. Yet people talk about reforms which are only workable if they can be adopted uniformly. For him, there should be a body which can impose its will on the universities and the other parties to the interaction between the universities and the schools. Elvin—echoed by Morrison—takes a diplomatic line. He describes how the determined pressure of opinion within universities can influence events. He thinks that

a great deal can be done by pure reason, especially if the threat of an imposed settlement is in the background.

Dr Nicol is unhappy about SCUE, because it is not fed from roots in the faculties. He would prefer a separate organization equipped to deal with problems of university entrance and problems of the first and second year courses in universities. Mrs Williams says that it is hard to see how such a system could arise without creating grave political questions concerning the relationship between the universities and the outside world. And in any case, she says, the problem may be complicated by the way in which the university teaching profession is much less homogeneous than that in the schools. Elvin has a tactful way out—let Nicol's agency deal with the transition from schools to universities, deal simply with admissions and not the broader question of the curriculum. Tester considers that if the universities do not create machinery for dealing with the problem, possibly within the Committee of Vice-Chancellors, somebody else will do it for them. But Elvin, returning to his argument that the first thing to press for is a treaty on admissions, argues that the machinery for discussion exists already—there is no reason why the vice-chancellors committee should not be asked if it will agree to put to its universities a request for an acceptance of what proposals may seem reasonable for the better regulation of the interaction between the schools and universities. It seems to be agreed that the universities could not be dealt with separately from the polytechnics and the colleges of education.

## DECLARATION

*The following statement was discussed at the final session of the conference, and may be taken as a representation of the common view rather than a form of words by each detail of which each participant is formally bound.*

It was agreed by all that a broadening of the curriculum in the sixth form is an urgent need.

For one thing, it is important to postpone for as long as possible the point at which a student at school must make decisions about what courses to follow in higher education. More important, a broad sixth form curriculum should be an educational objective in its own right. This is how best to serve the interests of students in the schools. This is how to provide a supply of educated people familiar with all aspects of the modern world. Entrants to science departments should have a better grounding in the arts. Entrants to arts departments need a core of science and mathematics.

One of the reasons why this problem has become urgent is that the size and diversity of interest of the sixth form population is growing rapidly, and sixth forms must provide recruits not merely for the universities but on an increasing scale for colleges of education, the polytechnics and for commerce and industry.

One practical benefit of a broader curriculum is that there might well result a greater supply of teachers able to teach science in the lower forms of secondary schools.

There were differences of opinion about the value and potential effect of proposals now being put forward by the Schools Council for a change in the pattern of sixth form education. The proposal is that no student should take more than two A-level examinations in any year, and that he should also follow in parallel a number of shorter courses contrasting with or supporting the A-levels and examined in some other way, together with general studies.

The schools look to this pattern not merely as a way of relieving the pressure of examinations but as a device for broadening the curriculum. There is, however, a fear that the pressure on the schools of the process of university

selection will perpetuate the present tendency to make decisions about future specialization early in the sixth form. To some, the proposals of the Schools Council would be more attractive if the character of the A-level examinations were modified.

In general, it was agreed to be desirable that students in sixth forms should be able to keep options open between the sciences. There was also wide support for the necessarily more distant objective of keeping options open between the arts and sciences.

Substantial progress towards these objectives can only be made if there are consequential changes in the pattern of university education—there must be a lengthening of university courses, a more efficient use of time at university or a decrease in the content of first degree courses. The universities may be more ready to consider the last of these possibilities because of the increasing tendency of graduates from the first degree to stay on at universities for at least one extra year.

Within a broader sixth form curriculum, mathematics needs special treatment. It is an essential part of education in engineering, the physical, biological and social sciences, and of a general education as well. There is a case for asking that a mathematics curriculum should be developed which will serve as a common core and particularly emphasize the applications of mathematics.

To make more effective the link between the schools and the universities, there is a need for some means by which university opinion on matters such as entrance can be made more univocal. The Standing Conference on University Entrance might be developed as a forum for discussion of and decision on the problems. If, however, it is to become this, some means must be found of giving it stronger roots in the university faculties and colleges.

## CAMBRIDGE CONFERENCE

## Sixth Form Studies

The following is an extract from a statement by Professor L. Rosenhead, one of the five members of the Dainton Committee soon to report.

THE material well-being of this country, as well as its intellectual strength, depends vitally on increasing the flow of young people into engineering, technology and science. Arrangements should be made for these young people to emerge from our educational machine with as good an education as can be devised, both in a general and in a specialist sense; and the graduated experts should be given satisfying and socially worth-while jobs.

Secondary education in England and Wales is more or less broadly based up to the end of the fifth form, and during that period students study eight subjects simultaneously. In the sixth forms, the number of subjects studied is drastically reduced to three (with a trivial amount of time devoted to non-examination subjects), the drastic nature of the reduction being a feature of the secondary education of only England and Wales. In all other educational systems there is some reduction in the number of subjects of study at this stage, but not as drastic as that which obtains in England and Wales. The time is now ripe for considering whether this educational practice is in keeping with the social and industrial demands of the final third of the twentieth century. In all fairness it must be said that, with the spread of the comprehensive system of education, some forward looking headmasters, acting on their own initiative, are completely transforming the pattern of sixth form education in their schools by devoting a great deal of school time to non-examination activities and studies, over and above the time devoted to three Advanced level subjects.

Students at the end of the fifth form in our secondary schools usually take O level examinations, and the available statistics on the number of passes at GCE O level, in mathematics and science subjects, have shown a steady uninterrupted increase from 1952 onwards. From 1952 to 1966 the number of passes in mathematics and science subjects has about trebled—both for boys and for girls. Expressed as a percentage of the subjects passed at O level, the proportion has increased—for boys—from about 34 to about 42 during this period; for girls the increase has been from 19 to 22 per cent. During the same period the relevant percentage in the traditional arts subjects has dropped from about 55 to about 45. The percentages for girls, though different, show the same trend.

In the sixth forms, however, the situation is completely reversed. Interest in science subjects is dropping, that in traditional arts subjects is increasing, and that in a new group, "Science-cum-Arts", is rapidly increasing. The facts are there, but the causes underlying the facts are matters to be brought into the open through discussion and investigation.

The Dainton enquiry is concerned with putting forward recommendations that will have the effect of increasing the flow of candidates into engineering, technology and science; but it is, of course, a matter for debate whether or not the flow into these fields should be increased. The Dainton report puts forward a number of recommendations, but they are largely long term in their implications.

I myself doubt whether any short term quick results can emerge, but we must try.

It is quite clear that while the output from our educational system is continuing to grow rapidly, the output of specialists in engineering, technology and science shows a quite different pattern. The annual age groups from which sixth formers are at present being drawn are appreciably smaller than those of the "bulge years" which immediately preceded them. There is now firm evidence to show that within these smaller age groups the science based activities of society are losing their hold on the imagination of our young people. The remarkable growth in the numbers enrolling for sixth form education in this country which has been achieved over the past decade seems to have occurred in those regions of study which, under present conditions, disqualify the students from entering higher education in engineering, technology and science.

Against this situation must be set the growing economic difficulties of this country, and the formidable developments in the science based industries of competitor countries such as the United States, the USSR and Japan. The new developments in those countries are associated with increased effectiveness in the exploitation of skilled manpower—whether in management, engineering or technology.

In such a developing situation, groups such as this must face the problem of whether to influence the choice and selection of individuals in relation to their studies and their future careers. The tradition of the personal freedom of the individual is deeply embedded in our educational as well as in our political traditions, and we must think very carefully before putting into train courses of action that will limit the freedom of individuals to choose their own careers. However, in educational matters, this freedom is more apparent than real. On the surface we may appear to compel our young people to decide, at the age of 16, between science based and arts based studies, but in actual fact the determining factors start to operate much earlier, probably at age 14 or even 13. This, it is now being said, is due primarily to the mechanics of school forward planning, and I am sure that this is an area that merits quick and immediate investigation. We must find out, quickly, whether it is or is not true that the processes of our educational system are compelling an increasing number of our young people to make decisions for or against science at an age when they know relatively little about it.

If we are to prepare our young people for the lives that they are going to live, it is obviously important that they must be exposed, from as early an age as possible, to talks and experiences which indicate the importance of the science based activities of society. Much has been done in this field—but much remains to be done. I am not at all convinced that the processes of formal teaching and examination are the best that can be devised if we wish to stimulate the imagination of our young people. The age of decision for young people, as between the

Table 1. NUMBER OF PUPILS ON A LEVEL COURSES IN FIRST YEAR OF SIXTH FORMS (ENGLAND AND WALES)

	January 1962	January 1963	January 1964	January 1965	January 1966
"Science" subjects only	32,708 (4.8)	35,880 (4.9)	40,091 (5.0)	38,363 (5.0)	37,429 (5.2)
"Arts" subjects only <sup>1</sup>	38,310 (5.6)	45,263 (6.2)	54,530 (6.8)	55,929 (7.2)	58,185 (8.0)
"Science cum Arts" <sup>2</sup>	7,754 (1.1)	9,430 (1.3)	12,404 (1.6)	14,275 (1.8)	15,260 (2.1)
Total	78,772 (11.6)	90,573 (12.5)	107,025 (13.4)	108,567 (14.0)	110,874 (15.3)
Estimated population of young people in England and Wales from which first year in sixth form was drawn	680,000 (100.0)	725,000 (100.0)	800,000 (100.0)	775,000 (100.0)	725,000 (100.0)

<sup>1</sup> The "arts" group has been taken to include (a) traditional arts subjects, and (b) social sciences subjects.

<sup>2</sup> At least one science subject and one arts subject.

sciences and the arts, should be postponed as far as possible.

We must bear in mind that national needs do, after all, determine opportunities for individuals, and through these opportunities they affect the pattern of the educational requirements of society. Curriculum organization in schools and universities, which has remained substantially unchanged for the past 40 to 50 years, is drastically in need of revision. The question therefore arises of whether school and university training be altered in such a way that they make provision for those who show themselves likely to become high specialists, as well as for those who show themselves suitable for the more broadly based activities of society. Further, what are the reasons underlying the growth of the "Science cum Arts" group in our schools? Is this to be encouraged or resisted by our schools? Can the universities accommodate such young people within their existing educational structure? If not, then in what way must the educational pattern be modified? If, as has been tentatively suggested, the growth of the "Science cum Arts" group is a result of the publicity given to the needs of management and management services, then are young people really preparing themselves for such careers by selecting randomly from science studies and from arts studies? Should not training for management services be planned more systematically?

There is no shadow of a doubt that the decision for or against mathematics at an early age has a strong

determining influence on the decision for or against careers in science based activities or arts based activities. At present, about two-thirds of pupils with O level passes in mathematics abandon the subject when they enter the sixth form. More people could study it to O level, and many more to A level.

Everyone seems to agree, though without demonstrable justification, that mathematics is a discipline for training in logical reasoning and thinking—and this has been emphasized so much by those who teach mathematics in schools and universities that the importance of mathematics as a means of communicating quantifiable ideas and information, and as a vital tool in the progress and practice of engineering, technology and science, has not been given adequate prominence in this country. In fact, I feel that, if the teaching of mathematics in schools and universities is to mirror the most exciting developments of the twentieth century, it must show the effects of bringing mathematics much closer to engineering, technology, science and economics than at present. The effect of presenting mathematics completely separated from the motivations that give rise to the need for mathematics would be psychologically disastrous. It would turn our independently minded young people away from the study and the use of mathematics even more than at present. It would probably attract those who wish to study mathematics as an end in itself, but would repel those who want to use mathematics to underpin studies in other fields.

## Recurrent Continental Drift of Gondwanaland

by  
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Palaeomagnetism shows the pattern of drift of Gondwanaland for the past 600 million years. There seem to have been at least four episodes of rapid drift, with little or no drift in between.

THE principal evidence that continental drift, with or without polar wandering, has been continuous or frequent during Phanerozoic time is that the mean palaeomagnetic poles for each geological period from Europe are different, and lie in chronological order on a fairly smooth curve starting near the Equator in the Lower Palaeozoic and finishing at the present pole<sup>1,2</sup>. The same observation was made on North American rocks<sup>1-3</sup>.

The purpose of this article is to point out that the palaeomagnetic data from the southern continents appear not to conform to this pattern (Table 1, Fig. 1). Taking each continent separately, the palaeomagnetic record is too sparse and in some cases the dating of magnetization of the rocks is too uncertain to make possible a determination of the rate or the continuity of movement. Exceptions to this are that in Australia and Africa there is known to be a rapid transition within the Carboniferous<sup>5,6</sup>, and in both these continents the close grouping of Mesozoic poles indicates the absence of large-scale movement during that interval (ref. 5 and unpublished results). But if all the data from the southern continents are taken together, on any set of continental reconstructions which is compatible with the palaeomagnetic data, the poles fall in groups corresponding to time intervals which do not coincide with geological periods. Within each group there is generally no significant difference between poles, and these do not lie in chronological order. The mean poles of each group, however, do lie in chronological order. The transitions between one group and the next appear to have been accomplished quickly compared with the span of time represented by each group. It is suggested that the groups of poles represent long intervals during which little or no lateral movement of continents occurred ("quasi-static

intervals") and that the transitions between groups represent comparatively short "drift episodes".

Fig. 2 is the reconstruction for the Palaeozoic which most nearly conforms with the Palaeozoic palaeomagnetic data. There are no data from Antarctica, which is placed with the best geometrical and geological fit to southern Australia, and none from India, which is placed close to Australia so that the Mesozoic poles from the two continents coincide. The palaeomagnetic poles fall in groups, except for a few poles which refer to limited periods of geological time and which fall in transitional positions between the groups. These are:

### Group 1

Poles A1, A2, A3, B1, B2, B3, C1 and C2. These come from rocks which range in age from about 600 million years (B1) to Lower Devonian (A3). The last three were derived from limited data with no laboratory tests of stability. Differences between poles of this group may therefore arise from errors in the positions of some of the poles (themselves arising from secondary magnetization in some of the rocks from which they have been deduced) or from departures of the geomagnetic field in the Lower Palaeozoic from the geocentric dipole configuration (which is the model on which the poles were calculated). If this is so, the poles may belong to a single population. Indeed, Fisher's analysis<sup>7</sup> gives a mean pole 27° N., 4° W. relative to the present co-ordinates of Africa, with a radius of 95 per cent confidence ( $\alpha$ ) equal to 15.1°. This could indicate that there was a quasi-static interval from 600+ to about  $390 \times 10^6$  yr. The interval may have persisted into the Middle Devonian, to include pole C3, in which case the mean pole is 25° N., 1° W. ( $\alpha = 16.3^\circ$ ) as illustrated in Fig. 2.

Because of the limitations of the data, the possibility cannot be excluded of some drift within this interval. (It may be noted that Cambro-Ordovician poles  $A1$ ,  $B1$ ,  $B2$  are some  $25^\circ$  away from Silurian poles  $A2$ ,  $B3$ .) It is, however, unlikely that the drift was as great as that during any of the major drift episodes.

#### Transition between Groups 1 and 2

This is ascribed to a Middle Devonian drift episode. Precise dating of this episode depends on confirmation of

the ages assigned to the poles which delimit it. It is younger than the "Devonian" red beds ( $A3$ ) but older than the "Upper Silurian to Devonian" rocks from Australia ( $C4$ ,  $C5$ ). It is probable that pole  $C3$  records an intermediate position during the transition. The mean poles of groups 1 and 2 are  $50^\circ$  apart. If the transition took  $20 \times 10^6$  yr, this corresponds to a drift rate of about  $2.5^\circ$  per million years, which is of the same order as that suggested by Irving<sup>5</sup> for the Upper Carboniferous.

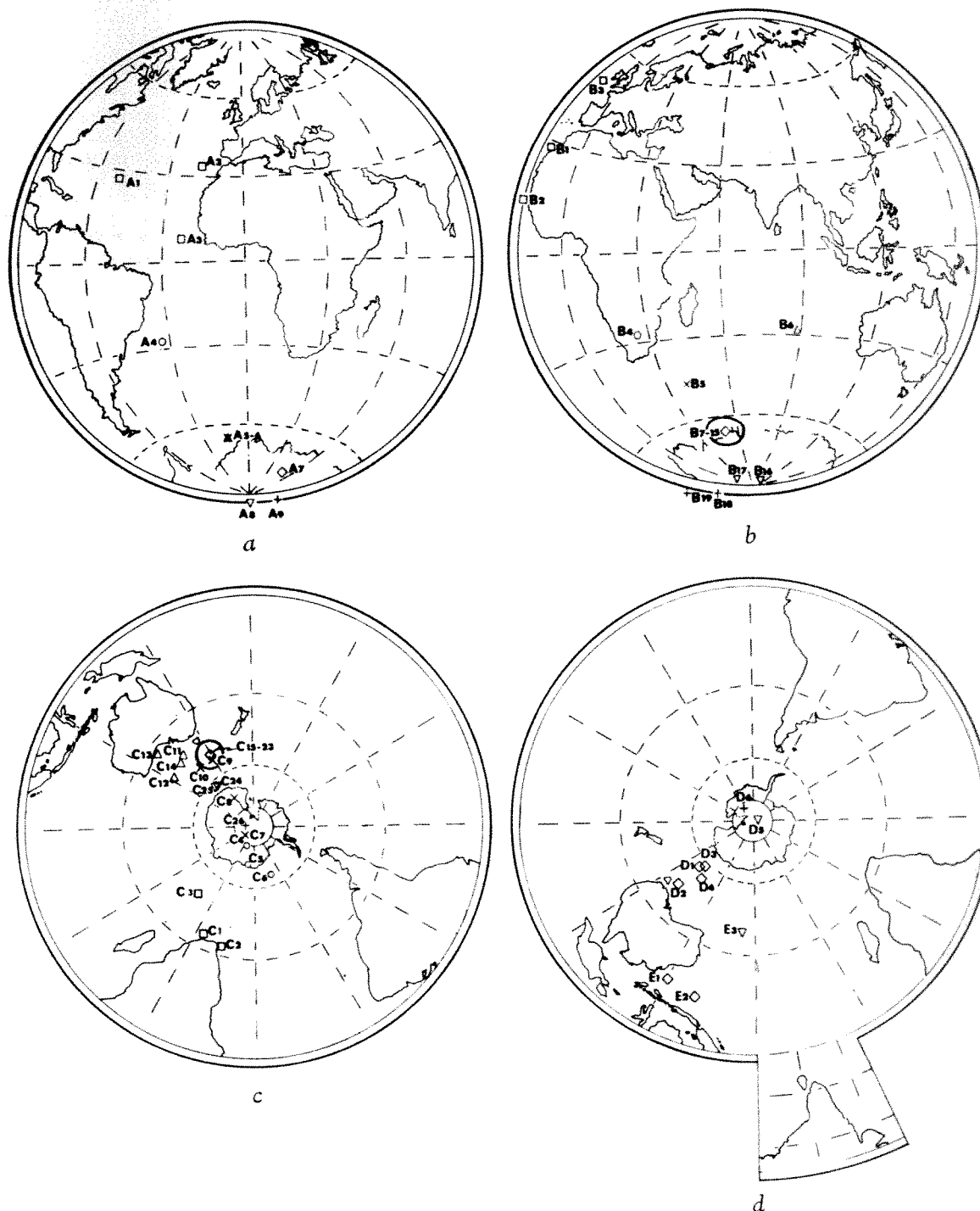


Fig. 1. Palaeomagnetic poles from Phanerozoic rocks from the Gondwana continents. (a) South America and (b) for Africa are on equal area meridional projections. (c) Australia and (d) for Antarctica and India are on polar stereographic projections. The poles are numbered in approximate chronological order within each continent and cross-referenced to Table 1. Separate symbols are used for each drift episode and quasi-static interval as follows:  $\square$ , 600+ to about  $390 \times 10^6$  yr (including pole C3);  $\circ$ , about 370 to  $320 \times 10^6$  yr;  $\times$ , Upper Carboniferous drift episode;  $\Delta$ , about 290 to  $240 \times 10^6$  yr;  $\diamond$ , about 220 to  $100 \times 10^6$  yr;  $\nabla$ , about 70 to  $20 \times 10^6$  yr;  $+$ , recent drift episode. In (b) and (c) the Mesozoic results are summarized by their mean and oval of 95 per cent confidence, but in (b) B7-15 has been plotted in the wrong longitude; it should be  $80^\circ$  E.



## Group 2

Poles A4, B4, C4, C5 and C6 range in age from "Upper Silurian to Devonian" (C4, C5) to Carboniferous (A4, B4). Their mean is at  $22\frac{1}{2}^{\circ}$  S.,  $20^{\circ}$  E. relative to the present co-ordinates of Africa and  $\alpha$  is about  $8^{\circ}$ .

This close grouping is taken to reflect a quasi-static interval from about  $370$  to  $320 \times 10^6$  yr. The interval could not have been longer than this unless the drift episodes which delimit it were very much more rapid than any previously suggested. But stratigraphic evidence indicates that the interval was not much shorter than that assigned to it here. Poles C4 and C5 are regarded as Devonian, and the Taiguati Formation (A4) and Dwyka varvoids (B4) are regarded as older than the Upper Carboniferous transition. Although four of the five poles in this group appear in Fig. 2 to lie in a line, they are not in chronological order and they are considered to form a group and not to represent slow drift.

## Transition between Groups 2 and 3

This is represented by poles A5, B5, C8 and probably C7, C9 and C10. They are all of Upper Carboniferous age and are taken to record an Upper Carboniferous drift episode. It was originally identified in New South Wales where the intermediate pole C8 was detected<sup>5</sup>, it has recently been found in Africa<sup>6</sup> and its existence is consistent with the South American data<sup>8</sup>. If the concept of Gondwanaland before the Carboniferous means anything, then the shift detected in Australia must be the same as is found elsewhere because it indicates drift of Australia towards the rest of Gondwanaland. Pole C11 may belong to the end of the transition, but it has been included in group 3 from which it is statistically indistinguishable.

## Group 3

Poles A6, B6, C11, C12, C13 and C14 are late Carboniferous and Permian. Except for A6 they are very closely grouped ( $\alpha = 5^{\circ}$ ) with a mean position relative to

Table 1. GONDWANALAND PALAEOMAGNETISM

		South America		Position		Africa		Position		Australia		Position
		Pole				Pole				Pole		
0	TERTIARY	Quaternary basalts	A9	83 S.	126 E.	Rift valley lavas	B19	74 S.	45 W.	Newer volcanics (Victoria)	C26	86 S.
						Nairobi lavas	B18	82 S.	41 W.			
						Turkana lavas	B17	85 S.	13 E.			
		"Kimeridgian" Red Beds	A8	86 S.	172 E.	Ethiopian basalt series	B16	87 S.	74 E.	Tertiary basalts (N.S.W.)	C25	63 S.
100	Cretaceous									Older volcanics (Victoria)	C24	63 S.
		Serra Geral lavas	A7	78 S.	54 E.	Lupata series volcanics	B15	61 S.	80 E.	Mt. Dromedary complex	C23	50 S.
	Jurassic					Mlanje Massif syenite	B14	60 S.	82 E.	Cygnat alkaline complex	C22	56 S.
						Karoo lavas (central Africa)	B13	57 S.	84 E.	Ginginbulen dolerite	C21	53 S.
						Stormberg lavas	B12	71 S.	89 E.	Noosa Heads complex	C20	36 S.
						Karoo dolerites	B11	65 S.	75 E.	Tasmanian dolerites	C19	51 S.
						Mateke Hills complexes	B10	59 S.	80 E.	Prospect dolerite	C18	51 S.
						Marangudzi complex	B9	70 S.	105 E.	Gibraltar microsyenite	C17	41 S.
200	Triassic					Red Beds, Zambia	B8	68 S.	50 E.	Brisbane tuff	C16	57 S.
						Shawa ijolite	B7	64 S.	86 E.	Narrabeen chocolate shale	C15	49 S.
300	Permian	Permian Red Beds	A6	65 S.	13 W.					Upper Marine latites	C14	44 S.
						K3 Red Beds, Songwe	B6	27 S.	89 E.	Moonbi lamprophyre	C13	35 S.
	Carboniferous									Lower Marine basalt	C12	46 S.
		Plau Formation	A5	65 S.	13 W.	K3 Red Beds, Galula	B5	45 S.	40 E.	Main glacial stage	C11	43 S.
		Taiguati Formation	A4	28 S.	34 W.	Dwyka varvoids	B4	26 S.	26 E.			
400	Devonian									Paterson toscanite	C9	53 S.
										Lower Kuttung lavas	C8	73 S.
	Silurian	Devonian Red Beds	A3	9 N.	22 W.	(Lower Table Mountain Series)	B3	50 N.	11 W.			
		Urucum Formation	A2	34 N.	16 W.					(Yalwal Stage basalts)	C6	65 S.
500	Ordovician									(Canberra volcanics)	C5	71 S.
										Mugga Mugga porphyry	C4	80 S.
	Cambrian									(Murrumbidgee series)	C3	49 S.
		Cambrian Red Beds	A1	27 N.	50 W.							
600	Lower Palaeozoic									(Elder Mountain Sandstone)	C2	34 S.
										(Antrim Plateau Basalts)	C1	36 S.
						Ntonya ring structure	B1	28 N.	15 W.			

Summaries of the data for South America have been provided by Creer<sup>8</sup>; for Africa by McElhinny *et al.* (unpublished); for Australia by Irving<sup>5</sup>; for India by Irving<sup>4</sup>. For chronology, see ref. 9.

the present co-ordinates of Africa at  $28^{\circ}$  S.,  $86.5^{\circ}$  E., although this statistic is dominated by the Australian results. The group is taken to reflect a quasi-static interval from about  $290$  to  $240 \times 10^6$  yr. The discrepancy of pole A6 is discussed later. It is notable that all the rocks in this group are reversely magnetized and it may be that this quasi-static interval corresponds approximately or exactly to the Kiaman magnetic interval as defined by Irving<sup>4,5</sup> during which the Earth's magnetic field retained reversed polarity for about  $50 \times 10^6$  yr.

#### Transition between Groups 3 and 4

The fact that Mesozoic poles are not well grouped on the Palaeozoic reconstruction (Fig. 2) or, to put it another way, that the Palaeozoic and Mesozoic reconstructions are different (Figs. 2 and 3) is taken to reflect another drift episode, close to the Palaeozoic-Mesozoic boundary, which is here called the Upper Permian drift episode. No intermediate pole positions have been recorded, which

may suggest that the episode was shorter than those already described. The distances involved in this episode are smaller (less than  $30^{\circ}$ ), but it is important because changes in the relative positions of the Gondwana continents are involved which did not, however, result in destroying the unity of the supercontinent.

#### Group 4

Fig. 3 is a reconstruction which satisfies all the Mesozoic palaeomagnetic data available from all five continents (but not west Antarctica). There are only Jurassic data from South America (A7), India (E1, E2) and east Antarctica (D1-4), but Australia (C15-23) and Africa (B7-15) are each represented by series of nine results spanning the time interval  $220$  to  $100 \times 10^6$  yr, another quasi-static interval ( $N = 25$ ,  $\alpha = 2.8^{\circ}$ ). This reconstruction is not unique because, unlike that in Fig. 2, it is only valid for one quasi-static interval so that relative longitude cannot be specified.

#### POSITIONS, CORRELATIONS AND DRIFT HISTORY

	Antarctica Pole	Position			India Pole	Position		
Volcanics (South Shetlands)	D6	82 S.	129 W.					RECENT DRIFT EPISODE
								QUASI-STATIC INTERVAL (about 70 to $20 \times 10^6$ yr.)
Andean intrusive suite	D5	86 S.	2 W.	Deccan Traps	E3	39 S.	96 E.	UPPER CRETACEOUS DRIFT EPISODE
Ferrar dolerites	D4	54 S.	136 W.					QUASI-STATIC INTERVAL (about 220 to $100 \times 10^6$ yr.)
	D3	59 S.	139 W.					
	D2	45 S.	141 W.	(Rajmahal Traps)	E2	13 S.	111 E.	
	D1	58 S.	142 W.	Sylhet Traps	E1	16 S.	120 E.	
								UPPER PERMIAN DRIFT EPISODE
								QUASI-STATIC INTERVAL (about 290 to $240 \times 10^6$ yr.)
								UPPER CARBONIFEROUS DRIFT EPISODE
								QUASI-STATIC INTERVAL (about 370 to $320 \times 10^6$ yr.)
								MIDDLE DEVONIAN DRIFT EPISODE
								QUASI-STATIC INTERVAL (600+ to about $390 \times 10^6$ yr.)

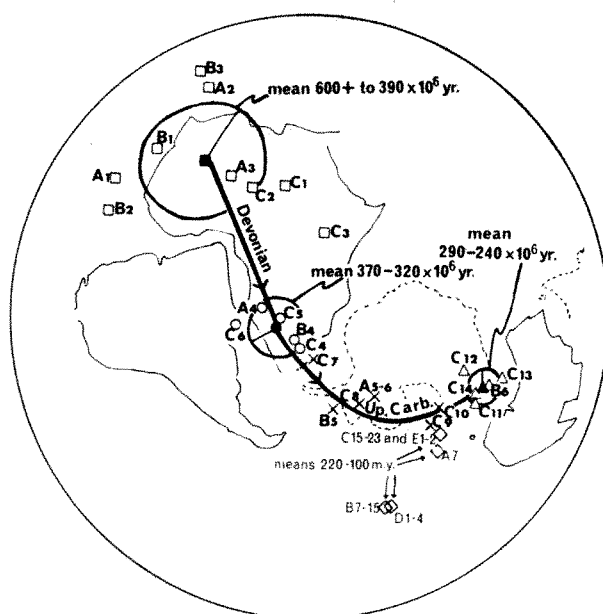


Fig. 2. Reconstruction of Gondwanaland for the Palaeozoic, together with the palaeomagnetic data. Equal area projection; notation as in Fig. 1 and Table 1. The solid symbols and thick ovals denote the mean pole and its 95 per cent confidence limits for each quasi-static interval. The thick lines are apparent polar wander curves representing movement during the drift episodes. Mean Mesozoic poles from each continent are added in lighter print.

### Transition between Groups 4 and 5

Tertiary poles are not well grouped on the Mesozoic reconstruction (Fig. 3) so that the Tertiary reconstruction is different (Fig. 4). This is a reflexion of the Upper Cretaceous drift episode—that which accounts for the major fragmentation of Gondwanaland. Separation could have begun earlier than this. Geological evidence of the influx of shallow seas along the developing fractures is likely to date from an early stage, but the divergence of polar wander curves from  $100 \times 10^6$  yr onwards (Fig. 3) shows that this was the time when large-scale lateral movements began. The limits of this episode cannot be precisely defined because of a gap in the palaeomagnetic record from about 100 to  $70 \times 10^6$  yr.

### Group 5

Fig. 4 is a reconstruction which satisfies all the poles ranging from about 70 to  $20 \times 10^6$  yr (A8, B16, B17, C24, C25, D5 and E3) with  $\alpha$  only about  $2^\circ$ . Relative longitudes are again arbitrary. Because there are only one or two results from each continent, it is not certain that the reconstruction is valid for the whole interval. South America, Africa and Antarctica may not have moved appreciably since the beginning of the Tertiary, but Australia and India could have moved more or less continuously through the past  $100 \times 10^6$  yr. On the other hand, the data are also consistent with a quasi-static interval from about 70 to  $20 \times 10^6$  yr. The older limit is uncertain because of the absence of data for the latest Cretaceous and the younger limit is vague because of another gap in the palaeomagnetic record from about 30 to  $10 \times 10^6$  yr. This interpretation is favoured, first, because it provides continuity with the pattern of stop-go drift observed in the Palaeozoic and Mesozoic and, second, because there is evidence from surveys of a change in magnetic anomaly patterns across the ocean floors corresponding to roughly  $20 \times 10^6$  yr ago<sup>10,11</sup> which may reflect the boundary between a quasi-static interval and a drift episode.

### Transition between Group 5 and the Present Pole

Poles A9, B18, B19, C26 and D6 come from rocks less than about  $10 \times 10^6$  yr old. They are close to the present pole (Fig. 1), but those from Australia and India

do not accord with the Tertiary reconstruction (Fig. 4). In conjunction with evidence for spreading of the ocean floor, which is believed to be a symptom of drift<sup>12,13</sup>, they are taken to indicate a recent drift episode during the past  $20 \times 10^6$  yr or so.

The reconstructions in Figs. 2 and 3 differ from previous ones only in detail. For example, Fig. 2 is very similar to King's<sup>14</sup> map for the Upper Carboniferous which was used by Irving<sup>5</sup> and McElhinny and Opdyke<sup>6</sup> in assessing the Australian and African data. Fig. 3 is almost identical with du Toit's<sup>15</sup> reconstruction which was found by Creer<sup>16</sup> to stand up well to comparison with palaeomagnetic data. The data presented here suggest that these reconstructions may have been valid for rather longer intervals of time than suggested by the original authors.

It remains true that an infinite set of reconstructions can be made to satisfy the palaeomagnetic data for any short period of geological time, for single palaeomagnetic studies do not permit evaluation of ancient longitude. But the reconstructions presented here are a very simple solution, satisfying the data with the minimum of drift and the minimum of relative movement. This does not of course prove that they are necessarily correct, and some modification is possible within the statistical uncertainties of the palaeomagnetic data. But, on any set of reconstructions which is generally consistent with the palaeomagnetic data and with geological evidence and geometrical constraints, the groups of poles and transitions between groups retain their integrity. Uncertainties still, of course, remain—notably the detailed history in the intervals between about  $600 \pm$  to  $390 \times 10^6$  yr and between  $100 \times 10^6$  yr and the present.

There is also to be considered an alternative interpretation of late Carboniferous and Permian data which stems from the discrepancy between the pole from some Permian red beds from South America (A6) and the other poles in group 3. There are several possible explanations. First is the possibility that the rocks involved are late Carboniferous, but this seems not to be upheld by geological evidence. The second is that pole A6 is not an accurate representation of the Permian field relative to South America, because of sampling inadequacy, partial instability of remanence or departures of the Permian field from the geocentric dipole configuration. A third possibility is that the position of pole A6 is a genuinely

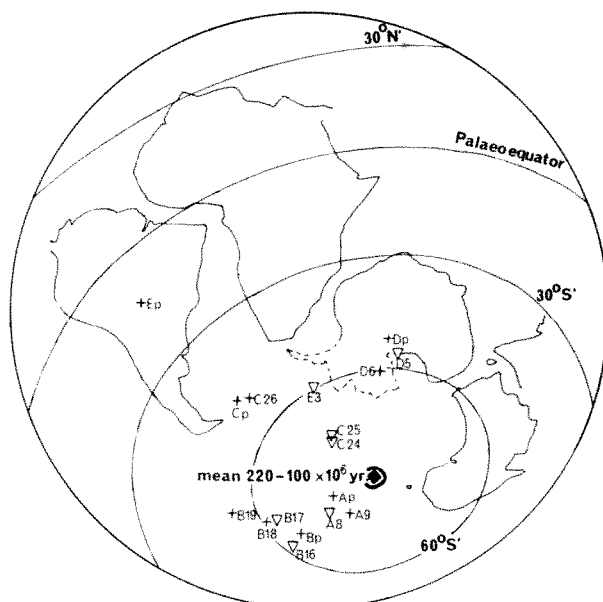


Fig. 3. A reconstruction of Gondwanaland for the quasi-static interval 220 to  $100 \times 10^6$  yr, with the palaeomagnetic poles and palaeolatitudes. Data for  $100 \times 10^6$  yr to the present are added in lighter print. Oblique equal-area projection; notation as in Figs. 1 and 2. In Figs. 3 and 4 relative longitudes are arbitrary and palaeolatitudes are denoted by primes.

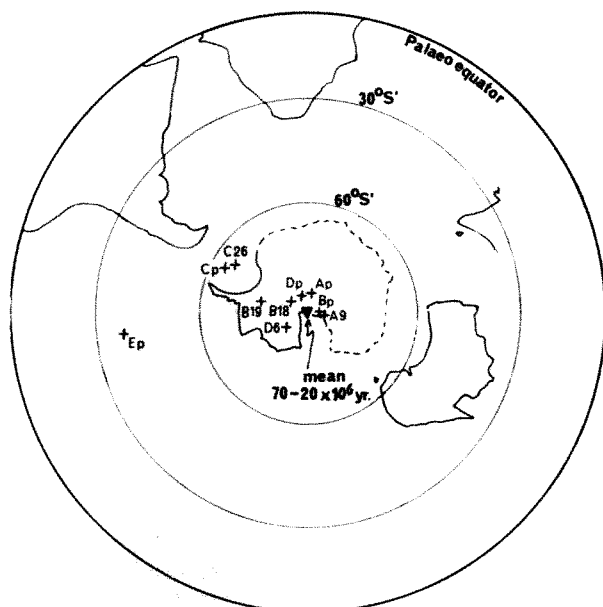


Fig. 4. A reconstruction for the quasi-static interval 70 to  $20 \times 10^6$  yr with palaeomagnetic poles and palaeolatitudes. Data for  $20 \times 10^6$  yr to the present are added in lighter print. Palaeopolar equal area projection. Notation as in Figs. 1 and 2.

accurate result, in which case the drift scheme set out here requires modification.

One such modification is that A6 could be reconciled with the other group 3 poles on the reconstruction in Fig. 2, which leads to a position of South America very far south relative to Africa and further south than during the Mesozoic (Fig. 3). This is a most unlikely answer. It is easier to defend the view that Fig. 3 (with very slight modification to compromise between poles A6 and A7) is the correct reconstruction for the late Carboniferous and Permian as well as for the Mesozoic. On this scheme, the Upper Carboniferous drift episode would be complicated by relative movements between the Gondwana continents (the differences between Figs. 2 and 3) and there would be no Permian drift episode. This is in accord with Irving's<sup>5</sup> observation that there is no statistical difference between poles C9-23 (late Carboniferous to Cretaceous) from Australia, and is not incompatible with the combined pole B5+B6 originally described by Opdyke<sup>17</sup> provided all the supposedly Permian red beds from Tanzania are actually Carboniferous. This would, however, imply that the transitional poles between groups 2 and 3 (now including B6) were much more scattered than in Fig. 2. The scheme presented here is favoured by the current data, and this alternative need only be substituted if further studies confirm that pole A6 is an accurate result for the Permian of South America.

Because of uncertainties such as this about the age and duration of the drift episodes and quasi-static intervals, informal names have been used and no apology is made for using geological terminology for drift episodes and an absolute age scale for quasi-static intervals. A formal system of nomenclature cannot be erected until the course of events is known much more precisely.

It is premature to do more than point out the apparent coincidence in timing of the drift episodes with well known orogenic episodes outside Gondwanaland, and also that the drift episodes appear to occur just before times of widespread extinction of a variety of faunas. But these coincidences may well be borne in mind in the light of the recent ideas of Irving<sup>5</sup> and Hide<sup>24</sup> on possible correlations between drift episodes and frequency of reversal of the Earth's magnetic field, coupled with the possibility of correlations between field reversals and evolutionary changes for which there is both theoretical and observational evidence<sup>18-20</sup>.

## Conclusions

The burden of this argument is to present the evidence for short drift episodes interrupting long periods of little or no drift. It has been possible to distinguish three episodes of "pre-Wegenerian drift", before the dispersal of Gondwanaland. Because the bulk of the movement during these older episodes affected all of Gondwanaland equally, it is fair to ask whether they may be episodes of polar wander rather than of continental drift. The answer must depend on an assessment of Palaeozoic data from Europe and North America, for polar wander can only be demonstrated palaeomagnetically by the finding of identical differences between corresponding pairs of poles from all continents. Creer<sup>25</sup> considers that such identical differences have been found in the Palaeozoic, but this conclusion leans heavily on the interpretation of two palaeomagnetic poles from North America derived from rocks which are known to be secondarily magnetized. The conclusion is not upheld by recent results from North America<sup>21,22</sup> and, on balance, it seems that the Upper Carboniferous and Middle Devonian drift episodes did not occur with the same timing, magnitude and direction as in the southern continents. It is therefore concluded that these episodes did at least contain a component of continental drift. At this stage it is unnecessary to invoke a second mechanism (polar wander) to explain the data from Gondwanaland.

The inference from North American and European data that large-scale drift and/or polar wander has been continuous or frequent conflicts with the conclusions presented in this paper, because it is clearly unlikely that entirely different patterns were followed by the northern and southern continents. More investigation is required to test which pattern of drift has really predominated, and the first requirement is for many demagnetization studies on Palaeozoic rocks from Europe and North America. One of the few such studies<sup>23</sup> so far has given a hint that drift relative to Europe may not have been as regular as had been thought.

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# Mitochondrial and Cytoplasmic Polysomes from *Neurospora crassa*

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Mitochondria from *Neurospora crassa* contain 73S ribosomes, which can be separated from 77S cytoplasmic ribosomes. Mitochondrial and cytoplasmic rRNA differ in sedimentation constants and base composition.

RECENTLY Stutz and Noll<sup>1</sup>, confirming earlier observations<sup>2-4</sup>, presented evidence that the chloroplasts of green plants contained polysomes consisting of 70S ribosomes in contrast to the 80S ribosomes which make up the polysomes of the surrounding cytoplasm. They also showed that chloroplast and bacterial ribosomes were indistinguishable both with respect to the sedimentation behaviour of the monomeric particle and the sedimentation coefficients (23S and 17S) of the RNA components corresponding to their 50S and 30S subunits. Observations of similar sensitivity to antibiotics<sup>5</sup> further indicated that ribosomes from bacteria and chloroplasts are very closely related. Analogous findings of a preferential inhibition of mitochondrial protein synthesis by chloramphenicol<sup>6-8</sup> and the apparent evolutionary parallelism led these authors to suggest that mitochondria, being another class of organelles containing DNA, also possess bacterial type ribosomes. We now confirm this hypothesis and present physical chemical evidence for the existence of a unique class of mitochondrial ribosomes engaged in mitochondrial protein synthesis.

In these studies, which were carried out with *Neurospora crassa*, we also examined the cytoplasmic ribosomes; the sedimentation coefficients corresponding to the monomer particles (77S) and their two RNA components ( $\alpha = 25S$ ,  $\beta = 17S$ ) were very similar to the values previously determined for cytoplasmic ribosomes of green plants<sup>1</sup>. These findings thus complement earlier observations from this laboratory and provide more evidence for the concept that there are at least three principal classes of ribosomes: organelle and bacterial, plant cytoplasmic and animal cytoplasmic ribosomes<sup>1</sup>.

Using zone velocity centrifugation in isokinetic sucrose gradients<sup>9</sup> in combination with a high resolution analysing system, we have been able to rank ribosomes from various sources in order of increasing sedimentation rate: bean chloroplasts, *Escherichia coli*, *Neurospora* mitochondria, *Neurospora* cytoplasm, bean cytoplasm, rat liver.

## Preparation of Cytoplasmic and Mitochondrial Ribosomes from *Neurospora crassa*

After preliminary experiments with rat liver cells we selected the fungus *Neurospora* as the most suitable source for the study of mitochondrial ribosomes for the following reasons: (a) under conditions of vigorous aeration these organisms grow rapidly and synthesize mitochondria at a high rate which results in the accumulation of relatively high concentrations of these organelles in the cell; (b) the cells are ruptured under relatively mild conditions and the mitochondria recovered in good yields and with high purity; (c) conditions affecting mitochondrial function can

be manipulated at will; and (d) much is known about the physiology and genetics of *Neurospora*.

Cells of *Neurospora crassa* (Em 5256, wild type strain) were grown and collected as described by Luck<sup>10</sup>. In order to isolate the mitochondria without degradation of mitochondrial ribosomes, we found it necessary to use buffer solutions with relatively high concentrations of magnesium and monovalent cations (AMT buffer: 0.1 molar  $\text{NH}_4\text{Cl}$ , 0.01 molar  $\text{MgCl}_2$ , 0.01 molar *tris*). Batches of seven Erlenmeyer flasks, each containing 200 ml. of minimal medium and an inoculum of  $1.4 \times 10^7$  conidia, were agitated in a reciprocating shaker and collected while still in the exponential growth phase after 15 h at 25° C. The cell mass (about 20 g wet weight) was collected on a Buchner funnel in the cold room, washed with two portions of 40 ml. 0.44 molar sucrose in AMT buffer, and, after mixing with 10 g of acid-washed sea sand, ground to a paste while gradually adding 30 ml. of sucrose-AMT. The extract was centrifuged for 10 min at 500*g* in a swinging bucket centrifuge and the pellet extracted with another portion of 25 ml. sucrose-AMT. The combined supernatants were then centrifuged for 20 min at 80 per cent line voltage (26,000*g*) in a Servall SS-1 centrifuge. The postmitochondrial supernatant was saved for the isolation of cytoplasmic ribosomes, the crude mitochondrial pellet (about 30 mg total protein) suspended in sucrose-AMT to a concentration of 10 mg/ml. and portions of 2 ml. layered over gradients consisting of seven 3-ml. steps of 2.1, 1.95, 1.8, 1.65, 1.5, 1.35, 1.2 molar and a 12 ml. top layer of 0.9 molar sucrose (made up in AMT) in 35 ml. swinging bucket tubes of a rotor SB-110 (International Equipment Company). After centrifugation for 60 min at 25,000 r.p.m. and 3° C, the yellowish mitochondrial band at the interphase between the 1.5 and 1.65 molar sucrose layers was removed and either used immediately for preparing ribosomes or stored at -20° C. The yield was about 25 mg protein from each batch of seven flasks.

To prepare ribosomes, the mitochondrial fraction was diluted with AMT to make it 0.44 molar with respect to sucrose and spun down by centrifugation. The mitochondrial pellet was taken up in AMT (1.0 ml./5 mg of protein) and lysed by adding 0.05 ml. of a 20 per cent solution of 'Triton X-100'/ml. of suspension and mixing with a hypodermic syringe. Treatment with 'Triton X-100' does not lyse present bacteria<sup>11</sup>. The clear lysate was either subjected directly to sedimentation analysis or used to isolate purified ribosomes by layering portions of 7 ml. over 3 ml. of 0.9 molar sucrose-AMT and centrifuging for 14 h at 40,000 r.p.m. and 3° C in an I.E.C. or Spinco angle head rotor. The translucent yellow pellets were rinsed quickly with AMT to remove a thin layer of a greenish-yellow precipitate from the surface and resuspended in

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AMT. After a clarifying spin the solution (about 1 mg RNA/ml.) was stored at  $-60^{\circ}\text{C}$ . The yield was about  $30\text{ }\mu\text{g}$  RNA/mg mitochondrial protein.

To isolate the cytoplasmic ribosomes, the postmitochondrial supernatant was made 0.5 per cent with respect to 'Triton X-100' and centrifuged to remove insoluble material as described by Stutz and Noll<sup>1</sup>. The supernatant was layered over 2 ml. of 1.0 molar sucrose-AMT, centrifuged for 2 h at 40,000 r.p.m. and  $3^{\circ}\text{C}$  and the resuspended pellets (5 mg/ml.) stored at  $-60^{\circ}\text{C}$ .

### Resolution of 73S Mitochondrial and 77S Cytoplasmic Ribosomes by Zone Velocity Sedimentation

To determine whether cytoplasmic and mitochondrial ribosomes are different, we examined the sedimentation behaviour of ribosome preparations from the two sources in sucrose gradients. Fig. 1 shows strip chart recordings of sedimentation patterns characteristic of cytoplasmic polysomes (A), mitochondrial lysates (B) and a mixture of the two preparations (C). The two preparations are strikingly different: the pattern of the cytoplasmic polysomes exhibits a strong monomer peak corresponding to 77S which is followed by clearly resolved polysome peaks ranging from dimer to hexamer, while in the mitochondrial lysate the polysome region is not resolved and the strongest peak sediments somewhat more slowly, corresponding to an *S*-value of 73. In addition, two prominent slower components corresponding to 50S and about 40S are always present in the mitochondrial lysate. The difference in sedimentation rate between the cytoplasmic and mitochondrial monomer species, although slight, is firmly established by the fact that the two components have been clearly resolved during co-sedimentation in the same tube (C). Indeed, the sedimentation pattern obtained with the mixture corresponds exactly to the summation curve produced by superimposing the tracings of the two components. Equally, separations, not shown here, were obtained when purified ribosomes from mitochondria (Fig. 2) were centrifuged after they had been mixed with cytoplasmic ribosomes. The difference between the sedimentation rate of the two ribosomes is only 5 per cent.

To evaluate the significance of the unexpected similarity in sedimentation rates of cytoplasmic and mitochondrial ribosomes from *Neurospora*, a more precise characterization of their sedimentation rates was carried out by comparing them under standardized conditions with representatives from other ribosome classes. In these experiments *E. coli* ribosomes with an assumed sedi-

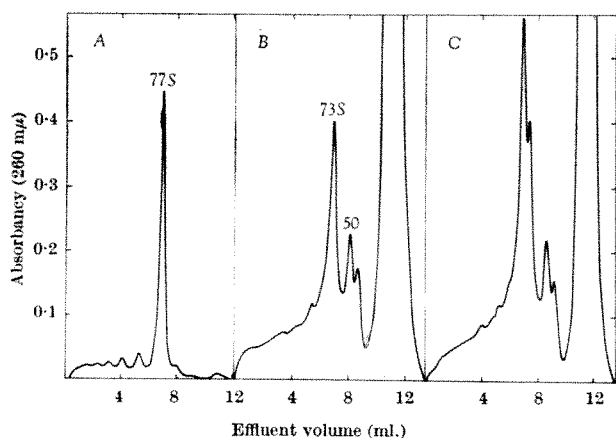


Fig. 1. Sedimentation pattern of a mitochondrial lysate and of cytoplasmic ribosomes from *Neurospora*. Cytoplasmic ribosomes and freshly prepared mitochondrial lysates were layered on 12 ml. convex sucrose gradients made up in AMT and centrifuged for 2 h at 41,000 r.p.m.,  $3^{\circ}\text{C}$ , in the rotor (SB-283, B-60 centrifuge, International Equipment Company). A, Cytoplasmic ribosomes (0.68  $A_{260}$  units equivalent to  $34\text{ }\mu\text{g}$  RNA in  $5\text{ }\mu\text{l}$ . AMT). B, Mitochondrial lysate (5 mg protein in 0.5 ml. AMT, containing 1 per cent 'Triton-X-100'). C, Mixture of A and B.

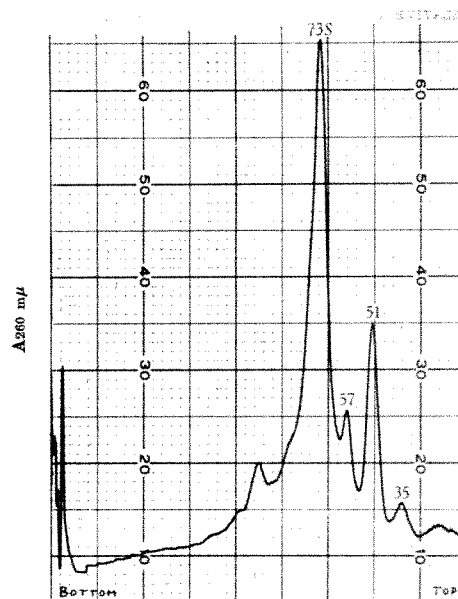


Fig. 2. Sedimentation pattern of isolated mitochondrial ribosomes. Freshly prepared mitochondrial ribosomes (1.8 mg RNA dissolved in 0.2 ml. AMT) were layered on a 12 ml. convex sucrose-AMT gradient and centrifuged for 2 h at 40,000 r.p.m.,  $3^{\circ}\text{C}$ . The ordinate corresponds to 0.625  $A_{260}$  units.

mentation coefficient of 70S served as calibrating standard. The samples were mixed in the combinations shown in Table 1 and, for optimal resolution, centrifuged in an isokinetic gradient<sup>9</sup> to a position about one quarter above the bottom of the long 12 ml. tubes of the I.E.C. SB-283 rotor. The mobilities of the 70/50/30S *E. coli* calibrating standards indicate that the particles sedimented at a perfectly constant rate. To the results in Table 1 we have added the previously published values for chloroplast ribosomes from beans<sup>1</sup>. Of particular interest is the finding that mitochondrial ribosomes from *Neurospora* have a markedly higher *S* value (73.2) than *E. coli* ribosomes and sediment at a rate intermediate between that of *E. coli* (70S) and *Neurospora* cytoplasmic ribosomes (77S). By contrast, the sedimentation rate of chloroplast ribosomes (67S) is lower than that of *E. coli* ribosomes by about the same amount.

We estimate that the relative *S*-values which establish the ranking order in Table 1 are accurate within about  $\pm 0.3$  units. Thus we believe that the figures are significant which indicate that the cytoplasmic ribosomes from *Neurospora* sediment somewhat slower and those from liver somewhat faster than bean cytoplasmic ribosomes. It should also be noted that the values obtained here by constant velocity sedimentation are in excellent agreement with published data determined with the analytical ultracentrifuge.

Table 1. RELATIVE *S*-VALUES OF RIBOSOMES FROM ORGANELLES AND CYTOPLASM OF MAJOR CLASSES DETERMINED BY ISOKINETIC SEDIMENTATION

Com- ponents in mixture No.	<i>E. coli</i> sub-units 30S 50S	Bean chloro- plasts 3	<i>E. coli</i> 70S 4	<i>Neurospora</i> Mito- chondria Cyto- plasm 5 6	Bean cyto- plasm 7	Rat liver cyto- plasm 8
1+2+5	29.0 50.0			73.2		
1+2+6	29.9 50.0			76.9		
4+6			70.0	76.9		
4+7			70.0		78.5	
5+6				73.2 76.9		
4+3*†		68	70.0			
4+7*			70.0		78.8	
3+7		66.8			78.8	
7+8*†					79	80
Synopsis	29.0 50.0	66.8	70.0	73.2 76.9	78.6	80

\* Data taken from ref. 1.

† Only partial separation of components; *S* values refer to positions of shoulders.

Table 2. RELATIVE *S* VALUES OF RIBOSOME MONOMERS AND POLYSOMES FROM VARIOUS SOURCES

Source: <i>n</i>	<i>E. coli</i>	<i>Neurospora</i>		Liver cytoplasm
		Mitochondria	Cytoplasm	
Monomer: 1	70	73	77	81
Polysomes: 2	102	103	108	119
3	129	134	140	152
4	155	160	167	180
5	179	186	192	207
6			215	230

The conclusions that the mitochondrial ribosomes in *Neurospora* are different from those in the cytoplasm is further supported by an examination of the polysomes from the two cell compartments. The sedimentation pattern of mitochondrial lysates often exhibits a number of polysome peaks visible as distinct shoulders above the leading edge of the predominant monomer peak (Fig. 6). It is evident that these peaks are more closely spaced than the polysome peaks in the sedimentation pattern of the cytoplasmic preparation (Fig. 1A). Thus, it is clear from the *S*-values compiled in Table 2 that the difference observed between the two types of monomer ribosomes is reflected and magnified in the *S*-values of the corresponding polysome series. For comparison we have included the analogous data for *E. coli* and rat liver cytoplasmic ribosomes.

### Subunits of Mitochondrial and Cytoplasmic Ribosomes

Sedimentation diagrams of the mitochondrial lysate always exhibit two closely spaced peaks in the 35 to 50S region (Fig. 1B). In preparations of purified mitochondrial ribosomes an additional, more rapidly sedimenting component is revealed (Fig. 2). The positions of the three subunit peaks in Fig. 2 correspond to 57-58, 51 and 35-36S. In a large number of patterns examined the 51S peak was always the strongest, whereas the intensity of the other two peaks relative to this principal peak varied considerably. We also noted that the increase of the 51S peak, which was particularly pronounced after thawing frozen samples of purified mitochondrial ribosomes, regularly occurred at the expense of the 73S monomeric ribosome unit. This indicates that the 51S peak corresponds to the large subunit of mitochondrial ribosomes. Because the conditions responsible for the variable amount of the 51S component evidently failed to affect the amounts of other subunit peaks, we conclude that these minor peaks are of a different origin, most likely the subunits of cytoplasmic ribosomes present in variable proportions as contaminants. In support of this interpretation it may be pointed out that the sedimentation constants of the minor peaks (57/35S) correspond closely to the values expected for the subunits of the 77S cytoplasmic ribosomes. The 35S peak was always much smaller, in relation to the larger subunits, however, than expected on the basis of an equimolar ratio. The failure to recover an equivalent proportion of the smaller subunits is a general observation which we attribute to the tendency of these particles to remain bound to larger aggregates because of their strong interaction with messenger RNA. The broad nature of the 35S peak further suggests that it arises from a mixture of the two smaller subunits (expected to sediment at about 36 and 30S) derived from both the cytoplasmic and mitochondrial ribosomes.

### Characterization of RNA from Mitochondrial and Cytoplasmic Ribosomes

Surveys carried out in several laboratories suggest that the ribosomes from a wide spectrum of organisms may be grouped into three broad classes (*A*, *B*, and *C*, Table 3) based on the size or derivative properties (sedimentation rate, electrophoretic mobility) of their ribosomal RNA. These relationships are summarized in Table 3 which compares the relative sedimentation rates of representatives of the three classes in the presence of either magnesium or sodium ions. To facilitate comparison we have

normalized all measurements using as calibrating standard the small component of *E. coli* (*C* $\beta$ ) and assigning it a value of 16S. It can be seen that regardless of ionic conditions members of the three classes differ most significantly among each other in the sedimentation rate of the large component relative to the *E. coli* 16S component. By adopting this reference system, the three classes may be characterized by the following generic sedimentation values: *A* = 30/18, *B* = 25/17, and *C* = 22/16.

Table 3. RELATIVE SEDIMENTATION RATES OF RIBOSOMAL RNA FROM THREE CLASSES OF RIBOSOMES IN THE PRESENCE OF Mg<sup>++</sup> OR Na<sup>+</sup>

rRNA components:		Relative <i>S</i> values				Ratio $\alpha/\beta$	
Class of ribosomes	Source	$\alpha$	$\beta$	$\alpha$	$\beta$	Mg <sup>++</sup>	Na <sup>+</sup>
		0.001 molar Mg <sup>++</sup>	0.01 molar Na <sup>+</sup>	0.01 molar Na <sup>+</sup>	0.01 molar Na <sup>+</sup>		
<i>A</i>	Rat liver cytoplasm	30.4	18.9	29.5	17.8	1.61	1.66
<i>B</i>	Bean cytoplasm	26.5	16.0	25.9	17.0	1.65	1.52
	<i>Neurospora</i> cytoplasm	25.8	16.5	25.0	17.4	1.56	1.44
<i>C</i>	<i>E. coli</i>	22.6	16.0	21.2	16.0	1.41	1.31
	Mitochondria ( <i>Neurospora</i> )	23.0	16.2	20.5	16.4	1.42	1.29
	Chloroplasts	22.6	15.9	20.8	15.7	1.42	1.33

The preparations were centrifuged in the I.E.C. SB-283 rotor for 12 h at 35,000 r.p.m. and 3° C. Relative *S* values were determined from the peak positions on the strip chart recordings with the aid of an empirical calibration curve. All values were normalized by arbitrarily setting *E. coli* rRNA  $\beta$  = 16.0. Following the convention proposed previously, we designate the larger rRNA component with the letter  $\alpha$ , the smaller with  $\beta$ .

In Table 4 we have listed the number and *S* values of clearly separated peaks observed in the sedimentation diagrams of mixtures containing ribosomes from two different classes in the three possible combinations. The analyses were carried out in the presence and absence of magnesium ions. It is interesting to note that the Na<sup>+</sup> system is capable of resolving the larger but none of the smaller components of all possible pairs (unpublished results of Stutz and Rawson). On the other hand, in some cases the system containing magnesium ions permits the separation of all four components. In other cases, exemplified by all mixtures containing type *B* + *C* ribosomes, this system fails to separate any of the components.

The results obtained with RNA from cytoplasmic and mitochondrial ribosomes (Tables 3 and 4 and Figs. 3-5) clearly show that the cytoplasmic RNA of *Neurospora* belongs to the 25/17S type (class *B*), whereas the mitochondrial RNA is most closely related to bacterial RNA (class *C*). In particular it is evident from Fig. 3 that in the presence of magnesium ions cytoplasmic RNA from *Neurospora* can be separated from the RNA of rat liver but not from that of bean cytoplasm. Fig. 4 shows that *Neurospora* cytoplasmic RNA sediments more rapidly than either *E. coli* RNA or mitochondrial RNA, although it cannot be separated when mixed with *E. coli* RNA. The sedimentation diagram of the mixture containing RNA from *E. coli* and *Neurospora* cytoplasm (Fig. 4D), however, exhibits a noticeable broadening of the two peaks, and the peaks of the mixture are located between the positions of the individual components, indicating

Table 4. RESOLUTION OF COMPONENTS IN rRNA MIXTURES PREPARED FROM TWO DIFFERENT RIBOSOME CLASSES

rRNA components: Mixture	Source	Relative <i>S</i> values		Ratio $\alpha/\beta$	
		$\alpha$	$\beta$	$\alpha$	$\beta$
		0.001 molar Mg <sup>++</sup>	0.01 molar Na <sup>+</sup>		
<i>A</i> + <i>B</i>	Rat liver + bean	4 peaks: 31.4 18.6 26.6 16.0	3 peaks: 29.5 17.3		
		3 peaks: ( $\beta$ -peaks partially resolved)			
	Rat liver + <i>Neurospora</i>	31.1 18.4 26.1	29.5 17.5 24.8		
		3 peaks:	3 peaks:		
<i>A</i> + <i>C</i>	Rat liver + <i>E. coli</i>	29.0 16.2 22.9	29.0 16.9 20.9		
		2 broad peaks:	3 peaks:		
<i>B</i> + <i>C</i>	Bean cytoplasm + <i>E. coli</i>	24.3 16.3	24.9 16.5 21.0		
	<i>Neurospora</i> cytoplasm + mitochondria	24.2 16.2	25.0 17.0 20.5		

that the two species interact reversibly during sedimentation. The fast moving peak in the mixture can be shown to result from incomplete disruption of the *E. coli* 50S subunit by the detergent sodium dodecylsulphate (unpublished results of Stutz and Rawson). Mitochondrial and *E. coli* RNA, on the other hand, sediment almost identically (Fig. 4B and C), except that the larger component of the mitochondrial preparation is slightly faster as expected from the somewhat larger *S* value of the parent ribosome. The nature of the minor fast component has not been elucidated. A comparison of the two types of RNA from *Neurospora* in the absence of magnesium ions (Fig. 5) further corroborates their distinct nature; the larger components of mitochondrial and cytoplasmic RNA are resolved as expected from the analogous behaviour previously reported for cytoplasmic and chloroplast RNA from green plants. It should be stressed that even our best RNA preparations from either chloroplasts or mitochondria have suffered at least partial degradation because of the ubiquitous presence of nucleases in plant material. This degradation is always more pronounced in the absence of magnesium ions which seem to protect the molecules by keeping them in a more compact conformation.

#### Base Composition of Mitochondrial and Cytoplasmic rRNA

In an attempt to confirm by chemical method the differences between mitochondrial and cytoplasmic ribo-

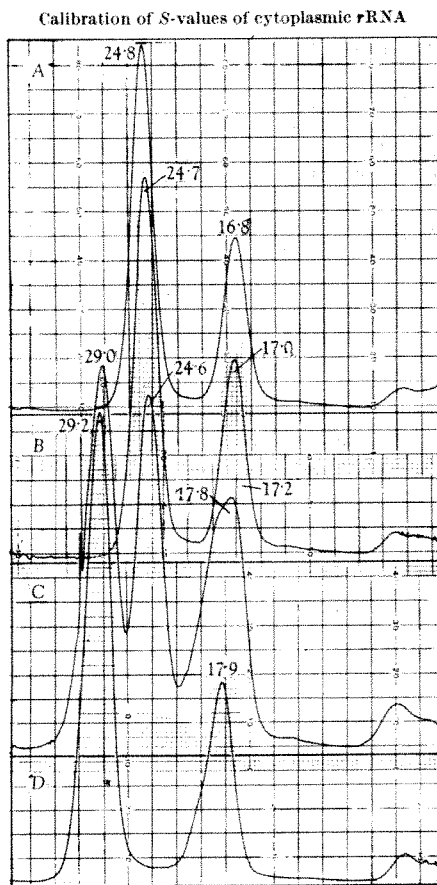


Fig. 3. Sedimentation pattern of rRNA from *Neurospora*, bean and rat liver cytoplasmic ribosomes. Ribosomes equivalent to 0.9 mg RNA were dissolved in 1 ml. of AMT, and a 10 per cent solution of SDS was added to a final concentration of 2 per cent. The mixture was incubated for 10 sec at 37° C. After chilling, 0.2 ml. of the mixture were layered immediately on 12 ml. convex sucrose gradients made up in 0.002 molar MgCl<sub>2</sub>, 0.001 molar tris-HCl, pH 7.5 and centrifuged for 13.5 h at 35,000 r.p.m., 3° C. The ordinate corresponds to 1.25 A<sub>280</sub> units. A, *Neurospora* 25/17S (0.2 ml.). B, *Neurospora* and bean 25/17S (each 0.1 ml.). C, *Neurospora* and rat liver 25/17S, 29/18S (each 0.1 ml.). D, Rat liver 29/18S (0.2 ml.).

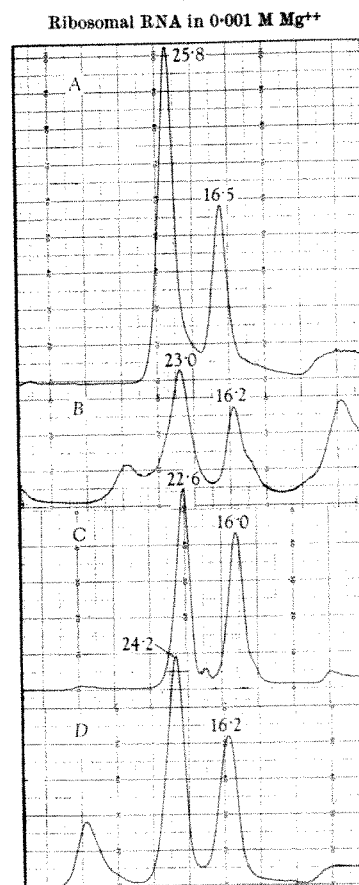


Fig. 4. Sedimentation pattern of cytoplasmic and mitochondrial rRNA from *Neurospora* and of *E. coli* rRNA. Ribosomes were treated with SDS as described in Fig. 3. 12 ml. convex sucrose gradients made up in 0.002 molar MgCl<sub>2</sub>, 0.001 molar tris-HCl, pH 7.5 were centrifuged for 12 h at 35,000 r.p.m., 3° C. The ordinate corresponds to 1.25 A<sub>280</sub> units. A, *Neurospora* cytoplasmic rRNA 25/17S (0.2 ml.). B, *Neurospora* mitochondrial rRNA 23/16S (0.1 ml.). C, *E. coli* 22/16S (0.2 ml.). D, A (0.1 ml.) + C (0.1 ml.).

somes established by the physico-chemical analyses, we examined the nucleotide composition of the ribosomal RNA from the two sources.

Suspensions of purified cytoplasmic or mitochondrial ribosomes labelled with phosphorus-32 were diluted to contain no more than 0.9 mg RNA/ml. The ribosomes were then disrupted in the presence of 2 per cent sodium dodecylsulphate (SDS)<sup>1</sup>. The lysate was centrifuged through a convex exponential sucrose gradient in the I.E.C. rotor SB-283 for 12 h at 35,000 r.p.m. and 3° C. The gradient fractions containing the two rRNA peaks were collected and pooled. The RNA was isolated, hydrolysed and analysed by high voltage paper electrophoresis as described in the legend to Table 5. The results reveal a striking difference in the nucleotide composition of the two RNA species: cytoplasmic RNA has a much higher GC-content ( $G + C/A + U = 1.02$ ) than mitochondrial RNA ( $G + C/A + U = 0.66$ ). Of particular interest is the unusually low proportion of CMP. Another characteristic feature of mitochondrial RNA is the relatively high proportion (5 per cent) of unidentified component with a mobility between that of AMP and GMP. An unknown product in hydrolysates of chloroplast rRNA from *Euglena* has been previously reported by Brawerman and Chargaff<sup>12</sup>.

#### Protein Synthesis by Mitochondrial Polysomes

We have tested the ability of intact mitochondria to incorporate amino-acids into protein. Purified mitochondria were incubated at 37° C with <sup>14</sup>C-leucine under the conditions described by Wheeldon and Lehninger<sup>8</sup> and after intervals ranging from 5 to 40 min samples of



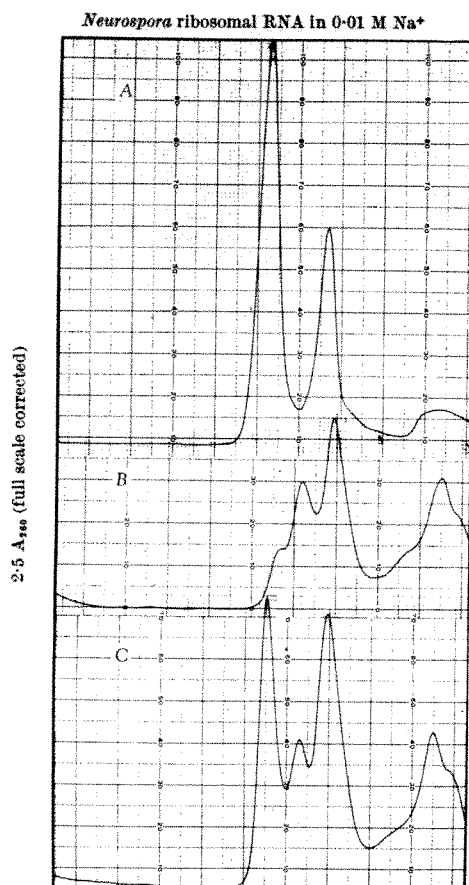


Fig. 5. Sedimentation pattern of cytoplasmic and mitochondrial rRNA from *Neurospora*. Ribosomes were treated with SDS as described in Fig. 3. 12 ml. convex sucrose gradients containing 0.01 molar NaCl, 0.005 molar *tris*-HCl, pH 7.5 were centrifuged for 12 h at 35,000 r.p.m., 3° C. The ordinate corresponds to 1.25  $A_{260}$  units. A, Cytoplasmic rRNA (0.2 ml.). B, Mitochondrial rRNA (0.2 ml.). C, Mixture of A+B.

0.5 ml. were withdrawn and diluted with ice-cold AMT-buffer. The mitochondrial pellets obtained after centrifugation were lysed with 'Triton X-100' and subjected to zone-velocity centrifugation in sucrose gradients. The contents of the tubes were analysed for absorbancy by automatic spectrophotometric scanning of the effluent and the fractions assayed for acid-insoluble radioactivity<sup>13</sup>. Fig. 6 shows the distribution of radioactivity and ribosomes observed after 5 and 20 min; the radioactivity is mainly associated with the polysome region, a smaller fraction with the supernatant. The proportion of acid-insoluble radioactivity in the supernatant fraction increases with time; some of the nascent chains originally associated

with polysomes are completed and released. Because there is little if any nascent protein associated with the monomer peak, the rather large proportion of monomers does not seem to result from degradation of polysomes by RNase action, but is more likely to be attributable to poor physiological conditions which usually have been found to cause polysome breakdown by a preferential inhibition of chain initiation. This explanation is supported by the observation that the mitochondria begin to lyse after the first 20 min of incubation.

Similarly, the low incorporation of radioactive amino-acids is probably caused by a large internal amino-acid pool and by unfavourable conditions for the transport of amino-acids through the mitochondrial membrane.

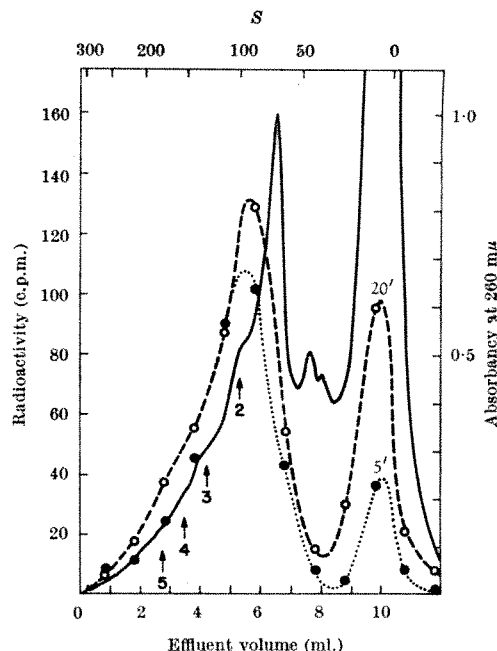


Fig. 6. Incorporation of  $^{14}\text{C}$ -leucine into mitochondrial protein. The incubation mixture contained (in  $\mu\text{moles/ml.}$ ): sucrose (440), KCl (100),  $\text{MgCl}_2$  (10),  $\text{KH}_2\text{PO}_4$  (10), proline (5), phosphoenolpyruvate, cyclohexylammonium salt (5), ATP (2), *tris*-HCl, pH 7.5 (33), amino-acid mixture minus leucine (0.1 each),  $^{14}\text{C}$ -leucine, specific activity 200 mc./mmole (3  $\mu\text{c.}/\text{ml.}$ ), pyruvate kinase (10  $\mu\text{g.}/\text{ml.}$ ) and purified mitochondria (10 mg protein/ml.). Incubation was at 37° C. 0.5 ml. aliquots were taken out as specified, diluted with 5 ml. ice cold 0.44 molar sucrose-AMT and centrifuged for 15 min at 85 per cent line voltage in the Servall SS-1. The pellets were lysed with 0.5 ml. AMT containing 1 per cent 'Triton X-100' and layered on 12 ml. convex sucrose gradients containing AMT. After centrifugation for 2 h at 40,000 r.p.m., 3° C. the gradients were collected in 1.2 ml. fractions. The fractions were assayed for radioactive protein as described previously<sup>12,13</sup>.

Table 5. BASE COMPOSITION OF CYTOPLASMIC AND MITOCHONDRIAL RIBOSOMAL RNA FROM *Neurospora* (In moles per cent)

	AMP	UMP	GMP	CMP	Unidentified
Cytoplasmic RNA: 25.8+17.8	24.1±0.5	24.3±0.8	28.1±0.2	21.1±0.3	2.3±0.6
Mitochondrial RNA: 21.8+16.8	27.2±0.5	29.8±0.7	22.9±0.5	14.8±0.8	5.7±0.1

Cells labelled with phosphorus-32 were obtained by adding 1.2 mc. sodium phosphate- $^{32}\text{P}$  (Abbott Laboratories) to 200 ml. minimal medium. The mitochondria were isolated and mitochondrial ribosomes were treated with SDS as described under Fig. 3 and layered over 12 ml. gradients (sucrose-AMT). After centrifugation for 12 h at 35,000 r.p.m., 3° C. the fractions containing the two rRNA peaks were collected and pooled. To precipitate the RNA 0.1 vol. of 1 molar potassium acetate pH 5 and 3 vol. of 95 per cent ethanol were added. The precipitate was dried, dissolved in 0.1 ml. 0.3 molar KOH/0.4 mg RNA and incubated at 30° C for 12 h. The hydrolysate was neutralized with 0.3 molar  $\text{HClO}_4$ , the potassium perchlorate was removed by centrifugation and 10  $\mu\text{l.}$  of the supernatant containing about 8,000 c.p.m. were applied on Whatman 1 MM paper and subjected to electrophoresis on a Savant flat plate system at 2,800 V, 30 mamp for 3.5 h. The electrophoresis buffer (pH 3.5) contained 0.6 molar acetic acid, 0.01 molar EDTA and 34 ml. pyridine/l. The dried electropherogram was cut in 6 mm strips and counted in a Packard liquid scintillation counter.

The significance of incorporation data obtained with mitochondria under non-sterile conditions has been questioned because of the possibility that bacterial contaminations might be the principal source of the incorporation activity. We therefore wish to point out that under our assay conditions the presence of bacteria would not affect the results, for they fail to lyse in the presence of 'Triton X-100' (ref. 11).

## Discussion

While it has been firmly established that mitochondria are endowed with their own DNA (ref. 14), the question whether these organelles also contain ribosomes and, if so, whether they are distinct from cytoplasmic ribosomes has remained an enigma. Several recent observations, however, have encouraged the search for specific mitochondrial ribosomes. Thus isolated mitochondria have been reported to be capable of incorporating amino-acids into their structural protein<sup>15</sup>. Other observations suggested that mitochondrial protein synthesis was suscep-

tible to inhibitors that fail to affect cytoplasmic protein synthesis<sup>6-8,16</sup>. Particularly persuasive are observations by Kirk and Juniper<sup>17</sup>, who found that streptomycin inhibited the development of both chloroplasts and mitochondria. The observations with chloroplasts seemed to find their explanation in the demonstration by Stutz and Noll<sup>1</sup> that these organelles contain bacterial type ribosomes, who also pointed out that chloroplast function and development seem to be affected by all those antibiotics that specifically inhibit protein synthesis on bacterial ribosomes. Most recently the isolation of a tRNA and aminoacyl-synthetase specific for mitochondria has been reported<sup>18</sup>.

The isolation of a 55S ribosome particle from rat liver mitochondria believed to be the active unit in mitochondrial protein synthesis was recently described<sup>19,20</sup>. No evidence was, however, given to rule out that it was not a subunit from a mitochondrial ribosome.

In this report we described the isolation of a new type of ribosome from *Neurospora* that meets some rigorous tests to qualify as the structural unit responsible for mitochondrial protein synthesis: (1) it occurs only in mitochondria; (2) it can be distinguished from cytoplasmic ribosomes by sedimentation analysis of either the intact particles or the corresponding RNA component and chemically by its unique base composition; (3) it exhibits the characteristic subunit structure and resembles bacterial ribosomes; and (4) it forms polysomes engaged in mitochondrial protein synthesis.

Although the mitochondrial ribosome clearly belongs to the bacterial class, we were surprised to find that, having an *S*-value of 73, it was distinctly larger than both chloroplast (67S) and most of the bacterial ribosomes (70S) (ref. 21). As the cytoplasmic ribosomes of *Neurospora* are somewhat smaller than expected, the actual difference of barely 4S makes it difficult to resolve a mixture of the two species by zone velocity centrifugation. The separation achieved here, though clear-cut and obtained reproducibly with specialized high resolution equipment, probably comes very close to the limit of the resolving power of the techniques used.

An intriguing aspect of our comparative sedimentation studies with ribosomes from widely differing sources is the

apparent increase in size accompanying evolution to higher forms. If we accept this as a rule to map the course of evolution, it is interesting to note that within the class of bacterial type ribosomes the chloroplast ribosomes rank at the very bottom together with the 66S ribosomes of the photosynthetic bacteria (*Rhodospirillum rubrum*)<sup>21</sup>, whereas the mitochondrial ribosomes rank at the top with 73S *Bacillus licheniformis*. It is tempting to speculate that endosymbiotic forms are relics of the distant past still reflecting the ancestors of the two organelles in the process of integration to form a eucaryotic cell. By the same token, the low position of the chloroplast ribosome could be taken as confirmation of the obvious postulate that photosynthesis must have its origin at the very threshold of life.

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## Molecular and Genetic Aspects of Human Blood-group Le<sup>b</sup> Specificity

by

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Chemical characterization of Le<sup>b</sup>-active fragments isolated from alkaline degradation products of human blood-group HLe<sup>b</sup> substance supports the idea that Le<sup>b</sup> specificity results from the action of two genes. These are *H* and *Le*, and they belong to independent blood-group gene systems, *Hh* and *Lele*.

THE chemical nature of the serologically reactive structures responsible for the A, B, H and Le<sup>a</sup> specificity of the blood-group active glycoproteins in human secretions and tissue extracts has been established and the inter-relationships of the different specificities are now largely understood<sup>1-3</sup>. The specific determinants were identified as carbohydrate structures in which the immunodominant sugar is a non-reducing residue present at the end of, or as a branching unit on, the carbohydrate chains. The overall structure of the glycoprotein molecules is not yet completely elucidated, but there is no doubt that in each

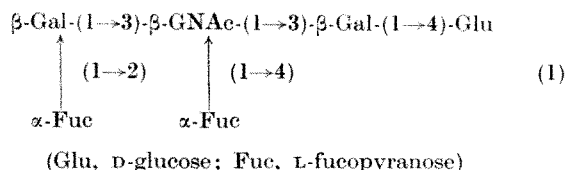
macromolecule there are many carbohydrate chains ending in blood-group active groupings. The available evidence also supports the view that A, B, H and Le<sup>a</sup> specificities arise from structures superimposed on a common precursor type of glycoprotein and that, according to the genetic make-up of the donor from whom the blood-group substance is obtained, some or all of these specificities occur on individual macromolecules.

The structures responsible for specificity were identified by the inhibition of specific haemagglutination<sup>4,5</sup> and precipitation<sup>6,7</sup> by sugar derivatives of known structure,

by the sequential degradation of the carbohydrate chains by specific enzymes<sup>8</sup> and by chemical identification of serologically active fragments arising from the partial acid hydrolysis<sup>9-13</sup> or alkaline degradation<sup>14-19</sup> of the group specific substances. Partial degradation products of A, B and H substances each yielded two different kinds of serologically active trisaccharide and on the basis of this finding it was proposed that in the precursor glycoprotein two types of carbohydrate chain-endings form the backbone of the serologically active structures<sup>20</sup>: type 1 chain-ending  $\beta$ -Gal-(1 $\rightarrow$ 3)- $\beta$ -GNac . . . and type 2 chain-ending  $\beta$ -Gal-(1 $\rightarrow$ 4)- $\beta$ -GNac . . . (Gal, D-galactopyranose; GNac, N-acetyl-D-glucosaminopyranose).

The two H-active trisaccharides<sup>16</sup> correspond to the two chain-endings with  $\alpha$ -L-fucosyl residues joined in (1 $\rightarrow$ 2) linkage to the non-reducing galactose end residues. In contrast only one active trisaccharide was obtained from Le<sup>a</sup> substance. This trisaccharide arises from the type 1 chain by the addition of an  $\alpha$ -L-fucosyl residue to the carbon-4 position in N-acetylglucosamine. In the type 2 chain the carbon-4 position of the subterminal N-acetylglucosamine is substituted by the terminal galactose, so that this type of chain cannot give rise to an Le<sup>a</sup>-active structure.

A fifth blood-group specificity associated with the secreted blood-group active glycoproteins is the Le<sup>b</sup> character<sup>21</sup>. Inhibition tests with oligosaccharides of known structure indicated the nature of the Le<sup>b</sup> determinant<sup>22,5</sup> but until now no direct chemical evidence in support of this structure was forthcoming. Of many carbohydrates tested only two, lacto-N-difucohexaose I (1)<sup>23</sup>,



and lacto-difucotetraose<sup>24</sup> inhibited in the Le<sup>b</sup> system, and it was therefore proposed<sup>22,5</sup> that the Le<sup>b</sup> determinant was constituted by two  $\alpha$ -L-fucosyl units joined by glycosidic linkages to each of two adjacent sugars, as in the tetrasaccharide moiety at the non-reducing end of lacto-N-difucohexaose I.

In this communication the isolation of two Le<sup>b</sup>-active oligosaccharides from a human HLe<sup>b</sup> substance is described and the significance of the structure of the Le<sup>b</sup> determinant is discussed in terms of the genetic control of its formation.

### Isolation and Characterization of Le<sup>b</sup>-active Oligosaccharides

A preparation of HLe<sup>b</sup> substance (No. 534, 10.2 g containing fucose 13.4, galactose 22.6, N-acetylhexosamine 25.7, amino-acids 25 per cent; molar ratio N-acetyl-galactosamine to N-acetylglucosamine 1:2.9) isolated from a single specimen of ovarian cyst fluid from a group O "secretor" person was dissolved in 850 ml. of a water soluble non-diffusible poly(vinylbenzyltriethyl) ammonium hydroxide resin<sup>25</sup> in its carbonate form (0.05 N; pH 8.5-8.8). The solution was pumped through a short coil of glass tubing at 100°C, and the mixture passed immediately into a coil (80 ft.) of 'Visking' tubing (8/32 in.) which returned to the heating coil, thus making a closed circuit. The dialysis tubing was inside a larger bore plastic tube carrying a slow stream of distilled water at 1-3°C. The reaction mixture was pumped continuously so that the whole of the mixture passed through the system every 2 h. This ensured that the longest heating time at 100°C for any portion of the mixture

before it passed to the dialysis system was about 20 min. Degradation continued for 90 days. The diffusate was evaporated under reduced pressure at a temperature not exceeding 40°C, and dried from the frozen state.

The diffusible material (5.2 g) dissolved in 20 ml. of water was passed through a column (81  $\times$  3.6 cm) of 'Sephadex G-15'. Fractions were examined for carbohydrate fragments by thin-layer chromatography on silica gel G, developed with *n*-propanol:ethyl acetate:water (6:1:3) (solvent *a*). Earlier inhibition studies indicated that an Le<sup>b</sup>-active fragment must be at least a tetrasaccharide, and the fractions were pooled according to their approximate molecular size, estimated by the movement on thin-layer plates of reference oligosaccharides of known molecular weight. Rechromatography of the selected materials on a column of 'Sephadex G-15' (157  $\times$  1 cm) gave a fraction with Le<sup>b</sup> activity which was shown by thin-layer chromatography in solvent (*a*) to contain components with  $R_{\text{lactose}}$  values varying from 0.45 to 0.9. Substances recovered (1.26 g) within this  $R_{\text{lactose}}$  range were chromatographed on a charcoal-celite column (59  $\times$  2 cm) using gradient elution with aqueous ethanol (0-20 per cent ethanol) maintained at pH 3 with formic acid<sup>26</sup>. The material eluted in the concentration range 2.5-4.0 per cent ethanol had Le<sup>b</sup> activity and contained two principal components. These fractions were pooled, neutralized by addition of 'Permutit De-Acidite FF-IP' (carbonate form), evaporated to a small volume and dried from the frozen state. The components in this mixture (72 mg) were separated by preparative chromatography on Whatman No. 40 paper developed with ethyl acetate:pyridine:water (2:1:2) (solvent *b*). The two principal fragments recovered had  $R_{\text{lactose}}$  0.27 (substance I; 14 mg) and 0.09 (substance II; 28 mg) on Whatman No. 4 paper in ethyl acetate:pyridine:water (10:4:3) (solvent *c*) and were both Le<sup>b</sup>-active. They each behaved as a single component when examined by chromatography on Whatman No. 4 paper in solvents (*a*), (*b*) and (*c*), by paper electrophoresis in borate buffer (pH 10) and by two-dimensional thin-layer chromatography in solvent (*a*) and butanol:ethanol:water (50:25:40). Substances I and II each yielded fucose, galactose and glucosamine on complete acid hydrolysis. The proportions of these sugars in the two substances (Table 1) and their chromatographic behaviour indicated that substance I was a tetrasaccharide and substance II a pentasaccharide.

The tetrasaccharide  $[\alpha]_D - 62^\circ$  (*c*, 1, water),  $R_{\text{lactose}}$  0.27 (solvent *c*),  $M_G$  0.25 (on paper electrophoresis in borate buffer at pH 10), did not react with alkaline triphenyltetrazolium chloride<sup>30</sup>, indicating that there was a substituent on carbon-2 of the reducing sugar unit. Products of reduction of the tetrasaccharide with sodium borohydride and hydrolysis with acid<sup>31</sup> contained no glucosamine, but yielded an equivalent amount (23.7 per cent) of glucosaminitol (estimation to be described elsewhere), indicating that the reducing sugar end unit in the tetrasaccharide was N-acetylglucosamine. The tetrasaccharide gave a negative indirect Ehrlich reaction<sup>32</sup> showing that there was a substituent on carbon-4 of the N-acetylglucosamine. Hydrolysis of the tetrasaccharide with N-acetic acid at 100°C for 2 h gave several sub-

Table 1. ANALYTICAL VALUES FOR THE Le<sup>b</sup>-ACTIVE TETRA- AND PENTASACCHARIDES BEFORE AND AFTER REDUCTION WITH SODIUM BOROHYDRIDE

	Tetrasaccharide				Pentasaccharide			
	<i>a</i>		<i>b</i>		<i>a</i>		<i>b</i>	
	%	Molar ratio	%	Molar ratio	%	Molar ratio	%	Molar ratio
Fucose	38.1	1.9	35.0	2.0	31.5	2.0	28.8	2.0
Galactose	21.5	1.0	18.9	1.0	32.4	1.9	21.1	1.3
N-Acetylglucosamine*	26.8	1.0	0	0	21.2	1.0	20.0	1.0

(*a*) Before reduction; (*b*) after reduction. Fucose was determined by the method of Gibbons<sup>37</sup>. For galactose, total sugar value was determined by the method of Dubois, Gilles, Hamilton, Rebers and Smith<sup>38</sup> after subtracting the amount of fucose in substance.

\* Calculated from the glucosamine value determined by the method of Rondle and Morgan<sup>39</sup>.

stances including the unchanged tetrasaccharide and fucose. A substance that gave a strong indirect Ehrlich reaction and a greenish colour with benzidine-trichloroacetic acid reagent<sup>33</sup> was chromatographically indistinguishable from lacto-*N*-biose I (*O*- $\beta$ -D-galactosyl-(1 $\rightarrow$ 3)-*N*-acetyl-D-glucosamine). *O*- $\beta$ -D-galactosyl-(1 $\rightarrow$ 6)-*N*-acetyl-D-glucosamine in the same conditions had a different  $R_{\text{lactose}}$  value and gave a reddish brown colour with the benzidine trichloroacetic acid reagent. *N*-acetyl-lactosamine (*O*- $\beta$ -D-galactosyl-(1 $\rightarrow$ 4)-*N*-acetyl-D-glucosamine) gave a golden yellow colour with this indicator, and did not react with Ehrlich's reagent after heating in alkali. A substance in the hydrolysis products ( $R_{\text{lactose}}$  0.95; Whatman No. 4 paper; solvent (c)) which gave a strong indirect Ehrlich reaction was believed, on the basis of its chromatographic behaviour, to be the H-active trisaccharide, *O*- $\alpha$ -L-fucosyl-(1 $\rightarrow$ 2)-*O*- $\beta$ -D-galactosyl-(1 $\rightarrow$ 3)-*N*-acetyl-D-glucosamine<sup>16</sup>. Another substance, also believed to be a trisaccharide ( $R_{\text{lactose}}$  0.57), gave no Ehrlich reaction after treatment with dilute alkali. This fact, combined with the observation that the acid hydrolysis products had  $\text{Le}^a$  serological activity not present before hydrolysis, suggested that it was the  $\text{Le}^a$ -active trisaccharide, *O*- $\beta$ -D-galactosyl-(1 $\rightarrow$ 3)-[*O*- $\alpha$ -L-fucosyl-(1 $\rightarrow$ 4)]-*N*-acetyl-D-glucosamine<sup>17</sup>.

Treatment of the tetrasaccharide with 5 per cent triethylamine in 50 per cent aqueous methanol at 80°C for 1 h gave products which failed to react with Ehrlich's reagent. With alkaline-silver nitrate reagent three spots, chromatographically identical with fucose,  $\alpha$ -L-fucosyl-(1 $\rightarrow$ 2)-galactose<sup>34</sup> and unchanged tetrasaccharide, appeared. An unidentified substance with  $R_{\text{lactose}}$  0.46 was also present. This evidence indicates the  $\text{Le}^b$ -active tetrasaccharide has the structure given in Fig. 1. Further evidence to support the structure was given by periodate oxidation and methylation. Oxidation with sodium metaperiodate<sup>35,36</sup> (Table 2) gave values for the consumption of periodate, production of the formic acid and the evolution of formaldehyde close to the expected values. After oxidation and reduction with sodium borohydride the tetrasaccharide gave 1 mole of formaldehyde. Methylation of the tetrasaccharide with methyl iodide in dimethyl formamide in the presence of BaO/Ba(OH)<sub>2</sub>·8H<sub>2</sub>O, by a modification<sup>37</sup> of the method of Kuhn *et al.*<sup>38,39</sup> followed by cleavage of the compound with 0.5 *N* hydrochloric acid in methanol at 80°C, gave a mixture of methylated glycosides which were examined by gas liquid chromatography. Peaks with retention times identical to the peaks given by authentic specimens of methyl-2,3,4-tri-*O*-methyl fucoside and methyl-3,4,6-tri-*O*-methyl galactoside were identified among the products, in agreement with the proposed structure.

The pentasaccharide ( $[\alpha]_D - 67^\circ$  (c, 1, water),  $M_G$  0.47,  $R_{\text{lactose}}$  0.09 on Whatman No. 4 paper in solvent (c)) was heated in *N*-acetic acid at 100°C for 5 h and the

Table 2. RESULTS OF THE OXIDATION OF THE  $\text{Le}^b$ -ACTIVE TETRA- AND PENTASACCHARIDES WITH 0.025 MOLAR SODIUM METAPERIODATE AT A pH OF ABOUT 5 FOR 24 H AT ROOM TEMPERATURE

Substance	Periodate consumed/mole of sample		Formic acid formed/mole of sample		Moles of formaldehyde released/mole of sample		Formaldehyde released/mole of sample after reduction	
	Found	Expected	Found	Expected	Found	Expected	Found	Expected
Tetrasaccharide	4.7	5.0	1.9	2.0	0.1	0.0	0.9	1.0
Pentasaccharide	6.1	6.0	2.0	2.0	0.3	0.0	1.9	2.0

products were run in solvent (c). Two spots reactive with the benzidine reagent<sup>33</sup> were identified as fucose and galactose respectively, and a third was chromatographically identical with the  $\text{Le}^b$ -active tetrasaccharide ( $R_{\text{lactose}}$  0.27 on No. 4 paper). A fourth substance ( $R_{\text{lactose}}$  0.49 on No. 1 paper) was considered on the basis of the following evidence to be the backbone trisaccharide, *O*- $\beta$ -D-galactosyl-(1 $\rightarrow$ 3)-*O*-(*N*-acetyl- $\beta$ -D-glucosaminyl)-(1 $\rightarrow$ 3)-D-galactose<sup>40</sup>. Hydrolysis of the pentasaccharide in 2*N*-acetic acid at 100°C for 18 h gave the same products as hydrolysis for 5 h and two additional substances with  $R_{\text{lactose}}$  1.15 and 1.49 (No. 1 paper). The faster running substance was chromatographically identical with lacto-*N*-biose I, and gave a strong indirect Ehrlich reaction. The slower running substance gave a positive indirect Ehrlich reaction, a positive reaction with triphenyl tetrazolium chloride reagent, and had the reported  $R_{\text{lactose}}$  value (1.15) of lacto-*N*-biose II (*O*-(*N*-acetyl- $\beta$ -D-glucosaminyl)-(1 $\rightarrow$ 3)-D-galactose).

The pentasaccharide heated with 5 per cent triethylamine in 50 per cent aqueous methanol<sup>40</sup> for 90 min at 80°C was largely degraded to *O*- $\alpha$ -L-fucosyl-(1 $\rightarrow$ 2)-galactose ( $R_{\text{lactose}}$  1.57; solvent (c)). In less severe conditions, at 40°C, the rate of degradation of the pentasaccharide was similar to that of the (1 $\rightarrow$ 3) linked disaccharide, *O*- $\alpha$ -D-galactosyl-(1 $\rightarrow$ 3)-D-galactose, and considerably faster than the rate of degradation of (1 $\rightarrow$ 4) linked lactose or (1 $\rightarrow$ 6) linked melibiose. The products of degradation after a 30 min run on No. 4 paper with the same solvent system gave some unchanged pentasaccharide ( $R_{\text{lactose}}$  0.08) and a substance ( $R_{\text{lactose}}$  0.26) chromatographically identical with the  $\text{Le}^b$ -active tetrasaccharide. More prolonged heating (3 h) in the same conditions gave *O*- $\alpha$ -L-fucosyl-(1 $\rightarrow$ 2)-D-galactose. The ready breakdown of the pentasaccharide to *O*- $\alpha$ -L-fucosyl-(1 $\rightarrow$ 2)-D-galactose in these mild alkaline conditions supports the conclusions reached on the basis of the evidence from the acid hydrolysis experiments that both glycosidic linkages in the principal chain are 1 $\rightarrow$ 3 linkages. None of the products of alkaline degradation gave positive reactions with Ehrlich's reagent. When the tetra- and the pentasaccharide were separately treated in the same mild conditions of degradation with alkali (1 h at 80°C with triethylamine), the tetrasaccharide no longer survived at a time when the pentasaccharide was still readily detectable.

In control experiments, lacto-*N*-difucohexaose I (I) heated with triethylamine at 65°C for 80 min gave products which chromatographically were shown to contain some unchanged lacto-*N*-difucohexaose I, and a substance with an  $R_{\text{lactose}}$  value identical with that given by the  $\text{Le}^b$ -active pentasaccharide run at the same time. More extensive degradation of the hexaose occurred on heating at 80°C for 1 h and the decomposition products examined by paper chromatography (solvent c) gave no spots with  $R_{\text{lactose}}$  values corresponding to the  $\text{Le}^b$ -active tetra- or pentasaccharide. In these conditions the hexaose was eroded as far as *O*- $\alpha$ -L-fucosyl-(1 $\rightarrow$ 2)-galactose.

The pentasaccharide was methylated<sup>37</sup> and the methanolysis products gave peaks on gas liquid chromatography with retention times identical with those detected in the methylation products of the tetrasaccharide, and, in addition, peaks corresponding to methyl-2,4,6-tri-*O*-

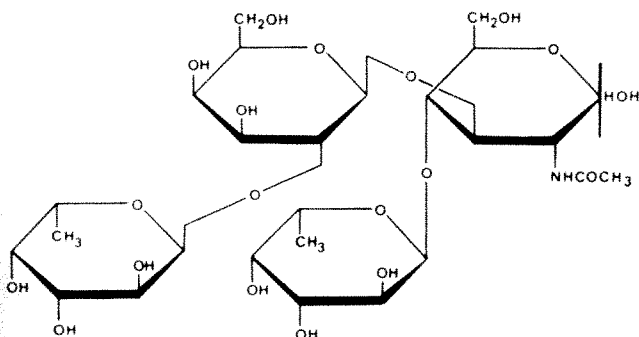


Fig. 1. Structure of the  $\text{Le}^b$ -active tetrasaccharide *O*- $\alpha$ -L-fucosyl-(1 $\rightarrow$ 2)-*O*- $\beta$ -D-galactosyl-(1 $\rightarrow$ 3)-[*O*- $\alpha$ -L-fucosyl-(1 $\rightarrow$ 4)]-*N*-acetyl-D-glucosamine.





isolation and characterization of the H and Le<sup>a</sup> determinants<sup>34,35</sup> established the positions and linkages of the fucose residues and indicated that the H gene controls the addition of fucose to the carbon-2 position of a terminal non-reducing  $\beta$ -galactosyl residue in either the type 1 or type 2 chains, whereas the Le gene transferase adds fucose to the carbon-4 position of the subterminal  $\beta$ -N-acetylglucosaminyl residue of the type 1 chain. The chemical characterization of the Le<sup>b</sup>-active oligosaccharides isolated from an HLe<sup>b</sup> substance (Figs. 1 and 2) as structures with two fucose units joined to adjacent sugars—one fucose linked as in H determinants and the second as it occurs in the Le<sup>a</sup> determinant—thus supports the interpretation that the Le<sup>b</sup> determinant is an interaction product of the H and Le genes. The structure formed by the presence of the two fucose residues, although compounded of the H and Le<sup>a</sup> active groupings, has neither H nor Le<sup>a</sup> specificity; instead it has the new specificity, Le<sup>b</sup>, which is different from that produced by either one of the H or Le genes acting in the absence of the other. An Le<sup>b</sup> gene is not therefore required to account for the appearance of Le<sup>b</sup> activity. It is to be noted that of the two types of chain known to occur in the blood-group specific glycoproteins, only one, namely the type 1 chain, can give rise to an Le<sup>b</sup> specific structure. In the presence of H and Le genes the type 2 chains have only the fucose added by the H gene, and are therefore H-active. Type 1 and 2 chains occur in the same macromolecule and so it is understandable that it has not been possible to isolate from secretions glycoproteins which have only Le<sup>b</sup> activity.

The first examples of red blood cell antigens that were peculiar to the hybrid and occurred in neither of the homozygous parental types were reported in doves<sup>17,18</sup>. Hybrid antigens have also been described in pigeons<sup>19</sup>, ducks<sup>20</sup> and rabbits<sup>21</sup>. In man there is evidence that antibodies are occasionally formed to compound antigens in the Rh blood-group system<sup>21</sup> and to interaction products of the gene I with the A, B or H genes<sup>21,22</sup>. The only hybrid blood-group active molecules that have been clarified in chemical terms, however, are the products formed in heterozygous group AB persons<sup>23</sup>, and the example described here. The human blood-group alleles A and B acting together in "secretor" persons of genotype AB give rise to a glycoprotein that has A and B specific structures in the same molecule<sup>23</sup> but the A and B characters are each expressed and no new serological specificity is formed. We believe that the reason why the serological A and B specificities remain distinct, even when superimposed on the same molecule, is that the A- and B-active structures cannot be present together at the end of the same carbohydrate chain<sup>20</sup>. The macromolecules therefore have some chains ending in A-active groupings, and others in B-active groupings, and the spatial pattern is presumably such as to allow access of the appropriate A- or B-specific antibodies. This type of hybrid molecule therefore differs from the one described in the present paper in which each gene, H and Le, acts independently to elicit its own specific structure but, presumably because the structural contributions occur in close proximity on the same carbohydrate chain, a new spatial pattern is formed that is reflected in a new antigenic specificity. By the same token the H- and Le<sup>a</sup>-specific antibodies are probably prevented sterically from approaching their homologous antigenic groupings, and thus the original specificities are lost.

The chemical characterization of the two types of hybrid molecules arising in the glycoproteins associated with ABH and Lewis blood-group specificity thus illustrate the diversity of products that may be expected for genetically controlled antigens in which the specific structures are carbohydrate. The tenet of "one gene, one antigen", at one time widely held in the field of human blood groups, has to be abandoned. Similarly the concept that a child cannot have a blood-group antigen not present in either

of its parents is seen to be only a partial truth. Nevertheless, the proposed mechanism for the formation of these hybrid antigens, whereby the individual genes give rise to specific enzymes responsible for single biosynthetic steps in the formation of complex carbohydrate structures, in no way conflicts with the present ideas on the molecular basis of gene action.

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# Protein Components of Encephalomyocarditis Virus

by

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Chemical and physical studies of protein extracted from highly purified EMC virus suggest that at least three sorts of polypeptide chain are present in EMC and perhaps other picornaviruses. They may therefore differ fundamentally in structure from icosahedral plant viruses of similar size, such as TYMV.

THE information contained in the RNA of a virus is not enough to code for a single protein the size of the virus shell, or capsid. Crick and Watson<sup>1</sup> thus originally suggested that such a shell must be built up of smaller, identical, sub-units, which have since been generally assumed to consist of single proteins. In support of this suggestion several plant viruses, including tobacco mosaic virus<sup>2</sup> and turnip yellow mosaic virus<sup>3</sup>, seem to contain only one kind of polypeptide chain. Small animal viruses, however, may not obey the same rules, for protein extracted from several members of the picornavirus family, notably poliovirus<sup>4</sup> and viruses of the Columbia SK group<sup>5,6</sup>, gave more than one component when examined by polyacrylamide gel electrophoresis. Electrophoretic heterogeneity might arise from one of the following causes. (1) The virus protein might be contaminated with cell protein or with proteins from mutant forms of the virus also present in the preparations. The components might be artefacts produced either by (2) chemical modification during investigation of certain amino-acids in the virus protein or (3) by aggregation or breakdown of a single protein. (4) The virus might have contained more than one sort of polypeptide chain.

The present work, which has been carried out using protein obtained from cloned, rigorously purified encephalomyocarditis (EMC) virus, confirms the presence of more than one electrophoretic component of distinct amino-acid composition. It has now been shown that these components are of approximately the same size, before or after electrophoresis, with no sign of the existence of a larger precursor. The evidence obtained therefore supports the contention that the virion of this picornavirus contains more than one distinct polypeptide chain.

EMC virus protein, like that from several icosahedral viruses<sup>3,6</sup>, was relatively insoluble in common aqueous buffers tending to produce aggregates, possibly by disulphide bond formation. The sulphhydryl groups of the protein used in the present study were therefore blocked by carboxymethylation by the presence of iodoacetate during phenol extraction of the protein from the virus<sup>6</sup>. At the same time a radiochemical marker was introduced into the protein by carboxymethylating either with  $1\text{-}^{14}\text{C}$ -iodoacetamide or with  $2\text{-}^3\text{H}$ -iodoacetic acid (Radiochemical Centre, Amersham). Radioactivity was measured either in a Packard 'Tri Carb' or in a Beckman scintillation spectrometer in a dioxan based solvent<sup>7</sup>. At least four components were revealed by amido black staining when this carboxymethylated (CM) protein was examined in a Shandon analytical polyacrylamide gel electrophoresis apparatus using either 7.5 per cent or 15 per cent gel in the pH 4.3 acetate system of Reisfeld *et al.*<sup>8</sup> with 8 M urea in all solutions except the top reservoir (Fig. 1a).

When an unstained gel, immediately after electrophoresis, was immersed either in 50 per cent ammonium sulphate (Dr R. Martenson, personal communication) or in acetate buffer (pH 4.8), the isoelectric point of EMC virus revealed at least three white bands of precipitated protein (Fig. 1b); with this procedure, there was no band corresponding to the band 3 revealed by amido black staining, which immediately suggests that it contains material different from that in the other three bands. The three components 1, 2 and 4 have been studied in the present work and were obtained by cutting out the white bands, homogenizing in 67 per cent acetic acid and, after removing the gel by filtration, precipitating with 6 volumes of ethanol. These results confirmed previous observations that EMC virus CM-protein show electrophoretic heterogeneity. The next step was to investigate the origin of this heterogeneity.

*Contamination with protein from mutants or host cells.* We used virus consisting of a large plaque variant derived by four successive plaque isolations from the K2 strain<sup>9</sup> of EMC virus. The virus was grown in suspension cultures of Krebs 2 ascites tumour cells<sup>10</sup> and purified by a method (to be published) involving acid precipitation followed by organic solvent extraction, enzyme treatment and dif-

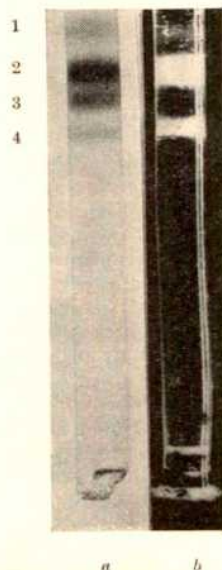


Fig. 1. Polyacrylamide gel electrophoretic patterns of EMC virus CM protein revealed (a) by staining with amido black; (b) by precipitation with acetate buffer, pH 4.8.

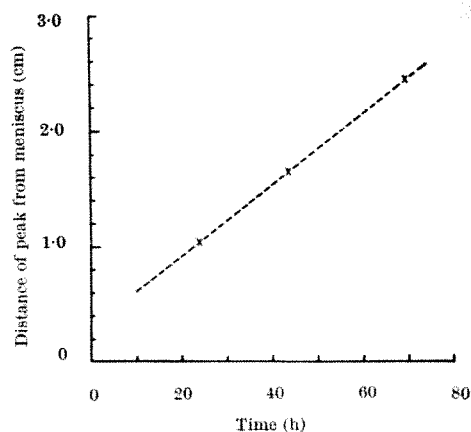


Fig. 2. Relationship between distance moved by serum albumin and time of centrifugation on a 5-20 per cent sucrose gradient containing 8 molar urea and 0.1 molar acetate buffer, pH 4.3.

ferential centrifugation. The extent of purification was monitored by the addition of non-infected cell material labelled either with  $^3\text{H}$  amino-acids or with  $^{32}\text{P}$ , as markers for cell protein or nucleic acid, respectively. As little as 0.014 per cent  $^3\text{H}$  and 0.0038 per cent  $^{32}\text{P}$  was recovered, but 60 per cent of the biological activity was recovered which indicates that a high degree of purity had been achieved. The use of virus purified both biologically and chemically in this way means that it is unlikely that the electrophoretic heterogeneity observed was due to contamination with protein from either host cell or mutant virus particles.

Table 1. AMINO-ACID COMPOSITION OF COMPONENTS OF EMC VIRUS CM PROTEIN SEPARATED BY POLYACRYLAMIDE GEL ELECTROPHORESIS

Amino-acid	Moles amino-acid per 100 moles amino-acid recovered		
	Component 1	Component 2	Component 4
Asp	8.9	10.1	8.6
Thr	7.0	10.0	9.6
Ser	13.0	10.6	8.7
Glu	10.7	9.5	9.0
Pro	5.3	8.8	7.1
Gly	15.1	10.5	9.8
Ala	6.7	7.3	8.4
Val	5.4	8.1	7.0
Ile	3.1	3.6	4.2
Leu	6.3	8.1	7.0
Tyr	1.7	3.4	3.2
Phe	4.4	8.1	5.4
Lys	4.9	5.2	4.5
His	3.0	2.1	2.4
Arg	4.4	4.1	4.1

**Chemical modification.** The alteration of a single amino-acid in part of the material might lead to charge heterogeneity which could be detected by gel electrophoresis and so be interpreted falsely as demonstrating the presence of more than one sort of polypeptide chain. Among the chief hazards in gel electrophoresis are oxidation of amino-acids such as tryptophan or cysteine by the persulphate<sup>11</sup> used in the polymerization, carbamylation<sup>12</sup> of the  $\epsilon$ -amino group of lysine by the cyanate which can contaminate the urea in electrophoresis buffers and desamidation of asparagine and glutamine residues. Precautions can be taken to reduce these hazards by running mercapto-ethanol in the gel to remove excess persulphate or by deionizing the urea immediately before use on a mixed bed resin such as Permutit "Deminrolit". If, however, the electrophoretic components differ in composition in those amino-acids which are not usually susceptible to modification, then presumably the components are distinct entities and are not artifacts of the handling procedure. Amino-acid analysis of the isolated components (Table 1) suggested that components 2 and 4, although broadly similar, differed in their content of phenylalanine—an amino-acid not susceptible to modification. Component 1,

however, differed considerably from components 2 and 4 and would appear to be an unrelated polypeptide. This evidence suggests therefore that EMC virus CM protein contains more than one kind of polypeptide chain. Differences have also been demonstrated by Rueckert and Duesberg<sup>13</sup> in amino-acid composition of electrophoretic components in ME virus protein.

**Aggregation or breakdown.** Proteins from poliovirus<sup>4</sup> and ME virus<sup>6</sup> have been reported as moving as a single peak when examined in alkaline buffers containing sodium dodecyl sulphate (SDS) in an analytical ultracentrifuge, thus presumably demonstrating the absence of aggregates and of possible breakdown products. The electrophoretic components had, however, been demonstrated in acetate buffer, pH 4.3 containing 8 M urea, so the possibility exists that heterogeneity in this buffer arose either from breakdown or from aggregation, but that such changes did not take place in the alkaline-SDS buffer used for analytical ultracentrifugation and so were not detected. This objection can be answered either by carrying out the electrophoresis and sedimentation studies in the same buffer or by isolating the separated components and demonstrating, by measuring their size, that aggregation or breakdown was not a factor leading to electrophoretic separation.

Examination of EMC CM-protein in sodium dodecyl sulphate buffer (0.1 per cent SDS-0.05 M sodium borate-0.1 M sodium chloride adjusted to pH 10.5 with sodium hydroxide) at 20° in a Beckman-Spinco Model E ultracentrifuge using schlieren optics showed a single, homogeneous peak of sedimentation coefficient 2.7S, a value identical to that obtained for ME virus CM protein by Rueckert<sup>6</sup> but which differs slightly from the figure of 2.0S obtained by Maizel<sup>4</sup>. This examination confirmed that the size of the protein extracted from EMC virus was similar to that obtained from other picornaviruses.

Because the electrophoretic components had already been demonstrated in acetate buffer, pH 4.3 containing 8 M urea, this solvent was also used for ultracentrifugal examination of EMC CM-protein. Zonal ultracentrifugation in a Beckman-Spinco Model L ultracentrifuge with an SW 39 rotor, using a serum albumin marker on sucrose gradients, was chosen, because it could give an estimate of size<sup>14</sup> and yield material for further study. Centrifugation for 72 h was necessary, and to ensure that the protein would not be retarded by the dense solvent used as it approached the bottom of the tube, a control investigation was carried out to show that a plot of the position of serum albumin against the time of centrifugation was a straight line over the period required (Fig. 2). The virus protein ran under the same conditions as a single peak of radioactivity of molecular weight  $30,000 \pm 3,600$  compared with serum albumin in the same tube (Fig. 3). This value

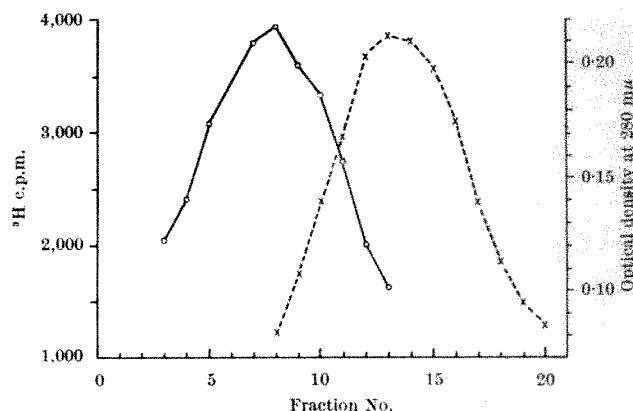


Fig. 3. Sucrose density gradient centrifugation in urea-acetate buffer of EMC virus  $^3\text{H}$ -CM protein (x---x) with serum albumin (O—O) as an optical density marker.



is similar to that obtained for poliovirus and ME virus proteins by Maizel<sup>4</sup> and Rueckert<sup>5</sup>, respectively, but disagrees with the figure of a molecular weight of 42,000 for poliovirus protein obtained by Boeyé<sup>15</sup>. These three previous determinations were made in SDS in alkaline buffer. Hamilton *et al.*<sup>16</sup> obtained a value of 60,000 for the molecular weight of an antigenically active sub-unit prepared from EMC virus and examined by agar gel diffusion.

Thus in the present work there was no sign of aggregation or breakdown in either SDS-buffer or in acetate-urea, and yet electrophoretic heterogeneity existed. It was decided therefore to examine the electrophoretic components individually on sucrose gradients to check whether the technique of electrophoresis was giving rise to artefacts.

Table 2. MOLECULAR WEIGHTS ESTIMATED BY SUCROSE DENSITY GRADIENT CENTRIFUGATION OF EMC VIRUS PROTEIN COMPONENTS SEPARATED BY ELECTROPHORESIS ON ACRYLAMIDE GEL

Component	Molecular weight
1	32,500 $\pm$ 3,400
2	29,300 $\pm$ 1,900
4	30,300 $\pm$ 3,000

Because components of different size were being sought, electrophoresis was performed using 15 per cent acrylamide gels on which separation is related more to size and shape rather than charge<sup>17</sup>. The three principal bands revealed by iso-electric precipitation (Fig. 1b) were individually centrifuged on sucrose density gradients in urea-acetate buffer. Although component 1 appeared to be consistently larger than components 2 and 4, the molecular weights thus obtained fell within the range of experimental error (Table 2). This result, together with the amino-acid differences (Table 1), would seem to exclude the possibility that the electrophoretic components were aggregates. It is difficult to be certain that these components did not arise from breakdown of a larger molecule. If, however, it occurs, conversion into smaller units must be remarkably complete, for no indication of a larger precursor molecule was seen; moreover, if breakdown takes place, all its products appear to be of similar size. Furthermore, Rueckert and Duesberg<sup>13</sup>

looked for, but could not find, N-terminal amino-acids in ME virus protein; breakdown of a polypeptide chain could be expected to yield detectable ends.

This evidence thus leads to the conclusion that EMC and perhaps other picornaviruses contain three or more types of polypeptide chains of similar size and therefore may differ fundamentally in structure from the icosahedral plant viruses of similar size, such as TYMV, which appear to have only one type of protein<sup>3</sup>. We cannot as yet equate these polypeptide chains with specific morphological structures within the picornavirus particle, nor is it known whether all the polypeptides detected comprise parts of the virus capsid or whether a core protein exists. The relationship between these polypeptides and the haemagglutinin which is found in many picornaviruses also remains uncertain. These latter problems are currently under investigation.

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## Secondary Crossing-over

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Secondary crossing-over between alleles seems to arise as a consequence of modifications of the normal crossover mechanism caused by a particular mutation. Interference between crossovers is believed to be polarized in relation to the centromere, but secondary crossovers are thought not to interfere with one another. Possible causes of postmeiotic segregation, which is sometimes associated with secondary crossing-over, are discussed.

RECIPROCAL recombination of linked mutants could arise in two ways. One possibility (crossing-over) is the inclusion in the interval between the mutant sites of the whole of the hybrid DNA segments which make up a crossover. This would be the usual explanation of reciprocal recombination of mutants well separated on a chromosome. The other possibility (reciprocal conversion) is the correction of mispairing in opposite directions in the two chromatids when a mutant site is within the hybrid DNA segments of a crossover. Evidence is presented here that when two allelic mutants show reciprocal recombination, this usually results from an entire crossover in the interval

between their sites, and not from reciprocal conversion at the site of one of them.

Evidence suggests that crossing-over is initiated from one end of a gene, but in this case an entire crossover between sites within a gene should be impossible. This paradox is resolved by regarding crossing-over between alleles as abnormal. The name secondary crossing-over is proposed for the process, because there is evidence that the crossover can be initiated normally and be modified subsequently by the presence within the gene of a particular kind of mutation.

I suggest that crossing-over is initiated when the phosphodiester backbones of newly synthesized nucleotide chains of DNA fail to join across the operator of a gene<sup>1,2</sup>.

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Hybrid DNA extends from this opening point at the operator over a part of or the whole gene in homologous chromatids. If a site of mutation, differing in each parent, was within the regions of hybrid DNA of the crossover, this would lead to mispairing in the DNA. Uncorrected mispairing would cause segregation of the corresponding character difference at first mitosis after meiosis (postmeiotic segregation). The mismatching might also be repaired by replacing one of the nucleotides of the pair by a nucleotide complementary to that in the other chain. In each chromatid this correction might be to mutant or to wild type, depending on which mispaired nucleotide was replaced in each molecule. If correction were in the same direction in both chromatids (that is, to mutant in both, or to wild type in both), the mutant would show non-reciprocal recombination with other mutants. For example, if two linked mutant differences are denoted by *A* and *a*, and *B* and *b*, respectively, and if the parental genotypes were *AB* and *ab*, hybrid DNA at the site of the *A/a* difference would give a tetrad of genotype *AB*, *A/a B*, *A/a b*, *ab*, initially. After correction of mispairing in the same direction in both chromatids, the four products of meiosis would be either *AB*, *AB*, *Ab*, *ab*, or *AB*, *aB*, *ab*, *ab*. The process giving rise to such non-reciprocal recombination has been called conversion.

If correction of mispairing took place in opposite directions in the two chromatids—to mutant in one and to wild type in the other—either a parental or a reciprocal non-parental arrangement with the other mutant would result; the four products of meiosis would be either *AB*, *AB*, *ab*, *ab*, or *AB*, *aB*, *Ab*, *ab*. This origin of reciprocal recombination is described here as reciprocal conversion, because the steps involved are essentially the same as those postulated to explain non-reciprocal recombination.

If the hybrid DNA segments of a crossover were of unequal length in the two chromatids, so as to reach a mutant site in only one of them, either a parental genotype or a non-reciprocal recombinant genotype for the tetrad would result. The alternatives depend on the direction of correction of the mispairing. Thus if the tetrad genotype were *AB*, *A/a B*, *ab*, *ab* after hybrid DNA formation, it would be either *AB*, *AB*, *ab*, *ab*, or *AB*, *aB*, *ab*, *ab*, after correction. Conversely, if the initial hybrid genotype were *AB*, *AB*, *A/a b*, *ab*, correction could give either *AB*, *AB*, *Ab*, *ab* or *AB*, *AB*, *ab*, *ab*. It is not possible to obtain reciprocal recombination of two mutant differences by this means.

Postmeiotic segregation and conversion, thought to be associated with a mutation within the hybrid DNA segments of a crossover, appear to occur also in the absence of crossing-over. A review of published data is illustrated by Table 1 (ref. 3), together with further data for the gene *pan-2* in *Neurospora crassa*<sup>8</sup>, and the extensive data for the gene *hi-1* in *Saccharomyces cerevisiae*<sup>13</sup>. The *hi-1* data show a progressive decline in the frequency of

parental combinations with proximity of the mutant site to the distal end of the gene with respect to the centromere. The frequencies of parental arrangements for the outside markers range from 36 to 64 per cent for the mutants given in Table 1 for which appreciable data are available. I consider<sup>1</sup> that these parental combinations are caused by a second or reverse crossover adjacent to the first one and involve the same two chromatids. Such a crossover would nullify the effects of the first one, as far as outside markers are concerned, and lead to parental arrangements of them.

Table 1. BEHAVIOUR OF OUTSIDE MARKER GENES WHEN A MUTANT SHOWS EITHER POSTMEIOTIC SEGREGATION OR NON-RECIPROCAL CONVERSION

Species	Locus and reference	Arrangement of outside markers			Per cent parental
		Parental	Recombinant	Total	
<i>Neurospora crassa</i>	<i>pdx-1</i> (ref. 4)	3	1	4	75
	<i>pyr-3</i> (ref. 5)	2	0	2	100
	<i>pan-2</i> (refs. 6-9)	17	15	32	53.1
	<i>cys</i> (refs. 10 and 11)	29	19	48	60.4
<i>Sordaria fimicola</i>	<i>g</i> (ref. 12)	78	44	122	63.9
<i>Saccharomyces cerevisiae</i>	Allele 315	227	168	395	57.5
	<i>hi-1</i> (ref. 13)	252	200	452	55.8
	204	10	14	24	41.7
	1	39	70	109	35.8

The data for each gene in *Neurospora* refer to more than one allele. Fogel and Hurst's data for *hi-1* in *Saccharomyces* refer in descending order to the alleles Nos. 315, 7, 204 and 1. This is the sequence of the sites within the gene, starting at the proximal end with respect to the centromere.

Two facts suggest that reciprocal recombination of alleles usually results from an entire crossover between the sites of the mutants, and not from reciprocal conversion. Reciprocal recombination of alleles seems to be associated most often with a non-parental outside marker arrangement, and, furthermore, with the particular arrangement expected from crossing-over in the interval between the sites of the allelic mutants. Published data on the behaviour of outside markers when alleles show reciprocal recombination are given in Table 2; they refer to three species of fungi and include mitotic as well as meiotic recombination. All results are similar and indicate that approximately 7 per cent of reciprocal recombinations of alleles show a parental arrangement of outside markers. This is in striking contrast to the data in Table 1, which show that postmeiotic segregation and non-reciprocal conversion are associated with parental marker combinations five to nine times as often. Reciprocal conversion is expected to be similar because, like postmeiotic segregation and non-reciprocal conversion, it is associated with the presence of a mutant within a region of hybrid DNA. More than 90 per cent of the reciprocal recombinations of alleles in Table 2 had the configuration expected if the recombination of the outside markers was caused by that of the alleles. It seems likely therefore that only about 10 per cent were caused by reciprocal conversion, and the remainder by an entire crossover in the interval between the sites of the allelic mutants.

Table 2. BEHAVIOUR OF OUTSIDE MARKER GENES WHEN ALLELES SHOW RECIPROCAL RECOMBINATION

Species	Locus and reference	Alleles crossed (proximal on left, distal on right)	Arrangement of outside markers			
			A Parental with extra recombination proximal to alleles	B Parental with extra recombination distal to alleles	C Crossover coinciding with alleles	D Crossover with extra recombination proximal and distal to alleles
<i>Aspergillus nidulans</i> (mitotic recombination)	<i>ad-8</i> (ref. 14)	20 × 8	0	0	3	0
		16 × 8	0	1	8	0
	<i>ad-9</i> (ref. 15)	33 × 13	0	0	1	0
		13 × 9	1	1	0	0
		13 × 32	1	1	4	0
		13 × 15	0	0	4	0
	<i>paba-1</i> (ref. 16)	5 × 2	0	0	1	3
		5 × 15	0	1	4	0
<i>Neurospora crassa</i> (meiosis)	<i>pan-2</i> (refs. 6 and 8)	5 × 18	0	1	8	0
		2 × 19	0	0	1	1
		5 × 3	0	0	2	0
		23 × 72	0	0	2	0
<i>Saccharomyces cerevisiae</i> (mitotic recombination)	<i>hi-1</i> (refs. 13 and 17)	7 × 1	0	0	12	1
		315 × 204	0	0	3	0
		315 × 1	1	0	21	0
		7 × 1	3	0	73	0
		Total	6	5	147	5

The pattern of hybrid DNA within the gene also suggests that reciprocal recombination of alleles is not usually brought about by conversion. Reciprocal conversion is, after all, only possible if, in both chromatids, there is hybrid DNA at the mutant site. At many mutant sites, however, the hybrid DNA seems to be confined to one chromatid. The work of Lissouba *et al.*<sup>18</sup> with ascospore colour mutants of *Ascobotus immersus* illustrates this. Mutants Nos. 63 and 138 in series 46 show only non-reciprocal recombination when crossed with one another. As indicated earlier, hybrid DNA confined to one chromatid at a mutant site would explain this (see Fig. 1a). If there was hybrid DNA in both chromatids at the site of 138, as in Fig. 1b, double mutant spores would arise when there was correction of mispairing to 138 instead of to wild type, but none was found. It is inferred that the lack of reciprocal recombinant asci results from the absence of reciprocal conversion, and that this is because

the hybrid DNA is confined to one chromatid at the site of 138. A similar argument applies to asci showing post-meiotic segregation from a cross between mutants Nos. 63 and 277. If there was hybrid DNA at the site of 277 in both chromatids, as in Fig. 1f, some asci with one or two double mutant spores would be expected when correction of mispairing failed to occur or went in favour of 277, but none was found.

An entire crossover, while seeming to be possible between alleles, appears to differ in at least four respects from the normal crossover. First, it seems to be specific to particular mutants, and there is no evidence that normal crossing-over shows any such specificity. Reciprocal recombination of alleles appears in general to be much less frequent than non-reciprocal. The most extensive data on the relative frequencies of the two phenomena are from the work of Lissouba *et al.*<sup>18</sup>, who showed that reciprocal recombination of mutants from the same series

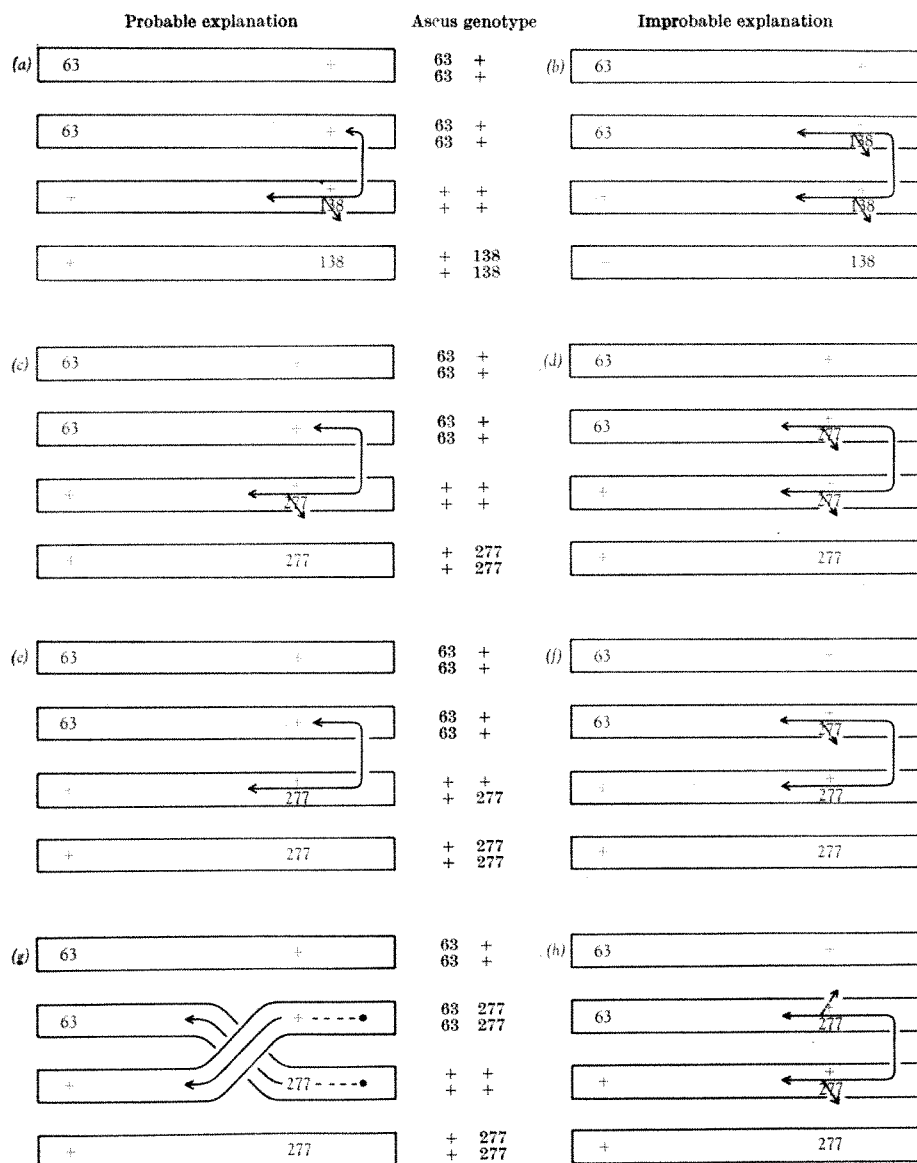


Fig. 1. Diagram of possible modes of origin of recombinant ascus genotypes obtained<sup>18</sup> by selecting asci with dark (wild type) spores from crosses between pale-spored mutants belonging to series 46 in *Ascobotus immersus*. The genotypes of the eight spores in the asci are shown in the centre column, and possible explanations of their origin are shown on either side, with a more probable alternative to the left and a less probable to the right in each instance. (a and b) Cross between mutants 63 and 138, (c-h) between mutants 63 and 277. The rectangles represent chromatids (which are considered to be equivalent to a DNA molecule), and the horizontal arrows show the extent of formation of hybrid DNA from an opening point at the right-hand end of the gene. This point is shown either by a dot or by a vertical line joining the two chromatids. Correction of mispairing is indicated by an oblique arrow through the excised allele. (g) Where there is secondary crossing-over, the broken lines show the region where hybrid DNA is thought to have existed only temporarily.

varies greatly in frequency from one pair of mutants to another. Many fail to show reciprocal recombination, and its occurrence seems to be characteristic of certain mutants. This is particularly clear with mutant 277 in series 46 already discussed. Similar variation is evident in the more limited data available from study of nutritional mutants in *Neurospora*. No reciprocal recombination of *cys* alleles was found<sup>11</sup>. On the other hand, frequent reciprocal recombination is a likely explanation for the high frequency of crossing-over for outside markers found when mutant No. 1710 at the *hist-3* locus was crossed with alleles and prototrophs were selected<sup>10</sup>.

Second, when crossing-over occurs between alleles, neither mutant site lies within hybrid DNA, because the mutants show neither postmeiotic segregation nor conversion. Evidently, the hybrid DNA segments may be quite short. A total of 256 recombinant asci of *Ascobolus*, heterozygous at three sites in series 75, have been analysed<sup>20</sup>. The two outer mutants were chosen because of their high frequency of reciprocal recombination with other mutants of the same series. All 256 asci showed reciprocal recombination of the outer mutants and sixty of them showed conversion at the intervening site. The hybrid DNA segments of a crossover between alleles may include the site of an intervening allele. This agrees with the fact that when mutants associated with reciprocal recombination were crossed individually with other mutants of the same series, the proportion of recombinant asci in which the recombination was reciprocal increased with increasing separation of the mutant sites. Studies of this kind should permit an estimate of the length of the hybrid DNA segments and their range of variation. A crossover between alleles may occupy a fraction of the length of a gene, which is probably considerably shorter than the usual length of hybrid DNA in a normal crossover, where the hybrid region is thought often to extend through one gene and into the next<sup>1</sup>.

Third, the hybrid DNA in a crossover between alleles does not extend to a normal opening point at the end of a gene. In normal crossing-over the data are consistent with dissociation of the DNA always being initiated only at an opening point at one particular end of a gene<sup>1</sup>.

Fourth, reverse crossovers do not seem to have taken place in association with crossovers between alleles. With normal crossing-over, reverse crossovers<sup>1</sup> are thought to occur regularly as a result of dissociation of the nucleotide-chains, chain synthesis and hybrid DNA formation from the same opening point as the primary crossover but in the opposite direction along the DNA molecule. As already discussed, this would explain intragenic recombination phenomena associated with a parental combination of outside markers. But with reciprocal recombination of alleles, evident from the data in Table 2, parental arrangements of outside markers are rare and can probably be explained by reciprocal conversion within the hybrid DNA regions of a normal crossover.

Despite these differences from normal crossing-over, there are two reasons for believing that crossing-over between alleles is initiated in the normal way. Mutant 277 in series 46 of *Ascobolus* shows reciprocal recombination with other mutants of the same series only when crossed with mutants placed to its left on the map<sup>18</sup>. When crossed with mutant No. 138, which to judge from recombination frequencies is to its right on the map (compare Fig. 1), the recombinant asci showed conversion at the site of 138 only (and once at the site of 277 only), but there was no reciprocal recombination of the mutants. Furthermore, in three-point crosses involving mutants 63, 277 and 138, there was no reciprocal recombination between any of the mutants in ten recombinant asci which were analysed: all showed conversion at the site of 138 only, as in the cross between 63 and 138 discussed here. For reciprocal recombination, it seems to be necessary to have the same condition which is needed with every series 46 mutant in order to obtain either postmeiotic

segregation or conversion at its site, namely that the mutant should be the right-hand member of those crossed. It is inferred from this that hybrid DNA is initiated only from the right-hand end of the gene, and that because of linked correction of mispairing (that is, correction of neighbouring sites to the same parent—compare ref. 8), selection for recombination of alleles implies selection for hybrid DNA ending before it has reached a left-hand site. It seems that, for the occurrence of the reciprocal recombination which is associated with 277 and which takes place to its left on the map, hybrid DNA has to arise in the normal way from the right-hand end of the gene.

The mutant specificity of crossing-over between alleles is the second reason for believing that this crossing-over is initiated in the normal way, through opening of the DNA at the end of the gene. It is difficult to understand how any peculiarity of a mutant could influence the course of the recombination process until hybrid DNA has formed, or attempted to form, because the chromatid carrying the wild type allele is necessarily involved as much as that with the mutant.

I conclude that in crossing-over between alleles the initial steps are normal, that is, the gene is opened from an end, synthesis of new chains occurs along the unbroken chains, the new chains dissociate from their templates, and then hybrid DNA begins to form, also from the end. The mutant may become incorporated into hybrid DNA, leading to either postmeiotic segregation or conversion (compare Fig. 1e and c). Alternatively, because of some peculiarity of the mutant, the process of crossing-over may take an abnormal course, with the mutant excluded from hybrid DNA. The details are uncertain, but the apparent result is that there is hybrid DNA on the far side of the mutant from the opening point, that is, between the sites of the two allelic mutants, while the parental situation, without hybrid DNA, is restored on the near side, that is, between the site of the first mutant and the opening point. This apparent reversal of hybrid DNA formation in the interval between the site of the first mutant and the end of the gene is indicated by broken lines in Fig. 1g. In view of these arguments for believing that crossing-over between alleles arises secondarily, as a consequence of modifications of the normal crossover mechanism caused by a particular mutant, it is proposed to call the process secondary crossing-over.

A peculiarity of secondary crossing-over is the lack of parental outside marker arrangements in conjunction with it. If with normal crossing-over parental combinations arise by reverse crossing-over, and if the reverse crossovers arise after the primary crossovers, it is likely that the mechanism of enzyme recognition of the primary crossover depends on the form which the nucleotide chains took at the opening point, because this is where the reverse crossover would be initiated. As indicated here, secondary crossovers are thought not to have the crossover configuration at the opening point, because the hybrid DNA seems to be confined between the sites of the mutations. It is possible therefore that a secondary crossover might not be recognized by the crossover-reversal enzyme. This would explain the lack of parental outside marker combinations with secondary crossing-over.

The places where DNA is opened for recombination seem to be distributed at random along the chromosome (apart from always being at the ends of genes)<sup>3</sup>, and interference seems to have been caused by potential crossovers in the neighbourhood of an existing crossover being influenced by it and switched to the double crossover path. According to this hypothesis of interference, secondary crossovers would be expected to show no interference with one another, assuming that reverse crossovers fail to form in association with them.

The occurrence of reciprocal recombination of alleles at the *hi-1* locus in *Saccharomyces* showed no interference with crossing-over in a region 2.4 units long adjacent to *hi-1* on its proximal side with respect to the centromere<sup>13</sup>.



On the other hand, there was strong interference with crossing-over in an interval 9.9 units long adjacent to *hi-1* on its distal side, and a significant though lesser reduction in crossing-over in a further region 19.3 units long beyond the 9.9 interval. This would be accounted for if the crossover-reversal enzyme always moved distally, that is, away from the centromere and towards the end of the chromosome arm. A crossover within *hi-1* would then be unable to reverse a crossover on its proximal side. Such polarity in interference would also explain the discovery<sup>21</sup> that in *Drosophila* crossovers seem to arise in sequence along each chromosome arm starting from the centromere. Furthermore, the relationship<sup>22</sup> between the position of crossovers induced by ultra-violet light and the time in the nuclear cycle of the irradiation could be similarly explained. Diploid cells of *Ustilago maydis* treated with ultra-violet light late in DNA synthesis, unlike earlier treatment, show mitotic crossing-over mostly near the centromeres. Holliday suggested that a particular phase of DNA replication was sensitive to the induction of crossing-over, and that this phase moved progressively from the end of each chromosome arm to the centromere. On the other hand, a crossover-reversal enzyme moving in the opposite direction would seem to offer an alternative explanation, because crossovers formed after its passage could not be reversed and so would accumulate near the centromeres.

The hypothesis that interference shows polarity determined by the centromere, and that secondary crossovers are immune to interference, could be tested if a paracentric inversion included the sites of a pair of alleles showing secondary crossing-over and if flanking markers were available.

To understand the process of secondary crossing-over, it is necessary to know the peculiarity of mutants which cause it. According to existing data<sup>18,23,24</sup> several of the ascospore colour mutants in *Ascomobolus* which show reciprocal recombination with members of the same series also show one or both of two other phenomena, that is, postmeiotic segregation, and the absence of recombination with two or more allelic mutants which nevertheless show recombination with one another. This lack of recombination is taken to mean that the mutant in question is caused by a multisite mutation, probably a deletion. Several of the pale-spored mutants of *Ascomobolus* are classified in Table 3 in terms of the recombination phenomena which they show. It is tentatively concluded that both secondary crossing-over and postmeiotic segregation are characteristic of multisite mutations, but that postmeiotic segregation occurs only when the extent of the mutant is short.

In attempting to explain these characteristics, multisite mutations (deletions or additions) of various sizes in bacteriophage T4 seem relevant. There is a characteristic frequency of formation of heterozygous DNA for each mutant, and this frequency is related to the length of the mutant segment in the DNA<sup>25</sup>. Drake concluded that extended mutants cannot easily be accommodated in DNA internal heterozygotes<sup>25</sup>. When they are included, they probably form single-chain loops, but with increasing length of the mutant there is a greater probability that internal heterozygotes will fail to form.

If the behaviour of DNA at meiosis is similar to that of phage T4, a short multisite mutation would be expected to give rise in some meioses to a single-chain loop when it lies within hybrid DNA, and in other meioses to prevent hybrid DNA from forming, while a long multisite mutation would regularly fail to be included in hybrid DNA. These expectations agree with the data in Table 3, because postmeiotic segregation and conversion, which are evidence of hybrid DNA formation at the mutant site, do not seem to take place (postmeiotic segregation) or only occur with low frequency (conversion) with long multisite mutations, while secondary crossing-over, which does not involve hybrid DNA at the mutant site, seems to occur with multisite mutations of all lengths.

Secondary crossing-over could therefore occur when a multisite mutation fails to enter hybrid DNA. X-rays and many other agents lead to the formation of interchanges between non-homologous chromosomes, and there is evidence that this process, and also the formation of aberrations within a single chromosome, may come about by a process analogous to normal crossing-over<sup>26,27</sup>. The non-homologous exchange process may be initiated by some event occurring independently in the two chromatids (or different parts of one)<sup>26,27</sup>. It is likely that this event is the breakage of one of the two nucleotide chains of the DNA. If a multisite mutation led to a failure of hybrid DNA formation at meiosis, nucleotide chains would be left unpaired. Possibly the presence of such single chains with free ends triggers off the process of secondary crossing-over in much the same way as is thought to happen in X-ray-induced exchanges. From evidence given here, secondary crossovers at meiosis seem to have relatively short hybrid DNA segments, occupying only a fraction of the length of a gene. If the non-homologous exchange process also depends on the formation of hybrid DNA, the hybrid segments are also likely to be short, because non-homologous chromosomes or different parts of a single chromosome would not be expected to have long segments with a nucleotide sequence in common. (Note added in proof. Berg and Curtiss<sup>30</sup> suggest that reciprocal exchange between non-homologous segments of the DNA of *Escherichia coli* to give transposition derivatives is the result of "mutual recognition" between regions of fortuitously similar nucleotide sequences. This is the same mechanism as I have proposed here to explain X-ray-induced exchanges in higher organisms.)

If secondary crossing-over is caused by multisite mutations, what is the explanation of the postmeiotic segregation which appears sometimes to be associated with it? Lacks<sup>28</sup> has found that short multisite mutations at the amylomaltase (*mal*) locus in *Diplococcus pneumoniae* show a high integration efficiency in transformation, whereas point mutations often show much lower integration efficiencies. He provides evidence that the efficiency of integration of point mutations depends on the nature of the mutation, transitions having the lowest value. Study of mutants at the aminopterin-resistance (*amiA*) locus has shown that high efficiency mutants are transmitted to daughter-cells at the third division after uptake of donor DNA and low efficiency mutants at the second division<sup>29</sup>. These are equivalent to postmeiotic segregation and conversion, respectively. At DNA replication in the recipient

Table 3. CLASSIFICATION OF SOME ASCOSPORE COLOUR MUTANTS OF *Ascomobolus immersus* TO SHOW THEIR BEHAVIOUR WHEN CROSSED WITH OTHER MEMBERS OF THE SAME SERIES, FROM THE DATA OF LISSOUBA *et al.*<sup>18</sup> AND MOUSSEAU<sup>23,24</sup>

Series	Mutant	Extent of mutant	Evidence of extent	Conversion	Postmeiotic segregation	Reciprocal recombination
Series 46	46	Single site or short multisite	No pairs of mutants known which show recombination with one another but not with one of these mutants	+	-	-
	63			+	-	-
	138			+	-	-
	277			+	+	+
Series 19 Group A	1,216	Short or intermediate multisite	Each mutant shows no recombination with a pair of mutants which nevertheless recombine with one another	+	+	+
	60			+	+	+
	1,028			(+)	-	+
	1,130			(+)	-	+
	49	Long multisite	Mutant shows no recombination with all group A mutants	(+)	-	+

+, Phenomenon given at the head of the column is shown by the mutant; -, not recorded; (+) indicates a low frequency. In assessing whether a mutant shows reciprocal recombination, the behaviour of the mutant with which it is crossed has also to be taken into account.

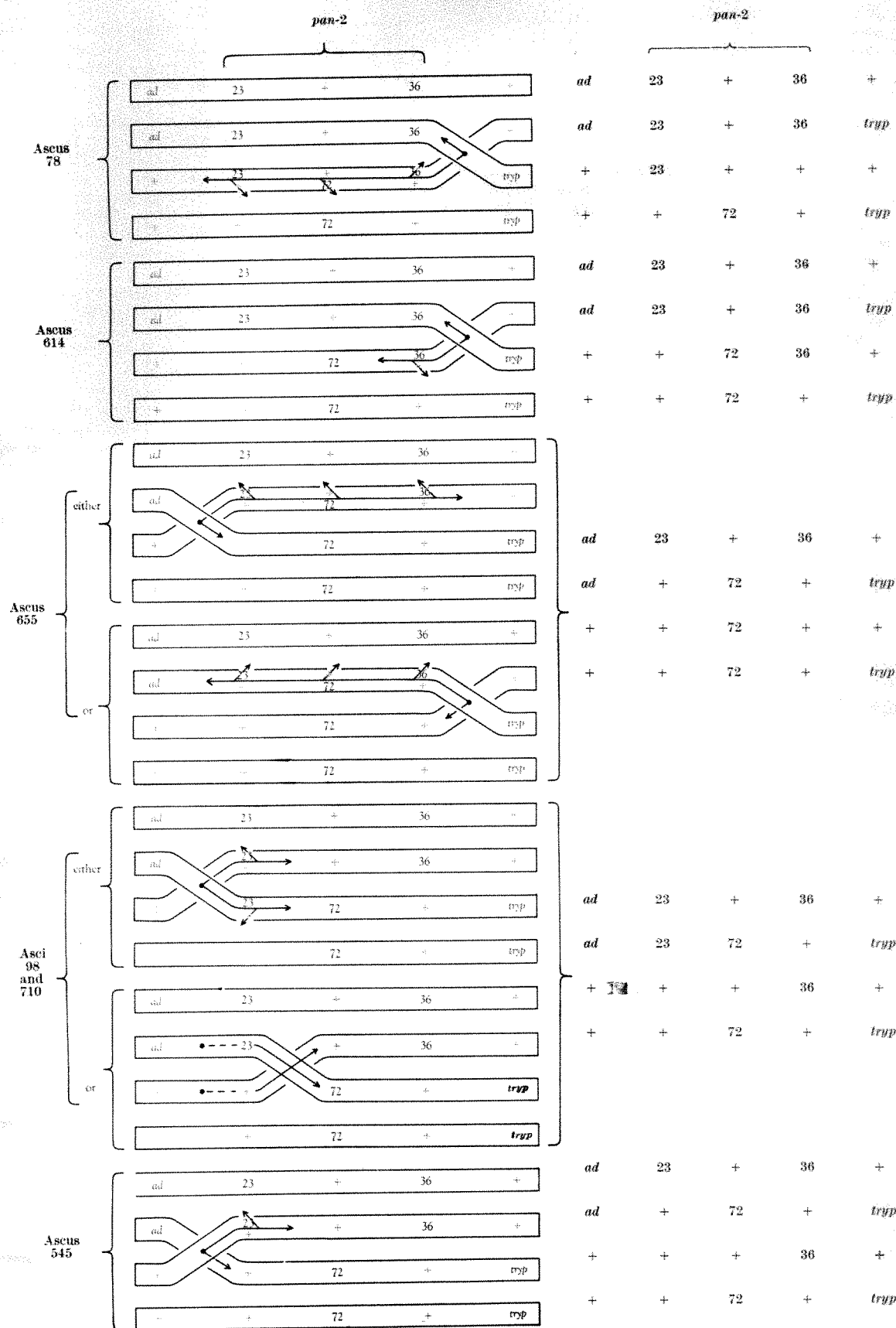


Fig. 2. Diagrams to illustrate possible modes of origin of six asci of *Neurospora crassa*<sup>a</sup> to show recombination within the *pan-2* locus, and crossing-over between outside markers *ad-1* and *tryp-2*. The genotypes of the four pairs of spores in each ascus are shown to the right of the corresponding diagram. The numbers 23, 72 and 36 denote the three mutant sites within *pan-2* by which the parents differed. The diploid nuclei in the young asci had the genotype  $\frac{ad-1 \ 23 \ + \ 36 \ +}{+ \ + \ 72 \ + \ tryp-2}$ . The components of the diagrams have the same meaning as in Fig. 1. Hybrid DNA can apparently enter *pan-2* from either end.

bacterium, the donor nucleotide chain could anneal with the homologous segment of the complementary chain of the recipient<sup>29</sup>. This would give a region of hybrid DNA in one of the daughter-molecules. With low efficiency mutants, an enzyme system then recognizes the mispairing in the DNA caused by the mutant, excises one chain and replaces it by a chain complementary to the other, the donor chain being excised more often than the recipient one<sup>29</sup>. If the enzyme system recognizes mismatched nucleotides in DNA, but often overlooks the lack of pairing with a multisite mutation (compare ref. 28), this would explain both the high integration efficiency and the postmeiotic segregation apparently found with short multisite mutations. The apparent absence of postmeiotic segregation with longer multisite mutants (Table 3) suggests that mutations of a sufficient length are recognized by the correcting enzyme system whenever they enter hybrid DNA, whereas short multisite mutations are not always

recognized and so can give rise to postmeiotic segregation as well as conversion.

The possible association between short multisite mutations and postmeiotic segregation could be tested by octad analysis using ascospore colour mutants of various lengths. There is no evidence that mutants 277 and 1216 in series 46 of *Ascobolus* are multisited although they show postmeiotic segregation (see Table 3), but in order to establish that a mutant occupies an extended length of the DNA it is necessary to find mutants which fail to recombine with it and yet recombine with one another. Furthermore, if a mutant was an addition of a few nucleotides rather than a deletion, it might map as a point mutation (compare ref. 25).

Some results obtained with *Neurospora crassa*<sup>8</sup> point to another possible cause of correction failure and thus of postmeiotic segregation. Thirteen asci were found in which recombination had occurred at the *pan-2* locus.

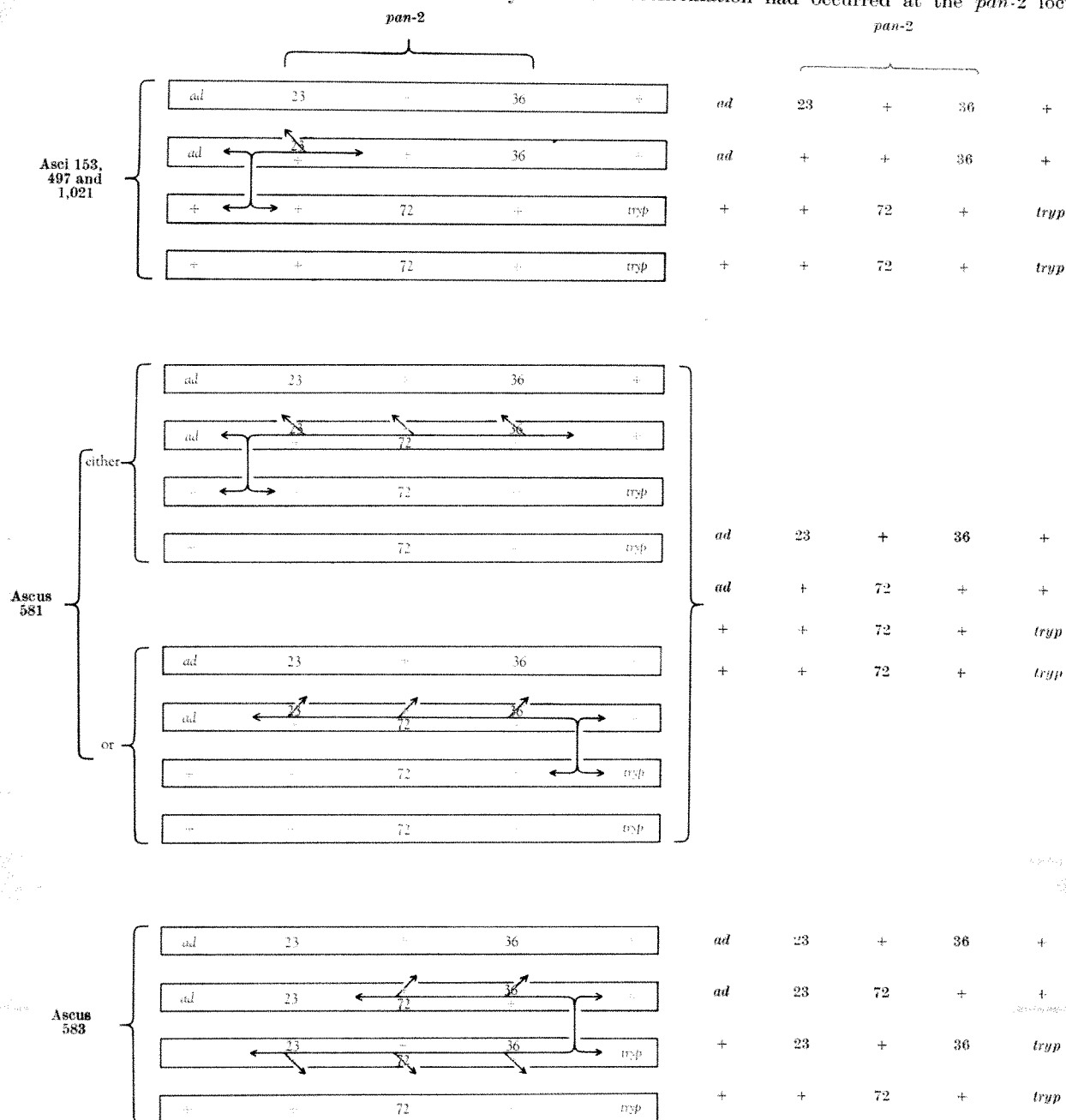


Fig. 3. Diagrams to illustrate possible modes of origin of five asci of *Neurospora crassa*<sup>8</sup> to show recombination within *pan-2*, and a parental arrangement of the outside marker genes. These asci were from the same cross as those shown in Fig. 2. The short horizontal arrows pointing away from *pan-2* indicate reverse crossovers. The other arrows have the same meaning as in Figs. 1 and 2.

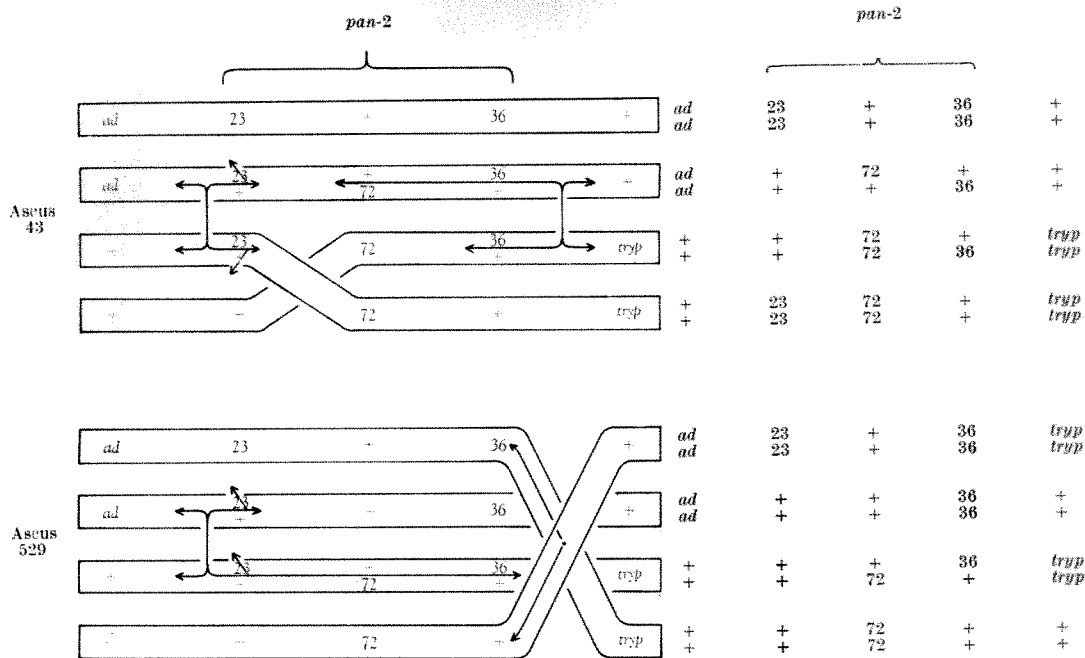


Fig. 4. Diagrams to illustrate possible modes of origin of two asci of *Neurospora crassa*<sup>8</sup> to show postmeiotic segregation within *pan-2*. These asci were from the same cross as those shown in Figs. 2 and 3. The genotype of the eight spores in each ascus is shown on the right. The arrows have the same meaning as in Figs. 1-3.

The parental strains differed at three sites within this gene, and also at single sites in marker genes (*ad-1* and *tryp-2*) on each side. Eleven of the asci showed 4:4, 6:2 or 2:6 ratios of wild type: mutant genotype at the three *pan-2* sites, implying that correction of mispairing had occurred at all the sites which had entered hybrid DNA. In each of these asci, two of the four pairs of spores showed the respective parental genotypes (Figs. 2 and 3). This means that the recombination was confined in the normal way to two of the four chromatids at meiosis.

In asci Nos. 98 and 710 it is uncertain whether the hybrid DNA included site No. 23, followed by correction in opposite directions (reciprocal conversion), or whether there was secondary crossing-over. These two asci are included in Table 2. Secondary crossing-over is the more probable explanation, because the outside markers show coincident crossing-over, but both alternatives are shown in Fig. 2. Ascus No. 581, like several others in Figs. 2 and 3, provides an example of linked correction, with neighbouring sites corrected to the same parent.

Two asci have been found in which postmeiotic segregation had occurred<sup>8</sup>. It is remarkable that in both of them there was such segregation at two of the three sites, and in both asci more than two chromatids were involved in recombination either within *pan-2* or in its vicinity. The genotypes of the eight spores in these asci are given in Fig. 4 together with a likely mode of origin. It seems that in ascus No. 43 hybrid DNA from double crossovers entered *pan-2* from both ends, but one of the chromatids involved at one end was not involved at the other. In ascus No. 529 a double crossover appears to have occurred at the proximal end and a crossover involving the other two chromatids beyond the distal end.

The association, evident from these asci, between multi-stranded recombination and postmeiotic segregation would be accounted for if distortions in a DNA molecule are recognized by an enzyme moving along it and if two recombination events (either a crossover and a double crossover, or two double crossovers) in proximity to one another and involving at least three of the chromatids prevent such movement. If the two events were not quite simultaneous, the second might interfere with the movement of the enzymes over the hybrid DNA regions of the first during a critical period. This would lead to a failure of correction where the hybrid DNA from one

recombination event came close to that from the other. The position of the sites showing postmeiotic segregation agrees with this hypothesis (Fig. 4).

The idea that multistranded recombination is a cause of postmeiotic segregation could be tested by study of recombination between allelic ascospore colour mutants, preferably with outside marker genes. The use of mutants the sites of which were near opposite ends of the spore colour locus would facilitate the recognition of multi-stranded recombination when two double crossovers were involved, as in ascus No. 43 discussed here.

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# X-Ray Diffraction Study of Fossil Elemis

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X-ray diffraction identification of  $\alpha$ -amyrin in guayaquillite, Highgate copalite and glessite places these fossil resins among the elemis in the Burseraceae family of angiosperm trees. Correlation of their geographical occurrences lends support to the belief that the so-called Amber Forests flourished during Eocene as well as Oligocene times.

RECENTLY<sup>1</sup> I suggested that there was a possible genetic relationship between the fossil resins guayaquillite and Highgate copalite and those of the modern genera *Protium* and *Bursera* in the Burseraceae family of angiosperm trees. This relationship was postulated on the basis of the similarity of their X-ray diffraction patterns, and the technique for obtaining these patterns was described. More recently the constituent represented by these patterns has been identified as  $\alpha$ -amyrin, a principal component of the elemis.

Elemi is the group name for the fragrant resin of several angiosperm trees of different botanical origins. According to Wehmer<sup>2</sup> this origin is not always certain, but Tschirch<sup>3</sup> assigned the elemis to various genera of the Burseraceae family, primarily *Canarium* and *Protium*. Some elemis come also from several species of *Amyris* and *Bursera*, and Tschirch classed the genus *Amyris*, in part, with the genus *Protium*. Elemis consist essentially of amyirin, resin acids, resenes and volatile oils and, in the narrowest sense, the true elemis are only those resins which contain crystalline amyirins. These are triterpenoid alcohols— $C_{30}H_{50}O$  (refs. 3 and 4). Both  $\alpha$ - and  $\beta$ -amyrin exist together in the resins, but the  $\alpha$ -amyrin is the predominant form. The amyrin was usually detected by its characteristic needle like crystals seen under the polarizing microscope, and chemical extraction followed by a melting point determination. In this investigation, the chemical extraction was not necessary as the high percentage of amyrin present, generally between 20–30 per cent, permitted its detection and identification in the resins by X-ray diffraction. Using pure  $\alpha$ -amyrin as a standard, I have identified it in a number of modern elemis, among which are: Yucatan copal from *Amyris elemifera*<sup>5</sup> or *Amyris plumieri*<sup>5</sup>; South American Tacamahaca resin from *Protium heptaphyllum*<sup>5</sup>; Brazilian elemi from *Protium icicariba* or *Protium heptaphyllum*<sup>6</sup>; and resin from *Bursera bipinnata*. Their X-ray diffraction patterns are shown in Fig. 1 and the  $d$ -spacings are given in Table 1.

The fossil resin guayaquillite from Ecuador is found in deposits of undetermined geological age. Johnston<sup>7,8</sup>, who first described it, suggested that it was of vegetable origin because, like amber, it occurred on the site of ancient forests of resiniferous trees. The X-ray diffraction pattern of guayaquillite (Fig. 1 and Table 1) shows the presence of  $\alpha$ -amyrin. Hence, it can be classed with the elemis.

Johnston<sup>9</sup> also described the Highgate resin or copalite which was found in the London Blue Clay of Eocene age. He compared this resin, on the basis of chemical analyses, with the anime-resin of a "Locust-tree" (Leguminosae) in French Guiana, South America. This was a false comparison, for, although the anime-resin comes from *Hymenaea courbaril*, a Leguminosae, the X-ray diffraction pattern of the Highgate copalite (Fig. 1 and Table 1), revealing the presence of  $\alpha$ -amyrin, indicates that it is an elemi. Reid and Chandler<sup>10</sup> mentioned fossil plant remains of various genera of the Burseraceae family found in the London Clay. The suggestion by Langenheim<sup>11</sup> that these plant remains present a possible botanical source for the Highgate copalite is now validated.

$\alpha$ -Amyrin was identified in still another fossil resin, the rare glessite (Fig. 1 and Table 1), found in the "Blue Earth" of the east Baltic along with Baltic amber. Although Paclt<sup>12</sup> classes glessite together with the Baltic ambers from coniferous trees, Helm<sup>13</sup>, who named and first described the material, claimed that under the microscope it showed a resemblance to modern Myrrh in the Bur-

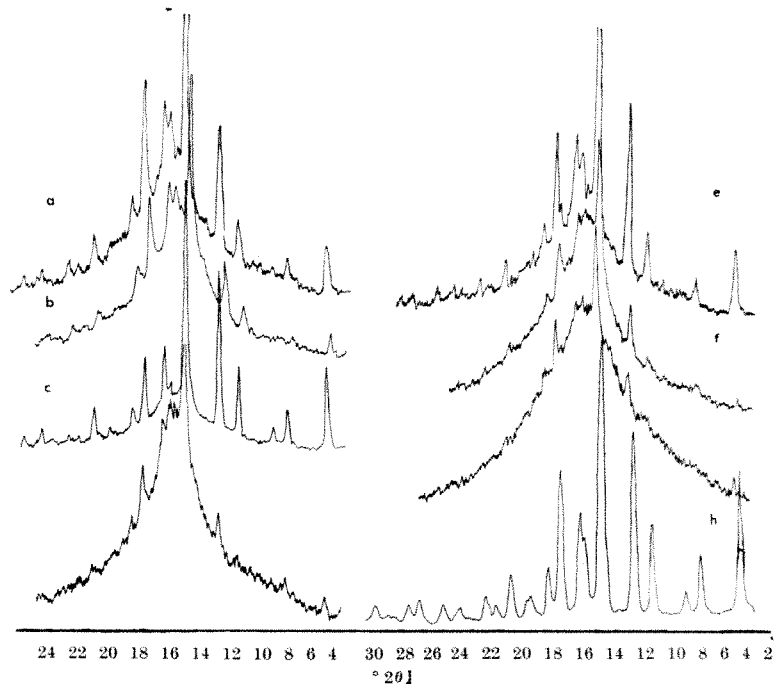


Fig. 1. X-ray diffraction patterns of some modern elemis, fossil elemis and  $\alpha$ -amyrin.

a. Yucatan copal;	} Modern elemis	e. Guayaquillite;	} Fossil elemis
b. Tacamahaca resin;		f. Highgate copalite;	
c. Brazilian elemi;		g. Glessite.	
d. <i>Bursera bipinnata</i> .		h. $\alpha$ -Amyrin.	



# Formation of Sparks by Several Electron Avalanches

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In a spark chamber, interaction between electron avalanches causes the resultant spark to incline along the particle track. At an angle of incidence greater than about  $35^\circ$  the sparks tend to follow the electric field.

THE formation and the appearance of sparks produced by the avalanche and streaming of a single electron have been extensively investigated<sup>1,2</sup>. A streamer originating from a single electron avalanche can propagate over long distances, even where the initial ionization is negligible; lightning discharges are an example<sup>3</sup>. In a spark chamber the ionization extends initially along the path of the triggering event and the spark, which is eventually visible, may be the result of several electron avalanches developing simultaneously. When these avalanches interact, sparks are inclined away from the direction of the applied field in a spark chamber. This article describes an extension of the existing theory including the effects of multiple electron avalanches.

According to the Meek criterion, a streamer begins when the field due to the intrinsic space charge inside the avalanche equals the electric field immediately outside it. The electric field is the vector sum of the field applied between the electrodes of the spark chamber,  $E_a$ , and the fields between adjacent avalanches,  $E_s$  (see Fig. 1). The most probable direction of the streamer and of the visible spark is determined by the direction of this field at the onset of the streamer.

The fields, at the head of one avalanche due to the negative charges located at the centres of adjacent electron

clouds, cancel if it is assumed that all of the avalanches grow at the same rate, the quantities of charge in each are equal, the avalanches are equally spaced, and the centres of each avalanche are aligned with each other. This cancellation does not occur near the end of the line of avalanches, and this can occasionally be observed near the electrodes as distortions in the line of the sparks<sup>4</sup>. It will be assumed that the effective centre of the positive charges, caused by ionization, is at the centre of each avalanche.

It has been shown<sup>2</sup> that the radius of the electron cloud at the head of an avalanche, at any moment up to the onset of the photo-ionization, is given by the distance diffused by an electron within the time interval from the start of the pulsed electric field. The forces due to the mutual repulsion of the electron space charge become appreciable only when the streamers propagate.

At any time  $t$ , therefore, the internal field of an avalanche is

$$E_i = \frac{nq}{4\pi\epsilon_0} (2D_i) \frac{V}{m}$$

where  $q$  is the charge of an electron and  $D$  is the thermal diffusion coefficient<sup>5</sup>.

Similarly, in the notation of Fig. 1, the space charge field is

$$E_s = \frac{nq}{4\pi\epsilon_0} \sum_{i=1}^2 \sum_{j=1}^m \frac{1}{(R_{ij})^3} R_{ij} \frac{V}{m}$$

where  $m$  is the number of effective avalanches on either side of the one under consideration. In these calculations  $m$  was taken to equal 5; larger values did not appreciably affect the results.

The angle of incidence of the resultant field, with respect to the applied field, at the moment when the Meek criterion predicted the onset of streamers is shown in Fig. 2. They are plotted as functions of the angle of incidence of the original ionized track and of the mean avalanche spacing. The angles connecting the inclinations of the resultant field vary when there is a variation of  $\pm 0.5$  nsec in the development time of the avalanche. It was assumed that the applied field increased linearly with time. It can be seen that as the avalanches come closer together, for example with an increase in the gas pressure, the sparks follow the particle track more faithfully. The mean spacing of the avalanches in an experimental gas mixture at atmospheric pressure was found to be between 0.4 mm and 0.5 mm (ref. 6).

Fig. 3 shows how the theoretical maximum inclination of the sparks and their formation times vary with the applied pulse parameters for an avalanche spacing of 0.4 mm. Fig. 4 compares the theoretical predictions with the experimental results for the sparks, in a 1 in. gap spark chamber, which were triggered by relativistic muons. As the angle of incidence increased beyond about  $35^\circ$  the space charge fields on either side of the avalanche tended to cancel each other out, so that the sparks tended to follow the direction of the applied field rather than the track of the muon.

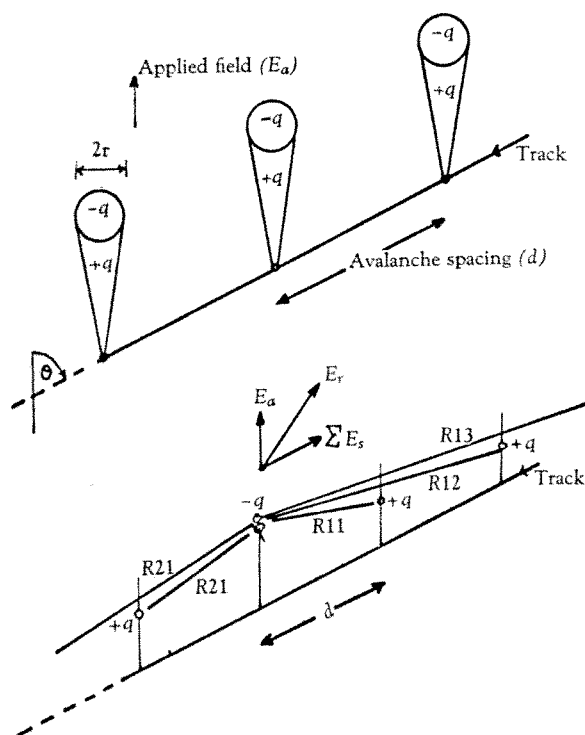


Fig. 1. Spark model.

The theoretical predictions here agree with experimental results reviewed elsewhere<sup>7,8</sup>. They all show the sparks following the particle track up to a maximum angle of incidence between 25°–35°, and thereafter following the applied field, although occasional sparks were inclined at larger angles. Because the space charge interaction is small at these large angles, the electron avalanches and streamers can develop individually and many closely spaced sparks can often be seen in the gap traversed by the particle.

The interaction between electron avalanches can be seen clearly when the resultant fields, acting at the head of each avalanche, are not aligned. If the subsequent streamers develop together and the misalignment is small the sparks may appear in steps or kinks<sup>9</sup>.

As can be seen from Fig. 2, fluctuations in the inclinations of the sparks could occur because of variations in the avalanche spacing and because of variations in the development time of each avalanche. These inherent angular fluctuations set an upper limit to the accuracy with which a particle track may be fitted to the sparks<sup>6</sup>.

The fluctuations can originate in two ways: first, the ionization density left by a relativistic particle follows a normal distribution, and small statistical variations in the spacings of the ions and of the electrons are very

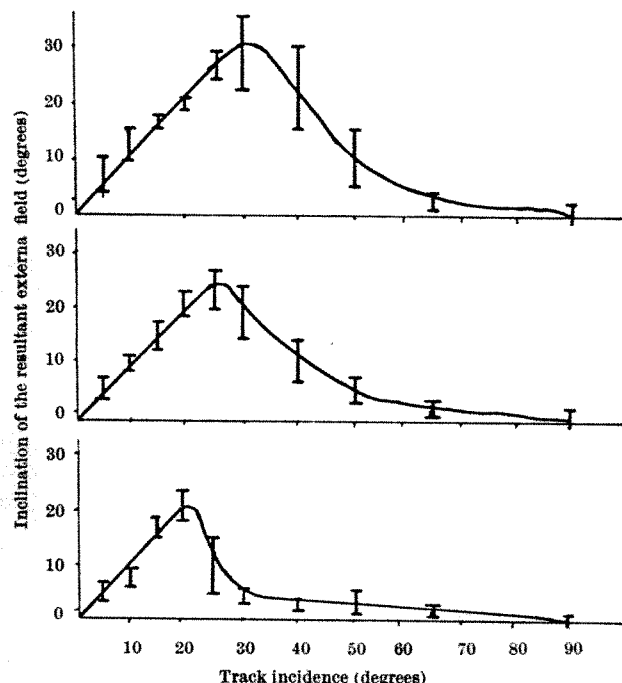


Fig. 2. Theoretical spark inclinations. Applied pulse, 1 kV/cm/nsec. Avalanche spacing: top, 0.3 mm; middle, 0.4 mm; bottom, 0.5 mm.

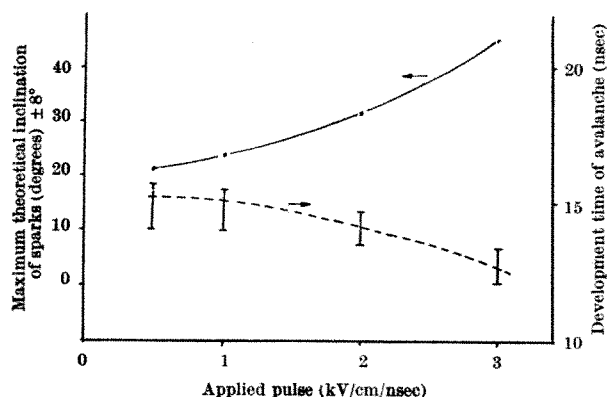


Fig. 3. Effects of the parameters of the applied field. Avalanche spacing of 0.4 mm.

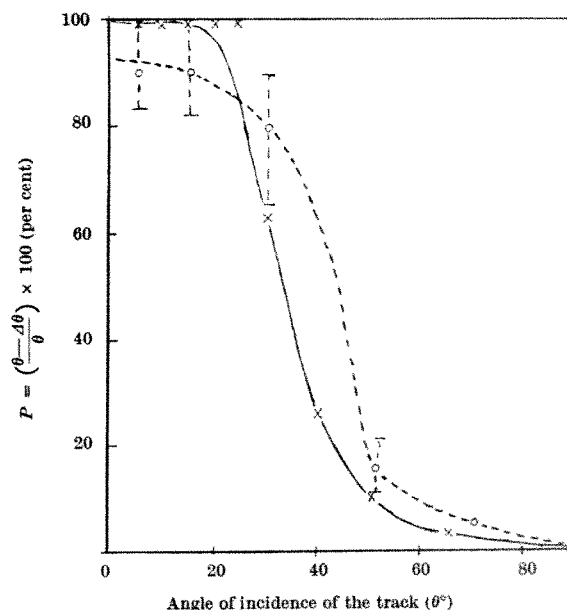


Fig. 4. Theoretical angular deviations of the sparks from the track.  $\times$ — $\times$ , Theoretical;  $\circ$ — $\circ$ , experimental values for 1 in. gaps.

probable<sup>10</sup>; second, at the onset of the streamers the rate of increase of the space charge fields is very large, and small variations in the spacing and in the development time have a marked effect on the relative delays between the propagation of the streamers from individual avalanches. These effects are magnified by the addition of alcohol vapours to the gas mixture. It has been shown that the addition of about 2 per cent of ethyl alcohol to a 90 per cent neon + 10 per cent helium gas mixture can increase the brightness and linearity of the sparks<sup>11</sup>.

As soon as one streamer develops it rapidly propagates along the direction of the resultant field at each point. The resultant ionization distorts the electric field around the avalanches so that they are absorbed or suppressed in their development. The luminous modulation along a spark, especially when the spark development has been curtailed<sup>8</sup>, corresponds to the regions of maximum ionization, one for each streamer. This is very noticeable in wide gaps operating with very short pulsed fields.

Up to the moment of writing no quantitative model of the propagation of streamers has been published. It is a stochastic process and would involve successive photo-ionization along the continually enhanced electric field, produced by the previous ionization and by the presence of the neighbouring avalanches. The most probable path of the streamers among the fluctuations of the space charge fields and the continuously varying successive photo-ionization might be evaluated by the Monte Carlo method.

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# LETTERS TO THE EDITOR

## ASTRONOMY

### Radio Source Spectra and the Cosmological Red-shift

RECENT spectral surveys of discrete radio sources have not revealed any significant dependence of spectral index on apparent intensity<sup>1-3</sup>. It is therefore of interest to determine the way in which the mean observed spectral index depends on flux density, when the effects of cosmological red-shift are taken into account. We shall choose, by way of example, a hyperbolic world model.

The observed flux density  $S(\nu)$  at a frequency  $\nu$ , from an isotropic source of emission of intrinsic luminosity  $P(\nu_e)$ , at a frequency  $\nu_e$ , in unit bandwidth over  $4\pi$  steradians, if red-shifted by  $z$  and located  $\omega$  from the observer in co-ordinate space, is given by

$$S(\nu) = \frac{P[\nu(1+z)]}{R_0^2(1+z) \sinh^2 \omega} \quad (1)$$

where  $\nu_e = \nu(1+z)$ ,  $R_0$  is the scaling factor in the Robertson-Walker metric equation

$$ds^2 = dt^2 - \frac{R^2(t)}{c^2} \{d\omega^2 + J^2(\omega)(d\theta^2 + \sin^2\theta d\varphi^2)\}$$

$R_0$  being evaluated at time  $t=t_0$  and

$$\omega = \frac{cT_0}{R_0} \int_0^z \frac{dz}{\{1+2(q_0+1)z+(q_0+1+3\sigma_0)z^2+2\sigma_0 z^3\}^{1/2}} \quad (2)$$

(see ref. 4). Let the number of sources,  $n$ , be in a unit co-ordinate volume independent of  $t$ , and the probability that a radio source has a spectral behaviour of the form  $P(\nu_e)z\nu_e^{-\alpha}$ , be  $p(\alpha)\delta\alpha$  in the range  $\alpha \pm \delta\alpha/2$ . The physical volume contained by a spherical shell of co-ordinate thickness  $\delta\omega$  and radius  $\omega$  is

$$4\pi R^2 \sinh^2 \omega \delta\omega$$

Thus the number of sources observed between  $\check{\omega}$  and  $\hat{\omega}$  having spectral indices  $\alpha \pm \delta\alpha/2$  is

$$\begin{aligned} & 4\pi n_0 \int_{\check{\omega}}^{\hat{\omega}} R_0 \sinh^2 \omega d\omega p(\alpha) \delta\alpha \\ &= 4\pi n_0 R_0^3 \left[ \frac{\sinh^2 \omega}{4} - \frac{\omega}{2} \right]_{\check{\omega}}^{\hat{\omega}} p(\alpha) \delta\alpha \end{aligned}$$

The values of  $\check{\omega}$  and  $\hat{\omega}$  are defined in terms of the observable flux density by equation (1), that is to say, if it is asked how many sources are observed to have a flux density between  $\check{S}$  and  $\hat{S}$  and a spectral index  $\alpha \pm \delta\alpha/2$  which have an intrinsic power  $P_0$  at a reference frequency  $\nu_0$  and dependence on frequency of the form  $\nu_0^{-\alpha}$ , then  $\check{\omega}$ , say, is defined by the equation

$$\check{S}(\nu) = \frac{P_0 \left( \frac{\nu}{\nu_0} \right)^{-\alpha} (1+z)^{-\alpha-1} (q_0+1-3\sigma_0)}{c^2 T_0^2 \sinh^2 \check{\omega}} \quad (3)$$

that is, in terms of  $T_0$ ,  $q_0$  and  $\sigma_0$ —the Hubble time, acceleration and density parameters, respectively<sup>4</sup>. Thus, the observed distribution of spectral indices in a flux density range  $\check{S}$  to  $\hat{S}$ , conditional on the parameters of the cosmology  $T_0$ ,  $q_0$ ,  $\sigma_0$ , and on the nature of the radio sources  $P_0$  and spectral distribution in co-ordinate space defined by  $p(\alpha|\check{\alpha}, \check{\sigma})$ , is, explicitly

$$F(\alpha|T_0, q_0, \sigma_0; \check{\alpha}, \check{\sigma}; \check{S}, \hat{S}) \delta\alpha$$

is proportional to

$$p(\alpha|\check{\alpha}, \check{\sigma}) \delta\alpha \left[ \frac{\sinh^2 \omega}{4} - \frac{\omega}{2} \right]_{\check{\omega}}^{\hat{\omega}} \quad (4)$$

where the probability distribution  $p(\alpha|\check{\alpha}, \check{\sigma})$  is characterized by a mean spectral index  $\check{\alpha}$  and spread  $\check{\sigma}$ . Clearly, then, the last term in equation (4) will modify the observed distribution of  $\alpha$  because of the functional dependence of  $\omega$  on  $\alpha$ —equation (3).

It is intuitively clear that in an expanding universe only sources with the flatter spectra (low  $\alpha$ ) will survive the red-shift effect in a given range of flux densities. Thus we might expect a shift in the mean observed spectral index,  $\check{\alpha}_0$ , say, in the sense that  $\check{\alpha}_0 < \check{\alpha}$ .

Let us consider by way of example<sup>4</sup>

$$\begin{aligned} 3 &\geq q_0 \geq 1 \\ 0.2 &\geq \sigma_0 \geq 0 \end{aligned}$$

$$h_1 = 100 \text{ km/sec/Mpc} = 1/T_0 \text{ (following ref. 5)}$$

$$\check{\alpha} = 0.75$$

$$\check{\sigma} = 0.20$$

$$(p(\alpha|\check{\alpha}, \check{\sigma}) \alpha \exp \left\{ -\frac{1}{2} \left( \frac{\alpha - \check{\alpha}}{\check{\sigma}} \right)^2 \right\})$$

The effects on the observed distribution of spectral indices of changes in  $P_0$ ,  $\sigma_0$  and  $q_0$  will now be investigated.

By numerical procedures, various of these equations necessary for the evaluation of  $\check{\alpha}_0$  were solved using an Atlas computer. The results for  $q_0 = 1.8$  and  $\sigma_0 = 0.2$  may be seen in Fig. 1. As expected,  $\check{\alpha}_0$  tends to decrease as the level of flux density is lowered: the higher the intrinsic luminosity ( $P_0$ , defined at 178 Mc/s), the more severe the effect of observational selection. For  $P_0 = 8 \times 10^{25} \text{ W (c/s)}^{-1} \text{ sterad}^{-1}$ ,  $\check{\alpha}_0$  deviates from  $\check{\alpha}$  by only 0.02 at  $S_{178}^* = 1.0$ ; for luminosities an order of magnitude higher:  $\check{\alpha} - \check{\alpha}_0 = 0.03$  at  $S_{178} = 2.0$ .

Altering  $q_0$  and  $\sigma_0$  within the ranges given here does not change  $\check{\alpha}_0$  by more than 0.005. No combination of  $q_0$  and  $\sigma_0$  makes the observed dispersion of spectral indices significantly different from  $\check{\sigma} = 0.20$ .

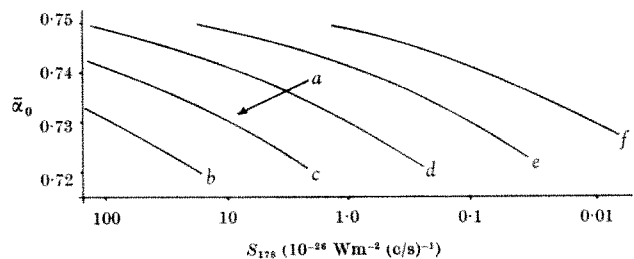


Fig. 1. Effect of observational selection on the mean observed spectral index. Model:  $q_0 = +1.8$ ;  $\sigma_0 = +0.2$ ;  $\check{\alpha} = 0.75$ ;  $\check{\sigma} = 0.20$ . a,  $P_0$  ( $\text{W(c/s)}^{-1} \text{ sterad}^{-1}$ ); b,  $P_0 = 8 \times 10^{27}$ ; c,  $P_0 = 8 \times 10^{26}$ ; d,  $P_0 = 8 \times 10^{25}$ ; e,  $P_0 = 8 \times 10^{24}$ ; f,  $P_0 = 8 \times 10^{23}$ .

It has been shown that for a range of feasible model universes uniformly populated with radio sources which have spectra of the form  $\nu^{-\alpha}$ , observational selection discriminates against high spectral indices.

Sources included in the 178 Mc/s spectral surveys<sup>1-3</sup> probably lie chiefly in the luminosity range  $8 \times 10^{24} < P_0 < 8 \times 10^{26} \text{ W m}^{-2} \text{ (c/s)}^{-1} \text{ sterad}^{-1}$ . No correlation has been observed between  $S_{178}$  and  $\check{\alpha}_0$ . The theoretical average value of  $\check{\alpha} - \check{\alpha}_0$  at  $S_{178} = 2.0$  is approximately 0.01, because of the selection effects discussed here, but no account has been taken of spectral curvature. This value of 0.01 is of the same order as the experimental uncertainty in  $\check{\alpha}_0$ ; the calculations given here are therefore consistent with the observations.

\*  $S_{178}$  in units of  $10^{-26} \text{ W m}^{-2} \text{ (c/s)}^{-1}$ .

A spectral survey of discrete sources down to about  $S_{178}=0.1$  may reveal quite significant changes in the average apparent spectral index. The direction of this change, however, will depend on the relative effects of spectral curvature, source evolution and distribution, together with geometry.

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### Relative Intensities of Hydrogen Recombination Lines in Gaseous Nebulae

RECENTLY, Gardner and McGee<sup>1</sup> have published measurements on the  $126\alpha$ ,  $159\beta$  and  $158\beta$  recombination lines of hydrogen in two galactic nebulae. These lines have frequencies differing by less than 2 per cent and are therefore observed with the same beamwidth. Their results are summarized in Table 1.  $T_L$  is the measured peak line temperature above the continuum.  $(T_{L\beta}/T_{La})_E$  is the predicted ratio of temperatures assuming thermodynamic equilibrium and equal line widths.

The effect of departures from thermodynamic equilibrium has been pointed out by Goldberg<sup>2</sup>. The solution of the transfer equation under these circumstances is

$$T_L = b_n \left( 1 + \frac{\gamma}{2} \tau_C \right) T'_L \quad (1)$$

where  $\tau_C$  is the optical depth in the continuum;  $\gamma = (kT_e/h\nu) (d \ln b_n/dn) \Delta n$ ;  $T'_L$  is the line temperature in thermodynamic equilibrium.  $\Delta n$  is 1 or 2 for an  $\alpha$  and  $\beta$  transition, respectively. Equation (1) is derived assuming that the optical depth in the line and the continuum is small. From equation (1), the ratio of the line temperature ratio out of thermodynamic equilibrium to that in thermodynamic equilibrium is simply

$$R = \frac{(T_{L\beta}/T_{La})}{(T_{L\beta}/T_{La})_E} = \frac{b_{n\beta} \left( 1 + \frac{\gamma_\beta}{2} \tau_C \right)}{b_{n\alpha} \left( 1 + \frac{\gamma_\alpha}{2} \tau_C \right)} \quad (2)$$

I have calculated values of  $R$  for  $T_e = 10^4$  °K from computations of  $(d \ln b_n/dn)$  as a function of electron density.  $\tau_C$  is calculated from<sup>3</sup>

$$\tau_C = 8.24 \times 10^{-2} T_e^{-1.35} \left[ \frac{\nu}{G \text{ c/s}} \right]^{-2.1} \left[ \frac{E}{\text{pc.cm}^{-6}} \right] \quad (3)$$

$E$  is the emission measure. In Fig. 1,  $R$  is shown as a function of emission measure for various densities. Also shown are the measured ratios and their uncertainties from Table 1. The emission measures for the two nebulae are taken from Mezger and Henderson<sup>3</sup>. The root-mean-square electron density of M17 is  $565 \text{ cm}^{-3}$  (ref. 3), and of the Orion Nebula is  $\geq 10^3 \text{ cm}^{-3}$  (ref. 4).  $R$  does not depend much on density for densities  $\geq 10^2 \text{ cm}^{-3}$ . The measured ratios are in only approximate agreement with the theory, but definitely show a trend to the occurrence of maser

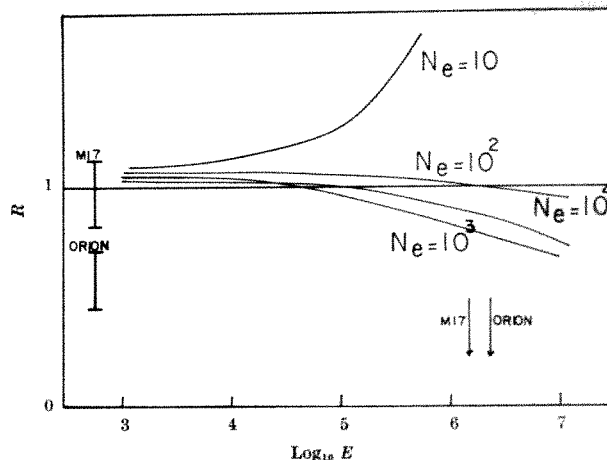


Fig. 1.  $R$  as a function of emission measure for various densities. The arrows give the emission measures of the two nebulae.

action. Computed values of  $R$  are about 10 per cent outside the uncertainty range for the Orion Nebula. This could be accounted for by broadening of the  $n=158$  quantum level. So far, however, there has been no definite detection of broadening in the higher quantum levels.

It is evident that a series of measurements of  $\alpha$  and  $\beta$  transitions of approximately the same frequency would be of great value in confirming the maser action. For the two lines discussed in this paper, regions of local density  $\sim 10 \text{ cm}^{-3}$  or  $\sim 10^3 \text{ cm}^{-3}$  would provide the optimum conditions in the sense of greatest deviation of  $R$  from unity.

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## PLANETARY SCIENCE

### Radius of the Earth's Core

ACCORDING to the modified Lomnitz law of imperfect elasticity, the response to a constant stress contains a term in  $t\alpha$ . I have pointed out<sup>1</sup> that in a vibration, according to this law, besides the damping of vibrations, there is a delay proportional to  $(\text{period})^\alpha$ ; this would be small for the  $S$  waves that I was considering. If, however,  $1/Q$  is the damping factor as usually defined, the delay lengthens the period by a factor  $(1/Q) \cot \frac{1}{2}\pi\alpha$ . For the slowest free vibration of the Earth as a whole  $Q$  appears to be about 400; with  $\alpha = \frac{1}{2}$ , as I have suggested, the factor would be about  $1/160$ .

Theoretical periods of free vibrations are systematically of the order of 1 per cent shorter than the observed ones. Suggested explanations have been a surprising constancy of density in the lower part of the shell<sup>2</sup> and an increase of about 15 km in the radius of the core<sup>3</sup>. It seems that my suggestion may provide an alternative.

I have analysed the observations of  $PcP$  (longitudinal waves reflected at the core boundary) given by Buchbinder<sup>4</sup> and find that they indicate an increase of the core radius by  $3 \pm 5$  km. Thus they would be consistent with my 1940 solution, the apparent standard error of

Table 1. OBSERVED AND PREDICTED TEMPERATURE RATIOS

Nebula	$T_{L\beta}/T_{La}$	$(T_{L\beta}/T_{La})_E$
Orion	$0.13 \pm 0.03$	0.224
M17	$0.22 \pm 0.04$	

which was 3.5 km, and rather against an increase by 10 km.

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## Age of the Irish Sea Glaciation of the Midlands

THE red till (or tills) with erratics from the Lake District and south-west Scotland, found in Lancashire, Cheshire, Staffordshire and Shropshire, has been ascribed to what has been called the Irish Sea Glaciation. That more than one ice advance could be represented by similar material has long been recognized, but the southward limit of the Lake District and Scottish boulders lay along a line Church Stretton-Wolverhampton-Burton on Trent, and this "Wolverhampton Line" could be taken as the southernmost extension of the Irish Sea Glacier<sup>1</sup>. The date of this advance has been variously claimed to be Late-Würm, Early-Würm and Riss (Saale). I have ascribed it to the Early-Würm<sup>2,3</sup> and accepted the view of Boulton and Worsley<sup>4</sup> that the Wrexham-Barr Hill Moraine was the most southerly extension of the Late-Würm glaciation as corroboration of this. Now, as the result of work here—stratigraphy by Mr A. V. Morgan, palaeontology by Dr G. R. Coope and radiocarbon dating by Mr R. E. G. Williams—it can be demonstrated that the advance of the Irish Sea Glacier to the Wolverhampton Line occurred in Late-Würm times, that is, it probably centred around a date of about 25,000 yr ago.

In the region of Four Ashes (*SJ* 9208) about 5 miles north of Wolverhampton and thus north of the Wolverhampton Line but well south of the Wrexham-Barr Hill Line, sheet 153 of the Institute of Geological Sciences shows an extensive cover of boulder clay. This is typical Irish Sea till with characteristic erratics, often very large. The map does not reveal the existence beneath this till of a great spread of water-deposited gravels and sands now worked commercially on a large scale. Working faces change rapidly but there is always a three-fold succession, in descending sequence, of till (from as little as 1 to as much as 8 ft. thick) on gravel and sand (4–15 ft. thick) on Upper Bunter Sandstone. The till undoubtedly overlaps the gravel and rests directly on the Bunter beyond the area of gravel working. Nowhere have we seen a till beneath the gravels, but it is significant that the latter, although almost entirely derived from Bunter Pebble Beds, contain rare pebbles of rhyolites, rhyolitic ashes and andesites, and also brown flints testifying to the presence, somewhere to the north, of an earlier source of Irish Sea material.

Interbedded with the gravels are layers of organic silt and peat, at the base in hollows on the Bunter Sandstone, and at various levels within the body of the gravels. Two peaty samples, taken from different layers within the gravels, have been dated at the Radiocarbon Laboratory here with the following results

Birm. 24    36,340 ± 770 years B.P.  
                  — 700

Birm. 25    30,655 ± 765 years B.P.  
                  — 700

These dates indicate that the gravels belong to the Mid-Würm or Upton Warren Interstadial<sup>3,5</sup>, a conclusion which I believe Dr Coope would have maintained in the absence of any dates, from a comparison of the large insect fauna from the organically rich layers with that from other

known sites of this interstadial. It therefore follows that the overlying till must be Late-Würm in age.

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## Nannofossils in Eocene Eugeosynclinal Limestones, Olympic Peninsula, Washington

LIMESTONE lenses occur within a thick sequence of Eocene volcanic rocks (known as the Crescent or Metehosin Formation) that border the northern, eastern and southern sides of the Olympic Peninsula in north-western Washington State. These carbonate rocks have previously been thought to have a largely inorganic origin<sup>1</sup>, but electron microscopy, which I report here, indicates the presence of abundant nannofossils and suggests a different origin.

The volcanic rocks are predominantly basalts, apparently principally the products of submarine eruptions, and they occur as pillow-lavas, flows, breccias and tuffs. Associated with them are discontinuous layers of argillite and hard, most commonly red to pink, and locally manganeseiferous limestone. Limestone bodies appear to be lensoid, between a few inches and 200 ft. thick, and with lateral dimensions from a few tens of feet to more than 2 miles. These bodies usually lie between volcanic layers, but limestone sometimes occurs within cores of basalt pillows or forms a matrix enclosing pillows. Limestone is also present as angular fragments in volcanic breccias and as xenolith like inclusions in basalt flows.

These close spatial associations of limestones and volcanics led Park<sup>1</sup> to propose a direct relationship between submarine volcanism and limestone genesis. Referring to an earlier hypothesis of Kania<sup>2</sup>, he suggested that agitation and heating of sea water above sites of submarine eruptions led to local inorganic precipitation of calcium carbonate, a process which was perhaps enhanced by release of calcium during early spilitization of the volcanic rocks.

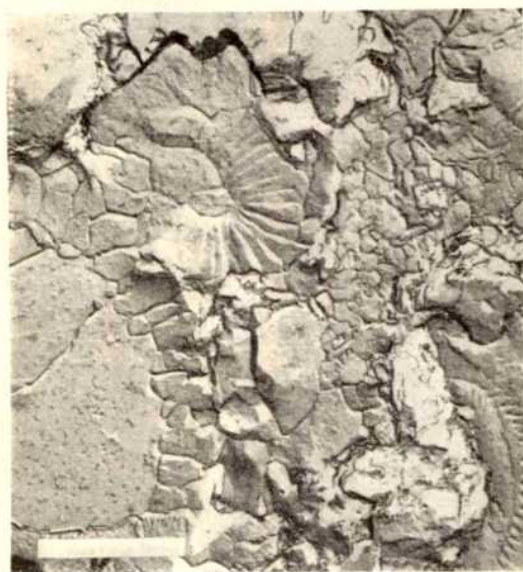
As seen in thin sections, many of the limestones comprise 5–15 per cent planktonic Foraminifera embedded in a fine-grained calcitic matrix. This matrix also contains a few dispersed coccospheres and pentoliths of the nannofossil genus *Braarudosphaera*, as well as tiny spherical shells which can be assigned to the coccolithophorid genus *Thoracosphaera*<sup>3</sup>. The remainder of the predominant matrix appears in thin sections and at high optical magnifications as a largely unresolvable mixture of abundant small calcite grains and smaller amounts of non-carbonate grains such as clay minerals, iron oxides, and secondary, microcrystalline quartz.

Electron microscopy confirms the presence of nannofossils and indicates that they are far more numerous than would be suspected from examination of thin sections alone. Ten limestone specimens from widely separated parts of the Olympic Peninsula were studied with the electron microscope using the two-stage peel-replica method of sample preparation<sup>4</sup>. Coccoliths and fragments of coccoliths (Fig. 1) are the most abundant form observed. Other common nannofossils are *Thoracosphaera* shells (Fig. 1a, left), *Braarudosphaera* pentoliths and discoasters (Fig. 1b). These forms are further illustrated by Fischer, Honjo and Garrison<sup>5</sup>. All of the specimens investigated contain abundant nannofossils, and the matrix in many comprises up to 60 per cent nannofossils with abundances of 3–4 × 10<sup>6</sup>/mm<sup>3</sup> of limestone. In some

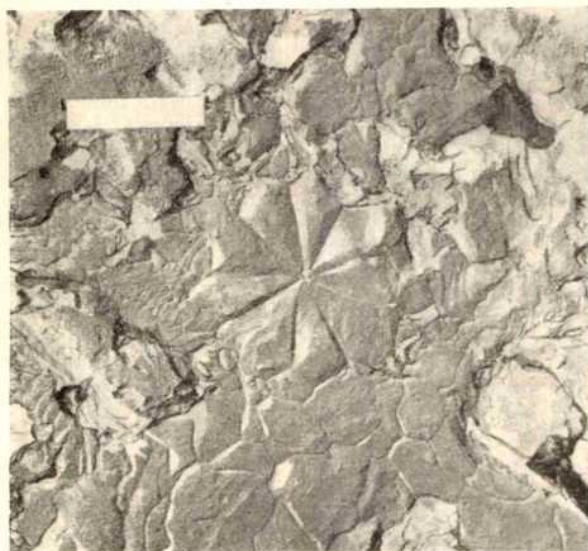


specimens recrystallization has partially destroyed many nannofossils, as is evident in Fig. 1a where the coccolith in the upper centre area is partly replaced.

The present work does not exclude the possibility that some of the calcite in the limestone specimens investigated was a primary inorganic precipitate. The observations so far, however, suggest that the limestones studied were pelagic oozes, composed chiefly of nannoplankton and planktonic foraminifers, and comparable with modern globigerinid oozes. Such oozes may have slowly accumulated on top of a submerged volcanic terrain during periods of volcanic quiescence. Subsequent, renewed volcanism apparently tended to disrupt the carbonate layers, incorporating them as matrix between pillows or as fragments within breccias and flows, and leaving only lensoid remnants of the original deposits.



a



b

Fig. 1a. Electron micrograph of an Eocene limestone, Olympic Peninsula, Washington. At left is part of a *Thoracosphaera* shell with blocky wall structure and interior filling of secondary sparry calcite. Toward the right are fragments of coccoliths, one of which (above centre) shows partial recrystallization. b. Well-preserved specimen of *Discoaster* cf. *D. barbadiensis* in centre. Fragments of coccoliths in surrounding grains and part of a *Thoracosphaera* shell at the bottom. (Bar scales indicate 5 $\mu$ .)

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## THE SOLID STATE

### High Pressure Transformations in Zinc Germanates and Silicates

THIS communication reports some observations of the behaviour of some zinc silicates and germanates at high pressures. The compounds investigated were  $\text{Zn}_2\text{SiO}_4$ ,  $\text{Zn}_2\text{GeO}_4$ , together with mixtures of pyroxene compositions— $\text{ZnGeO}_3$  and  $\text{ZnSiO}_3$ . The results are of a preliminary nature, and the principal objective of this communication is to record the occurrence of transformations in these compounds, and where possible to indicate the nature of these transformations.

The compounds  $\text{Zn}_2\text{GeO}_4$  and  $\text{Zn}_2\text{SiO}_4$  were prepared by intimately mixing the constituent oxides in the requisite proportions, pelletizing, and heating for 2–3 h at temperatures between 50° and 200° C below the respective solidus temperatures. After heat treatment, the tablets were crushed and checked for homogeneity by X-ray diffraction and microscopical examination in immersion liquids. If not homogeneous, the powders were remixed, pelletized and reheated. This procedure sufficed to produce homogeneous single phases. The runs on  $\text{ZnSiO}_3$  and  $\text{ZnGeO}_3$  compositions were carried out on unfired mixtures of ZnO with silicic acid and hydrous germania, respectively.

Samples so prepared were subjected to controlled high pressure-temperature conditions in two types of apparatus. The first was a piston-cylinder apparatus as described before<sup>1,2</sup>. All samples were subjected initially to a pressure of 35 kbar at 1,100° C for 2 h. If transformations were observed, the samples were run at successively lower pressures, but with the same temperature/time conditions. All samples were also subjected to desired pressures in the range 60–150 kbar at 900°  $\pm$  200° C for 5 min in a Bridgman anvil apparatus equipped with an internal heater<sup>3</sup>. After completion of runs, the samples were quenched under pressure, removed from the apparatus and examined by microscopic and X-ray diffraction methods. The following results were obtained.

For  $\text{Zn}_2\text{GeO}_4$ , which has the phenacite structure at atmospheric pressure there was no change at a pressure of 10 kbar, 1,100° C; however, at pressures of 20 kbar and 35 kbar (1,100° C) and at 110 kbar (900° C) it transformed completely to a slightly distorted spinel structure (pseudocubic lattice parameter 8.3 Å).

According to Hayashi *et al.*<sup>4,5</sup>  $\text{Zn}_2\text{SiO}_4$  (willemite), which also has the phenacite structure at atmospheric pressure, transforms to an olivine structure at 1,500° C and 42 kbar. We did not observe a transformation at 35 kbar, 1,100° C, but at 110 and 130 kbar, 900° C, there was complete transformation into a denser, birefringent phase. The maximum and minimum refractive indices of this phase were 1.75 and 1.77 ( $\pm$  0.005). The principal lattice spacings are given in Table 1. This phase may be



Table 1. X-RAY DIFFRACTION DATA FOR HIGH PRESSURE  $\text{Zn}_2\text{SiO}_4$  PHASES

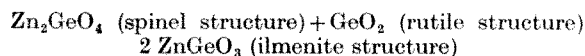
Phase A 110 kbar, 120 kbar, 900° C		Phase B 150 kbar, 900° C	
d Å	I	d Å	I
5.46	1	6.78	4
3.75	1	4.74	7
3.60	6	3.48	1
3.11	4	3.24	<1
2.972	5	2.870	<1
2.815	10	2.712	5
2.766	10	2.641	5
2.621	6	2.507	2
2.568	3	2.454	10
Four weak lines omitted		2.366	3
2.244	5	2.256	<1
		2.215	3
		2.094	3
		2.029	8
		Several weak lines omitted	
		1.458	7
		1.435	3

I, Estimated relative visual intensities.

an olivine, but the resemblance of the X-ray diffraction pattern to those of other olivines is not striking. A further run was carried out on  $\text{Zn}_2\text{SiO}_4$  at a higher pressure—150 kbar. Complete transformation into an even denser birefringent phase with a mean refractive index of about 1.87 was observed. From the difference of refractive indices, this phase is estimated to be about 23 per cent denser than willemite. The X-ray diffraction pattern is complex—the spacings of the principal reflexions are given in Table 1.

A mixture of  $\text{ZnO}$  and  $\text{SiO}_2$  in equimolar proportions crystallizes at one atmosphere to  $\text{Zn}_2\text{SiO}_4$  (willemite) +  $\text{SiO}_2$ , but at 30 kbar at 500° C. Hayashi *et al.*<sup>4,5</sup> reported that the mixture crystallizes to a pyroxene structure. We have confirmed this synthesis. At 35 kbar, 1,100° C, and at 120 kbar, 900° C, the mixture crystallized to a pyroxene like structure.

An oxide mixture of  $\text{ZnGeO}_3$  crystallizes at one atmosphere to  $\text{Zn}_2\text{GeO}_4$  (phenacite structure) +  $\text{GeO}_2$ . This was also found to be the stable assemblage at 10 kbar, 1,100° C, but at 20 kbar, 35 kbar (1,100° C) the mixture crystallized to  $\text{Zn}_2\text{GeO}_4$  (spinel structure) +  $\text{GeO}_2$  (rutile structure). A further transformation was found in the 110 kbar, 900° C run. The  $\text{ZnGeO}_3$  composition was found to crystallize completely to an ilmenite structure, similar to the high pressure modifications of  $\text{MgGeO}_3$  and  $\text{MnGeO}_3$ . Thus, in this case, we have the reaction



occurring with increase of pressure from 35 kbar to 110 kbar.

These results contribute towards a broader understanding of the behaviour at high pressures of  $\text{M}(\text{SiGe})\text{O}_3$  and  $\text{M}_2(\text{SiGe})\text{O}_4$  compounds ( $\text{M} = \text{Mg}, \text{Fe}, \text{Ni}, \text{Co}, \text{Mn}, \text{Ca}, \text{Cd}, \text{Zn}$ ) which have formed the subject of many previous investigations in this laboratory<sup>6</sup>. Nearly all these compounds which have been investigated so far display major phase transformations at high pressures. A systematic understanding of these transformations has important applications to studies of the constitution of the Earth's mantle.

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<sup>5</sup> *J. Amer. Ceram. Soc. Abstracts*, 26 (January 1967).

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## PHYSICS

### Aging and Degradation in Dilute Polymer Solutions

WE have studied the properties of dilute solutions of polymers which have been found to reduce friction in turbulent flows<sup>1</sup>. The substances tested, in solution in tap water, were guar gum, polyethylene oxide (Union Carbide 'Polyox' WSR 301) and polyacrylamide (Dow 'Separan' AP 30). It had been previously found that solutions of 'Polyox' and 'Separan' exhibit normal-stress difference effects<sup>2</sup>. Correspondingly for 'Polyox' solutions, it has been reported<sup>3</sup> that the pressure increment at the mouth of a pitot tube, or open-ended tube facing into the flow, can be reduced below the value  $\frac{1}{2}\rho V^2$  normal for Newtonian liquids, where  $\rho$  is density and  $V$  is speed. The present experiments began as a further investigation of this loss of pitot pressure. It was found that the pitot loss reduced with time, and that with 'Polyox' solutions other normal-stress difference effects showed a similar aging, without apparently impairing the ability of the liquid to produce turbulent drag reduction. Continued exposure of the liquid to intense turbulence does, however, lead to a degradation of the drag reduction.

The apparatus used was as in Fig. 1. Liquid in a header tank *H* could have an air pressure *p*, positive or negative relative to the atmosphere, applied to its upper surface. The liquid descended through a nozzle *N* and emerged through a smoothly rounded orifice *O*, of 2.5 mm narrowest diameter, to form a jet, which flowed over a pitot tube, of 0.9 mm outside diameter, and then into a receiver tank *R*, from which the fluid was returned by a peristaltic pump to the header tank. The liquid pressure in the header tank was observed in an open-ended manometer tube, in which the liquid level rose, of course, by an amount *p* above the level in the header tank. The level to which the liquid rose in a manometer tube attached to the pitot enabled the pitot loss *L* to be determined as a fraction of the true pitot pressure *P*.

Typical results are shown in Figs. 2 and 3. In Fig. 2 results for two solutions of 'Polyox', both of 70 p.p.m. concentration, are shown. One was tested 4 days after mixing, and the other within 3 h of mixing. Pitot loss is shown as a fraction of time after starting up the flow in the apparatus, with *P* held constant at 34 cm ( $V = 260$  cm/sec in the jet at the pitot mouth). The liquid level in the pitot manometer tube was made equal to that in the header tank tube at the beginning. The time taken for *L* to reach a maximum represents the response time for adjustment of the pitot manometer level by outflow through the small bore (0.6 mm diameter) of the pitot tube. The maximum loss with the fresher solution is greater than that with the older, but the aging process is evidently accelerated by the flow in the apparatus.

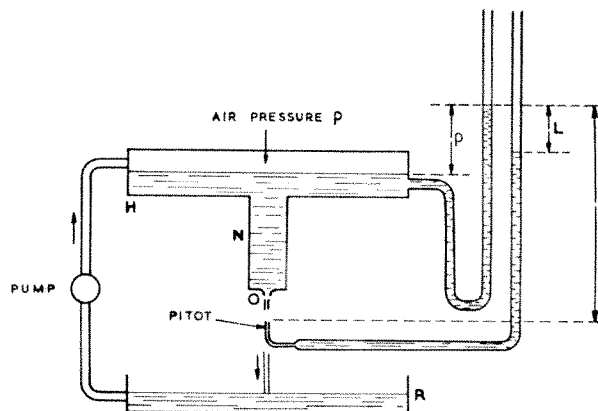


Fig. 1. Pitot tube apparatus.

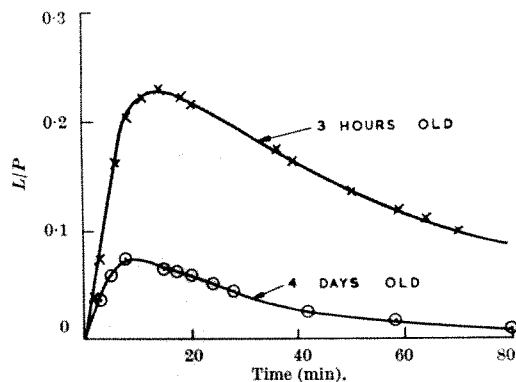


Fig. 2. Pitot loss as a function of time.

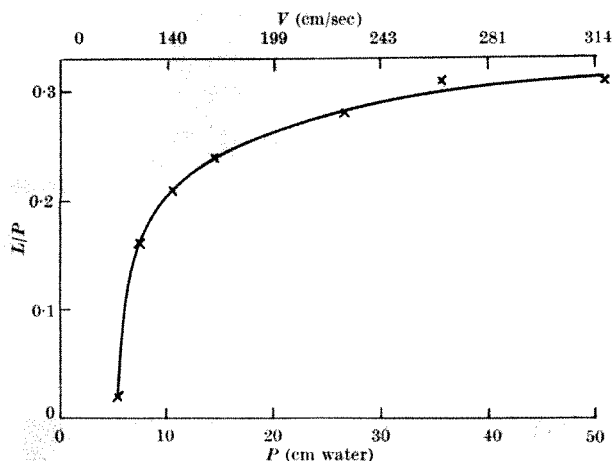


Fig. 3. Pitot loss as a function of speed.

More dilute 'Polyox', of 20 p.p.m. concentration, shows only a very small pitot loss even when fresh, but at 50 p.p.m. concentration the loss is about half as great as at 70 p.p.m. Results for 'Separan' solutions of 100 and 130 p.p.m. concentration are similar to those for 'Polyox', except that the pitot loss does not diminish with aging to zero, but attains a final limiting value smaller than the maximum for fresh solution. Guar gum solution of 160 p.p.m. concentration shows no pitot loss at all.

For 70 p.p.m. 'Polyox' solution tested within 2 h of mixing the maximum pitot loss is shown as a function of pitot pressure  $P$ , or equivalently of jet speed  $V$ , in Fig. 3. At very low speeds there is no effect. Goren<sup>3</sup> suggests that at higher speeds than those of the present experiments the pitot loss may fall off, to diminish to zero at sufficiently high speeds. The reduction of pitot loss, however, with increasing speed which Goren<sup>3</sup> reported might be due to accelerated aging at the higher speeds in the turbulent flows of those experiments.

Turbulent drag reduction was measured in the rotating wheel rig we described elsewhere<sup>4</sup>. A flanged wheel rotates in a casing containing liquid, and the frictional torque exerted on the casing is measured and compared with the torque for the same speed of rotation when the liquid is replaced by the same amount of water. 'Polyox' solutions, aged in the pitot rig so as to show no remaining pitot loss, produced just as much drag reduction as freshly mixed solutions, with no greater tendency to degradation with time.

The viscosities of various solutions, some of which had been aged in the pitot rig of Fig. 1, and others of which had been degraded in the turbulent friction apparatus, were measured. A capillary instrument was used in which the wall shear rate was about 1,200 reciprocal seconds. Results are shown in Table 1. The measured viscosities can be compared with the value of 1.0038 centistokes

Table 1. VISCOSITIES OF VARIOUS SOLUTIONS

Case No.	Fluid	Kinematic viscosity (centistokes) at 20° C
1	130 p.p.m. 'Separan' aged in pitot rig	1.158
2	Same mixture not put into pitot rig	1.162
3	70 p.p.m. 'Polyox' aged in pitot rig	1.056
4	Same mixture not put into pitot rig	1.055
5	70 p.p.m. 'Polyox' degraded in turbulent flow rig	1.036
6	Same mixture not put into turbulent flow rig	1.056
7	70 p.p.m. 'Polyox' filtered 1 h after mixing, and viscosity of filtrate measured 5 h after mixing	1.081
8	Same fluid, 5 days later	1.064

for distilled water at 20° C. Concentrations quoted are only approximate.

In cases 1 to 6, the fluids were stored at rest for 5 or more days before the measurements were made. Evidently, aging in the pitot rig does not make the viscosity any different from that of liquid kept for several days at rest, but degradation in turbulent flow (so that the resulting fluid produces much less drag reduction than when fresh) reduces viscosity, probably as a result of molecular scission. Fresh solution has a higher viscosity than aged solution.

The apparent viscosity of both fresh and aged solutions in filtration is much greater than the figures given here. It was measured by the following procedure: 50 ml. of water was put into a funnel lined with wet fine grade filter paper, and the time for the first 30 ml. to pass through was found. When all the water had drained through, 50 ml. of the solution at the same temperature was put in and the time for the first 30 ml. to pass through again was found. The average value from several tests of the ratio of these times is the apparent viscosity ratio, because for a completely homogeneous fluid containing nothing to block the filter pores the time of filtration would be proportional to viscosity. For fresh 70 p.p.m. 'Polyox' solution, which had been previously filtered once to remove any undissolved impurities, the ratio was about 2.3, and for the same solution, aged either by leaving it to stand for five days or by circulating it in the pitot rig for three hours, it was about 1.4. After degradation in the turbulent flow rig, so that the drag-reducing effectiveness was only a quarter of its initial value, the ratio was about 1.1. The average filter pore size is of the order of  $3\mu$ , so at this scale the fluid evidently cannot be regarded as a continuum with the same rheological properties as for larger-scale flows. Presumably molecules or molecular aggregates of a size comparable with the pore diameter are present in the fluid.

The second normal-stress difference, between directions perpendicular to the streamlines, was investigated with the concentric-tube instrument we described elsewhere<sup>2</sup>. It had been found that 'Polyox' solutions, unlike solutions of guar gum or 'Separan', exhibited a significant second normal-stress difference. Further tests have now shown that this difference is less for solutions mixed 1-3 days previously than for freshly mixed solutions, while solutions aged in the pitot rig, so as to have zero pitot loss, also have zero normal-stress difference.

As described earlier<sup>4</sup>, a laminar jet of coloured 'Polyox' solution emerging from a capillary tube into uncoloured fluid and impinging obliquely on a flat surface may remain as a narrow jet over this surface, whereas a corresponding water jet spreads out into a wedge-shaped region. It now appears that narrow jets, probably attributable<sup>5</sup> to non-Newtonian tensile stresses along the streamlines, are characteristic only of fresh 'Polyox' solutions, for aged 70 p.p.m. solution has been found to behave like water. Thus here is another instance where non-Newtonian effects disappear with aging.

We have noted<sup>4</sup> that jets of coloured 'Polyox' solution squirted from a fine bore capillary into uncoloured 'Polyox' solution, so that the initially laminar jet became turbulent, in some cases showed a greatly modified turbulence structure. Other experiments on turbulent jets<sup>3,5,6</sup> at higher Reynolds numbers have suggested, however, that

the turbulence is unaffected by the additive. This discrepancy may be due to accelerated aging in these flows at higher Reynolds number which are initially turbulent rather than laminar, as it has now been found that even initially laminar jets of 'Polyox' solution, aged in the pitot rig, show much less drastically modified turbulence than jets of fresh solution.

Many of the anomalous non-Newtonian effects found for some dilute polymer solutions seem to diminish or disappear with aging, which may be accelerated by gentle mixing such as that which takes place in the pitot apparatus. This aging may simply represent a more complete dissolving of the additive, though Pruitt *et al.*<sup>7</sup> attributed it to polymer adsorption on the walls of the containing vessel, and Shin<sup>8</sup> to biological attack. Shin<sup>8</sup>, however, showed that while biological degradation could evidently be important over longer time periods, for periods of less than a week, over which large reductions of viscosity could occur, the turbulent drag reduction was little affected, which is in agreement with the present findings. The suggestion of polymer adsorption also seems hard to reconcile with undiminished turbulent drag reduction, which is more consistent with the suggestion, made to explain certain anomalous turbulence measurements by Fabula<sup>9</sup>, that molecular aggregates occur in freshly mixed solutions but tend to break up with aging. These molecular aggregates would appear to be the cause of some normal-stress difference effects in fresh solutions. 'Polyox' solution, which for the concentration range 50–100 p.p.m. shows marked non-Newtonian effects when fresh, is, when aged and thoroughly dissolved, not very different from water in all viscometric and rheogoniometric measurements so far tried, except those for which the flow passages are only a few microns wide. Thus it may well be impossible to find constitutive equations which are significantly non-Newtonian for such aged liquids. Because these liquids are still fully effective in producing turbulent drag reduction, it would seem to be difficult to explain that phenomenon in terms of non-Newtonian equations.

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### Anomalies in Detonation Initiation of Liquid Explosives

ATTENTION has recently been directed to the study of low velocity detonations (LVD) (refs. 1 and 2), which propagate at velocities only slightly supersonic with respect to the unreacted liquid and may be very easily initiated in some liquids. It is the purpose of this communication to show that both the shape and material of the container and the presence of a witness plate affect LVD initiation. Theories proposed for LVD (refs. 1–3) require that the container sound speed be greater than that of the liquid explosive. We have tested this prediction by using lead tubes with 1,2-difluoroaminopropane, a liquid known to sustain normal and low velocity detonations<sup>4</sup>. Because of the low sound velocity, we expected that weak initiating shocks would not yield LVD in lead though identical shocks would in steel. Unexpectedly, there was definite

evidence of LVD propagation. The experiments were repeated and photographed with a high speed framing camera. The lead tubes used were 1.27 cm inner diameter  $\times$  0.635 cm wall  $\times$  10.4 cm long, and the shock source consisted of an exploding bridge-wire detonator, a small ( $\sim 1$  g) RDX pellet, two pressed tetryl pellets (each 3.87 cm diameter  $\times$  2.54 cm long; density  $\sim 1.51$  g/cc; weight  $\sim 90$  g), and a 'Plexiglas' attenuator (3.87 cm diameter  $\times$  30.5 cm long). The shock strength at the end of the attenuator was about 1 kbar compared with about 60 kbar required to initiate normal detonation.

Frames selected from the photographic record are shown in Fig. 1. The experiment was backlit with an electronic flashgun and the principal features appear as shadows. These include a transparent ruler and a notched strip to serve as fiducial markers, the lead tube (the large black rectangle in the upper half of each frame), and the 'Plexiglas'. Because of its shape the 'Plexiglas' transmitted a thin strip of light parallel to its axis. Irregularities at the 'Plexiglas'–lead interface arise from bits of cement used in assembling the shot. Numbers below each frame are the lapsed time from initiation of the detonator. Fig. 1(a) shows the still unshocked system. From independent measurements the time at which the shock reaches the 'Plexiglas'–lead interface is known. Then the lead expands and wrinkles (Fig. 1(b)). The air shock from the donor (marked by arrows) is apparent in the discontinuities across the ruler and the light strip along the 'Plexiglas' axis. (This situation may not be evident in the half-tone; a limited number of photographic prints are available from the author on request.) At 267  $\mu$ sec after initiation (Fig. 1(c)) the air shock has reached the lead which has now separated from the 'Plexiglas'. The

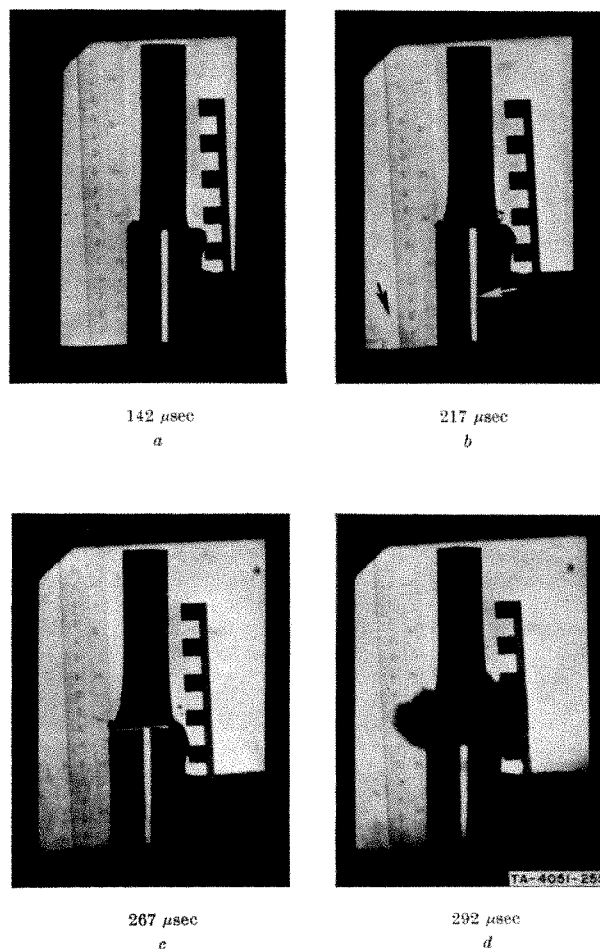


Fig. 1. Low velocity detonation in lead.

subsequent detonation is detectable in the very next frame but is more apparent at 292  $\mu$ sec (Fig. 1(d)).

These experiments show that a second mechanism may also produce LVD. In the low yield strength lead containers the imposed weak shock caused tube expansion and fragmentation of the liquid; the subsequent reaction is induced by the air shock. This sequence is similar to that proposed elsewhere<sup>2</sup> except that a high sound speed container is not required.

Experiments employing very weak shocks (too gentle to initiate either normal detonation or Mach zone LVD), steel tubes and thin steel witness plates were photographed in the same fashion. Still another mechanism of LVD initiation was discovered. It was calculated that the weak shock travelled barely supersonically along the container walls and was reflected by the terminal witness plate; on reaching the attenuator-liquid interface, LVD initiated and propagated in the direction of the original shock. LVD did not occur when the same tests were run without the witness plate.

In summary, LVD in high sound speed containers may arise from shock wave (Mach) interactions along the charge axis<sup>4</sup>, or reflexion of the shock wave from the witness plate. In low yield strength containers, LVD may be initiated subsequent to tube expansion, liquid fragmentation and air shock arrival.

Because of these many causes of detonation propagation, the results of routine detonability testing<sup>5</sup> are of limited value in establishing safe handling criteria.

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### Theoretical Collision Efficiencies for Small Cloud Droplets in Stokes Flow

WHEN a cloud forms in the atmosphere, water vapour condenses on aerosol particles to form a relatively homogeneous distribution of small droplets, typically a few microns in radius. A principal problem in cloud physics is to understand the growth of droplets from this initial stage into drizzle drops with radii of several hundred microns. Under many conditions the most important growth process is that of collision and coalescence; in warm clouds all droplet growth subsequent to condensation proceeds in this way. It is therefore reasonable to emphasize the coalescence growth mechanism in numerical cloud models<sup>1-3</sup>.

The most widely accepted theory of small cloud droplet collisions under electrically neutral conditions is that of Hocking<sup>4</sup>, who treated interacting cloud droplets as spheres sedimenting in still air under conditions of stationary Stokes flow. One striking result of his analysis was the prediction that no collisions between cloud droplets can occur if both radii are less than 19 $\mu$ . But because the droplet population after initial condensation very often fails to contain a significant number of droplets with radii larger than this cut-off, it is difficult to understand how the droplet growth process gets started<sup>5</sup>. In order to

grow large drops in numerical cloud models it has been necessary to postulate an initial distribution containing droplets with radii larger than 19 $\mu$  or to provide some *ad hoc* mechanism to initiate the coalescence process.

We have re-examined the theory of the collisions of small cloud droplets, assuming, with Hocking<sup>4</sup>, that steady Stokes flow conditions apply. In contrast with Hocking's results, we do not find any cut-off in droplet collisions, even for droplets with radii as small as 5 $\mu$ .

We treated the problem of small droplet collisions by assuming that for droplets with radii smaller than 30 $\mu$  the steady-state Stokes hydrodynamic theory would be adequate to determine drag forces. The droplets were assumed to be rigid spheres, which should be valid for the very small droplets of interest, because of their high internal fluid pressure. Electrical effects were omitted. Collision efficiencies were computed by integrating the equations of motion of pairs of droplets sedimenting in still air;  $y_c$ , the initial horizontal off-set resulting in a grazing trajectory, was found by a successive approximation process, as in Hocking<sup>4</sup>. A "collision" was taken, by convention, to be any approach to within 0.001 times the radius, or less, of the larger droplet. This procedure took account approximately of short-range forces at small separations of the droplet surfaces; it was found that the results were relatively insensitive to the exact cut-off distance used.

We have developed independently the drag forces on the two spheres. One approach (RAND) was to use the Stimson and Jeffery<sup>6</sup> and Maude<sup>7</sup> solution for the component of motion along the line of centres, together with Hocking's expressions, corrected for the effect of rotation, for motion perpendicular to the lines of centres. A preliminary report<sup>8</sup> on this approach has pointed out that several of Hocking's drag expressions are in error when the spheres are close together. The other method (NCAR) was to extend Hocking's expressions by including many more terms, giving particular care to considerations of numerical stability. We used these forces in separate integration routines to derive collision efficiencies<sup>\*</sup>. Our separate calculations predict collision efficiencies that differ from each other by no more than a few per cent.

Our results (from the RAND calculations) appear in Fig. 1, which also shows Hocking's results for 30 $\mu$  and 20 $\mu$  drops for comparison (he predicted no collisions for 10 $\mu$  drops). Differences include the disappearance in our results of the 19 $\mu$  cut-off, as well as the prediction of finite collision efficiencies both for droplets that differ considerably in size and for those which are nearly the same size; in fact our calculations showed finite collision efficiencies for all values of  $(b/a) > 0$ . Another difference evident in Fig. 1 is that the maxima of our collision efficiency curves are systematically somewhat smaller than Hocking's for  $a \geq 20\mu$ . We do not have a qualitative explanation for the confluence of our curves to a finite value for  $b/a \rightarrow 1.0$ .

Numerical studies suggest that Hocking would not have found a 19 $\mu$  cut-off in collision efficiencies had he used the Stimson-Jeffery<sup>6</sup> solution for that part of the problem which they treated, rather than his own solution (or if he had extended his expressions to a higher order). We are confident that the Stokes hydrodynamic theory does not predict such a cut-off for the Stimson-Jeffery theory is well substantiated both theoretically and experimentally<sup>9</sup>. Sensitivity studies indicate that varying such parameters as the cut-off distance used as a collision criterion can lead to differences in computed collision efficiencies of up to 20 per cent of the reported value. Although we believe that our values plotted in Fig. 1 are more reliable than this, it is possible that further studies may show discrepancies of this magnitude. Application of these results to studies of droplet growth requires knowledge of the

\* The definition of collision efficiency used here is that used by Hocking:  $(y_c/a)^2$ , the square of the ratio of the initial horizontal off-set for a grazing trajectory to the radius of the larger drop.



## CHEMISTRY

## Gas-Solid Chromatography on Organic Crystals

THE full potential of gas-solid chromatography has not yet been revealed. Theoretically it could be used to obtain much greater selectivity and higher efficiency than gas-liquid chromatography<sup>1-4</sup>. So far the characteristics required of an adsorbent have limited the use of gas-solid chromatography.

For successful analyses an adsorbent should have a homogeneous surface, a relatively small adsorption enthalpy, a convenient surface area and should be easily prepared. A heterogeneous surface will give rise to tailing peaks. A large adsorption enthalpy requires that separation be performed at a high temperature, when the thermal stability of the compounds may be poor. If the surface area is too large the result is the same and if it is too small, efficiency is poor. Usually a specific surface area of 4 to 10 m<sup>2</sup>/g is satisfactory.

Until recently only graphitized carbon black, which has a high specificity in various separations, met these requirements<sup>2,3,5</sup>. This contrasts with the versatility of gas-liquid chromatography using several hundred stationary phases. If gas-solid chromatography is to develop further a method for preparing large numbers of adsorbents which fulfil the requirements for high efficiency must be found. We propose that crystallized organic compounds coated on graphitized carbon black be used for preparing suitable adsorbents of various selectivities for gas-solid chromatography.

Many organic compounds have a high melting point, a small vapour pressure and show good thermal stability. Phthalocyanines, quinaeridones and other types of dyes—polynuclear hydrocarbons—can withstand temperatures greater than 300° C, and only sublime slowly. If lower temperatures only are required there are hundreds of possible compounds.

It is impossible, however, to use columns packed with pure organic crystals: crystals of most compounds have

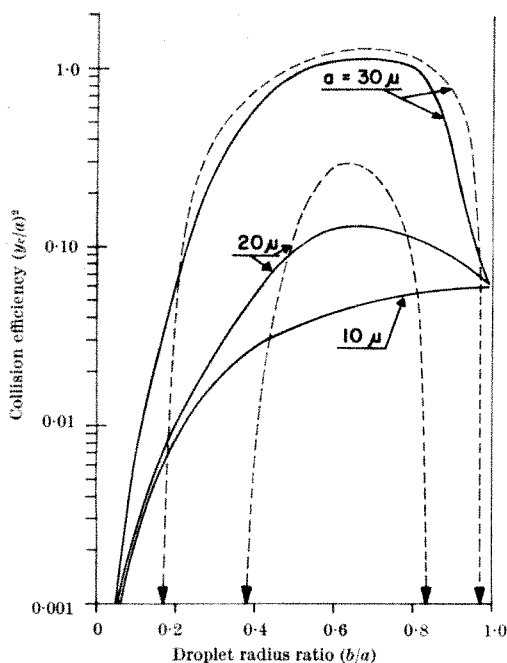


Fig. 1. Collision efficiency,  $(y/a)^2$ , versus the droplet radius ratio,  $(b/a)$ , for several values of the larger radius,  $a$ . —, Present results; ---, Hocking (ref. 4). The arrowheads at the points where Hocking's curves touch the bottom of the figure indicate that his curves go to zero at these points.

probability that two colliding droplets will coalesce. The available evidence suggests that coalescence probability is near unity for small cloud droplets<sup>10</sup>, but the question merits further investigation. Probably the most important reservation about our results comes from our use of time-independent Stokes hydrodynamics. Further improvement awaits a more general solution for fluid flow around two spheres.

Recent experimental studies of small droplet collection efficiencies (collision followed by coalescence) are summarized in the paper of Woods and Mason<sup>10</sup>. They were able to set an approximate upper limit experimentally on collection efficiencies for droplets with radii 17–19  $\mu$  interacting with droplets of radii 10–14  $\mu$ . Their limit of 0.1 (expressed according to our definition of a "collision efficiency") does not disagree with our findings, for we predict a finite collision efficiency of about 0.1 for this case. Our findings are also in qualitative agreement with their observation of finite collection efficiencies for  $(b/a) \leq 0.1$ .

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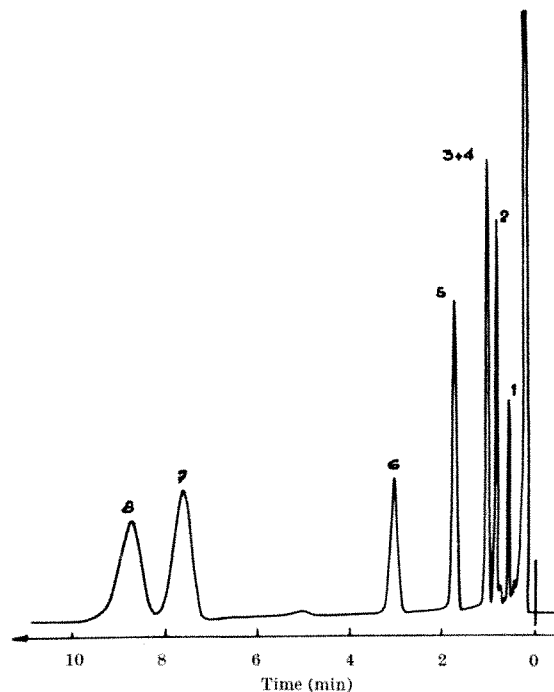


Fig. 1. Analysis of a mixture of polynuclear aromatic hydrocarbons. The column was 1 m long with an internal diameter of 2 mm, and was packed with 1 per cent copper phthalocyanine on graphitized carbon black Sterling M T (Cabot). Temperature 265° C. Carrier gas was hydrogen, with an outlet velocity of 25 cm/sec. (1) Naphthalene; (2) diphenyl; (3) 1-methylnaphthalene; (4) 2-methylnaphthalene; (5) acenaphthene; (6) fluorene; (7) phenanthrene; (8) anthracene.

no mechanical stability; their surface area is very small, which gives very small column efficiency and loadability, as shown by the previous results with crystallized copper amine complexes and several pure phthalic derivatives used as column packing<sup>6-9</sup>.

A solid support with a function similar to that of a catalyst is needed. In gas-solid chromatography, however, it is impossible for the solid support to be completely inert, and we can only try to minimize its influence. Because surface homogeneity is essential graphitized thermal carbon black is much better than 'Chromosorb' for the analysis of polar compounds, as shown by the results obtained with 'Chromosorb' coated with benzophenone<sup>10,11</sup> and copper amine complexes<sup>12</sup>.

Excellent chromatographic results, however, can be obtained with anthraquinone<sup>13</sup> or copper phthalocyanine (Fig. 1) coated on graphitized carbon black. With the latter compound high efficiency has been obtained for various phenols<sup>14</sup>, aniline and nitrobenzene.

The high retention of anthracene relative to phenanthrene (1-16 at 265° C) is also unusual as is the high specificity to xylene isomers, which is much larger than the specificity of uncoated carbon black. The column is stable at 300° C for several weeks at least.

Results obtained with columns of other metal phthalocyanines are similar to previous ones<sup>15</sup>, but with a higher specificity, better thermal stability and more efficiency.

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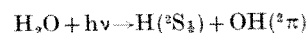
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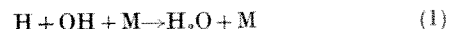
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### Rate of H-abstraction by OH from Hydrocarbons

FLASH photolysis of mixtures of water and argon can produce hydroxyl radicals with a spectroscopically detectable lifetime sufficient to allow kinetic investigations to be carried out. This was achieved by Black and Porter<sup>1</sup> and Greiner<sup>2,3</sup> with a vacuum ultra-violet flash photolysis apparatus; in our case a standard apparatus was modified by using a flash lamp and a reaction vessel made of Spectrosil quartz (which transmits ultra-violet radiation down to 1620 Å (ref. 4)) and by flowing dried nitrogen through the reflector for about 1 h before and during each experiment to displace atmospheric oxygen and water vapour. Water was used as the precursor of hydroxyl radicals because H-abstraction by OH cannot affect the OH concentration, whereas H-abstraction from any other substrate results in rapid OH-decay. Furthermore, the system is simple, because radiation absorbed by the first absorption region of water vapour (1400-1850 Å) causes only one photochemical reaction<sup>1,5,6</sup>.



The following reactions of OH can then occur



Black and Porter<sup>1</sup> determined third order rate constants in various gases for reactions (1) and (2) at room temperature. The relative values for these rate constants are valid, but the absolute values are low because they were based on  $k_1 + 2k_2 = 1,400 \text{ mm}^{-2} \text{ sec}^{-1}$ , obtained by Oldenberg and Rieke<sup>7</sup>, who used an electrical discharge through water as the source of hydroxyl radicals in a flow system. Kaufman and Del Greco<sup>8</sup> have shown that such a discharge is a poor source of OH, as the observed OH was produced not only in the discharge, but throughout the length of the flow system, probably by the reactions  $\text{H} + \text{O}_2 + \text{M} \rightarrow \text{HO}_2 + \text{M}$  and  $\text{H} + \text{HO}_2 \rightarrow 2\text{OH}$ . Absolute rate constants for reactions (1) and (2) have recently been obtained by Caldwell and Back<sup>9</sup> by comparing the rates of these reactions with the rate of reaction (3), as determined by Del Greco and Kaufman<sup>10</sup>. Bearing in mind that the correction factor derived by Caldwell and Back<sup>9</sup> is too high by a factor of 2, because the rate constant  $k_3$  was defined differently by Black and Porter<sup>1</sup> and Del Greco and Kaufman<sup>10</sup>, it is possible to calculate the overall rate constant for the decay of OH by reactions (1)-(3) after flash photolysis of mixtures of water and argon at room temperature.

We have found that flash photolysis of 8 mm H<sub>2</sub>O/300 mm Ar mixtures produced OH which underwent second order decay with a detectable lifetime of greater than 2 msec (see Fig. 1). The relative concentrations of OH were determined from OH spectral intensities, using a calibration curve prepared by the conventional two path length method<sup>11</sup>. From the gradient of a second order plot and the calculated total rate constant at 25° C

$$\{k_1(\text{Ar}) + 2k_2(\text{Ar})\} [\text{Ar}] + \{k_1(\text{H}_2\text{O}) + 2k_2(\text{H}_2\text{O})\} [\text{H}_2\text{O}] + 2k_3 = 2.2 \times 10^{10} \text{ l. mole}^{-1} \text{ sec}^{-1} \quad (i)$$

the OH concentration at 100 μsec was determined as  $5.5 \times 10^{-7} \text{ mole l.}^{-1}$ . The decay of hydroxyl radicals was studied as a function of temperature in the range 25°-150° C

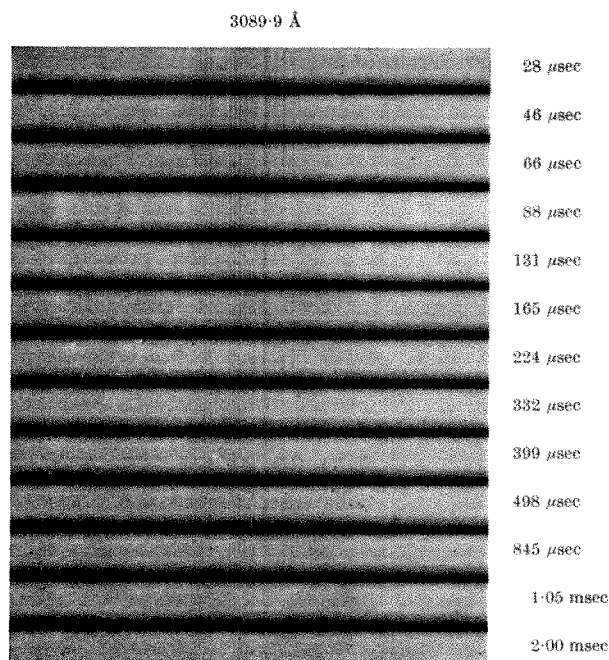
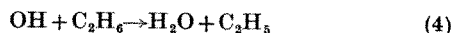


Fig. 1. Decay of OH after flash photolysis of H<sub>2</sub>O (8 mm) and Ar (300 mm) at 23° C.

and the resultant Arrhenius plot of the overall rate constant was found to be curved. The "activation energy" was approximately  $-2$  kcal/mole at  $25^\circ\text{C}$  and between  $-1$  and  $0$  kcal/mole at  $150^\circ\text{C}$ . The curvature of this plot arises because of the opposition of reactions (1) and (2), which have negative activation energies, and reaction (3), which has a positive activation energy,  $E_3 = 1.0 \pm 0.5$  kcal/mole<sup>12</sup>. The relative magnitudes of the various terms in equation (i) show that the contribution from the third order reactions (1) and (2) to the overall decay rate at  $25^\circ\text{C}$  is 93 per cent, whereas at  $150^\circ\text{C}$  reactions (1) and (2) are relatively less important, though still predominant as shown by the negative temperature coefficient.

These results characterize the water/argon system, and so it is now possible to investigate the reaction of hydroxyl radicals with certain hydrocarbons by photolysing  $\text{RH}/\text{H}_2\text{O}/\text{Ar}$  mixtures. We wish to report here an estimate of the rate constant for the reaction



The addition of less than 1 mm  $\text{C}_2\text{H}_6$  to 8 mm  $\text{H}_2\text{O}/300$  mm Ar mixtures at room temperature resulted in a greatly increased rate of OH decay which was close to first order. Reactions of radicals derived from ethane are excluded because ethane does not absorb light above  $1620 \text{ \AA}$  (ref. 13), the limit of Spectrosil transmission<sup>4</sup>.

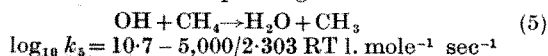
The decay of OH is therefore adequately described by the kinetic equation

$$-\frac{d[\text{OH}]}{dt} = k_1[\text{OH}][\text{C}_2\text{H}_6] + \left\{ k_1(\text{Ar}) + 2k_2(\text{Ar}) \right\} [\text{Ar}] + \left\{ k_1(\text{H}_2\text{O}) + 2k_2(\text{H}_2\text{O}) \right\} [\text{H}_2\text{O}] + 2k_3 [\text{OH}]^2$$

The results were plotted in the form  $-d \ln[\text{OH}]/dt$  v.  $[\text{OH}]$ . Fig. 2 shows such a plot for the decay of OH at  $25^\circ\text{C}$ . Because the rate constant for the decay of OH by reactions (1)–(3) at  $25^\circ\text{C}$  had already been calculated as  $2.2 \times 10^{10} \text{ l. mole}^{-1} \text{ sec}^{-1}$ , see equation (i), insertion of this value for the gradient in Fig. 2 reduced the error in determining the intercept. Similar plots were constructed for the decay of OH in  $\text{C}_2\text{H}_6/\text{H}_2\text{O}/\text{Ar}$  mixtures in the temperature range  $25^\circ\text{--}150^\circ\text{C}$ . At each temperature, the previously determined rate constant for the decay of OH by reactions (1)–(3) was inserted as the gradient. The values of  $k_4$ , obtained from the intercepts of these plots, gave a linear Arrhenius plot and  $k_4$  was determined as

$$\log_{10} k_4 = (11.1 \pm 0.7) - (3,600 \pm 600)/2.303 \text{ RT l. mole}^{-1} \text{ sec}^{-1}$$

Similar but less numerous experiments on the methane/water/argon system led to an approximate estimate of the rate constant for the corresponding reaction



These values for  $k_4$  and  $k_5$  are significantly different from those reported by Avramenko and Kolesnikova<sup>14</sup>,

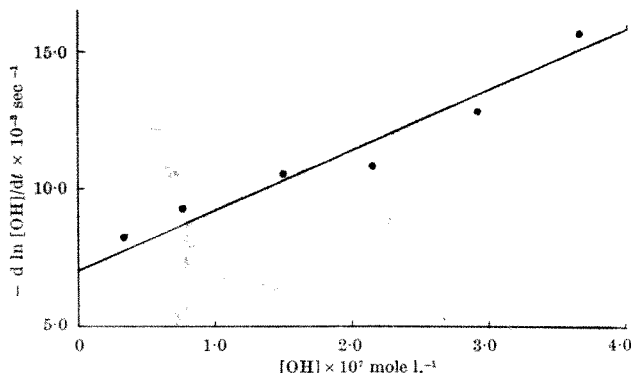


Fig. 2.  $-\frac{d \ln [\text{OH}]}{dt}$  plotted against  $[\text{OH}]$  for a mixture of 0.4 mm  $\text{C}_2\text{H}_6$ , 8 mm  $\text{H}_2\text{O}$  and 300 mm Ar at  $25^\circ\text{C}$ .

$\log_{10} k_4 = 11.1 - 5,500/2.303 \text{ RT l. mole}^{-1} \text{ sec}^{-1}$  and  $\log_{10} k_5 = 11.4 - 8,300/2.303 \text{ RT l. mole}^{-1} \text{ sec}^{-1}$ . They used, however, an electrical discharge through water as the source of OH radicals, a system which has been criticized by Kaufman and Del Greco<sup>8</sup>. On the other hand, the values at room temperature obtained by Greiner<sup>2,3</sup>,  $k_4 = 1.76 \times 10^{11} \text{ l. mole}^{-1} \text{ sec}^{-1}$  and  $k_5 = 5.3 \times 10^9 \text{ l. mole}^{-1} \text{ sec}^{-1}$ , are 40 per cent and 55 per cent lower than the results presented here.

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## BIOCHEMISTRY

### Counter-current Distribution of Rapidly Labelled RNA from Rats treated with Hormones

WE have failed to confirm the report<sup>1</sup> that the injection of several hormones into rats is followed by rapid and specific alterations in the profile obtained by counter-current fractionation of "rapidly labelled RNA" from the liver. We consider that the alterations of profile described could have resulted from slight differences in the procedures for isolating RNA from experimental and control rats. In our work, we have used double labelling to eliminate the possibility of such differences: animals treated with hormones received a tritium precursor while control animals received a carbon-14 precursor and the livers were combined before isolation and fractionation of RNA.

Counter-current distribution was performed over fifty or eighty exchanges in a hand operated machine containing 1.5 ml. of each phase of solvent system 150/12.5, essentially as described by Kidson and Kirby<sup>1</sup>.  $^{14}\text{C}$  and  $^3\text{H}$  in the fractions were counted simultaneously in a Beckman liquid scintillation spectrometer.

Fig. 1 shows the radioactivity profiles of the labelled RNA isolated by the method of Kidson, Kirby and Ralph<sup>2</sup> from the livers of two rats, one of which had been given insulin and  $^3\text{H}$ -orotic acid and the other  $^{14}\text{C}$ -orotic acid without insulin. The tritium and carbon-14 counts have been plotted on scales that give the same area under each profile. There are no essential differences between the two profiles, and there was none in another similar experiment, or in experiments in which hydrocortisone (5 mg) or thyroxine (30  $\mu\text{g}$ ) were injected 50 or 35 min respectively before death and in which labelled orotic acid was injected 20 min before death.

In further experiments, liver slices were incubated in Ringer-bicarbonate buffer<sup>3</sup> with a hormone and  $^3\text{H}$ -adenine, while control slices were incubated with  $^{14}\text{C}$ -adenine without hormone, and the labelled RNA isolated by Method II of Kirby<sup>4</sup>. Fig. 2 shows a typical

experiment in which there are again no differences between the two profiles, although the labelled RNA has not travelled so far along the apparatus as that in Fig. 1. Similar profiles were obtained in six similar experiments in which corticosterone (500  $\mu$ g), oestradiol (50  $\mu$ g), or 3,3',5-triiodothyronine (50  $\mu$ g) was added for 30 min.

The movement of the rapidly labelled RNA can be expressed on a linear scale between 0 and 1 by the following "index of travel". The tube is found where one-half of the radioactivity in the apparatus is in tubes later than itself. The number of this tube is divided by the total number of exchanges. In eight experiments in which the RNA was isolated as in Fig. 1 (ref. 2), the mean index of travel was  $0.36 \pm 0.020$  (S.E.M.). In six experiments in which the RNA was isolated as in Fig. 2 (ref. 4), the mean was  $0.22 \pm 0.008$ . Thus it is clear that large changes in profile can result from a slightly altered method of preparation. Hence, smaller changes could well result from smaller and unavoidable differences in preparation between experimental and control rats.

The labelled RNA in Fig. 1 probably moves more rapidly than that in Fig. 2 because it is degraded more by an intrinsic ribonuclease. Thus when material prepared as in Fig. 2 was stored at 2°C for some weeks its index of travel increased from 0.19 to 0.33. Also, predigestion with pancreatic ribonuclease (10  $\mu$ g for 60 min at 37°C) increased the index for both materials to about 0.52. The material of Fig. 1 contains DNA but little ribosomal RNA while that of Fig. 2 contains ribosomal

RNA but little DNA. Association of the rapidly labelled RNA with DNA or ribosomal RNA, however, does not seem to account for the different rates of movement. Thus the rates were unaltered by adding calf thymus DNA to the material of Fig. 2 or by adding ribosomal RNA to that of Fig. 1 or predigesting it with deoxyribonuclease (ribonuclease-free).

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### Insulin in the Cerebrospinal Fluid

STUDIES involving injection of labelled insulin have been interpreted as evidence that insulin does not pass into brain<sup>1,2</sup> or cerebrospinal fluid (CSF)<sup>3</sup>. Neither was any significant amount of insulin detected in human CSF using the rat adipose tissue bioassay technique<sup>3,4</sup>. We have reinvestigated the question of whether insulin passes the blood-CSF barrier using the more sensitive and specific radioimmunoassay technique.

Experiments were carried out on male and female dogs (10–15 kg) which had been fasted for 18–20 h and anaesthetized with sodium pentobarbital (30 mg/kg, intravenously). Samples of cerebrospinal fluid (1.5 ml.) were taken from the cisterna magna through a No. 22 spinal needle, which was left in place when the concentrations of insulin in the CSF were followed over a period of time. Blood samples were taken from the jugular vein through a polyethylene catheter. Crystalline beef insulin and glucose were infused into the saphenous vein. Concentrations of insulin in the plasma and CSF were determined by an adaptation of the radioimmunoassay of Yalow and Berson<sup>5</sup> to the double-antibody immunoassay method of Morgan and Lazarow<sup>6</sup>. Because we anticipated that the concentration of insulin in the CSF in the basal state would be very low, larger samples were taken of CSF (0.4 ml.) than of plasma (0.05 ml.) for the assay. In this way, the absolute amount of insulin in each assay tube was usually greater for CSF than for plasma.

Insulin was infused at 0.2 or 1.0  $\mu$ /kg/h. Glucose was also infused, at rates which either just prevented hypoglycaemia (about 750 mg/kg/h for 0.2  $\mu$ /kg/h of insulin) or produced hyperglycaemia (0.9–1.5 g/kg/h for both the high and low insulin dosage). All dogs had a normal basal concentration of plasma glucose ( $109 \pm 13$  mg per cent).

Insulin was found in the cerebrospinal fluid of all twenty-two normal dogs examined after treatment (Table 1). The average concentration of insulin in the CSF was 3  $\mu$ /ml., or 27 per cent of the average concentration of plasma insulin (11  $\mu$ /ml.) observed in these animals. When concentrations of plasma insulin were increased by intravenous infusion of insulin, the CSF insulin values rose between two- and seventy-fold. Although the greatest increases in CSF insulin were obtained when

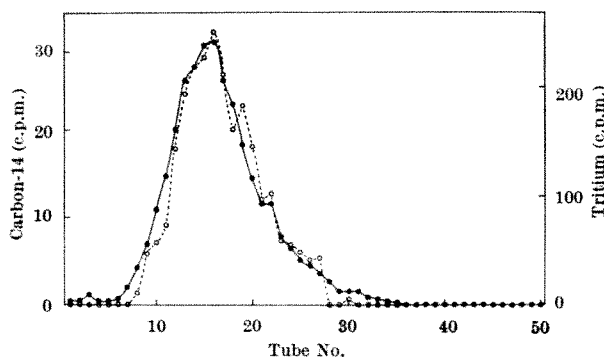


Fig. 1. Counter-current distribution profile of rapidly labelled RNA from livers of control rats and rats treated with insulin. One rat received insulin (1 mg in 0.5 ml. of 0.003 normal HCl) intraperitoneally 35 min before death followed by <sup>3</sup>H-uric acid (200  $\mu$ c.; 0.22  $\mu$ mole in 0.45 ml. of 0.15 molar NaCl) 15 min later; the other control rat received HCl alone 35 min before death, followed by <sup>14</sup>C-uric acid (10  $\mu$ c.; 0.22  $\mu$ mole) 15 min later. Livers of the two rats were combined before RNA isolation<sup>2</sup>. ○ — ○, Carbon-14 counts (control); ● — ●, tritium counts (insulin treated).

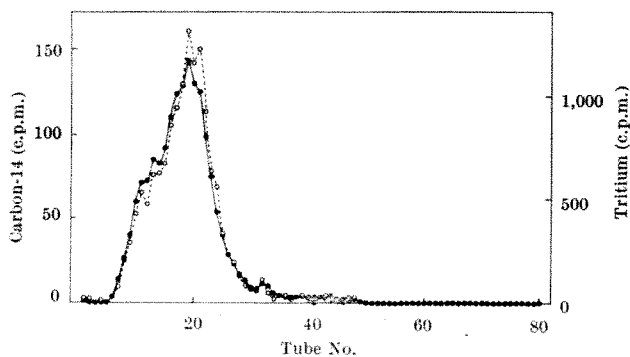


Fig. 2. Profiles of rapidly labelled RNA from control slices of rat liver and slices incubated with insulin. One sample of slices (1 g) was incubated at 37°C for 1 h under oxygen (95 per cent) and carbon dioxide (5 per cent) in Ringer-bicarbonate buffer (10 ml.) containing glucose (30 mg) and insulin (50  $\mu$ g); <sup>3</sup>H-adenine (200  $\mu$ c., 0.35  $\mu$ mole) was added for the last 30 min. The control slices were treated identically except that no hormone was added and <sup>14</sup>C-adenine (10  $\mu$ c., 0.35  $\mu$ mole) replaced <sup>3</sup>H-adenine. The slices were combined before RNA isolation<sup>4</sup>. ○ — ○, Carbon-14 counts (control); ● — ●, tritium counts (with insulin).

Table 1. CONCENTRATION OF INSULIN IN PLASMA AND CEREBROSPINAL FLUID OF DOGS

	Insulin concentration (mean $\pm$ S.E.M.)	
	Plasma	CSF
	$11 \pm 1.2$ $\mu$ /ml.	$3 \pm 0.3$ $\mu$ /ml.
	(N = 22)	



plasma insulin values were raised to 1,000–2,000  $\mu\text{U}/\text{ml.}$ , even in the presence of plasma insulin concentrations in the more physiological range of 150–300  $\mu\text{U}/\text{ml.}$ , the CSF insulin concentration increased up to ten-fold (Fig. 1).

The concentration of plasma glucose did not appear to affect the passage of insulin from plasma to CSF, and although very high concentrations of plasma insulin were maintained for several hours, the CSF concentration of insulin did not increase proportionally (Fig. 2). This suggests that insulin passes into the CSF by way of a saturable transport system rather than by passive diffusion.

The reports that insulin penetrates only slightly, if at all, into the brain and CSF, have been based either on the detection of radioactivity after injection of labelled insulin or on the measurement of "insulin like activity" using the adipose tissue bioassay technique. Both these approaches have limitations. In one study<sup>2</sup> the concentration of radioactivity in brain 15 min after intravenous injection of insulin labelled with iodine-131 was lowest of any tissue examined with the exception of erythrocytes. Skeletal muscle and adipose tissue, however, both of which are sensitive to insulin, had only slightly greater amounts of radioactivity. Insignificant amounts of radioactivity were found in lumbar CSF up to 90 min after intravenous injection of labelled insulin<sup>3</sup>. These studies are difficult to interpret as evidence either for or against the passage of insulin across the blood-brain barrier. Considering the rapid metabolism of insulin<sup>5</sup> and its slow passage across the blood-CSF barrier as shown here, measurable amounts of labelled insulin are not likely to be found in the brain or CSF after a single injection.

In studies on CSF, Mahon *et al.*<sup>3</sup> used the rat adipose tissue bioassay technique to determine "insulin like activity" (ILA). They reported an average serum ILA of 371  $\mu\text{U}/\text{ml.}$  in five subjects, with less than 31  $\mu\text{U}/\text{ml.}$  in the CSF. This concentration was the lowest point on their standard curve. Schrader and Weinges<sup>4</sup> found serum concentrations of ILA averaging 320  $\mu\text{U}/\text{ml.}$  and

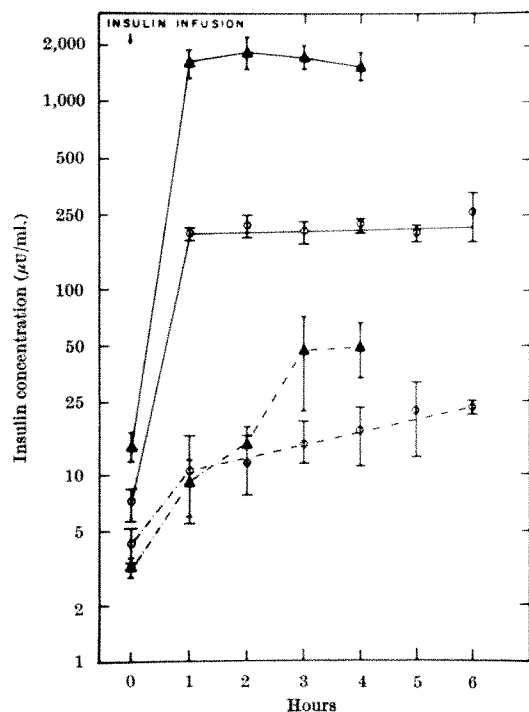


Fig. 1. Effect of infusion of intravenous insulin on insulin concentration in plasma (—) and CSF (---).  $\circ$ , Values obtained during infusion of 0.2  $\text{U}/\text{kg}/\text{h}$ ;  $\blacktriangle$ , values obtained during infusion of 1  $\text{U}/\text{kg}/\text{h}$ . Insulin concentration is plotted on a logarithmic scale.

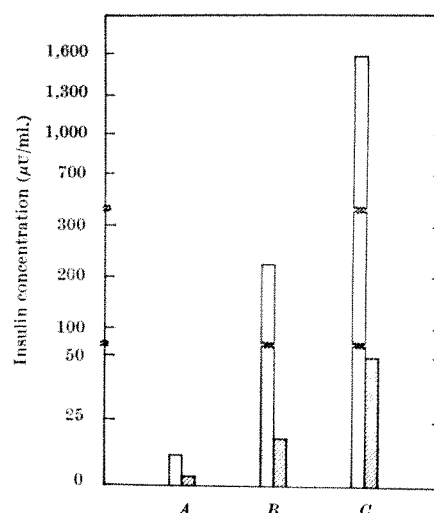


Fig. 2. Relationship between plasma (white columns) and CSF (hatched columns) insulin concentrations at three concentrations of plasma insulin. A represents the basal state, B and C the situation 4 h after insulin infusions of 0.2 and 1  $\text{U}/\text{kg}/\text{h}$ , respectively.

less than 10  $\mu\text{U}/\text{ml.}$  of ILA in the CSF in ten metabolically normal subjects. Although the adipose tissue bioassay may be useful in studies of metabolism, it lacks the specificity and sensitivity of the radioimmunoassay. The ILA in the cerebrospinal fluid in the studies referred to represents less than 3 per cent<sup>4</sup> and 8 per cent<sup>3</sup> of the serum ILA. Our value of 3  $\mu\text{U}/\text{ml.}$  for the average CSF insulin concentration, although small in absolute terms, nevertheless represents a substantial fraction (27 per cent) of the true plasma insulin concentration.

This work also reveals that concentrations of CSF insulin increase after an increase in plasma insulin. The entry of insulin would appear to be quite slow, however, judging from the 3–4 h required for insulin levels to stabilize in the CSF during continuous intravenous infusion of insulin (Fig. 1). Although these experiments indicate that insulin is able to cross the blood-CSF barrier (and presumably also the blood-brain barrier), the physiological significance of this remains to be elucidated.

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### Distribution of Acid Glycosaminoglycans in Human Articular Cartilage

THERE is evidence<sup>1,2</sup> that the distributions of chondroitin sulphate and keratansulphate in both articular and costal cartilage are different and change with age. The concentration of keratansulphate has been shown to increase with distance from the periphery of the cartilage using 'Alcian Blue' (8GX, I.C.I. Ltd.) in graded concen-

trations of magnesium chloride, according to the "critical electrolyte concentration" technique<sup>3</sup>, with or without hyaluronidase digestion. In mature human articular cartilage, chondroitin sulphate is predominantly territorial while keratansulphate is characteristically interterritorial, except in the deepest zones (adjacent to the calcified layer) of older tissue, where keratansulphate is localized close to the chondrocyte.

It is necessary to compare these histochemical findings with biochemical measurements of the relevant materials. The superficial, middle and deep zones of articular cartilage are easily separable by sectioning tangential to the articular surface, and because, histochemically, the concentrations of chondroitin sulphate and keratansulphate vary considerably between these layers, it seemed worthwhile to analyse them by micro-biochemical methods. Macroscopically normal articular cartilage from human post-mortem femoral condyles was cut tangentially into sections about 0.2 mm thick, each section being about 15 mg wet weight. The tissue was digested at 65°C with papain<sup>4</sup> and polyanions were precipitated with 1 per cent cetyl pyridinium chloride (CPC) at 0.35 molar sodium chloride. The CPC complex was dissociated with either 2 molar sodium chloride or 4 normal hydrochloric acid, and CPC was removed by chloroform extraction<sup>5</sup>. Uronic acid was determined by the borate modification of the Dische carbazole method<sup>6</sup>, and hexose by the method of Dische<sup>7</sup>, on samples of the polysaccharide solution. Ratios of glucosamine to galactosamine were determined on 4 normal hydrochloric acid hydrolysates (100°C for 8 h) of the polysaccharide, by the method of Scott<sup>8</sup>, using naphthyl isothiocyanate. Sections 10 µm thick of tissue adjacent to that used for quantitative analysis were stained in 0.1 per cent w/v 'Alcian Blue' in 0.4 molar and 0.9 molar magnesium chloride<sup>1</sup>.

Table 1. HUMAN POST-MORTEM ARTICULAR CARTILAGE

Depth from surface (ratio to total depth)	Uronic acid as glucuronic lactone (% dry wt.)	Hexose as galactose (% dry wt.)	Molar ratio: hexose/hexose + uronic acid	Molar ratio: glucosamine/glucosamine + galactosamine
0.1	0.41	0.20	0.32	0.32
0.3	1.51	0.77	0.32	0.18
0.4	1.91	0.87	0.29	0.24
0.6	2.07	1.13	0.33	0.33
0.7	2.07	1.34	0.38	—
0.8	1.80	1.43	0.42	0.32
0.9	1.76	2.30	0.53	0.34
0.95	1.28	2.08	0.59	0.41

Medial femoral condyle. Male aged 31 yr. Analytical values for successive tangential sections.

Table 2. HUMAN POST-MORTEM ARTICULAR CARTILAGE

Region in cartilage	Uronic acid as glucuronic lactone (% dry wt.)	Hexose as galactose (% dry wt.)	Molar ratio: hexose/hexose + uronic acid
Superficial zone (SZ) (<0.25 mm from articular surface)	0.77 ± 0.30	0.45 ± 0.20	0.34 ± 0.064
Middle zone (MZ)	2.13 ± 0.60	1.46 ± 0.54	0.39 ± 0.042
	Difference from SZ: $t = 5.0, P < 0.001$	Difference from SZ: $F = 18.0, P < 0.01$	
Deep zone (DZ) (<0.20 mm from calcified layer)	1.25 ± 0.61	1.72 ± 0.60	0.56 ± 0.070
	Difference from MZ: $t = 2.53, P < 0.05$	Difference from MZ: $t = 5.3, P < 0.001$	

Mean values ± 1 standard deviation.

Medial femoral condyle. Mean values for superficial, middle and deep zones in six adult cases aged 31–78 yr.

The results of a representative case are shown in Table 1, and mean values for six adult specimens are shown in Table 2. The histochemical findings (see Fig. 1A and B) are supported by the present biochemical investigations. (1) Uronic acid (representative of chondroitin sulphate) is maximal in the middle zone, while hexose (representative of keratansulphate) increases with distance from the articular surface. (2) The ratios hexose: hexose + uronic acid and glucosamine: glucosamine + galactosamine both rise sharply in the deep zone.

Glucosamine and hexose are less reliable indicators of keratansulphate than are galactosamine and uronic acid of chondroitin sulphate. Thus galactose and xylose are present in the linkage region of chondroitin sulphate to protein<sup>9</sup> while the exact composition of keratansulphate is uncertain<sup>10</sup>. Precipitation of the CPC complexes at 0.35 molar sodium chloride probably eliminates severe contamination by non-sulphated polyanions while minimizing losses of low molecular weight acid glycosaminoglycan.

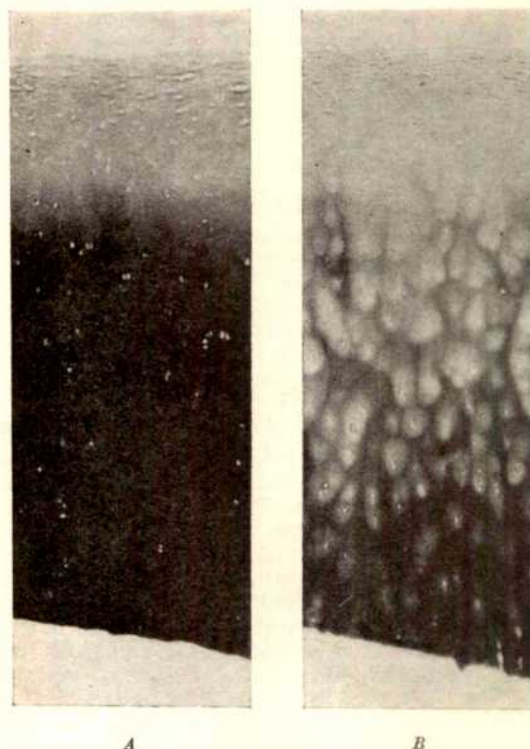


Fig. 1. A, Human articular cartilage. Medial femoral condyle. Aged 31 yr. 0.1 per cent 'Alcian Blue' at 0.4 molar magnesium chloride, pH 5.7. Staining attributed to chondroitin sulphate and keratansulphate. ( $\times 50$ .) B, as A but stained at 0.9 molar magnesium chloride. Staining attributed to high molecular weight keratansulphate; note increase with depth from articular surface. ( $\times 50$ .)

Szirmai *et al.*<sup>11</sup> found a similar pattern of distribution in horse nasal cartilage, apparently with much less keratansulphate. The various types of hyaline cartilage appear to differ in the proportion of keratansulphate in the total glycosaminoglycan content. Thus human costal cartilage has a higher proportion of keratansulphate<sup>12,13</sup> than either horse nasal<sup>11</sup> or human articular cartilage. It is possible that the differences in the composition of these skeletal elements may be associated with differences in their physical properties—for example, permeability.

In adult articular cartilage, the variation of keratansulphate distribution with depth from the joint surface is similar in form to that in costal and horse nasal cartilage with respect to distance from the perichondrium. There is evidence that the synovial fluid is the principal source of nutrition of adult articular cartilage<sup>14</sup>. If this is so, the distribution of keratansulphate in this tissue is in agreement with the speculation made by Stockwell<sup>1</sup> that an increase in the distance from a source of oxygen supply is a principal determining factor for an increase in the ratio of keratansulphate to total acid glycosaminoglycan.

This appears to be the first time that a relatively specific histochemical staining technique for acid glycosaminoglycans has been correlated with the results of microchemical analysis. The two sets of data are compatible



and therefore substantiate the claims made for the histochemical method.

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## Changes in Nucleic Acid Metabolism of Tumour Cells under High Pressure Oxygen

THE radiosensitizing effect of oxygen<sup>1-6</sup> has been extensively studied not only in radiobiology but also in clinical radiotherapy, but knowledge of the biological action of high pressure oxygen *per se* is rather scanty. We have examined the effects of high pressure oxygen on the nucleic acid metabolism of Yoshida ascites hepatoma cells *in vitro* and Ehrlich ascites tumour cells *in vivo*.

Yoshida ascites hepatoma cells (AH 130) were inoculated into the peritoneal cavity of male rats of the Donryu strain weighing 200 g. On the seventh day after inoculation, ascites tumour cells were collected, washed in phosphate buffered saline and suspended in 10 volumes of a tissue culture medium consisting of lactalbumin hydrolysate, yeast extract and 10 per cent calf serum. Cell suspensions were pressurized in a small oxygen chamber and irradiated with a deep therapy machine, 300 kV, General Electric 'Maxitron', operated at 20 m.amp, filtered through 2.0 mm copper, which resulted in a half value layer of 1.77 mm copper and a dose rate of 240 r./min at a distance of 35 cm. After irradiation, tritiated thymidine (thymidine-methyl-T, 5 c./mmole) or <sup>14</sup>C-thymidine (thymidine-2-<sup>14</sup>C, 35 mc./mmole) plus tritiated uridine (uridine-5-T, 3c./mmole) were added, high pressure oxygen or nitrogen was again introduced and the cells were incubated for 30 min at 37° C with constant shaking. After incubation 1/10 volumes of cold 50 per cent trichloroacetic acid were added and nucleic acid fractions were separated according to the method of Schmidt and Thannhauser<sup>7</sup>. The radioactivity of the nucleic acid fractions was determined by a liquid scintillation spectrometer using a dioxane-toluene phosphor<sup>8</sup>. DNA was measured according to the method of Burton<sup>9</sup> and RNA according to Dische<sup>10</sup>.

Table 1 shows the effects of pressurization on the *in vitro* incorporation of tritiated thymidine into DNA of the ascites hepatoma, and Table 2 shows the effects of irradiation under high pressure oxygen on the *in vitro* incorporation of <sup>14</sup>C-thymidine and tritiated uridine into DNA and RNA of the ascites hepatoma cells. The effects of irradiation under high pressure oxygen were slightly greater than those of irradiation in air when the enhancement of incorporation by high pressure oxygen *per se* was taken into account.

Similar experiments have also been carried out *in vivo*. Because of the limited size of the oxygen chamber, we

used mice as the experimental animals. Ehrlich ascites tumour cells were inoculated into the peritoneal cavity of ddD strain male mice of 25 g body weight. On the eighth day after inoculation, all the animals were given an intraperitoneal injection of 25 µc. of tritiated thymidine. Experimental animals were put into the chamber and high pressure oxygen was introduced. The animals were exposed to high pressure oxygen for about 50 min and then were killed exactly 60 min after the injection of tritiated thymidine. Ascites cells were collected and the specific activity of DNA was measured by the same method as before.

Table 1. EFFECTS OF EXPOSURE TO HIGH PRESSURE OXYGEN AND NITROGEN ON THE INCORPORATION OF TRITIATED THYMIDINE INTO DNA OF THE ASCITES HEPATOMA CELLS *in vitro*

	Specific activity (c.p.m. × 10 <sup>-3</sup> /mg DNA)	Per cent of control
Control (in air)	301	100
Oxygen, 1 atmosphere	371	123
Oxygen, 3 atmospheres	363	121
Oxygen, 5 atmospheres	365	121
Nitrogen, 3 atmospheres	317	105
Nitrogen, 5 atmospheres	293	97

Cells were incubated for 30 min at 37° C with 1 µc./ml. of tritiated thymidine under different atmospheric conditions. Mean of two experiments.

Table 2. EFFECTS OF EXPOSURE TO HIGH PRESSURE OXYGEN ON THE INCORPORATION OF <sup>14</sup>C-THYMIDINE AND TRITIATED URIDINE INTO DNA AND RNA OF THE ASCITES HEPATOMA CELLS *in vitro*

	<sup>14</sup> C-TMP incorp. (c.p.m. × 10 <sup>-3</sup> /mg DNA)	Per cent of control	<sup>3</sup> H-UMP incorp. (c.p.m. × 10 <sup>-3</sup> /mg RNA)	Per cent of control
Control (in air)	329	100	184	100
Oxygen, 3 atmospheres	409	124	189	103
X-ray (in air), 2,000 r.	257	78	190	103
Oxygen, 3 atmospheres, X-ray, 2,000 r.	260	79	199	105
X-ray (in air), 4,000 r.	260	79	187	99
Oxygen, 3 atmospheres, X-ray, 4,000 r.	196	60	171	91

After irradiation in air or under high pressure oxygen at 3 atmospheres, cells were incubated under the same atmospheric conditions in the presence of 0.1 µc./ml. of <sup>14</sup>C-thymidine and 1.0 µc./ml. of tritiated uridine for 30 min at 37° C.

Mean of two experiments.

Table 3. EFFECTS OF BREATHING HIGH PRESSURE OXYGEN OR IRRADIATION UNDER HIGH PRESSURE OXYGEN ON THE INCORPORATION OF TRITIATED THYMIDINE INTO DNA OF EHRlich ASCITES TUMOUR CELLS *in vivo*

	Specific activity (c.p.m. × 10 <sup>-3</sup> /mg DNA)	Per cent of control
<i>a</i>		
Control (in air)	111.4	100
Oxygen, 1 atmosphere	148.3	133
Oxygen, 3 atmospheres	145.0	130
<i>b</i>		
X-ray, 1,000 r. (in air)	55.2	50
Oxygen, 1 atmosphere, X-ray, 1,000 r.	44.6	40
Oxygen, 3 atmospheres, X-ray, 1,000 r.	29.8	27

*a*, Immediately after the injection of tritiated thymidine (25 µc), animals were exposed to high pressure oxygen for about 50 min, and were killed exactly 60 min after the injection.

*b*, After irradiation under different atmospheric conditions, tritiated thymidine (25 µc.) was injected intraperitoneally and animals were left in air for 60 min and then killed.

Mean of two experiments.

The upper half of Table 3 shows the effects of exposure to high pressure oxygen on the incorporation of tritiated thymidine into DNA of Ehrlich ascites tumour cells *in vivo*. The results are in accordance with those obtained *in vitro*.

In another experiment, animals were exposed to high pressure oxygen for 30 min and a whole body irradiation of 1,000 r. was then given under the same conditions as the *in vitro* experiments except that the dose rate was 65 r./min and the distance 70 cm. After irradiation, 25 µc. of tritiated thymidine was injected and the animals were killed 60 min later. The results are shown in the lower half of Table 3. Irradiation under high pressure oxygen produced a greater inhibition of DNA synthesis as compared with irradiation in air.

Several authors have reported toxic effects of high pressure oxygen on mammalian cells both *in vitro*<sup>11</sup> and *in vivo*<sup>12-15</sup>. Regression of experimental tumours which had been exposed to high pressure oxygen was

observed<sup>12-15</sup>. High pressure oxygen may alter the state of oxygenation of ascites tumour cells, thereby producing changes in the metabolism of the cell, but the reason for, and possible significance of, the increased incorporation of radioactive precursors into DNA exposed to high pressure oxygen both *in vitro* and *in vivo* are not yet clear.

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### Effect of Cysteine on Acetylcholine Synthesis

CHOLINE acetyltransferase (choline acetylase; acetyl-CoA: choline O-acetyltransferase, *EC* 2.3.1.6) (ChA) is a sulphhydryl enzyme responsible for the synthesis of acetylcholine (ACh) in nervous and other tissues. Early attempts to purify ChA from rat brain and calf caudate nucleus showed that this enzyme is unstable and attempts were made to protect it by addition of cysteine to the medium. It was observed, however, that high concentrations of cysteine decreased the synthesis of ACh. Similar findings were recently reported by Morris, Hebb and Bull<sup>1</sup> in Torpedo Marmorata and rabbit brain extracts. They attributed the decreased synthesis of ACh to the inhibition of ChA by cysteine. Subsequently, Morris<sup>2</sup> studied the effect of a number of sulphhydryl compounds on ChA activity. He demonstrated that their mode of action was not directly on ChA but that they acted by depletion of the acetyl-CoA in the system. We have been able to produce evidence to support Morris's suggestion that the reaction involved is a spontaneous transacetylation between acetyl-CoA and cysteine. This mechanism presumably applies to the other sulphhydryl compounds which he studied.

In our investigations, the source of ChA used was a 100,000g supernatant of homogenate of rat cerebral cortex diluted 1:30 with distilled water. ChA activity was assayed by a modification of the radioactive method of McCaman and Hunt<sup>3</sup> and the electrophoretic method described here. All cysteine solutions were prepared from the free base immediately before use and adjusted to pH 7.7 for addition to the ChA assay medium. Addition of cysteine to the assay in concentrations above  $10^{-3}$  molar produced inhibition of ACh production (Fig. 1).

To examine the reaction products in the presence of cysteine the protein was removed by trichloroacetic

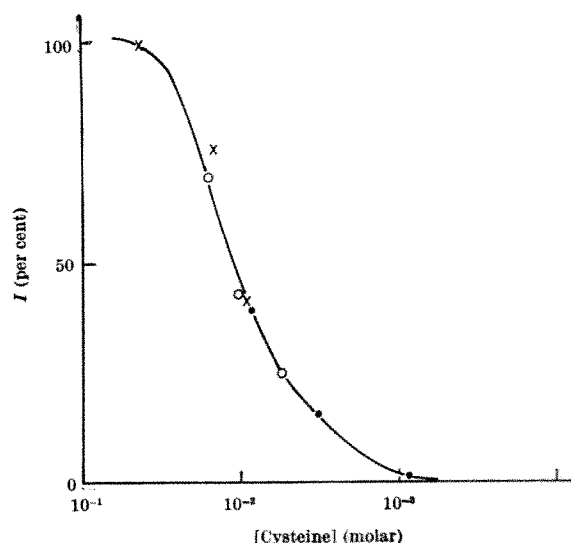


Fig. 1. The formation of *S*-acetylcysteine from cysteine and acetyl-CoA in the absence (○) and presence (●) of brain extract containing choline acetyl transferase. The inhibition of acetylcholine production in the complete incubation medium (x) is also shown.

acid precipitation and the supernatant was subjected to horizontal paper electrophoresis at 500 V in formic acid, acetic acid, water (1:3:36) (ref. 4). Using an 'Atomic Accessories' paper strip scanner, three radioactive spots were observed. Cysteine was located by staining with ninhydrin. The mobilities of the various components relative to acetyl-CoA are shown in Table 1. Spot 1 could be identified as unreacted acetyl-CoA by showing its mobility to be identical to that of acetyl-CoA applied directly to the paper. Spot 4 was of identical mobility to acetyl-1-<sup>14</sup>C choline and to non-radioactive ACh stained by exposure to iodine vapour. Spot 3 showed the mobility of cysteine. Incubation of acetyl-1-<sup>14</sup>C CoA and cysteine without rat brain homogenate produced only spots 1 and 2 after electrophoresis and strip scanning, and spot 3 on ninhydrin staining.

Table 1. RELATIVE ELECTROPHORETIC MOBILITIES OF SOME COMPOUNDS STUDIED

Spot No.	Compound	Relative cathodal mobility*
1	Acetyl-CoA	1.0
2	<i>S</i> -acetylcysteine	1.3
3	Cysteine	1.7
4	Acetylcholine	2.4

The spots are numbered in order of increasing cathodal mobility.  
\* The mobility of acetyl-CoA is taken as 1.0.

The most likely position of transfer of the <sup>14</sup>C acetyl group of acetyl CoA is to the free sulphhydryl of cysteine. When acetyl CoA was incubated with  $8 \times 10^{-3}$  molar cysteine for 60 min at room temperature at pH 9.0 virtually all the radioactivity was found in spot 2 after electrophoresis. At pH 8.0 about 50 per cent of the radioactivity was found in spot 2. The *pK* value of the sulphhydryl group of cysteine is about 8.1.

Spot 2 has a mobility different from that of *N*-acetyl cysteine stained with ninhydrin. *N*-acetyl cysteine, used in place of cysteine, inhibited ACh production in the enzyme preparation and gave rise to a radioactive spot following electrophoresis, in both the enzyme and non-enzyme incubation, different in mobility from spot 2 and presumed to be *S*-*N*-diacetyl cysteine. When cysteine was replaced by either *S*-methylcysteine or *S*-ethylcysteine, no radioactive spot other than acetyl-CoA was seen after electrophoresis of the non-enzyme incubation. These compounds did not inhibit the enzyme synthesis of acetylcholine by ChA. No radioactive spot other than acetyl-CoA and acetylcholine was seen after electrophoresis of the enzyme incubation.



It may therefore be concluded that cysteine causes apparent inhibition of ChA by consumption of the acetyl-CoA substrate by non-enzyme transacetylation. The rate of *S*-acetyl cysteine formation, both in the presence of enzyme and the complete incubation mixture, and in a mixture of acetyl-CoA and cysteine (Fig. 1), paralleled the reduction in the rate of ACh formation.

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### Use of Urine to Serum Fluoride Concentration Ratios to confirm Serum Fluoride Analyses

IN 1966 I published<sup>1</sup> results showing that the normal concentration of fluoride in human serum is about one-tenth the generally accepted value—that of Singer and Armstrong. Part of the evidence supporting my results was the “at least five-fold discrepancy” that could be shown in their data<sup>2</sup> which is most easily explained by an error in the measurement of the serum fluoride. The discrepancy involves the ratio of urine to plasma fluoride concentrations when determined by fluorine-18 as compared with stable fluoride analyses. Because the concentration of fluoride in urine is well established in contrast to that in serum where analysis is much more difficult, it is reasonable to question the latter when there is a discrepancy. Singer and Armstrong<sup>3</sup>, however, in their recent rebuttal, think that I came to erroneous conclusions because I directed attention to only two of their analyses, which they now consider aberrant.

In summarizing the data as a whole they calculate that the average concentration of fluoride in the urine from one subject (C.H.C.) was 90  $\mu$ molar (1.7 p.p.m.) and from the other (W.D.A.), 179  $\mu$ molar (3.4 p.p.m.). They state that these concentrations are reasonable because I indicated that up to 150  $\mu$ molar (3 p.p.m.)<sup>1</sup> might be expected in the two samples where there was a very concentrated urine. Such a comparison completely overlooks the effect of the flow rate of urine on the expected concentration of fluoride. In the case of subject C.H.C., 1,888 ml. of urine was collected in 321 min, or 5.9 ml./min, in contrast to the 0.42 and 0.57 ml./min of the two “aberrant” samples. If the rate of flow of urine had no effect on clearance of fluoride from the blood then there would be a direct inverse relationship between concentration and rate of flow. Because the average rate of flow is increased ten-fold over the “aberrant” samples, the expected concentration in the urine for the samples as a whole would be one-tenth as much, or up to 15  $\mu$ molar (0.3 p.p.m.) rather than 150  $\mu$ molar (3.0 p.p.m.). In subject C.H.C., increasing the rate of flow twenty-fold doubled the clearance of fluorine-18, so there would be some increase in the expected concentration, but not to more than 23  $\mu$ molar (0.43 p.p.m.).

My original estimate of up to 150  $\mu$ molar (3.0 p.p.m.) fluoride for the two “aberrant” samples was based on observations<sup>4</sup> of persons drinking slightly different amounts of water than those used by Carlson *et al.*<sup>2</sup>. In order to substantiate better the expected urine fluoride concentration in the conditions used by Carlson, Armstrong and Singer, I ingested fluoride and water in the same pattern and collected 1,025 ml. of urine in 255 min or 4.0 ml./min. After thoroughly mixing the urine, five samples were analysed by Singer and Armstrong's

method<sup>5</sup> and  $21 \pm 1.5$   $\mu$ molar (0.40 p.p.m.) fluoride were found. The urine collected before any water was ingested showed  $78 \pm 8$   $\mu$ molar (1.47 p.p.m.) fluoride by the same analysis. Analysis of the same samples by my method gave mean values within 3 per cent of those analysed by Singer and Armstrong's method.

If my flow rate had been the same as that of subject C.H.C. then the urine fluoride concentration would have been lower, possibly as low as 14  $\mu$ molar (0.27 p.p.m.). Thus a four- to six-fold discrepancy exists between their calculated concentration (90  $\mu$ molar) and what I measured. This does not differ much from the “at least five-fold discrepancy” that I originally pointed out using just the two “aberrant” samples. No data on the volume of urine are given for the other subject (W.D.A.) so a comparable analysis cannot be made, but there would appear to be at least as great a discrepancy, for the concentration as calculated by Singer and Armstrong is twice as great as that for C.H.C. with the same range of urine flow rates. As indicated, the most logical explanation of this discrepancy is to assume that their serum fluoride values are in error. Certainly, they are not measuring what the kidney sees as fluoride.

There is a simple way to show what the normal serum fluoride should be in humans drinking fluoridated water. The average data for clearance of fluorine-18 for all Singer and Armstrong's samples with a flow rate of less than 2 ml./min, which involves four samples for each subject, are 56 and 49 ml. plasma cleared/min for the two subjects. If a person is excreting 50  $\mu$ molar (1 p.p.m.) fluoride in his urine at a flow rate of 1 ml./min then the plasma fluoride concentration must be 0.9–1.0  $\mu$ molar (0.018 to 0.02 p.p.m.), which is very close to the 0.7  $\mu$ molar I reported.

The inability of Singer and Armstrong<sup>5</sup> to reproduce my fusion data certainly cannot be taken as a refutation of my findings<sup>1</sup>. First, they fused sodium hydroxide rather than converting it to the carbonate-chloride salt. This makes a difference in the fusion temperature because pure sodium hydroxide fuses at 318°C while the carbonate fuses at 851°C. Second, they only examined serum from one individual and a single individual cannot be assumed to be representative of normal.

The accurate determination of the ratio of fluoride in the urine to serum by means of fluorine-18 has made it possible to confirm measurements of serum fluoride concentration of only 0.1  $\mu$ molar (0.002 p.p.m.) in pups that have been raised on a milk diet. Extension of this principle makes it possible to estimate serum fluoride concentrations as low as 0.01  $\mu$ molar, if the need should arise.

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### Traumatic Acid : an Accelerator of Abscission in Cotton Explants

WE have been investigating the physiology of bud abscission induced by the feeding action of lygus bugs, *Lygus hesperus* Knight, because explants are used for biological assays<sup>1</sup>. In this bioassay, various extracts are applied in agar to the stumps of the cotyledonary petioles and the time for 50 per cent of the petioles to abscise is used for comparative purposes. In the course of these experi-

ments in which lygus bugs were fed a water extract made from lyophilized 'Kentucky Wonder' green beans, *Phaseolus vulgaris*, we discovered that the bean extract itself contained something which would greatly accelerate abscission of the explants. Many substances are known to accelerate abscission<sup>2</sup>; especially active is the plant hormone abscisin II (ref. 3). Physiologically, however, the cotton explants react quite differently to bean extract and abscisin II. With abscisin II, no well defined separation layer is formed<sup>4</sup> and little colour change occurs in the petioles before abscission. When the extract from 0.1 mg of dried beans was applied to each petiole, a pronounced separation layer was present after 24 h and the distal 3 mm of the petioles had turned brownish. (We used explants having petioles 6 mm long in contrast to the petioles 3 mm long used by Addicott *et al.*<sup>1</sup>) Abscission induced by bean extract was physiologically similar to that caused by gibberellic acid ( $GA_3$ ), in which a separation layer is formed accompanied by an increase in cell number<sup>5</sup>. With both  $GA_3$  and bean extract but not with abscisin II, a hyaline layer appeared in the abscission zone 12–24 h before the formation of the separation layer.

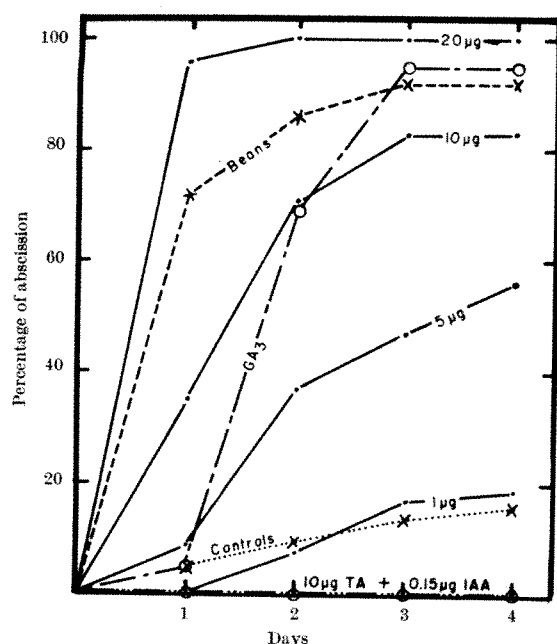


Fig. 1. Abscission of cotyledonary petioles of cotton explants in response to (1) traumatic acid (TA), applied at the rates of 20, 10, 5 and 1  $\mu$ g/abscission zone; (2) gibberellic acid ( $GA_3$ , 0.01  $\mu$ g/zone); (3) water extract from 0.1 mg lyophilized green beans; (4) controls (agar only); and (5) a mixture of 10  $\mu$ g TA plus 0.15  $\mu$ g indoleacetic acid (IAA) per zone.

Subtle yet significant differences exist in the reactions caused by  $GA_3$  and bean extract. Bean extract caused separation within 24 h, a browning of the distal portion of the petioles, few if any tyloses and proliferation of the cells in the protective layer after separation had occurred. After 48 h this proliferation of cells appeared as an undifferentiated cellular growth similar to callous tissue and arose from both the stump region and proximal surface of the separated petiole.  $GA_3$  induced separation after 48 h (none occurred after 24 h) but caused no browning of the petiole tips. It resulted in significant tylose formation<sup>5</sup> with no tissue growth following separation.

F. T. Addicott (personal communication) observed that these effects were like those of the wound hormone, traumatic acid (TA), which stimulates callus growth and was first isolated from 'Kentucky Wonder' beans<sup>6,7</sup>. Forty milligrams of commercially obtained TA were dissolved in 1 ml. of warm ethanol. This solution was then

mixed with 4 ml. of warm water, followed by the addition of 5 ml. of 3 per cent hot agar solution. This formed a suspension of TA in agar which was taken up in a syringe and 5  $\mu$ l. of the cooled suspension (containing 20  $\mu$ g traumatic acid) was applied to the tips of the cotyledonary petioles. Appropriate dilutions were made to yield concentrations of 20, 10, 5 and 1  $\mu$ g/abscission zone. Each concentration was tested at least three times using a minimum of twenty explants/concentration. The results (Fig. 1) show that all concentrations tested, except 1  $\mu$ g/zone, accelerated abscission in comparison to the controls. Ten micrograms of TA/zone accelerated abscission about as much as 0.01  $\mu$ g of  $GA_3$ . The abscission accelerating effects of TA can be significantly reduced by including 0.15  $\mu$ g of indoleacetic acid (IAA)/abscission zone (Fig. 1). A histological examination of paraffin sections stained with safranin fast green revealed no detectable difference between petioles treated with TA or bean extract. With TA, the first cellular changes associated with abscission were detected after 18 h, a slight amount of separation was evident at 24 h and separation was usually completed by 48 h.

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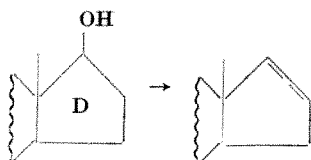
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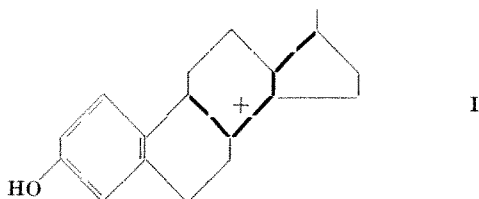
### Specificity of the Kober Reaction

THE specificity of the Kober<sup>1,2</sup> reaction for oestrogen assay has been studied as part of a general investigation of the mechanisms of steroid acid colour reactions. Exactly what constitutes a positive Kober reaction has never been defined; in this work, a Kober positive steroid is regarded as one which produces pink chromogen absorbing maximally in the 500–555 m $\mu$  region by the Brown<sup>3,4</sup> or Itrich<sup>5</sup>–Bradshaw<sup>6</sup> procedures. This chromogen we have designated  $\chi_4$  because at least three other chromogens absorbing at shorter wavelengths are produced by the Kober reaction with various oestrane derivatives. On this basis, from a study of 116 relevant steroids, the following features of the steroid molecule were found to be simultaneously necessary if the steroid is to give a positive Kober reaction. An oxygen function (carbonyl or hydroxyl) is essential at either C16 or C17 and is permissible at both. The only exceptions are the  $\Delta^{16}$ -steroid discussed later and 16-oxoestrone; oestrone-16 is only feebly positive. Of the fifteen possible combinations of oxygen function at C16 and/or C17, thirteen have been tested and all except the two just mentioned are strongly positive and give a pink chromogen with  $\epsilon_{\max}$  of 30,000 to 60,000.

The oestrogen with a C16–C17 double bond and no ring D oxygen function is strongly positive, but if ring D is saturated and possesses no oxygen function the oestrane derivative is Kober negative. This supports Boscott's<sup>7</sup> suggestion that the mechanism of the reaction involves dehydration at the C16–C17 positions as the initial step.



If this is accompanied by protonation and rearrangement of the double bond and angular methyl group at C13, as has frequently been observed with steroids reacting in acidic conditions<sup>8</sup>, a carbonium ion is produced which is capable of undergoing resonance by a hyperconjugation which involves several adjacent tertiary carbon sites and is represented by structure I.



An oxygen function (hydroxyl or methoxyl) must be present in ring A.

The steroid must possess an aromatic ring A. Androstane derivatives with a saturated ring A are Kober negative. Provided there is a C17 oxygen function in the molecule, 19-norandrogens with 4-en-3-one groups are feebly Kober positive, while androsta-1,4-dien-3-ones are strongly positive. It is suggested that the latter compounds undergo the dienone-phenol rearrangement, the 19-norandrogens dehydrogenation at C1-C2 and then rearrangement to the phenol structure. Experimental support for this theory has been obtained by measurements in the ultra-violet region.

Absence of the angular methyl group at C13 does not block the first stage of reaction—formation of the yellow chromogen  $\chi_3$ , which absorbs maximally at 450–500 m $\mu$ —but the conversion of  $\chi_3$  to  $\chi_4$  in the second stage is inhibited in the absence of the methyl group.

A yellow chromogen ( $\chi_2$ ) absorbing in the region 400–440 m $\mu$  is frequently formed as a product of the Kober reaction and appears to be derived from  $\chi_3$  as an alternative to its conversion into  $\chi_4$ .  $\chi_1$  is a chromogen absorbing at 350–370 m $\mu$  formed at either stage of either procedure from some of the oestrane derivatives examined.

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### Artefact of Hydrolysis of Glucosamine Derivatives

AN unusual amino sugar derivative has been reported in acid hydrolysates of the cell wall of *Penicillium notatum*<sup>1,2</sup>, and we report here the isolation of an apparently identical compound in acid hydrolysates of pure *N*-acetyl glucosamine.

*N*-acetyl glucosamine (100 mg) was heated in 10 ml. of 4 normal hydrochloric acid at 100° C for 20 h in a silicone greased, evacuated hydrolysis tube. The material

remaining after hydrolysis was recovered by evaporation of the centrifuged solution over phosphorus pentoxide and potassium hydroxide in a vacuum desiccator previously purged with nitrogen. This procedure is identical to that described for hydrolysis of the cell walls of *P. notatum*<sup>2</sup>.

Chromatography of the product on Whatman 3 MM paper using *n*-butanol, acetic acid and water (4:1:5) showed traces of a compound at an *R* glucose value of 1.65. After chromatography the area corresponding to this spot was eluted and just less than 1 mg of material was isolated. The compound gave a positive reaction to the silver nitrate<sup>3</sup>, aniline phosphate<sup>4</sup>, ninhydrin and Elson-Morgan sprays<sup>4</sup>, had the same *R* glucose values in *n*-butanol, pyridine and water (6:4:3) as the previously reported compound, and co-chromatographed with it in *n*-butanol, acetic acid and water (4:1:5) and *n*-butanol, ethanol and water (4:1:1).

Acid hydrolysis of the compound in 2 normal hydrochloric acid at 100° C gave glucosamine, identified as described before<sup>1,2</sup>. *N*-acetylation of the compound gave an *N*-acetyl derivative which co-chromatographed with the previously reported *N*-acetylated derivative in *n*-butanol, acetic acid and water (4:1:5) and *n*-butanol, ethanol and water (4:1:1).

An apparently similar compound can be detected in acid hydrolysates of lobster chitin and of the chitinous cell walls of *Penicillium roquefortii* and *Penicillium patulum*, but whereas it is produced in easily detectable amounts from the cell walls of the *Penicillium* species only traces can be produced from the other sources, including *N*-acetyl glucosamine.

The amino sugar derivative previously reported is therefore probably an artefact of acid hydrolysis of *N*-acetyl glucosamine or its derivatives. The extreme acid lability of the compound may mean that the aniline phosphate and Elson-Morgan reactions are spurious and caused by acid hydrolysis to glucosamine. No structural data are available on the small amounts of materials isolated. The compound is being described because other workers may detect it in hydrolysates of glucosamine containing polysaccharides.

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### Role of Cyclic 3',5'-Adenosine Monophosphate in the Release of Growth Hormone *in vitro*

CYCLIC 3',5'-adenosine monophosphate (3',5'-AMP) may be involved in the synthesis or release of several hormones. Corticotropin increases the concentration of cyclic 3',5'-AMP in adrenal cortex<sup>1</sup>, and cyclic 3',5'-AMP stimulates the synthesis of adrenal corticosteroids<sup>2</sup>. Luteinizing hormone increases the concentration of cyclic 3',5'-AMP in beef corpus luteum<sup>3</sup>, and cyclic 3',5'-AMP stimulates the synthesis of progesterone<sup>4</sup>. Theophylline (1,3-dimethyl-xanthine), which increases the intracellular concentration of cyclic 3',5'-AMP by inhibition of cyclic nucleotide diesterase<sup>5</sup>, increases the secretion of insulin *in vivo*<sup>6</sup>. Glucagon and epinephrine, which may affect the rate of synthesis of cyclic 3',5'-AMP<sup>7</sup>, also influence the release of insulin<sup>8</sup>. The suggestion that cyclic 3',5'-

AMP participates in the release of other peptide hormones<sup>6</sup> led me to investigate the effects of theophylline, epinephrine and glucagon on the release of growth hormone *in vitro*. I have already described a system for the study of the release of growth hormone using radio-immunoassay<sup>10</sup>.

Pituitary glands were obtained from heifers within 5 min of the death of the animal. The diaphragma sellae was dissected from the pituitary and the posterior lobe and the stalk removed. The anterior lobe was washed at 37° C in bicarbonate buffered salt solution<sup>11</sup> which had been gassed with a mixture of oxygen and carbon dioxide (95:5) and which contained bovine plasma albumin (2 mg/ml.) and glucose (1 mg/ml.), and sectioned in a Stadie-Riggs microtome. Slices were washed, and incubated at 37° C in 20 ml. of the same medium during transfer to the laboratory. They were then divided along the mid-line (so that one half served as a control for the other) and incubated with shaking in 5 ml. bicarbonate medium at 37° C for 30 min. The slices were then transferred to 5 ml. bicarbonate medium containing either epinephrine (200 µg/ml.), glucagon (5 µg/ml.) or theophylline (6.7 mmolar), and incubated with shaking at 37° C for 2 h. At intervals of 30 min, 100 µl. samples were removed and diluted in 10 ml. of phosphate buffer (40 mmolar phosphate, pH 7.4) containing albumin (1 mg/ml.) and thiomersal (0.25 mg/ml.). Growth hormone concentrations were determined by immunoassay<sup>10</sup>.

Table 1. EFFECT OF GLUCAGON, EPINEPHRINE AND THEOPHYLLINE ON THE RELEASE OF GROWTH HORMONE

Medium	30 min	60 min	90 min	120 min
Glucagon (5 µg/ml.)	—	103 ± 7%	—	107 ± 7% (11)
Epinephrine (200 µg/ml.)	—	94 ± 22%	—	86 ± 16% (8)
Theophylline (6.7 mmolar)	137 ± 8%*	140 ± 10%*	124 ± 6%*	122 ± 7% (35)†

Slices were incubated in 5 ml. bicarbonate buffered medium containing bovine plasma albumin (2 mg/ml.) and glucose (1 mg/ml.). The output in the presence of glucagon, epinephrine or theophylline was expressed as a percentage of the output by the paired control slice, and the mean and standard error are given. Figures in parentheses give the number of slices: five to six slices were obtained from each pituitary.

\*  $P < 0.001$ .

†  $P < 0.01$ .

As shown in Table 1, glucagon and epinephrine did not alter the rate of output of growth hormone, but theophylline increased the rate significantly ( $P < 0.001$ ). The mean stimulation by theophylline (31 per cent) was of similar magnitude to the stimulation observed in this system with hypothalamic extracts<sup>10</sup>. These data suggest that release of growth hormone is stimulated by cyclic 3',5'-AMP, and that pituitary  $\alpha$ -cell adenylyl cyclase is not stimulated by either glucagon or epinephrine. Stimulation of adenylyl cyclase is a possible mechanism for the effect of the hypothalamic growth hormone releasing factor.

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## Effect of $\gamma$ -Rays on the Action of an Enzyme

LITTLE is known about the effects of high energy radiation on the mechanism of enzyme action. Charlesby<sup>1</sup> investigated the decrease of activity of alcohol dehydrogenase irradiated with  $\alpha$ - and  $\gamma$ -rays and found a relatively high resistance. We have been interested, however, in the effects on the reaction of enzymes such as alterations in reaction rates, specificity and structure of products. We chose to use dextran sucrose because this enzyme is available in relatively pure preparations<sup>2</sup> and its reaction mechanism is well known from this kind of kinetic work<sup>3</sup>. It is of advantage that dextran sucrose catalyses a poly reaction and the values of the molecular weights of the products are a further parameter of the reaction. Dextran sucrose from *Leuconostoc mesenteroides* B 512F has been used, converting sucrose into fructose and high polymer dextran. The irradiations were carried out with a cobalt-60 source of about 20,000 c. First results showed that the radiation resistance of the enzyme depends a great deal on the purity of the preparation. In our experiments the enzyme concentration was between 12 and 50  $\mu$ /ml. and irradiation usually lasted for 1 h.

The decrease in the activity of the enzyme in relation to the dose of irradiation is shown in Fig. 1. The resistance of the enzyme is very high, 800 krad being necessary for a 50 per cent reduction of activity, and the enzyme is completely destroyed by 2.5 Mrad. Because of the high radiation resistance we were able to use a convenient range of radiation doses.

In the first series of experiments, we studied the dependence of the reaction rates at different substrate concentrations (5, 10 and 15 per cent) and doses of irradiation. In all cases a decrease in the reaction rate was observed with an increase in the dose of irradiation. At low substrate concentrations and with small doses of radiation the reaction is more strongly inhibited in relation to the deactivation of the enzyme. With higher doses of radiation, however, the reverse effect was observed (Fig. 1). It is interesting that the reaction rate of the dextran formation in the 15 per cent substrate solution is faster than in the solutions of lower concentrations. In our next experiment, we determined the reaction rate with and without irradiation (Fig. 2). A 5 per cent substrate solution was used and enough enzyme was added for a 100 per cent conversion within 3 h. The reaction took place under irradiation for the first and the third half hour, and in these periods the reaction rate was depressed. In the intervals without irradiation, however, the reaction rate was about as fast as in the blank experiments.

The results of all our experiments can be explained by the reaction mechanism of the enzyme we proposed earlier<sup>4</sup>. In this mechanism the dextran chain is propagated by an insertion type poly reaction. The enzyme and the growing chain form a relatively stable complex. Within one growing cycle the substrate reacts bifunction-

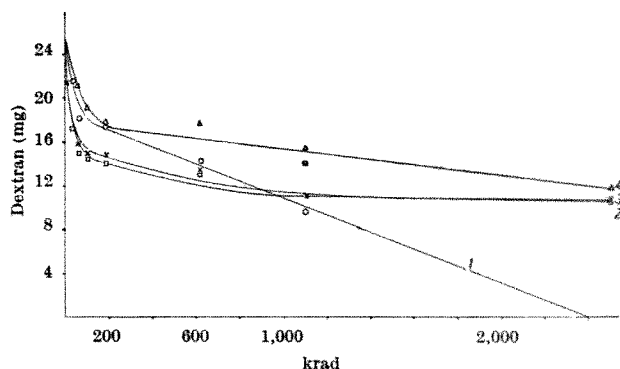


Fig. 1. Substrate concentrations: 4, 15 per cent; 3, 5 per cent; 2, 10 per cent. 1, Enzyme activity.



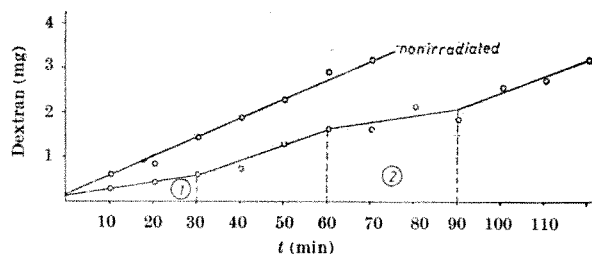


Fig. 2. 1, First radiation period; 2, second radiation period.

ally with this complex forming a Michaelis complex. The latter reacts with liberation of a molecule of fructose and the polymer chain adjacent to the enzyme is lengthened



where  $S$  is sucrose,  $F$  is fructose,  $E$  is enzyme and  $P_n$  is polymer dextran.

Considerations of the molecular mechanism<sup>4</sup> indicated that for the stability of the complexes the polymer chain must be connected to the enzyme at several sites along the chain. In pure enzyme preparations the free form exists. We assume that the adjacent polymer chain has a stabilizing influence and increases the resistance of the enzyme to radiation. The other results can be explained if the Michaelis equilibrium (1) is displaced in the direction  $EP_n + S$ . In this case the maximum rate is then reached only at higher substrate concentrations and an increase of the rate would be expected within a range in which the non-irradiated reaction is already at a maximum. Displacement of equilibrium can also be the reason for the strong inhibition with low radiation doses, for this effect would be expected to be bigger at lower substrate concentrations. This agrees with our experiments. Similarly, the experiments with the alternating irradiation periods can be interpreted as follows. The concentration of the  $ESP_n$  complex is decreased by the irradiation and therefore also the reaction rate ( $v = k_2 [ESP_n]$ ).

The interaction of the radiations on the Michaelis complex which affect the displacement of the equilibrium are not easy to interpret. Possibly oscillations of rather slow frequencies are induced by the irradiation with the result that the substrate molecule cannot be so strongly attached to the enzyme complex. The Michaelis complex then loses stability in favour of the enzyme complex. With this model the protection effect of the adjacent chain can be understood because the polymer chain is a stabilizing factor of the secondary structure of the enzyme.

If all this is true then radiation can also affect the secondary structure of enzymes, causing alterations in the elementary steps in the reaction. Such effects would lead to a fully reversible inhibition, which cannot be explained by changes in the primary structure of enzymes.

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## New Pyrimidine Pathway involved in the Biosynthesis of the Pyrimidine of Thiamine

OUR studies with mutants of *Salmonella typhimurium* LT2 have revealed that the pyrimidine moiety of thiamine is synthesized by a new biosynthetic route using intermediates from the purine biosynthetic pathway. Radioactive labelling has indicated that the conventional nucleic acid pyrimidine biosynthetic pathway is not used for biosynthesis of the pyrimidine of thiamine. David, Estramareix and Hirshfeld<sup>1</sup> showed that <sup>14</sup>C-formate was incorporated into the C-4 of the pyrimidine moiety of thiamine while the C-4 of nucleic acid pyrimidines was derived from aspartate. Moreover, neither <sup>14</sup>C-aspartate nor <sup>14</sup>C-uracil, which are precursors of nucleic acid pyrimidines, was incorporated into the pyrimidine of thiamine<sup>2,3</sup>.

The finding of the new pyrimidine pathway originated with the isolation and study of single site mutants of *S. typhimurium* LT2 which had a double growth requirement for a purine plus the pyrimidine moiety of thiamine (called ath mutants in distinction from simple purine requirers called Ad mutants). Such ath mutants have been previously used in genetic studies by Yura<sup>4</sup>. A study of the distribution of ath and Ad mutants in relation to the purine biosynthetic pathway revealed that all non-leaky mutations before aminoimidazole ribonucleotide (AIR) were ath mutants and, except for one group, all mutations after AIR were Ad mutants. The mutants of the exceptional ath group, which were blocked after 4-aminoimidazole-5-carboxamide ribonucleotide (AICAR) and which seemed identical to the mutants of the ath B group in the classification of Yura<sup>4</sup>, were shown, however, to be only phenotypically like ath mutants. The ath-like character seemed to result from the production of an inhibitor of pyrimidine moiety biosynthesis. If this inhibitor was washed free they formed large amounts of the pyrimidine until the inhibitor again accumulated.

The location of all true ath mutations on the purine pathway was therefore prior to AIR and this suggested the hypothesis that the early part of the biosynthetic pathway of the pyrimidine moiety of thiamine might be common to the early part of the purine biosynthetic pathway and that AIR was the last common intermediate.

Confirmation of this hypothesis was obtained from <sup>14</sup>C-labelling experiments. Using a mutant of *S. typhimurium* LT2 which, because of its inability to join the pyrimidine and thiazole moieties of thiamine, excreted the pyrimidine moiety into the medium, it was found that radioactivity from both carbon atoms of glycine was incorporated into the pyrimidine moiety. When experiments of this sort were done with mutants which were also auxotrophic for glycine, then pyrimidine moiety biosynthesis was strictly dependent on the presence of added glycine, and <sup>14</sup>C was incorporated from both carbon atoms of glycine with little or no dilution of specific activity. This indicated that, just as for purine biosynthesis, both carbon atoms of glycine were directly incorporated into the pyrimidine of thiamine.

The hypothesis was also confirmed by finding that the ribonucleoside form of AIR (AIR<sub>s</sub>) could satisfy both the requirement for a purine and the pyrimidine moiety of thiamine in an ath mutant. Normally, purine intermediates excreted by mutants (with the exception of 4-amino-5-imidazole carboxamide) cannot be re-utilized for purine synthesis by mutants blocked earlier on the purine pathway<sup>5</sup>. An ath mutant was isolated, however, which was able to use AIR<sub>s</sub> (rigorously purified) in place of its purine and pyrimidine moiety requirements. The AIR<sub>s</sub>, moreover, removed the glycine requirement for pyrimidine synthesis for derivatives of this mutant which require glycine.

The final test of the hypothesis was the conversion of <sup>14</sup>C-labelled AIR<sub>s</sub> to labelled pyrimidine moiety. A mutant able to use AIR<sub>s</sub> and which also excreted the

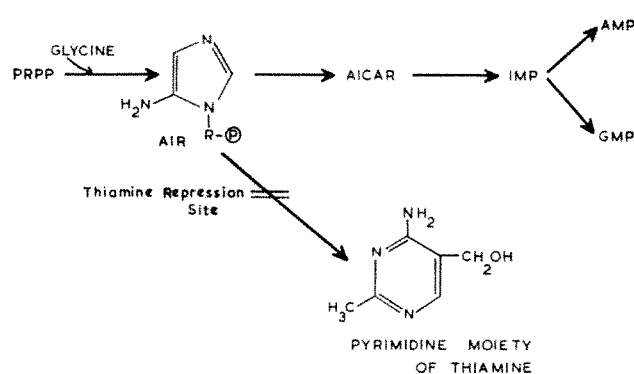


Fig. 1. Simplified scheme of the purine biosynthetic pathway showing the proposed branch leading to the pyrimidine moiety of thiamine. PRPP, 5-phosphoribosyl-1-pyrophosphate; AIR, 4-aminoimidazole ribonucleotide; AICAR, 4-amino-5-imidazole carboxamide ribonucleotide; IMP, inosine-5'-monophosphate; AMP, adenosine-5'-monophosphate; GMP, guanosine-5'-monophosphate.

pyrimidine moiety into the medium was found to convert chromatographically pure  $^{14}\text{C}$ -labelled AIR<sub>8</sub> into the pyrimidine moiety of thiamine with virtually no dilution of specific activity. This provided strong evidence that this purine intermediate is also an intermediate in the biosynthesis of the pyrimidine of thiamine.

Thiamine, which is known to repress the overall biosynthesis of the pyrimidine of thiamine<sup>6</sup>, was also found to repress the conversion of radioactive AIR<sub>8</sub> to radioactive pyrimidine moiety. This result indicates that the thiamine repression site is localized between AIR and the pyrimidine.

It is concluded that the pyrimidine moiety of thiamine is synthesized from the purine intermediate AIR and this pathway is subject to repressive control by thiamine (Fig. 1). Although AIR<sub>8</sub> was the compound excreted and absorbed, it must have been derived from AIR (the ribonucleotide form) and we therefore suggest that AIR is the actual branch point intermediate.

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### Ascorbic Acid in Steroidogenesis

THE adrenal glands contain the highest concentration of ascorbic acid of any tissue in the body. Changes in the concentrations of ascorbic acid and cholesterol are related to steroid secretion in the adrenal<sup>1</sup>. Although it has been suggested that ascorbic acid might play a primary part in steroidogenesis<sup>2</sup>, its physiological role in the adrenal has not been established.

It has been suggested that ascorbic acid has been involved in the electron transport system in porcine adrenal microsomes<sup>3</sup> as well as in the  $\beta$ -ol-dehydrogenase action in rat adrenal<sup>4</sup>. If such were the case in the *in vivo* system, one would expect ascorbic acid in the adrenal to facilitate production and release of steroid in con-

ditions in which the adrenals are stimulated. No such direct *in vivo* evidence is available at this time. On the contrary, accumulated indirect evidence points to an inhibitory role for ascorbic acid. A different and new hypothesis is offered here to explain some of the recent findings in the literature with regard to the role of ascorbic acid in steroidogenesis. Three lines of evidence support this hypothesis. The high concentration of ascorbate in the adrenal in the resting state exerts a "braking influence" on steroid biosynthesis. This inhibition is chiefly effected through the adrenal hydroxylase systems. Under the condition of adrenocorticotrophic hormone (ACTH) stimulation, the adrenal releases a considerable amount of ascorbate, thus reversing hydroxylase inhibition with resulting facilitation of steroidogenesis.

Lipscomb and Nelson<sup>5</sup>, using a rat adrenal vein cannulation method, demonstrated a temporal relationship between the release of ascorbic acid and steroids *in vivo*. They showed that ascorbic acid release precedes corticoid output, that concentrations of ascorbic acid in the adrenal vein effluent fall as steroid levels are rising and that no significant ascorbic acid release was observed in the absence of steroid release.

Studies in scorbutic guinea-pigs, in which progressive reduction of adrenal ascorbate was produced, indicated that no steroid inhibition occurred<sup>6</sup>. In fact, some transient stimulation of steroid production was noted in the early part of the deficiency state.

Rosenfeld<sup>7</sup>, using the perfused beef adrenal gland preparation, studied steroid biogenesis under conditions where the glands were devoid of ascorbic acid. The addition of ACTH to these preparations stimulated steroidogenesis in spite of lack of ascorbic acid in blood and adrenals.

Hayano *et al.*<sup>8</sup> demonstrated an inhibitory effect of ascorbate on steroid  $11\beta$ -hydroxylase of beef adrenal *in vitro*. The concentration of ascorbic acid in these preparations, however, was high. Recently, I have been able to demonstrate the same inhibitory phenomenon on this enzyme with a lesser amount of ascorbic acid<sup>9</sup>.

Cooper and Rosenthal showed that C-21 hydroxylase of beef adrenal is also inhibited by catalytic amounts of ascorbate<sup>10</sup>. This inhibition is associated with lipid peroxidation<sup>11</sup> and arachidonate reduction in phospholipid fraction of beef adrenal microsomes<sup>12</sup>.

Recent studies indicate that ascorbic acid released from the adrenal could be recovered quantitatively in the adrenal vein effluent<sup>13</sup>. The recovered compound is not ascorbic acid but other metabolic products of ascorbic acid, that is, dehydroascorbic acid or diketogulonic acid<sup>14,15</sup>.

Although the exact mechanism for the function of ascorbate is not known, it is possible that ascorbate in the adrenal may lead to rearrangement of the lipids and cholesterol esters. Ascorbate is a known catalyst for lipid peroxidation and is an inhibitor of another microsomal enzyme, gulonolactone oxidase, in rat liver microsomes<sup>16</sup>. The alteration of unsaturated fatty acid composition could conceivably lead to inhibition of steroidogenesis through alteration of steroid sulphhydryl complex ( $-\text{SH}-\text{C.M.S.}$ ) which has recently been postulated for steroid hydroxylase system<sup>17</sup>. Furthermore, it has recently been shown that addition of ACTH to the adrenal preparations of rats, sheep, and calves causes increase in free fatty acid content of adrenal<sup>18</sup>. It is tempting to suggest that in circumstances in which the availability of free fatty acids in the adrenal is increased, the production of cholesters of polyunsaturated fatty acids, which are known to be preferentially incorporated into steroids<sup>19</sup>, may predominate. In such conditions, a high concentration of ascorbic acid may prove deleterious to steroidogenesis through peroxidation of unsaturated lipids unless ascorbic acid is released from the adrenal before fatty acid accumulation.

If the hypothesis that the high concentration of ascorbate in the adrenal prevents steroidogenesis is correct

and that during stress or stimulation with ACTH the adrenal must first release the bulk of its ascorbate before stimulation can commence, with experimental intervention it may be possible to maintain high concentrations of adrenal ascorbate in the treated animal and demonstrate inhibition of steroidogenesis; this information is not available in the literature.

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## IMMUNOLOGY

### N- and C-terminal Sequences of a Heavy Chain Disease Protein and its Genetic Implications

A VARIANT of myeloma has been described<sup>1,2</sup>; it is known as heavy chain disease, and is characterized by the presence of a homogeneous serum and urinary protein bearing only the antigenic determinants associated with the heavy or  $\gamma$ -chain of human IgG immunoglobulin. Five such cases have been described, but the protein of two patients, Zu<sup>1</sup> and Cr<sup>2,3</sup>, have been more extensively investigated because of the availability of material and appear to share common structural features. The proteins are symmetrical dimers, stabilized by disulphide bonds, with molecular weights in the range of 50,000–55,000. Peptide maps reveal they contain the Fc portion of the heavy chain as a chief structural unit<sup>3</sup>. This conclusion is supported by the content of carbohydrate<sup>3</sup>, the presence of Gm specificities<sup>4</sup>, and biological activities<sup>5,6</sup> of these proteins, properties also attributed to the C-terminal half of the heavy chain.

The sequences of the C-terminal octadecapeptide of a  $\gamma_{2b}$  pathological IgG immunoglobulin and normal human IgG immunoglobulin have been reported<sup>7,8</sup> as shown in Fig. 1. A C-terminal octadecapeptide of homologous but not identical sequence was established for the urinary heavy chain disease protein of the patient Zu and a 7S myeloma protein, both of the  $\gamma_{2c}$  sub-class, as shown in

Fig. 1 (refs. 9 and 10). This observation provides chemical evidence that the biosynthesis of the Zu protein proceeded to a termination which has a corollary in intact heavy chain and infers that the cistron transcribed in the biosynthesis of the C-terminal region of myeloma proteins and this variant were structurally identical.

In both Zu and Cr antigenic identity has been demonstrated between the serum and respective urinary heavy chain disease protein of each individual, and in the case of Cr Franklin<sup>3</sup> has reported similar molecular weights, amino-acid and carbohydrate compositions for both the urinary and serum heavy chain disease proteins. These observations imply that passage of the proteins through the kidney was not accompanied by detectable degradation. Furthermore the serum of Cr was shown to be devoid of unusual proteolytic activity toward normal IgG, and the half life of administered <sup>131</sup>I-IgG was within the lower normal limits in this patient<sup>3</sup>. Although no evidence of unusual proteolytic activity was observed, the possible formation of the 3-6S serum heavy chain disease protein by fragmentation of an unusually fragile 7S precursor cannot be ruled out. The presence of Fc'-like material in normal urine<sup>11,12</sup> demonstrates the *in vivo* formation of such fragments and their ability to be excreted by the kidney.

The presence of a mixed N-terminal sequence would strongly support the degradative origin of the protein, and so I have investigated the N-terminus of the heavy chain disease protein of Zu. Totally reduced and alkylated Zu protein was treated with 2,4-dinitrofluorobenzene in a solution of potassium bicarbonate and guanidine hydrochloride<sup>13</sup>. After hydrolysis of the DNP-protein in 6 normal hydrochloric acid at 110° C for 18 h only DNP-Val and DNP-Leu (0.03 mole and 0.04 mole/26,000 g of protein respectively, uncorrected) were identified in the ether soluble phase and  $\epsilon$ -DNP-lysine (7.5 moles/26,000 g of protein, uncorrected) in the water soluble phase. No DNP-cysteic acid was observed in the acid hydrolysate of unreduced dinitrophenylated Zu protein which had subsequently been oxidized with performic acid<sup>14</sup>. Because of the destruction of DNP-glycine, DNP-proline and DNP-tryptophan during acid hydrolysis, and the difficulty in identifying *bis*-DNP-histidine, the N-terminus of the Zu protein was also examined by the cyanate method as described by Stark and Smyth<sup>15</sup>. By this procedure glycine, alanine and aspartic acid (0.05 mole, 0.03 mole and 0.08 mole/26,000 g of protein, respectively) were found, as well as valine and leucine (0.04 mole and 0.08 mole/26,000 g of protein, respectively). The significance of the glycine and alanine is difficult to assess, as the artefactual formation of these amino-acids has been observed in the conditions described<sup>15</sup>. Possibly the presence of aspartic acid here represents the release of a small amount of an aspartic-glycopeptide, for dinitrophenylation had not revealed aspartic acid as an N-terminal residue. Thus it would appear that no dominant free N-terminal amino-acid residue could be established for the Zu heavy chain disease protein, nor could the N-terminus be accounted for by the summation of various N-terminal residues.

Press *et al.*<sup>7</sup> have reported the identification of a pyroglutamic acid (PCA) blocked N-terminus on the heavy chain of a  $\gamma_{2b}$  pathological human IgG immunoglobulin. The tripeptide PCA-Val-Thr was isolated from a 'Pronase' digest of the heavy chain, and this sequence has been extended using the cyanogen bromide fragments of the chain<sup>7,16</sup>. A limited heterogeneity of PCA blocked N-terminal sequences also have been identified on the heavy chain of normal rabbit IgG immuno-

7S Myeloma Daw<sup>7</sup>  
Normal human IgG<sup>8</sup>

$\gamma_{2b}$  Met-His-Glu-Ala-Leu-His-Asn-His-Tyr-Thr-Gln-Lys-Ser-Leu-Ser-Leu-Ser-Pro-Gly

Zu Heavy chain disease protein<sup>9,10</sup>  
7S Myeloma Mar<sup>9,10</sup>

$\gamma_{2c}$  Met-His-Glu-Ala-Leu-His-Asn-Arg-Phe-Thr-Gln-Lys-Ser-Leu-Ser-Leu-Ser-Pro-Gly

Fig. 1. The C-terminal sequences of human  $\gamma_{2b}$  and  $\gamma_{2c}$  heavy chains. Italics indicate the difference between the two chains.

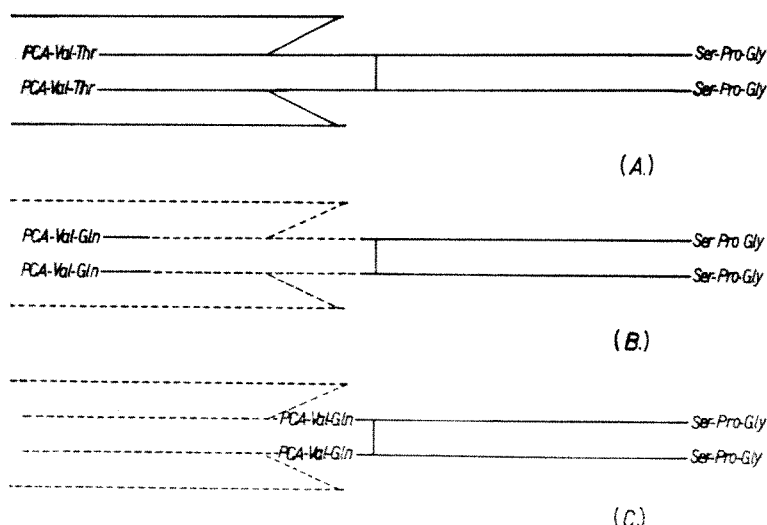


Fig. 2. Illustrative model of (A) 7S myeloma protein based on the data of Press *et al.*<sup>17</sup>; (B) cistronic deletion of the Fd area of heavy chain disease protein following initiation; (C) transposition of the site of initiation to an internal position with loss of the Fd area according to Askonas and Williamson<sup>20</sup>.

globulin<sup>17</sup>, which include PCA-Glu, PCA-Ser-Leu and PCA-Ser-Val. The Zu heavy chain disease protein was examined for a blocked N-terminus in the same manner as described by Press *et al.*<sup>7</sup> for their immunoglobulin and by Ikenaka, Bammerlin, Kaufmann and Schmid for orosomucoid<sup>18</sup>. A 'Pronase' digest of 50 mg of totally reduced and alkylated Zu protein was run down a column of 'Dowex 50' ( $\times 2$ ; H<sup>+</sup> form), and the unabsorbed peptides eluted with water; the eluate was concentrated and subjected to high voltage paper electrophoresis at pH 3.5. A ninhydrin stain was negative, but staining by the hypochlorite/starch-iodide technique revealed a neutral and an acidic band, the latter of which migrated with a mobility of 0.70 relative to synthetic PCA-Val used as a marker. Both bands were eluted, hydrolysed and analysed on the amino-acid analyser. The neutral band was found to contain aspartic acid, threonine, serine and hexosamine, and probably represented a glycopeptide. The acidic band contained glutamic acid and valine in a molar ratio of 1.97 to 1.00; the yield of tripeptide at this stage was 0.6 mole/26,000 g of Zu heavy chain disease protein. The acidic peptide was digested with carboxypeptidase A at pH 8.0 overnight at 25°C, and the digest electrophoresed on paper at pH 6.5. The hypochlorite/starch-iodide stain revealed an acidic and a neutral band, but only the neutral band gave a positive staining reaction with ninhydrin. On hydrolysis only glutamic acid was found in the neutral band, which therefore must have been present as glutamine in the original tripeptide. The acidic peptide possessed the same mobility as the synthetic PCA-Val marker when subjected to high voltage paper electrophoresis at pH 6.5, 3.5 and 1.9 and the same  $R_F$  on paper chromatography in butanol-acetic acid-water (4:1:5), and on hydrolysis and analysis yielded glutamic acid and valine in equimolar quantities. Hydrazinolysis of the dipeptide released only valine, establishing the N-terminus of the Zu heavy chain disease protein as PCA-Val-Gln. This same tripeptide has been identified as the N-terminus of one, and only one, of the fragments obtained by cyanogen bromide cleavage of the Zu protein.

It seems unlikely that this unique N-terminal sequence should arise from enzyme degradation of a precursor molecule, because this would predicate the selective cleavage leading to the new N-terminal glutamic acid or glutamine residue with subsequent cyclization to PCA. But obviously this possibility cannot be excluded. In view of the close homology to the N-terminus reported for a pathological human IgG (Fig. 2A) and the recent report

that 20–40 per cent of the IgG molecules of pooled normal human serum have the N-terminal sequence PCA-Val-Glu(NH<sub>2</sub>) on the heavy chain<sup>19</sup>, a more intriguing interpretation suggests itself. Perhaps, following the site of initiation of the  $\gamma$  chain, the cistron corresponding to the chief portion of the Fd area of the chain has been deleted, biosynthesis recommencing in the Fc area of the chain (Fig. 2B). The deletion of the Fd area and the heavy-light disulphide bond may partly account for the lack of light chain associated with the heavy chain disease protein, the persisting unutilized light chain suppressing the further production of light chain such that no significant quantity of circulating free light chain is observed. An alternative hypothesis has been put forth by Askonas and Williamson<sup>20</sup> proposing the transposition of the initiation site to a new position in the interior of the cistron by a mutation which permits an internal codon to be read as an initiator codon (Fig. 2C). A distinction between these alternatives may be possible if an extended homology of the N-terminal sequence of the Zu heavy chain disease

protein and 7S myeloma can be established, and a switch point to the Fc structure identified.

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## PATHOLOGY

### Metabolism of Tritium Labelled Dopamine in Schizophrenic Patients

THE metabolism of dopamine has been of interest in research on schizophrenia since Friedhoff and Van Winkle<sup>1</sup> announced that they had found 3,4-dimethoxyphenylethylamine (DMPEA) in the urine of schizophrenic patients but not in the urine of normal persons.



In 1963 they<sup>2</sup> administered labelled dopamine, the presumed precursor of DMPEA, to schizophrenic patients and reported that they found DMPEA, and 4-hydroxy-3-methoxyphenylethylamine and the two corresponding acetic acids excreted in the urine.

Later investigations<sup>3,4</sup> have, however, shown that their original method for identification of DMPEA was not specific. Several substances such as *p*-tyramine<sup>5</sup>, bacterial intestinal products<sup>6</sup> and drug metabolites<sup>4</sup> may interfere with the identification of DMPEA in the different analytical methods.

This uncertainty has led us to infuse tritium labelled dopamine in schizophrenic patients in the same way as Friedhoff and Van Winkle in order to study the metabolic conversion of dopamine.

The material consists of one normal control and four chronic schizophrenic patients, whose main symptoms were severe withdrawal, hallucinations and delusions. They had all been in hospital for many years and were on maintenance therapy with phenothiazines. The treatment was stopped 22–80 days before the investigation.

The patients were in bed and fasting during the investigation. At 9 a.m. they voided and labelled dopamine dissolved in 10 ml. of physiological sodium chloride solution was injected intravenously, the injection time being 2.5 min. Immediately after the injection they drank 400 ml. water. The urine produced for the next 3 h was collected.

The injected dopamine was 3,4-dihydroxyphenylethyl-2-<sup>3</sup>H-amine, specific activity 1.84 c/mmol. The amount injected was 176  $\mu$ C. dopamine-<sup>3</sup>H + 0.0146 mg dopamine.

The collected urine samples were investigated for the following acids: 3,4-dimethoxyphenylethylacetic acid (DMPAA), homovanillic acid (HVA) and vanilmandelic acid (VMA), and for the following amines: 3,4-dimethoxyphenylethylamine (DMPEA), 4-hydroxy-3-methoxyphenylethylamine (HMPA) and normetanephrine (NM).

One sixth of the collected urine was used for estimation of the acids, and to this part of the urine was added unlabelled carrier material: 600  $\mu$ g of DMPAA and HVA, and 300  $\mu$ g of VMA.

The acids were isolated on paper chromatograms as described by Armstrong *et al.*<sup>7</sup>. The spots were localized by scanning in shortwave ultra-violet light, cut out and extracted with ethanol and the amount of tritium in the extract was estimated in a liquid scintillation counter. To the rest of the urine which was used for the estimation of the amines 250  $\mu$ g of unlabelled carrier substance of each of the three amines were added.

The occurrence of the amines was investigated in both hydrolysed and unhydrolysed urine. The urine samples were subjected to three fractionations following each other: chromatography through a 'Dowex' column, one way paper chromatography of eluates from the column and two way thin-layer chromatography of eluates of the spots on the paper chromatogram as described by Faurbye and Pind<sup>8</sup>.

The thin-layer chromatograms were developed by ninhydrin spraying, the spots with the three amines were scraped off and extracted with ethanol, and the tritium content of the ethanol solution estimated in the liquid scintillation counter.

The recovery of the amines was between 64 and 86 per cent, and the values in Table 1 are corrected to 100 per cent recovery. The method allows estimation of labelled substances containing about 0.06 parts per thousand of administered tritium in both hydrolysed and unhydrolysed urine.

Table 1 shows that there is no difference between the control and the schizophrenics. The excretion of DMPEA, if any, must have been less than 0.06 parts per thousand of the administered labelled material.

The radioactivity extracted from the DMPAA spots was so slight that it was significant in only two of the schizophrenics. Whether this weak radioactivity was

Table 1. EXCRETION IN THE URINE OF LABELLED DOPAMINE METABOLITES IN NUMBER OF COUNTS/1,000 COUNTS OF ADMINISTERED LABELLED DOPAMINE, OF WHICH 176  $\mu$ C. WERE GIVEN INTRAVENOUSLY TO ONE CONTROL PERSON AND FOUR SCHIZOPHRENIC PATIENTS

Initials	Control person	Schizophrenic patients				
	K. P.	G. A.	P. J.	E. H.	O. D. J.	
Total amount of labelled material in urine	440	400	410	380	540	
DMPAA	0.072 ( <i>P</i> = 0.025)	0.074 ( <i>P</i> = 0.005)	0.066 ( <i>P</i> < 0.001)	0	0.043 ( <i>P</i> = 0.05)	
HVA	70	40	75	58	55	
VMA	4.7	3.5	1.8	1.5	0.97	
DMPEA, unconjugated	0	0	0	0	0	
HMPA	3.5	3.8	2.7	12	1.6	
NM	0.44	0.56	0.19	0.47	0.31	
DMPEA, summary of conjugated and unconjugated	0	0	0	0	0	
HMPA	7.1	12	7.7	17	3.5	
NM	0.62	0.97	0.31	0.76	0.51	

caused by DMPAA or another substance at the same place in the chromatogram is not known.

The obtained pattern of dopamine metabolites indicates that the main metabolite of dopamine is HVA (4-hydroxy-3-methoxyphenylacetic acid), and it seems likely that the DMPAA found in our experiment was formed by 4-methoxylation of HVA. Friedhoff and Van Winkle<sup>1,2</sup> have suggested that there is another pathway through DMPEA to DMPAA. The occurrence of dimethoxylation has been confirmed by Kuehl *et al.*<sup>9</sup>, who demonstrated the presence of DMPAA in both schizophrenic and normal urine in about equal amounts.

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## Carcinogenicity of the Herbicide Maleic Hydrazide

MALEIC hydrazide, 1,2-dihydropyridazine-3,6-dione, was synthesized in 1947 (ref. 1) and has since become an important herbicide with uses including inhibition of sprouting in vegetables and stored root crops, prevention of sucker production in tobacco plants and growth control of grass, and foliage<sup>2</sup>. Maleic hydrazide is claimed to be selectively toxic to plants but not to bacteria, fungi, rodents or dogs<sup>3</sup>; it is, however, mutagenic to *Drosophila melanogaster*<sup>4</sup> and *Bacillus megatherium*<sup>5</sup>, it reduces the fertility of pea aphids<sup>6</sup>, and produces cytotoxicity, mitotic inhibition and unbalanced growth in cultured mammalian cells<sup>7</sup>. The finding that maleic hydrazide induced breakage of heterochromatin besides mitotic inhibition in *Vicia faba*<sup>8</sup> prompted this warning: "Since nearly all chromosome-breaking agents have so far proved to be cancer-producing as well, we must hope that the agricultural use of this new agent will not be encouraged before suitable tests are made." Later tests claimed to show that maleic hydrazide is non-carcinogenic<sup>9</sup>. This conclusion seems at variance with the data on which it was based; for example, after weekly injections of 5 mg of maleic hydrazide as the diethanolamine salt during 14 months, an "unusually high" incidence of local sarcomas, in three out of fifty-two rats at risk with none in controls, was noted<sup>9</sup>. In other experiments, a total of 260 mg of maleic hydrazide in arachis oil was injected subcutaneously during 65

weeks, and three local sarcomas, one with a hepatoma, developed in three of six rats; it was concluded that the herbicide was clearly carcinogenic<sup>10</sup>.

We report here a high incidence of remote tumours, hepatomas, after subcutaneous injections of maleic hydrazide in infant mice. Swiss mice (*ICR/Ha*) were injected subcutaneously with aqueous solutions or tricapylin suspensions of maleic hydrazide as the free acid, batch No. Q5199 containing about 0.4 per cent hydrazine as an impurity, or with solvent in volumes of 0.1, 0.1, 0.2 and 0.2 ml. at 1, 7, 14 and 21 days following birth, respectively. (MH is the usual commercial formulation marketed by US Rubber and is stated to contain 42 per cent inert ingredients and 58 per cent diethanolamine salt of maleic hydrazide, equivalent to 30 per cent of this herbicide. MH-30 contained about 0.07 per cent hydrazine with respect to maleic hydrazide.) Results are shown in Table 1. Details of injection techniques, animal care and autopsy procedures have been described before<sup>11,12</sup>. Of the mice, 97 per cent based on numbers at risk at weaning were autopsied; the remainder were autolysed or cannibalized. Experiments stopped when mice reached 49–51 weeks old (49+).

Mortality before weaning was generally limited to the first 3 days of life and was high after injections of 5 or 10 mg of maleic hydrazide on day 1, 55 per cent and 100 per cent respectively, in contrast with controls, 14 per cent (Table 1). After weaning, substantial sex differences in survival developed because of a non-specific syndrome of obstructive uropathy the incidence of which, based on numbers of males alive at weaning and subsequently autopsied, was high in all groups. In controls it was 38 per cent, and 57 per cent and 52 per cent respectively in groups given 3 and 55 mg of the herbicide. Relative to controls, there was evidence of limited weight loss (about 5 per cent) in mice at 49+ weeks in the maleic hydrazide suspension group.

Numerous hepatomas were noted at the termination of experiments in males receiving a total dose of 55 mg of maleic hydrazide compared with controls; the incidences of hepatomas, based on mice alive at 49+ weeks, are 73 per cent for the suspension of maleic hydrazide versus 8 per cent for the solvent controls (Table 2). These differences are very significant, continuity-corrected  $\chi^2 = 24.27$ ,  $P < 0.000001$ ; analysis of variance to take possible litter influences into account, because tumours were not randomly distributed between various litters, yields similar significance,  $F(1, 23) = 33.86$ ,  $P < 0.00001$ . The hepatomas were solitary or multiple, ranging in size from the microscopic to larger than the normal liver, and were generally of the classical trabecular type with conspicuous hyaline

inclusion bodies. No metastases were noted. The high incidence of hepatomas in the maleic hydrazide suspension group, and to a lesser extent the lower incidence of tumours in the maleic hydrazide solution group, was associated with numerous atypia in the livers of the remaining non-tumour bearing male mice. These atypia featured zonal necroses and histiocytic infiltrations, nuclear and cytoplasmic inclusion bodies, increased cytoplasmic basophilia and cellular pleomorphism. If the advanced atypia, as possible pre-neoplastic lesions, are grouped with the hepatomas, then differences between test and control groups are further emphasized. Of the other tumours (Table 2), it may be noted that one fibrosarcoma developed in each of the maleic hydrazide groups in contrast with none in controls.

These data clearly indicate the high carcinogenicity of maleic hydrazide for male mice. The total dosage bringing about liver tumours in the suspension group was 55 mg; the four individual doses, of 5, 10, 20 and 20 mg, are equivalent to 2,500, 2,000, 1,000 and 667 mg/kg on days 1, 7, 14 and 21, if the corresponding animal weights are approximated as 2, 5, 10 and 15 g respectively.

While human populations in general are continuously exposed to maleic hydrazide, it is difficult to compute the level of this exposure. It can be assumed that each day an adult male ingests approximately 1.5 mg of maleic hydrazide from potatoes and onions and inhales 0.27 mg from forty cigarettes, assuming residues of 10 p.p.m. in potatoes<sup>13</sup> and onions and 132 p.p.m. in tobacco of which 6.7 per cent is found in smoke<sup>14,15</sup>. A resulting annual exposure to about 630 mg of maleic hydrazide is some twelve times greater than doses found here to be very carcinogenic. Additionally, it may be noted that because an adult man weighs about 3,000 times more than an adult mouse, on an mg/kg basis, this factor should be applied to the 55 mg of maleic hydrazide given as a carcinogenic dose to a mouse. Many drugs, however, on an mg/kg basis, are more toxic in man than mouse, generally by a factor of 10–15 (ref. 16), so that the 3,000-fold factor becomes reduced to about 200. Thus based on FDA tolerances of 50 p.p.m. (ref. 17), about five times greater than residues generally found, human ingestion of maleic hydrazide from potatoes alone would reach concentrations comparable with the dose of 55 mg carcinogenic to a mouse in about 4.5 yr. Protection in man, however, might be afforded by certain offsetting factors; much human exposure to maleic hydrazide is in the diets of children or adults, in contrast to the present experiments where suspensions of the compound were administered parenterally to infant mice. Intestinal absorption, however, seems high; after an oral dose of 100 mg/kg in rabbits, 43–62

Table 1. TOXICITY INDUCED IN SWISS MICE BY SUBCUTANEOUS INJECTIONS OF MALEIC HYDRAZIDE IN INFANCY

Treatment group	Drug dosage on specified days (mg)				Total dosage (mg)	No. of mice injected (No. litters)	Per cent mortality before weaning	Sex	No. of survivors at 49+ weeks	
	1	7	14	21					at weaning	at 49+ weeks
Controls	—	—	—	—	—	170 (16)	14	M	78	48
								F	69	68
Maleic hydrazide (solution)	0.5	0.5	1.0	1.0	3.0	67 (6)	5	M	35	17
								F	29	26
Maleic hydrazide (suspension)	5.0	10	20	20	55	193 (16)	53	M	46	26
								F	45	43
Maleic hydrazide (suspension)	10	—	—	—	10	11 (1)	100	—	—	—

Table 2. TUMOURS AND OTHER LESIONS INDUCED IN SWISS MICE BY INJECTIONS OF MALEIC HYDRAZIDE IN INFANCY

Treatment group	Initial drug dosage on day 1 (mg)	Sex	No. at risk at 49+ weeks	Hepatic lesions			Miscellaneous tumours* No.
				Moderate atypia	Advanced atypia	Hepatomas	
Controls	—	M	48	2	0	8	4*
		F	68	1	1	0	3†
Maleic hydrazide (solution)	0.5	M	17	0	6	18	1‡
		F	26	0	4	0	3§
Maleic hydrazide (suspension)	5.0	M	26	4	16	73	3
		F	43	16	2	0	6¶

\* Three solitary pulmonary adenomas and one histiocytic thymic lymphoma. † One mammary carcinoma and two solitary pulmonary adenomas. ‡ One reticulum cell sarcoma. § One subcutaneous fibrosarcoma, one lymphatic leukaemia and one solitary pulmonary adenoma. || One subcutaneous fibrosarcoma, one granulocytic leukaemia and one solitary pulmonary adenoma. ¶ Two mammary carcinomas, three solitary pulmonary adenomas and one reticulum cell sarcoma.

per cent can be recovered from urine within 48 h<sup>9</sup>. The importance of the age factor is questionable, for root crops are common baby foods.

It seems unlikely that maleic hydrazide presents a high carcinogenic hazard; but the risks are the result of the ubiquity of the herbicide and the progressive exposure of entire populations. Such hazards can only be justified if applicable to very small population samples, particularly if there is justification such as unmatched therapeutic benefits. It would thus seem appropriate to reduce tolerance levels of maleic hydrazide or ban its use, except when human exposure can be minimized.

The complete absence of pulmonary carcinomas and multiple adenomas in these experiments makes it improbable that the carcinogenicity of maleic hydrazide is caused by a presumptive ring scission *in vivo* to yield hydrazine or to trace contamination with this compound which is a potent lung carcinogen in several species of rats and mice, although also hepatocarcinogenic in *CBA* mice<sup>18</sup>. We do not yet know whether the carcinogenicity of maleic hydrazide depends on modification of nucleic acid synthesis, as demonstrated in plants<sup>19,20</sup> and cultured mammalian cells<sup>7</sup> or on other unrelated mechanisms.

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## CYTOLOGY

### Inhibition of DNA Synthesis of Target Cells *in vitro* by Sensitized Lymphocytes

SEVERAL experimental systems have been developed to test the cytotoxic effect of sensitized lymphocytes on target cells *in vitro*<sup>1-7</sup>. In a previous report<sup>8</sup> we presented evidence for the inhibitory effect of lymphocytes on DNA

synthesis in the target cells. Although this effect is strongest for the sensitized lymphocytes, the fact that it is also shown by the non-sensitized lymphoid cells casts some doubt as to its specificity. A further examination of this phenomenon was therefore undertaken.

The experimental methods have been described elsewhere<sup>8</sup>. Briefly, monolayer cultures were made with mouse cells of embryo or of sarcomas induced by methylcholanthrene and maintained by serial transplantation. Twenty-four to forty-eight hours later, a known number of cells from the peripheral lymph nodes of allogeneic mice sensitized by the subcutaneous inoculation of either the tumour cells or the splenic cells of normal mice were added to the target cells. Lymph node cells of untreated allogeneic or syngeneic mice served as controls. After a further 16-24 h of incubation, 0.1  $\mu$ Ci of thymidine-2-<sup>14</sup>C was added to each culture tube. The target cells, together with the added lymphocytes, were collected 4-68 h, 24 h or less in 85 per cent of experiments; after the addition of thymidine, they were washed with Hanks' salt solution, dissolved in formic acid and then dried on a planchet. Their radioactivity was measured by a thin windowed Geiger-Müller tube. The radioactivity was confined to the nuclear portion, indicating participation in the DNA synthesis by the labelled thymidine, as shown in autoradiographs prepared from parallel experiments labelled with tritiated thymidine. In prolonged incubation, there must have been some degradation of thymidine. This factor was probably insignificant in view of the continued incorporation of labelled material into cells in such cultures. Furthermore, in most of the experiments the incubation period was rather short, 24 h or less in 85 per cent and 12 h or less in 37 per cent of experiments.

The sources of allogeneic lymphocytes for (AXDBA/2)*F*<sub>1</sub> (H2<sup>a</sup>XH2<sup>a</sup>) target cells were C57BL (H2<sup>b</sup>), A.SW (H2<sup>s</sup>), ABY(H2<sup>b</sup>) and A.CA(H2<sup>f</sup>) mice, for A.CA target cells were ABY and A.SW mice, for C57BL target cells were A.CA and (AXADBA/2)*F*<sub>1</sub> mice, for ABY target cells were A.CA, A.SW and A(H2<sup>a</sup>) mice and for A target cells were ABY mice. A total of thirty-nine groups of experiments were performed. In some the target cells

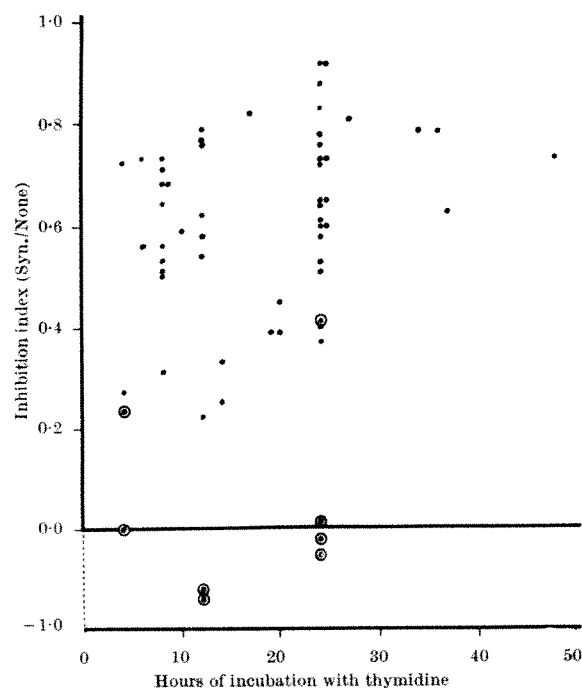


Fig. 1. Inhibition indices Syn/None calculated from the radioactivities of target cells after incubation with thymidine-2-<sup>14</sup>C for various periods of time. The lymphocytes were added to the target cells 16-24 h earlier. The experiments in which the old culture medium was replaced by fresh medium is indicated by a circle.

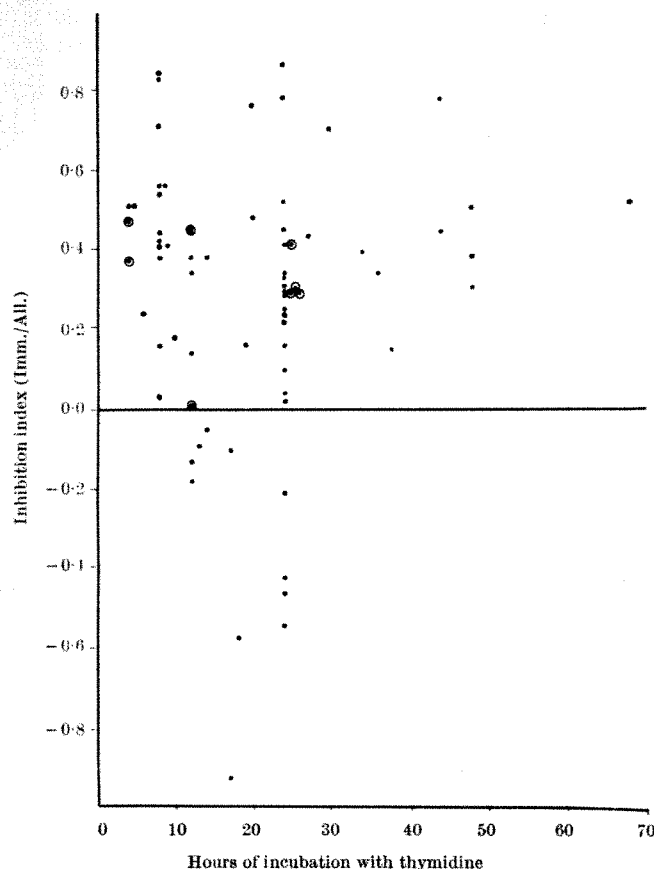


Fig. 2. Inhibition indices Imm./All. The experiments in which the old medium was replaced by fresh medium is indicated by a circle.

were collected after incubation with thymidine for various lengths of time. Thus in such cases there were several separate experiments within the same group. There were seventy-two individual experiments. Allogeneic lymphocytes were always used, but syngeneic cells were used in only sixty-two experiments. The lymphocytes to target cell ratio varied from 2:1 to 100:1; in 67 per cent of the experiments, however, it was between 5:1 and 10:1.

Radioactivities were measured in c.p.m. In order to evaluate the specificity of the lymphocytic effect, the results were expressed in terms of inhibition indices. The inhibition index Imm./All. is calculated as follows: the radioactivity of target cells treated with sensitized allogeneic lymphocytes was subtracted from the corresponding value of target cells treated with non-sensitized allogeneic cells and divided by the latter. It indicates the effect of sensitization. The inhibition index All./Syn. is calculated from the radioactivity of target cells treated with allogeneic or syngeneic lymphocytes of normal mice and indicates the effect of histoincompatibility. The inhibition index Syn./None, using the radioactivity of untreated target cells as control, indicates the non-specific effect of syngeneic lymphocytes.

All lymphocytes, irrespective of their sources, showed an inhibitory effect on DNA synthesis in the target cells. The lymphoid cells of normal syngeneic mice exhibited the least effect. Even with these cells the inhibition was strong (Fig. 1), corresponding roughly with the duration of incubation and with the number of lymphocytes. After the old medium was replaced by fresh culture medium at the time of addition of thymidine, this inhibition was largely eliminated; in some instances the target cells treated with lymphocytes grew even better than the untreated target cells (Fig. 1), indicating an exaggerated recovery from the lymphocytic effect.

The sensitized lymphoid cells from the allogeneic mice showed the greatest inhibition. It was more pronounced than the inhibition by non-sensitized allogeneic cells in 60 or 83 per cent of seventy-two experiments (Fig. 2). In ten experiments, however, the inhibition index was 0.20 or less and in six other experiments it was between 0 and -0.20. These sixteen experiments were considered inconclusive. In six others (8 per cent of the experiments) the inhibition index was less than -0.20, suggestive of either inadequate sensitization or excessive toxicity of normal lymphocytes. In fifty experiments (70 per cent), the inhibition index was over 0.20. The percentage of positive experiments increased to eighty-six when the old culture medium was replaced by a fresh one, as was done in seven experiments. There is no apparent correlation between the inhibition indices and the duration of culture. These results might be interpreted as indicative of the effect of sensitization and a manifestation of immune reaction.

In sixty-two experiments, the radioactivity of target cells treated with allogeneic lymphocytes from normal mice was compared with that obtained with syngeneic cells. The results (Fig. 3) indicated a higher degree of inhibition by the allogeneic cells and the degree of inhibition corresponded to some extent with the duration of incubation. In thirty-six experiments (58 per cent) the inhibition indices were within the range of 0.20 and -0.20, however. In only nineteen experiments (31 per cent) were the indices over 0.20, possibly the result of histoincompatibility. The interpretation is by no means certain, however, because by changing to fresh medium this effect was eliminated.

Because the radioactivity was measured on all the cells in the same culture, part of it must be in the lymphocytes. The mouse lymphocytes did not proliferate well, however,

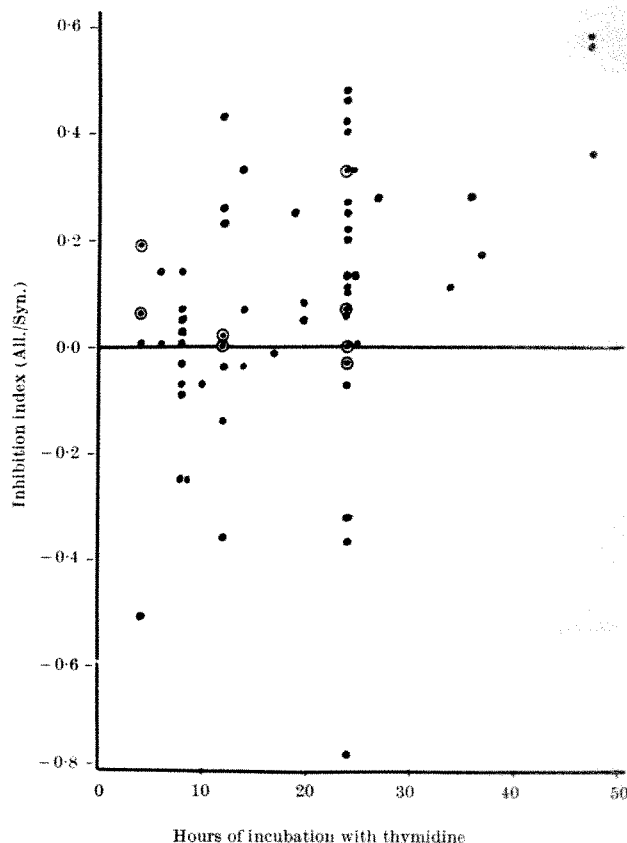


Fig. 3. Inhibition indices All./Syn. The experiments in which the old medium was replaced by fresh medium is indicated by a circle.



in this system, either incubated alone or with the target cells (especially in culture of long duration) and accounted for less than 5 per cent of the total radioactivity<sup>8</sup>. A significant amount of radioactivity in the lymphocytes would obscure its inhibitory effect on the target cells and this may well be the cause of the apparent lack of inhibition in some of the short term cultures.

The results of these experiments indicate a universal inhibitory effect of lymphocytes on the synthesis of DNA in the target cells. The non-specific inhibition by normal lymphocytes appears to be affected by the condition of the culture medium, whereas the inhibition by sensitized lymphoid cells appears to be cell bound. Whether the non-specific effect is the result of competition between the utilization of isotope labelled thymidine and the utilization of DNA or its derivatives released from the lymphocytes requires further investigation.

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### Splitting Line of Bacterial Spore

THE mechanisms involved in the germination of bacterial spores have been studied extensively from the biochemical and morphological points of view<sup>1,2</sup>. Details of morphological changes occurring during germination, however, have not yet been demonstrated clearly. It is not known whether or not, when a new vegetative cell emerges from the spore, the spore coat splits by some specific mechanism.

During electron microscopic studies of the structure of the spore coat of *Bacillus subtilis* we found that there was a fissure on laminated layers of the spore coat. We thought that the spore coat might split at the site of the fissure, when a vegetative cell emerges.

Spores of *Bacillus subtilis* Marburg strain, collected from a nutrient broth culture, were sectioned with an ultra-microtome and observed with a JEM-7 type electron microscope. Procedure was as follows: the spores were fixed with osmium tetroxide, embedded in epoxy resin and sectioned. Photographs of the cross section are shown in Figs. 1 and 2, of which the latter shows an enlarged picture of a fissure in the laminated layers illustrated in Fig. 1.

The intact spore was slightly disrupted in a cell disintegrator with fine glass beads to remove the contents of the spore. The spore coat thus prepared retains its initial shape and is more transparent, and more clearly shows its surface structure, as compared with the intact

spore. One of such direct images of the spore coat is shown in Fig. 3, and reveals the presence of an equatorial band opaque to electrons on the surface of the spores.

Fig. 1 indicates that the spore coat is composed of three layers: a membranous outermost layer, an electron opaque outer layer, and an inner laminated layer. We think, however, that these layers are made up of essentially the same material; the difference in appearance between them seems to result from some artefact produced during fixation.

As reported before<sup>3</sup>, we have shown by electron diffraction that the spore coat lamellae of *Bacillus subtilis* are single crystals. It is also known that the spore coats of bacilli are mainly proteins<sup>4,5</sup>. These facts suggest that the spore coat which wraps the cortex and core is composed of about fifteen lamellae, and that each of the lamellae is a thin single crystal of structural protein. Each lamella is about 33 Å thick.

If this conception is correct, it seems unlikely or impossible that various kinds of organic or inorganic substances

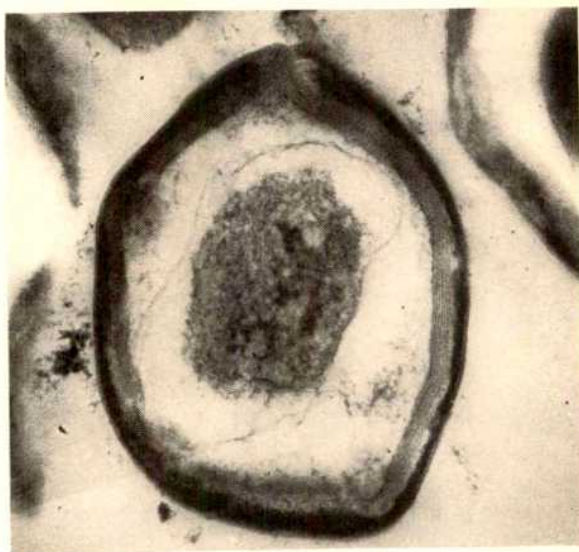


Fig. 1. Cross section of the spore of *Bacillus subtilis*, showing the outer layer structure of the spore coat with a fissure.



Fig. 2. Enlarged picture of the fissure in the laminated layers illustrated in Fig. 1.



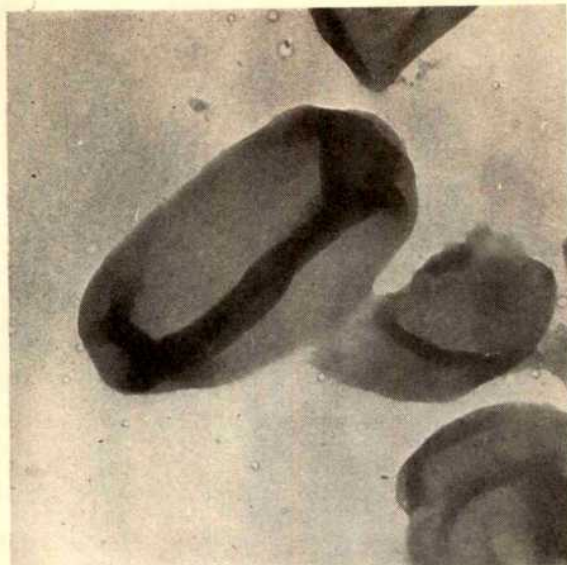


Fig. 3. Direct image of the partially disrupted spore of *Bacillus subtilis*. The electron opaque band is seen on the spore surface.

permeate through these crystal lamellae of the spore coat. It is, however, well known that water, amino-acids (especially L-alanine) and other substances actually penetrate into the spore core, and that dipicolinic acid is discharged from the spore in the early stage of germination. There must be therefore some passage through which these substances were carried into or out of the spore, and it seems not unreasonable to consider that the fissure of the laminated layer of the spore coat which is shown in Fig. 2 serves for the passage of these substances.

As for the swelling of the spore during germination, Rousseau *et al.*<sup>2</sup> found that an increase in volume of the spore was caused by the swelling of the cortex and core, and at the same time as these spore contents were swelling an expansive force was delivered to the spore coat. From the mechanical point of view, the part of the spore coat least resistant to this force is undoubtedly where the laminated layer is fissured. The electron opaque band, shown in Fig. 3, seems to have a ridge in the direction of the principal equator. We think that this ridge corresponds to the fissure shown in the cross section. Along this fissured and ridged line, the spore coat may split by mechanical pressure and allows the vegetative cell to emerge. Pictures resembling ours have been obtained by several investigators with spores of *Bacillus megaterium*<sup>5,6</sup>. We suppose therefore that such lines of splitting usually exist in the spores of bacteria belonging to the genus *Bacillus*.

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## Genetic Recombination in Germinated Oospores of *Phytophthora infestans*

THE sexual process of the heterothallic fungus, *Phytophthora infestans* (Mont.) De Bary, in Mexico<sup>1,2</sup> has long been suspected of being an important source of variation in the pathogenesis of new isolates to resistant varieties of potato<sup>3</sup>. Although various investigators<sup>2-6</sup> have reported a low percentage of germination of oospores from controlled crosses in the laboratory, there is little information about the role of sexuality. In some of our experiments as much as 64.0 per cent of the oospores germinated. In this communication, we will briefly describe the variation in pathogenesis and compatibility type of progeny from single germinated oospores.

Oospores were produced by pairing strains of the fungus of different pathogenicity and compatibility type on unclarified V-8 juice agar (V-8A), clarified V-8 juice agar (CV-8A), or CV-8A fortified with  $\beta$ -sitosterol, and incubating in darkness at 18°C. Plates containing oospores were refrigerated at 4°C for between 13 and 45 days. Oospores were separated from the agar by passage through a fine plastic sieve and from mycelium by allowing the oospores to settle in water and decanting off the supernatant. Clonal cultures from single oospores germinated in double-distilled water under diffuse fluorescent light were established by incubating germ-sporangia on tomato juice (5.0 per cent)-vancomycin (200 p.p.m.)-agar (TVA)<sup>7</sup> at 20°C. Germ-sporangia germinated directly to produce germ-mycelia. The percentage germination was determined by observing 500 or more oospores/sample. The pathogenicity of thirty of these cultures was determined by inoculating foliage of differential varieties of potato, employing a detached leaf technique in Petri dishes. Compatibility type of clonal cultures was determined by crossing them with two strains of known compatibility type, 445 (A2) and 473 (A1).

The production and germination of oospores were partially dependent on the substrate, for example, more oospores were produced on V-8A than on CV-8A, but when 0.3 mg of  $\beta$ -sitosterol was added to 15 ml. of CV-8A, the number of oospores produced was as great as on V-8A.

Germination of oospores produced on CV-8A, V-8A, and CV-8A supplemented with  $\beta$ -sitosterol (0.3 mg/15 ml. of medium), exposed to light for 1 h a day for 8 days, and then refrigerated at 4°C for 13 days was 20 per cent, 30 per cent and 34 per cent, respectively. In a further experiment, 48 per cent of the oospores produced on CV-8A plus 0.45 mg of  $\beta$ -sitosterol, exposed to fluorescent light for 1.5 h daily for 8 days, and refrigerated at 4°C for 45 days germinated. In another experiment, 64 per cent of the oospores formed on V-8A fortified with 0.3 mg of  $\beta$ -sitosterol germinated.

When oospores were obtained from isolates 445  $\times$  473 or from 477  $\times$  473 grown on CV-8A plus 0.45 mg of  $\beta$ -sitosterol and refrigerated for 45 days at 4°C, approximately 48 per cent germinated, but when oospores were obtained from 60A  $\times$  63B only 6 per cent germinated. This suggests that there is probably a genetically controlled tendency for oospores from different crosses to germinate. Considering possible evolutionary processes, this sort of variation might be expected. Perhaps isolates such as 60A  $\times$  63B might produce more persistent oospores in nature than those producing readily germinable oospores. This concept warrants more study and should contribute to a greater understanding of the epidemiology of late blight. For genetic studies of this genus, however, it is important to select isolates with as high a potential to germinate as possible so that selected clonal isolates from single oospores will represent a normal population.

When leaves of differential varieties of potato were inoculated with clonal isolates from single oospores, pathogenic races different from the parental isolates were identified, suggesting that genetic recombination occurred. From the cross 445 (race 0)  $\times$  473 (race 1, 2, 3, 4) race 1,



race 2, race 3, race 4, race 1, 3, race 1, 4, race 1, 3, 4, and race 2, 3, 4 were identified by their ability to infect varieties of potatoes with corresponding single dominant genes. Thus new races may arise through the sexual stage of the fungus in Mexico, the only country in the world where two sexual compatibility types are known<sup>1,2,9,10</sup>. In compatibility tests twenty-three cultures from single oospores were A1 and only six were A2, indicating that the inheritance of compatibility factors depends on a more complex mechanism than that of a single pair of alleles. In addition to variation in pathogenicity and compatibility, variation in rate of mycelial growth, type and colour of mycelium, and amount of sporulation was also manifested by clonal cultures on CV-8A.

No segregation among progeny from any single oospore was noted. This suggests that only one genotype is present in each single oospore culture and agrees with the findings of Galindo<sup>11,12</sup> and Satour<sup>8</sup>.

Oospores, apparently parthenogenic, were produced in an unpaired culture by the isolate 445. Progeny from germ-mycelia derived from four of these oospores showed the same pathogenic character and compatibility type as the parental isolate 445 (race 0, A2) suggesting that parthenogenic oospores may not be a source of variation.

This study confirms previous reports<sup>2,6,10</sup> that genetic recombination does occur in the sexual cycle of *P. infestans* and that this process is an important factor influencing the appearance of new pathogenic races of the fungus.

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## PHYSIOLOGY

### Electroretinographic "Threshold" in the Isolated Rabbit Retina

BASED on Sickel's technique<sup>1</sup> in the isolated frog retina a perfusion method has been developed during the last few years to keep the isolated warm blooded retina alive. By this method the negative *a*-wave and positive *b*-wave of the electroretinogram can be preserved<sup>2-4</sup>, and the isolated retina of the rabbit can be used to study physical and chemical effects. The preparation has always been assumed to retain normal light sensitivity, and we have tested this assumption.

The retinas of twelve rabbits were prepared in deep red light, freed of pigment epithelium and perfused by a technique described before<sup>2</sup>. The retinas were then tested with standard light flashes (0.05 lm/m<sup>2</sup> intensity and 0.1 sec duration). Two of the twenty-one retinas prepared produced an electroretinogram amplitude of less than 150  $\mu$ V and were discarded. In the remaining nineteen retinas the standard amplitude of the electroretinogram was between 150 and 650  $\mu$ V ( $372 \pm 130$   $\mu$ V).

After recording the electroretinographic response to a single standard flash, the responses to repeated weak stimuli were added by computer (CAT 1000; 120 responses to each intensity; stimulus duration 0.3 sec; stimulus interval 6 sec; white light of 3,200 °K). A suprathreshold reaction was assumed if the average amplitude of a response was more than 1  $\mu$ V. The threshold was lower than  $5 \times 10^{-5}$  lm/m<sup>2</sup> in all retinas. In fifteen retinas the threshold was lower than  $3 \times 10^{-5}$  lm/m<sup>2</sup>, and in twelve retinas it was lower than  $1 \times 10^{-5}$  lm/m<sup>2</sup>. No response was obtained with a stimulus intensity of less than  $1 \times 10^{-6}$  lm/m<sup>2</sup> (Fig. 1).

The tests were repeated after light adaptation of the retina (5 min, 50 lm/m<sup>2</sup>). In these conditions the electroretinogram was strongly reduced showing that the positive response was not an artefact (Fig. 1).

The isolated retina therefore seems to be fairly sensitive to light. Direct comparison with the electroretinogram of the intact animal is difficult because the signal to noise ratio is much lower without isolation. A luminance of 1 cd/m<sup>2</sup> in the stimulus field produces a retinal illumination of approximately  $0.24 \pm 0.08$  lm/m<sup>2</sup> ( $n=6$ ) in the dilated rabbit's eye (for a pupil of diameter 10 mm). In a sensitive isolated preparation  $4 \times 10^{-6}$  lm/m<sup>2</sup> is sufficient to elicit an electroretinogram (Fig. 1); this retinal illumination would correspond to a luminance of  $17 \times 10^{-6}$  cd/m<sup>2</sup>. The brightness threshold of the rabbit is unknown; it may be mentioned, however, that the brightness threshold in man was found to be  $0.85 \times 10^{-6}$  cd/m<sup>2</sup> (ref. 5). Our results with the rabbit are slightly more than 1 log unit higher. The electroretinogram "threshold" of the isolated frog retina was measured with monochromatic light (507 m $\mu$ ): with  $5.68 \times 10^{-14}$  W/cm<sup>2</sup> it was found to be slightly more than 1 log unit higher than the human brightness threshold<sup>6</sup>.

The relationship between the amplitudes of stimulus and the electroretinogram response has been found to be very similar in the isolated retina of the rabbit and in the intact animal<sup>7</sup>. The low electroretinogram "threshold" described in the present study also supports the assumption that the light sensitivity of the isolated preparation is nearly normal.

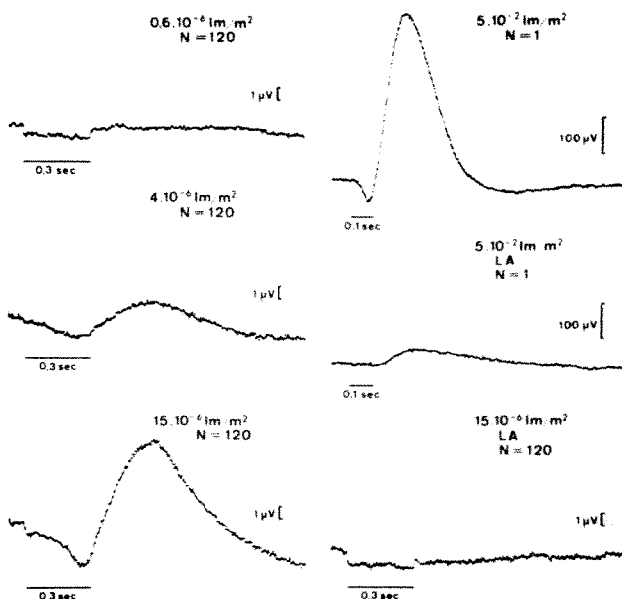


Fig. 1. Electroretinograms evoked from the isolated perfused rabbit retina with flashes of light (0.1 or 0.3 sec). Single responses ( $N=1$ ) and summated responses ( $N=120$ ). Intensity of retinal illumination is given in lm/m<sup>2</sup> (= lux). LA: Light adaptation (after 5 min illumination with 50 lm/m<sup>2</sup>). Negativity downwards. The electroretinogram threshold is somewhere between the first and the second record in the left row. In the summated responses a small negative stimulus artefact can be seen.

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## Electrical Potential across the Rat Small Intestine stimulated by Adenosine Triphosphate

TRANSPORT processes in the small intestine are dependent on energy which suggests the participation, directly or indirectly, of adenosine triphosphate (ATP). No evidence has, however, been produced that exogenous ATP can influence intestinal transport processes, which may well result from the difficulty of getting the unstable ATP molecule to the correct place in the cellular organization before its hydrolysis occurs. A way around this difficulty is offered by the study of electrical potentials in the intestine, which are known to be associated with intestinal transfer of various substances. Potential changes can be measured within a few minutes of setting up the intestinal preparation, and appear within seconds after addition of transferable substances. They therefore appear to offer an ideal method for studying the effect of ATP on intestinal transfer.

The electrical potential was recorded across the wall of rat intestine<sup>1</sup>. Experiments were carried out in pairs, using two adjacent 6 cm sacs from the everted mid-jejunum, bathed in 30 ml. of bicarbonate saline<sup>2</sup>. In this preparation substances can be added to the mucosal fluid in which the sac is suspended, and this can easily be changed for a fresh solution. Sacs were incubated for 15 min before ATP was added, and the following results were obtained.

Addition of 1 mmolar ATP to the mucosal fluid caused a rise in potential after 15 sec, which reached a peak in 60–70 sec and fell to the original level after about 6 min. The mean potential increase was 2.85 ( $\pm 0.20$ ) mV, the serosal side being positive. This change is considerably larger than that obtained from addition of transportable hexoses or amino-acids at the same concentration. A concentration of 0.1 mmolar caused a potential of about 1.6 mV and even 0.01 mmolar produced a potential of 0.6 mV; hexose and amino-acid at a concentration of 0.01 mmolar had no detectable effect on the potential. After the initial response to 1 mmolar ATP subsequent addition of ATP produced a much smaller response.

Addition of AMP or ADP (1 mmolar) produced much smaller effects, which suggests that ATP is specific and probably supplies energy for an ionic transport process. (A slight contamination of the AMP and ADP with ATP could be responsible for these changes.)

We also studied a number of inhibitors, with the following results. Phlorrhizin ( $2 \times 10^{-4}$  molar) had no effect on the ability of ATP to stimulate the potential. This is of interest in relation to the conclusion<sup>3</sup> that although phlorrhizin inhibits the stimulation of fluid transport by mucosal glucose it does not affect the coupling of hexose metabolism to fluid transport.

Addition of 2:4-dinitrophenol ( $2 \times 10^{-4}$  molar) prevented the development of a potential by ATP. The

transfer potential caused by galactose was found to be inhibited by 2:4-dinitrophenol<sup>1</sup> and the present observation raises the question whether DNP uncouples the action of ATP in generating the potential, as distinct from uncoupling the generation of ATP by oxidative phosphorylation.

Ouabain ( $10^{-3}$  molar) in the serosal fluid caused approximately 50 per cent inhibition of the potential generated by ATP but had no effect when present in the mucosal fluid. This is of interest in connexion with the well known inhibitory effect of ouabain on the sodium pump, which depends on membrane ATPase activity.

The effect of ionic environment was studied by replacing the sodium chloride in the bathing fluids by mannitol, choline chloride or potassium chloride, thereby reducing the concentration of sodium to 25 mequiv./l. Sacs were pre-incubated 20 min before the addition of 1 mmolar ATP.

Replacement with potassium chloride prevented the potential which is stimulated by ATP, but replacement with mannitol or choline chloride increased the ATP potential to about 6 mV. This is of interest in relation to the finding that replacement of sodium chloride with potassium chloride abolished the hexose transfer potential but replacement with mannitol did not<sup>4</sup>. We are unable at the moment to offer an explanation for the stimulation of the ATP potential in the presence of mannitol or choline chloride, but it may be related to alteration in the ratios of sodium to potassium in the system.

In the presence of galactose, ATP caused an increase in potential identical to that in the absence of galactose—that is, 2 mmolar galactose gives a potential of 2 mV, whereas 2 mmolar galactose together with 1 mmolar ATP gives 5 mV. In the presence of glucose (2 mmolar), however, a smaller increase (1.5 mV) is observed when ATP is added.

These results show that ATP in low concentrations stimulates the potential across the rat small intestine, and are in keeping with the action of ATP on a sodium pump. The experiments seem to provide the first demonstration that utilization of ATP plays a direct part in the intestinal transfer process.

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## Effect of Lithium on the Uptake of Noradrenaline by Synaptosomes

THERE is indirect evidence of a relationship between serious disorders of mood, such as mania and depression, and amines, particularly noradrenaline<sup>1,2</sup>. This is based in part on the observation that two classes of drugs which deplete the brain of noradrenaline (reserpine and alpha-methyl-DOPA), acting through different mechanisms, can cause depression in some patients. The imipramine class of drugs and the monoamine oxidase inhibitors have been shown in controlled studies to benefit many clinical depressions. The monoamine oxidase inhibitors have been found to block an important breakdown pathway of



noradrenaline<sup>1</sup>, while the imipramine type drugs block the re-uptake of noradrenaline into the adrenergic tissues<sup>1</sup>. It has been suggested that both classes of drugs increase "functional" noradrenaline at the receptor sites, and that this effect is related to their antidepressant actions.

Well controlled studies indicate that lithium is a highly effective treatment for mania<sup>3,4</sup>. There is evidence that lithium maintenance treatment can prevent the recurrence of manic or depressive episodes in patients with a history of recurrent mania, depression or both<sup>5,6</sup>. Depression may be associated with altered metabolism of noradrenaline and function, and depression and mania are clinically related, and so it seemed reasonable to investigate whether chronic lithium administration would modify the function of noradrenaline. The hypothesis tested is whether or not lithium pretreatment would affect the uptake of noradrenaline by isolated nerve endings.

A group of eighteen Sprague-Dawley rats were given 2 mequiv of lithium carbonate in their food each day, while 11 untreated animals were maintained as controls in similar conditions. This dosage was sufficient to maintain serum lithium levels between 1 and 2 mequiv./l. The treatment period varied between 5 and 7 days because a similar time is required for improvement to occur with the clinical use of lithium in mania.

The nerve endings "synaptosomes" were prepared by the subcellular fractionation technique of Whittaker<sup>7</sup>. The animals were decapitated and the brains were quickly removed, chilled, weighed, and then ground in nine volumes of 0.32 molar sucrose in a special homogenizer. The homogenate was centrifuged at 900*g* for 15 min to remove the nuclei as a pellet, and the supernatant was centrifuged at 17,000*g* for 30 min, leaving the microsomes in the supernatant and the myelin, synaptosomes, and mitochondria in the pellet. The pellet fraction was resuspended in 8 ml. of 0.32 molar sucrose, layered on a discontinuous sucrose gradient with 8 ml. layers of 1.2 molar and 0.8 molar sucrose and centrifuged at 50,000*g* for 90 min. The synaptosomal fraction (middle) was then recovered with a special tube-slicing device (Beckman Instruments) and diluted to 52 ml. with Krebs bicarbonate buffer<sup>8</sup>.

Tritiated noradrenaline, 0.2  $\mu$ c. (*dl*-noradrenaline-7-<sup>3</sup>H acetate, New England Nuclear Corp.) was added without carrier to the buffered nerve ending fraction, resulting in a final concentration of noradrenaline in the incubation medium of 1.2  $\mu$ g/ml. The synaptosomal fraction from each animal was divided into eight parts (6 ml. each), providing samples which were usually run in duplicate for four periods of incubation (0, 4, 8, 12 min) at 37° C. All samples were then centrifuged at 100,000*g* for 10 min, washed with 8 ml. of buffer and recentrifuged. The resulting pellets were extracted with 0.25 ml. of 0.4 normal perchloric acid and centrifuged at 50,000*g* for 10 min, 0.2 ml. of aliquot being transferred to a scintillation mixture for counting. (The mixture consisted of: 10 ml. toluene; 4 ml. of ethanol containing 0.4 per cent 2,5-diphenyloxyazole; and 0.01 per cent 1,4-bis-2-(phenol-oxazyl)-benzene.)

The quenching for each sample was observed with an external Beta monitor in a Model 4000 Packard scintillation spectrometer. The addition of the perchloric acid extract from our samples gave essentially the same quenching as perchloric acid alone—about 20 per cent. The range of quenching between the individual samples was only 3–4 per cent. Paper chromatography of the perchloric acid extracts in a butanol:acetic acid:water system (25:4:10) revealed that 90 per cent of the radioactivity travelled with an authentic noradrenaline carrier, indicating that most of the radioactivity was due to noradrenaline rather than breakdown products of noradrenaline. Concentrations of lithium in blood and tissue were determined by atomic absorption (Perkin-Elmer Model 303), with lithium carbonate as the reference compound.

Fig. 1 shows the uptake of noradrenaline by the synaptosome fraction after incubation at 37° C for 0, 4, 8

Table 1. NET UPTAKE OF NORADRENALINE BY SYNAPTOSOMES WITH AND WITHOUT LITHIUM PRETREATMENT

Incubation period* (min)	Net uptake and method error (c.p.m.)				Net uptake minus zero uptake/time period		Uptake increase with lithium pretreatment (per cent)
	Control Net uptake	Method error† (per cent)	Lithium pretreatment Net uptake	Method error† (per cent)	Control	Lithium	
0	2,144	10.5	2,600	4.2	—	—	—
4	7,357	6.4	9,431	5.6	5,213	6,831	31
8	13,993	4.8	17,978	6.7	11,849	15,378	30
12	20,326	2.8	25,219	5.1	18,182	22,619	24

\* The differences between time periods 0–4, 4–8, and 8–12 min were significant at the 0.0001 level for both lithium and controls.

† The percentage method error was calculated by dividing the mean difference between duplicate measurements by the net uptake for a given time period. For the twenty earlier experiments samples were run in duplicate. The latter nine experiments were not run in duplicate for the method variance was considerably smaller than the variance between animals.

and 12 min. Chronic lithium pretreatment increases the net uptake by approximately 30 per cent at 8 and 12 min (see Table 1). This increase in net uptake in the lithium pretreated rats is significant at the 4 (0.02), 8 (0.001) and 12 (0.001) min periods (*t*-test based on mean of duplicate determinations). On each experimental day control rats were compared with one or two rats pretreated with lithium, and so a comparison between lithium and control can be made for each day. In every case the mean lithium value for that day showed greater uptake than the control value, a finding itself significant at the 0.002 level for each period (Wilcoxon signed-ranks test).

The concentration of noradrenaline in the incubating medium was about 6,200 c.p.m./0.1 ml., whereas the concentration in the synaptosomal fraction after 12 min incubation was approximately 60,000 c.p.m./0.1 ml. This ten-fold increase in concentration of the synaptosomal fraction over the medium would argue against simple diffusion as the sole explanation for the results. When small amounts of lithium, ranging from 0.5 to 5 mequiv./l., were added to the incubation medium, no increase in uptake by the synaptosomes was observed in the control animals or the animals treated with lithium. Lithium levels in the serum were 1 to 2 mequiv./l., and in the whole brain were 0.5 mequiv./kg. Lithium concentrations in the synaptosomes and microsomes were 16 per cent and 10 per

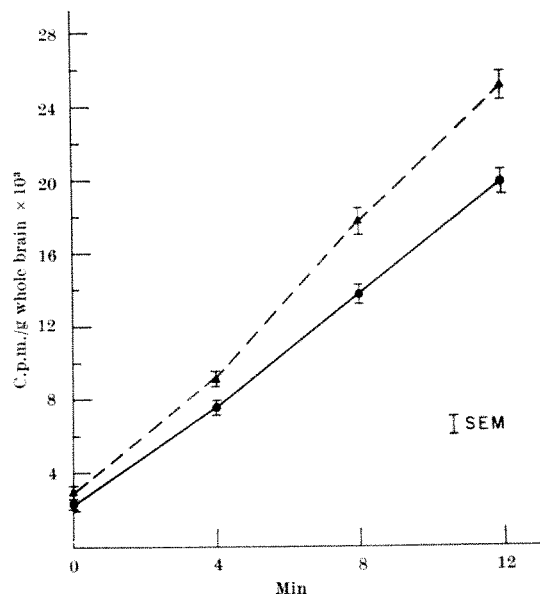


Fig. 1. Uptake of noradrenaline by synaptosomes isolated from lithium and control animals.  $\Delta$ — $\Delta$ , Treated;  $\bullet$ — $\bullet$ , control; S.E.M., standard error of the mean for all the lithium and control samples at the four time periods. The numbers of animals used in determination of each point for 0, 4, 8, and 12 min were eighteen, seventeen, eighteen, seventeen and eleven, eleven, ten, nine, respectively, for treated and untreated animals.

cent of the whole tissue levels, whereas only 5.8 per cent and 2.5 per cent, respectively, were found in the myelin and mitochondria.

Synaptosomes isolated from the brains of rats pretreated with lithium have been shown to take up noradrenaline against a concentration gradient to a significantly greater extent than control animals. That this effect may be due to the lithium directly is consistent with the findings of relatively high concentrations of lithium in the synaptosomes. The mechanism by which lithium increases net "uptake" in this system, however, is not yet clear. It may alter the membrane directly, interact with an enzyme and/or transport mechanism, deplete endogenous norepinephrine levels, affect adrenergic binding sites or interact with cations as they affect amine uptake<sup>9-13</sup>.

The use of the synaptosomal fraction has the advantage of providing a system consisting primarily of nerve endings, and hence reduces the possibility that non-specific diffusion, binding or uptake by other subcellular particles could obscure or confound small but real differences in synaptosomal uptake. In an *in vivo* experiment, Schanberg, Schildkraut and Kopin<sup>14</sup> found that acute lithium pretreatment altered the metabolism of tritiated noradrenaline in rat brain, increasing the ratio of deaminated to *o*-methylated metabolites. Our data suggest that these results could be explained if lithium pretreatment increased re-uptake of noradrenaline *in vivo*.

We have seen that the relative rates of release and re-uptake of noradrenaline at the central adrenergic nerve ending may be related to alterations in mood states<sup>1,15</sup>. The data reported here suggest a hypothesis for further study: that the therapeutic efficacy of lithium in mania may in part be a result of an increased re-uptake of noradrenaline, with a resultant decrease in the noradrenaline available for interaction with the receptor sites.

Electrolytes as well as noradrenaline have been implicated in mood disorders<sup>16-18</sup>. Furthermore, clinical evidence<sup>19</sup> suggests that mania and depression are not in all respects opposite, nor may biogenic amines and electrolytes be involved in any simple way. The experiments reported here describe an *in vitro* system for precise study of the inter-relationships between electrolytes and amine function—inter-relationships which may be important to the understanding of affective disease.

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## Technetium-99m(V)-Citrate Complex for Estimation of Glomerular Filtration Rate

THE standard compound used for estimating the rate of glomerular filtration is inulin, but its chemical determination is tedious and time consuming. The technique for estimating the filtration rate with inulin requires constant infusion and serial urine collections. <sup>14</sup>C-carboxyl-, <sup>131</sup>I- and <sup>125</sup>I-allyl-inulin have been suggested, but chemical instability, cost and lack of rapid and simple methods of analysis have limited their use<sup>1-3</sup>. A compound labelled with a gamma emitting isotope and excreted entirely by glomerular filtration would make the study of renal function much easier, and <sup>131</sup>I- (also <sup>125</sup>I-) diatrizoate<sup>4,5</sup>, <sup>131</sup>I- (also <sup>125</sup>I-) iothalamate<sup>6</sup>, <sup>57</sup>Co-vitamin B<sub>12</sub> (refs. 7-9), and <sup>51</sup>Cr-ethylenediamine tetraacetate<sup>10</sup> have received attention. There are, however, disadvantages in using these labelled compounds for estimating the rate of glomerular filtration when successive examinations are attempted in the presence of poor or rapidly declining renal function. In these conditions, the radioactivity of plasma tends to increase to levels great enough to reduce the accuracy of the measurements, because of the relatively long half life of these nuclides. <sup>57</sup>Co-vitamin B<sub>12</sub> has two further disadvantages: it is appreciably bound to plasma protein<sup>11</sup>, and it has a long residence time in the liver so that its repeated use in a patient with seriously impaired renal function would be undesirable.

Yeh and Kriss<sup>12,13</sup> described physiological investigations with a new and easily synthesized compound, technetium-99m(V)-citrate complex. Distribution and scintiphographic studies indicated that the drug was rapidly excreted by the kidney as the intact complex. The physiological behaviour of the complex plus the favourable physical characteristics of technetium-99m (ref. 14), that is, its short physical half-life (6 h) and low gamma energy (140 KeV), encouraged us to study the mechanism of its renal excretion by both the single injection<sup>15</sup> and standard clearance techniques.

The renal clearances of <sup>131</sup>I-diatrizoate (specific activity about 200  $\mu$ c. <sup>131</sup>I/mg; each dog received 2  $\mu$ c. of <sup>131</sup>I/kg of body weight) and technetium-99m(V)-citrate complex were compared simultaneously in ten conscious dogs with weights of between 17 and 24 kg. (Technetium-99m(V)-citrate complex was prepared by the method of Yeh and Kriss<sup>12,13</sup>. To obtain better reduction of <sup>99</sup>Tc from +7 to +5 oxidation state, 1.5 normal hydrochloric acid was used. Subsequently the citrate concentration increased to 0.25 molar. The final product had specific activity 125-250  $\mu$ c./ml. depending on the age of the <sup>99m</sup>Tc generator, and consisted of citric acid of approximately 40 mg/ml. The purity of the material was examined by ascending paper radiochromatography. The free <sup>99m</sup>Tc-pertechnetate was less than 2 per cent. Each dog received 200-400  $\mu$ c. The compound did not bind to red blood cells. The animals had no acute toxic reactions and were observed for several weeks and none showed any late toxic effects.) After a single intravenous injection of a mixture of <sup>131</sup>I-diatrizoate and technetium-99m(V)-citrate complex, heparinized samples of venous blood were collected at 5-10 min intervals for 90-140 min. The counts of each isotope contained in 1 ml. of plasma were determined in a well-type scintillation counter, using appropriate gamma-ray energy discriminator settings. A sample of the injected dose was diluted and 1 ml. was analysed in the same manner. The clearances, distribution volumes and intercompartmental clearances were calculated by a computer, using the formulae derived by Sapirstein *et al.*<sup>16</sup> as applied to the plasma <sup>131</sup>I-diatrizoate disappearance curve by Blafox *et al.*<sup>17</sup>. They showed no statistical difference in glomerular filtration rates when simultaneously determined in dogs by the techniques of a single injection of <sup>131</sup>I-diatrizoate and the constant infusion of

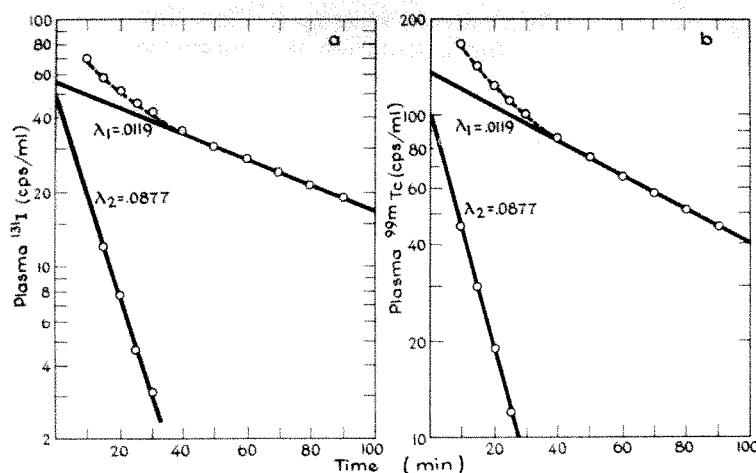


Fig. 1. Plot of typical plasma disappearance rates of  $^{131}\text{I}$  (a) and  $^{99\text{m}}\text{Tc}$  (b) after a single intravenous injection of a mixture of  $^{131}\text{I}$ -diatrizoate and technetium-99m(V)-citrate complex. The curves are resolved by eye-fit into their two slopes,  $\lambda_1$  and  $\lambda_2$ , which results in their respective intercepts  $B$  and  $A$ .

creatinine. The computer was programmed to perform calculations based on the following equations

$$C = \frac{D \cdot \lambda_1 \cdot \lambda_2}{A \cdot \lambda_1 + B \cdot \lambda_2} \quad (1)$$

$$V_1 = \frac{D}{A + B} \quad (2)$$

$$\alpha = \frac{V_1(A \cdot \lambda_2 + B \cdot \lambda_1)}{A + B} - C \quad (3)$$

$$V_2 = \frac{\alpha \cdot C}{V_1 \cdot \lambda_1 \cdot \lambda_2} \quad (4)$$

where  $C$  is renal clearance;  $D$  is dose;  $A$  and  $B$  are the intercepts of the fast and slow components of the plasma disappearance curve;  $\lambda_2$  and  $\lambda_1$  are the slopes of the fast and slow components;  $V_1$  is the volume of compartment 1 into which doses were injected;  $\alpha$  is intercompartmental clearance; and  $V_2$  is the volume of compartment 2 which is made up of the rest of the apparent distribution volume. The terminal slope was taken from the plasma radioactivity between 40 and 80 min. The second slope was determined from the difference in a line drawn between the 40–80 min points and the plasma radioactivity between 5 and 25 min.

The renal clearances were also determined by plotting the plasma radioactivity against time on semilogarithmic paper and resolving the disappearance curve by eye-fit. Fig. 1 shows typical plasma disappearance curves from a single intravenous injection of a mixture of  $^{131}\text{I}$ -diatrizoate and technetium-99m(V)-citrate complex. The slopes of the curves are identical. The ratio of the renal clearance of  $^{131}\text{I}$ -diatrizoate : renal clearance of technetium-99m(V)-citrate complex for the ten dogs averaged  $1.003 \pm 0.002$  standard error (Fig. 2a). The coefficient of correlation between the clearance rates of the two compounds with this technique was 0.99. The calculated distribution volumes and intercompartmental clearances of the two compounds were also nearly identical (Table 1).

The renal clearances were also determined in a well hydrated dog under anaesthesia. The urine was collected by bladder catheter during ten 15 min periods. A priming dose of a mixture of  $^{131}\text{I}$ -diatrizoate and technetium-99m(V)-citrate complex was injected intravenously, followed by constant infusion of the mixture. A venous

blood sample was taken at the mid-point of each collection period. The radioactivity in 1 ml. of plasma and urine was determined. The renal clearance rates were calculated by the standard formula  $UV/P$ . In these conditions, the clearance rates of the two substances were identical (Fig. 2b).

The plasma disappearances of  $^{131}\text{I}$  and  $^{99\text{m}}\text{Tc}$  following a single intravenous injection of a mixture of  $^{131}\text{I}$ -diatrizoate and technetium-99m(V)-citrate complex were determined in one dog immediately after bilateral nephrectomy had been performed. The plasma disappearance curve of  $^{99\text{m}}\text{Tc}$  is shown in Fig. 3. The  $^{131}\text{I}$  plasma disappearance curve was quite similar. The slight decline observed after the initial mixing phase was most likely caused by excretion into the bile. Aspiration of the gall bladder 60–70 min after the injection revealed small amounts of both  $^{131}\text{I}$  and  $^{99\text{m}}\text{Tc}$  in the bile. The biliary concentration of the two nuclides as compared with their respective concentrations in the injection

mixture represented identical degrees of dilution. Inulin is also excreted in small quantities into the bile<sup>18</sup>. These observations suggest metabolic inertness and absence of other mechanisms of excretion.

Table 1. COMPARISON OF DISTRIBUTION VOLUMES AND INTERCOMPARTMENTAL CLEARANCES OF TECHNETIUM-99m(V)-CITRATE COMPLEX AND  $^{131}\text{I}$ -DIATRIZOATE

Dog No.	Weight (kg)	Technetium-99m(V)-citrate complex			$^{131}\text{I}$ -diatrizoate		
		$V_1$ (l.)	$V_2$ (l.)	inter-comp. clearance (ml./min)	$V_1$ (l.)	$V_2$ (l.)	inter-comp. clearance (ml./min)
1	16.8	2.75	40	0.80	2.68	43	0.88
2	20.5	3.40	112	1.91	3.36	108	1.94
3	21.8	3.99	69	1.77	4.03	74	1.68
4	21.4	2.49	84	1.60	2.44	90	1.62
5	31.4	3.45	111	1.82	3.55	118	1.69
6	16.8	2.66	37	1.08	2.81	46	0.92
7	21.4	2.93	99	1.78	3.04	92	1.71
8	18.2	3.83	112	2.27	3.78	121	2.34
9	24.5	4.50	153	2.25	4.37	146	2.07
10	24.1	3.54	189	2.73	3.68	164	2.59
Mean	21.7	3.36	101	1.80	3.37	100	1.74

$V_1$ , Volume of compartment 1 into which doses were injected;  $V_2$ , volume of compartment 2 which is made up of the rest of the apparent distribution volume.

The average calculated technetium-99m(V)-citrate complex and  $^{131}\text{I}$ -diatrizoate space in the ten dogs were 23.8 per cent and 23.5 per cent of body weight, respectively. These values are not significantly different from the inulin space of dogs reported by Gaudino *et al.*<sup>19</sup>. We consider it unlikely that the identical values for renal clearance of the two labelled compounds could have resulted from a fortuitous combination of filtration plus

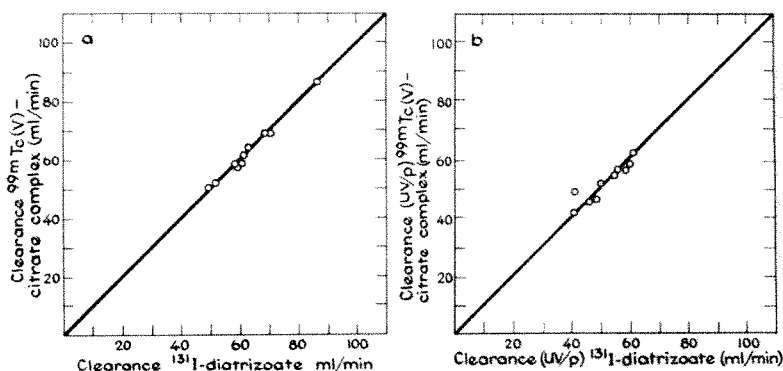


Fig. 2. Correlations between the calculated renal clearances by the single injection technique in ten dogs (a), and the measured renal clearances by the constant infusion technique for ten clearance periods in one dog (b) of  $^{131}\text{I}$ -diatrizoate and technetium-99m(V)-citrate complex.

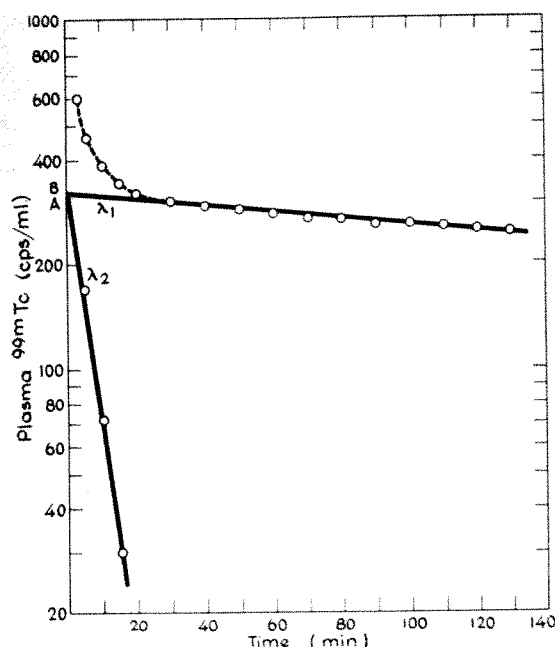


Fig. 3. Plot of plasma-disappearance rate of  $^{99m}\text{Tc}$  after injection of a mixture of  $^{131}\text{I}$ -diatrizoate and technetium- $^{99m}\text{(V)}$ -citrate complex in a dog immediately after bilateral nephrectomy. It is noted that the slopes  $\lambda_1$  and  $\lambda_2$  have common intercepts. The curve is corrected for decay during counting.

identical rates of tubular secretion and re-absorption of technetium- $^{99m}\text{(V)}$ -citrate complex.

Advances in the diagnosis and treatment of renal diseases and the need for a more precise tool to monitor the immunological reaction in clinical renal allotransplantation have stimulated interest in gamma emitting isotopes for the serial and simultaneous determinations of glomerular filtration rate and effective renal plasma flow<sup>20-22</sup>. It would be especially valuable to have techniques which would eliminate the need for collections of urine. The combination of  $^{131}\text{I}$ - (or  $^{125}\text{I}$ -) diatrizoate and  $^{125}\text{I}$ - (or  $^{131}\text{I}$ -) hippuran has been applied<sup>23,24</sup>, but the long half lives of these nuclides allow accumulation of plasma radioactivity in the presence of poor or rapidly declining renal function. Because of the short half life of  $^{99m}\text{Tc}$  and the physiological behaviour of the easily prepared technetium- $^{99m}\text{(V)}$ -citrate complex, the agent is well suited for repeatedly determining glomerular filtration rate by the single injection technique, especially when renal function is poor.

Our results suggest that technetium- $^{99m}\text{(V)}$ -citrate complex, like  $^{131}\text{I}$ -diatrizoate, fulfils the requirement necessary for a compound used for estimating glomerular filtration rate and should be valuable in the investigation of renal function.

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### Change in the Concentration of Lactic Acid in the Rat and Hamster Brain during Natural Sleep

Richter and Dawson<sup>1</sup> first reported that young rats killed by immersion in liquid nitrogen during natural sleep showed a decrease in the concentration of lactic acid in the brain when compared with control animals in the normal waking state, but experimental confirmation of this finding has not been published. Shimizu *et al.*<sup>2</sup> have recorded a failure to confirm it in experiments involving brief periods of sleep.

I have now reinvestigated the lactic acid content of the brain in small rodents under various physiological conditions and have confirmed the original observation that there is a significant decrease in the concentration of lactic acid in the brain during natural sleep.

Adult male Syrian hamsters and MRC  $T_1$  male albino rats between the ages of 10 and 60 days were used. The rats and hamsters were left undisturbed to sleep naturally for a period of at least 30 min before they were killed. The animals slept in a conical polythene container from which they were tipped after the required time into liquid nitrogen. When completely frozen, the heads were broken from the carcasses and kept in liquid nitrogen. The brains were chiselled free and homogenized at  $-10^\circ\text{C}$  in 6 ml. of 1 normal perchloric acid. The homogenate was kept at  $-25^\circ\text{C}$  overnight. On thawing, the homogenate was centrifuged at  $3,000g$  for 10 min in a bench centrifuge and the supernatant decanted. The residue was washed with 2 ml. of normal perchloric acid, centrifuged as before and the supernatant combined with the first. The acid extract was neutralized at  $4^\circ\text{C}$  with 12.5 normal potassium hydroxide. The solution was frozen and then allowed to thaw at  $4^\circ\text{C}$  to attain maximum precipitation of potassium perchlorate. When complete, the potassium perchlorate crystals were separated by centrifugation, washed with ice-cold water and discarded. The water wash was combined with the original extract and the whole made to 10 ml. Volumes of 200 and 400  $\mu\text{l}$ . were taken for the assay of lactic acid, which was measured enzymatically by a method based on that described by Bergmeyer<sup>3</sup>. The incubation mixture was 1 molar glycine buffer, pH 9.5, containing 2 mmolar DPN, 0.4 molar hydrazine sulphate and 5 mmolar EDTA.



Table 1. BRAIN LACTIC ACID CONCENTRATION DURING SLEEP

Animal	Control	Sleep	P
Rat, young (50 g)	1.46 ± 0.103 (24)	1.23 ± 0.108 (18)	0.05
adult (150 g)	3.14 ± 0.141 (30)	2.25 ± 0.173 (25)	0.0005
Hamster, adult (100 g)	3.11 ± 0.130 (35)	1.97 ± 0.086 (26)	0.0005

Results expressed as  $\mu\text{moles/g}$  of lactic acid  $\pm$  standard error of the mean. The number of animals is in parentheses.

In preliminary experiments it was found that the concentration of lactic acid in the rat brain changes with age from about 0.9  $\mu\text{moles/g}$  at 1 day old to the adult level of 3.14  $\mu\text{moles/g}$  at 35–40 days old. Some difficulty was experienced at first in inducing natural sleep in rats, because the experiments were carried out with adult rats weighing 150 g and I found that adult rats are very easily aroused. A difference between sleeping and waking animals was obtained with young rats of 50 g body weight, and this could also be shown in adult rats providing the rats were left to sleep undisturbed for at least 30 min. The same change could be shown in adult hamsters. Table 1 gives the results from such experiments. In the young rat the content of lactic acid in the brain decreased by 15 per cent during sleep, while in the adult it decreased by 28 per cent. The adult hamster showed a decrease of 36 per cent.

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### "Afterpotential" in the Cochlear Response

THE existence of a maintained summing potential (SP) seen in the avian<sup>1</sup> and mammalian<sup>2</sup> cochleas during strong tonal stimulation is a well established phenomenon and can be explained in terms of non-linear movements of the cochlear transducer mechanism<sup>3</sup>. Transients are seen at the onset and termination of such stimuli and can be studied with the aid of averaging techniques<sup>4</sup>. These transients are also asymmetric, their mean values being represented as deflexions of the SP which are of the same polarity (negative-going) both at the onset and cessation of the tone burst. This is, of course, the result to be expected if the asymmetry of the transducer mechanism were such as to produce a negative SP.

This response is, however, seen only at rather short durations of stimulation (20–50 msec) (Fig. 1). If the length of the tone burst is increased to, say, 500 msec, the off-transient is obscured by a positive-going "slow-wave", the amplitude and duration of which are dependent on the duration of the preceding stimulus. The slowwave is unaffected by section of the middle ear muscles and presumably cannot therefore arise from a reflex of the middle ear. Both the negative transients and the positive slowwave are seen equally well in the cat,

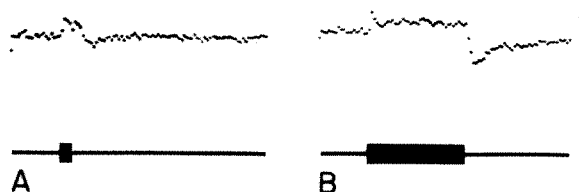


Fig. 1. The summing and transient potentials recorded from the pigeon cochlea in response to 1,600 c/s tone bursts of (A) 20 msec and (B) 400 msec duration. Mammalian responses are similar. Upward deflexion represents relative positivity in the scala tympani ("negative summing potential"). Averages of 160 sweeps with dwell times of 5 and 10 msec/point, respectively. Calibration, 10  $\mu\text{V}$ .

guinea-pig and pigeon and whether recorded from the scala tympani or from the intact round window.

The negative-going transients are influenced by the times of rise and fall of the stimulus and are most marked when the stimulus rises abruptly to its full value in one half cycle of the stimulus. They are almost completely abolished when the rise time is of the order of 50 msec, although the precise values are dependent on other factors, such as intensity and frequency of stimulus. They thus have the properties of true system transients.

By contrast, the positive slowwave seems to be entirely uninfluenced by the rate of turn-off, and is affected solely by the amplitude and duration of the previous stimulus. I have been unable to devise any explanation of this potential in terms of the mechanics of the basilar membrane, and the only possible explanation seems to be that it is associated in some way with the hair cell generator mechanism. Stimulation of the olivo-cochlear bundle, although it influences the size of the whole electric response, does not selectively influence the positive slowwave, and it seems unlikely that the latter could be due to a delayed efferent volley. In any case, the recent observations by Fex<sup>5</sup> show that potentials produced by olivo-cochlear activity are of the opposite polarity to the response which we are observing. The similarity of shape and behaviour between this potential and the afterpotential seen in neurones following repetitive firing suggests that this wave may be of similar origin and may represent an afterpotential of the hair cells themselves or of the hypothetical afferent transmitter mechanism.

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### Possible Role of Alveolar Surfactants in the Uptake of Inhaled Gases

MOST inhaled anaesthetic gases and many other physiologically important gases are non-polar and have very poor solubility in highly polar water. At a temperature of 37° C there is a water vapour pressure in the alveolar area of 47 mm mercury, which implies the presence of a water layer on the alveolar membrane. The polarity of this water forms a barrier to the migration of non-polar anaesthetic and other non-polar gases through the alveolar membrane.

Simple partition and diffusion mechanisms cannot possibly account for the profuse amounts of non-polar gas which are transported through lung tissue to the blood stream. We suggest here that solubilization through the formation of micelles is responsible for the rapidity of absorption. Solubilization<sup>1</sup> has been defined as the spontaneous dissolving of a normally water-insoluble substance by an aqueous solution of surfactant. This solubilization effect becomes significant when the amount of individual surfactant molecules in solution exceeds a critical concentration and the molecules aggregate to form groups called micelles. This concentration of surfactant molecules is referred to as the critical micelle concentration (CMC). Each surfactant has its own characteristic CMC.

The presence of surfactant material in the alveolar matrix has been demonstrated many times<sup>2–4</sup>. Bondurant and Miller<sup>5</sup> found a phospholipid, predominantly phosphatidylcholine (lecithin), in lung surface active material

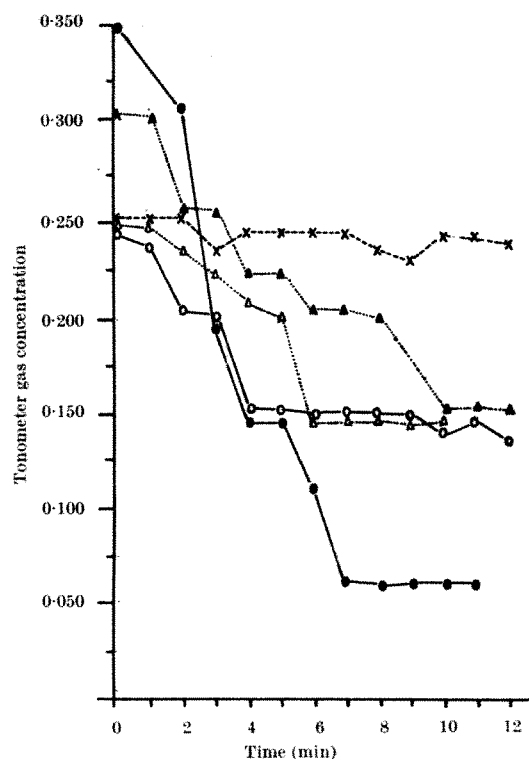


Fig. 1. Inability of homogenized lung without surfactant to solubilize halothane as compared with the marked ability of crude lung surfactant, and 'Tween 80' (a synthetic surfactant at concentrations of 3 and 30 per cent), to absorb the gas. Addition of 30 per cent 'Tween 80' to lung homogenate shows marked absorption as compared with lung homogenate alone, but less absorption as compared with 30 per cent 'Tween 80' alone. x---x, Lung homogenate; ○—○, crude surfactant extract; Δ...Δ, lung homogenate with 30 per cent 'Tween 80'; ●...●, 3 per cent 'Tween 80'.

and Brown<sup>6</sup> isolated dipalmitoyl lecithin as a lung surfactant. The characteristics of the micelles formed by the lecithin surfactants have recently been reviewed<sup>7</sup>, and their ability to solubilize material insoluble in water has been discussed<sup>8</sup>. DePalma *et al.*<sup>9</sup> have proposed that lecithin is a necessary component of these complexes.

In order to test our hypothesis, we prepared surfactant material, using the method of Bondurant and Miller<sup>5</sup>, by instilling normal saline into the lungs of dogs, ventilating them and extracting the foamy solution by suction. This procedure was repeated several times until no more foamy fluid could be obtained. The lung was then homogenized in saline in a Waring blender, centrifuged and the supernatant separated from the homogenate. The supernatant was then tested in the same way as the crude surfactant material and the lung homogenate.

The materials obtained were placed individually in a modified tonometer (150 ml. capacity) which had an extraction port fitted with a rubber septum for sampling gas. Halothane vapour, 1 per cent in oxygen, was admitted into the chamber, closed off and 0.1 ml. samples of the gas were extracted at intervals of 1 min for 20 min.

The samples were analysed by gas chromatography (katharometer) and quantified by comparison with freshly prepared standards.

The result of these tests is graphically summarized in Figs. 1 and 2. Lung homogenate (dog or human), prepared in saline to render it more fluid, showed virtually no absorption of halothane gas. Crude surfactant extract absorbed the gas very rapidly reducing the concentration of halothane in the tonometer by 40 per cent in 3–4 min. The absorption pattern observed when lung surfactant was added to homogenized lung did not differ significantly from that of lung surfactant alone. Lung homogenate did not improve the ability of surfactant to solubilize the gas.

A synthetic surface active agent, 'Tween 80', was tested for comparison at concentrations of 3, 30 and 70 per cent, at which increasingly larger aggregations (micelles) occur, as demonstrated by the sudden increase of halothane absorption (Table 1). 'Tween 80' at 3 per cent (Fig. 2) and at 30 per cent (Fig. 1) showed absorption characteristics that paralleled those of lung surfactant. Because 'Tween 80' at 70 per cent forms a gel, no attempt was made to mix it with lung homogenate.

When plotting surface tension against area in a film balance using crude extracts of lung surfactants, an area of hysteresis is obtained<sup>3</sup>. The hysteresis loop occurs on expansion and contraction of the film by the migration of surfactant molecules to the surface in sufficient concentration to form micelles and then, on the reverse cycle, the

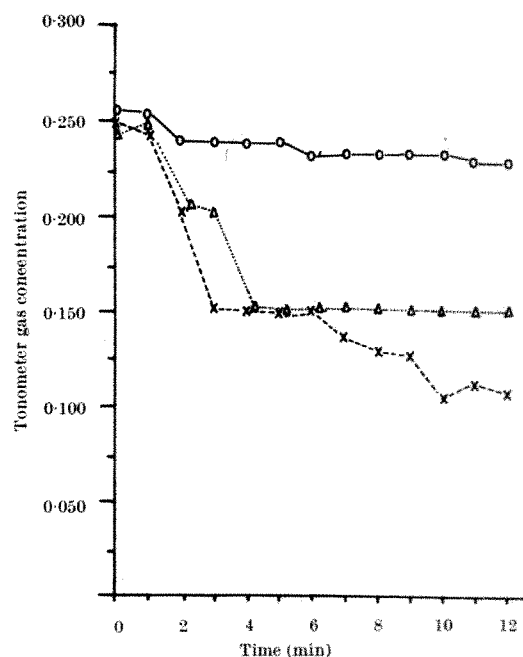


Fig. 2. Minced human lung without surfactant (○—○) shows very poor absorption of halothane vapour. Crude surfactant extracted from it (x---x) and minced lung with crude surfactant (Δ...Δ) demonstrate excellent absorption of gas. Surfactant alone is better than surfactant added to the minced lung.

Table 1. UPTAKE OF HALOTHANE BY 'TWEEN 80' IN VARIOUS CONCENTRATIONS

Approximate concentration of 'Tween 80' in water	Starting conc. 0	1	2	3	4	5	6	7	8	9	10	Total uptake in 20 min (mg)
0.100	0.3350	0.3350	0.2526	0.2531	0.2504	0.2531	0.3480	0.2500	0.2520	0.2500	0.2380	0.0970
0.50	0.3090	0.3040	0.2532	0.2520	0.2516	0.2440	0.2440	0.2400	0.2400	0.2280	0.2280	0.0810
1.00	0.3340	0.3340	0.3200	0.2542	0.2532	0.2524	0.2490	0.2490	0.2490	0.2490	0.2490	0.0850
3.00	0.3040	0.3040	0.2526	0.2500	0.2340	0.2420	0.2160	0.2100	0.2050		0.1545	0.1495*
5.00	0.3080	0.2518	0.2510	0.2380	0.2160	0.1545	0.1539	0.1522	0.1512	0.1512	0.1501	0.1579
7.00	0.3140	0.3040	0.2520	0.2280	0.2080	0.1545	0.1522	0.1512	0.1506	0.1430	0.1400	0.1740
10.00	0.3360	0.2544	0.2516	0.2270	0.2100	0.1540	0.1522	0.1512	0.1400	0.1370	0.1300	0.2060
30.00	0.3480		0.3100	0.2000	0.1524	0.1500	0.1200	0.0548	0.0526	0.0536	0.0522	0.2858*
50.00	0.2523	0.2380	0.1538	0.1140	0.0542	0.0520	0.0510	0.0490	0.0430	0.0380	0.0320	0.2203
70.00	0.3360	0.2537	0.2110	0.1512	0.1300	0.0547	0.0528	0.0526	0.0510	0.0504	0.0504	0.2756*

\* At these concentrations there was a sudden marked ability to solubilize gas demonstrating "critical micelle concentration" (CMC).

return of the micelles into the solution where the surfactant molecules disperse.

We have suggested that a circulation of surfactant molecules takes place between the alveolar cell and the alveolar surface lining with changes caused by respiration. Thus with inspiration, increased pressure exerted on the alveolar cell increases the membrane surface and thereby forces more surfactant to migrate to the surface, instantly causing the formation of micelles which can readily solubilize non-polar gas or inhaled anaesthetics in the quantities observed. With release of pressure on exhalation, these micelles find their way back into the subphase and disaggregate, releasing the anaesthetic or molecular gases to be absorbed by the blood stream.

The marked ability of both crude lung surfactant and a 30 per cent concentration of 'Tween 80' to absorb or solubilize halothane in the presence or absence of underlying lung homogenate is highly indicative of solubilization by micelles. It is not inconceivable that some similar mechanism may play a part in the uptake of molecular gases by the red blood cell and plasma proteins.

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## HAEMATOLOGY

### Second Kininase in Human Blood Plasma

COHN fraction IV-1 of human plasma contains an enzyme, carboxypeptidase N, which inactivates bradykinin and kallidin<sup>1,2</sup> and also cleaves smaller substrates such as hippuryl-L-lysine (HLL)<sup>3</sup>. The kininase also has an esterase activity—it hydrolyses the ester substrate hippuryl-L-argininic acid (HLAa)<sup>4</sup>. For the sake of simplicity in this communication, we shall refer to this enzyme as kininase I.

During purification of human carboxypeptidase N, we noticed the presence in plasma of a second bradykininase (kininase II) (refs. 3 and 4). This enzyme preparation inactivated bradykinin, although it did not hydrolyse HLL. This report describes the characterization and partial purification of kininase II.

The starting material for the experiments was obtained by means of gel filtration of fresh, heparinized human plasma on a 'Sephadex G-200' column. The peptidase and esterase activities, as measured with HLL and HLAa substrates, were found in a single protein peak (Fig. 1). The bradykininase gave a broader activity maximum, however, than the carboxypeptidase. When this plasma protein fraction containing the bradykininase activity was adsorbed on a DEAE-'Sephadex A-50' column and eluted stepwise, two bradykininases were separated (Fig. 2).

The peak that was eluted with 0.3 molar sodium chloride contained carboxypeptidase N, which hydrolysed HLL,

HLAa and bradykinin. This kininase I inactivated bradykinin by breaking the carboxyl terminal Phe<sup>8</sup>-Arg<sup>9</sup> bond of the peptide.

The peak which emerged during elution with 0.15 molar sodium chloride contained kininase II. It did not cleave HLL and hydrolysed HLAa only slightly but inactivated bradykinin when tested on the isolated rat uterus (Fig. 2).

The site of attack of kininase II in the bradykinin peptide was found by the combined use of thin-layer chromatography and the dansyl (1-dimethyl-amino-naphthalene-5-sulphonyl chloride) fluorescent labelling technique of Prado *et al.*<sup>5</sup>, by descending paper chromatography and by means of peptide mapping<sup>2</sup>. Thin-layer chromatography was performed on glass plates coated with silica gel G in an *n*-butanol : acetic acid : water (4 : 1 : 5 or 50 : 1 : 50) solvent. Paper chromatography was done on Whatman 3MM paper in the same solvent. Peptide mapping was used as previously described<sup>2</sup>. The split products of the reaction of kininase II with the substrates were separated by means of these techniques. These

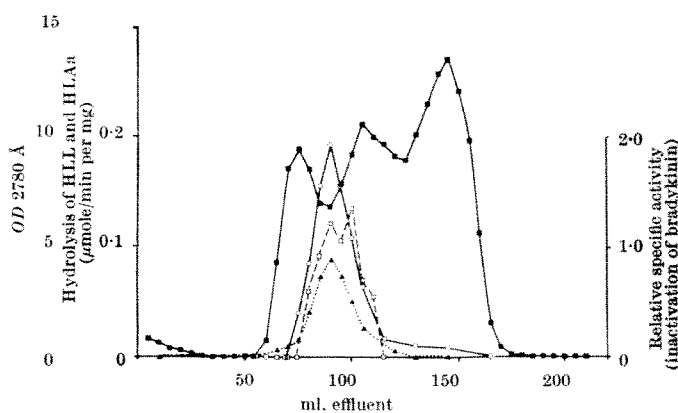


Fig. 1. 'Sephadex G-200' filtration of human plasma kininases. 15 ml. of fresh heparinized human plasma was filtered through a 3.5 × 46 cm. column. A 0.05 molar phosphate buffer, pH 7, containing 0.5 moles/l. of sodium chloride was the eluent. 5 ml. fractions were collected every 30 min. HLL, Hippuryl-L-lysine; HLAa, hippuryl-L-argininic acid. —●—, Protein; —□—, hydrolysis of bradykinin; —▲—, hydrolysis of HLL; —○—, hydrolysis of HLAa.

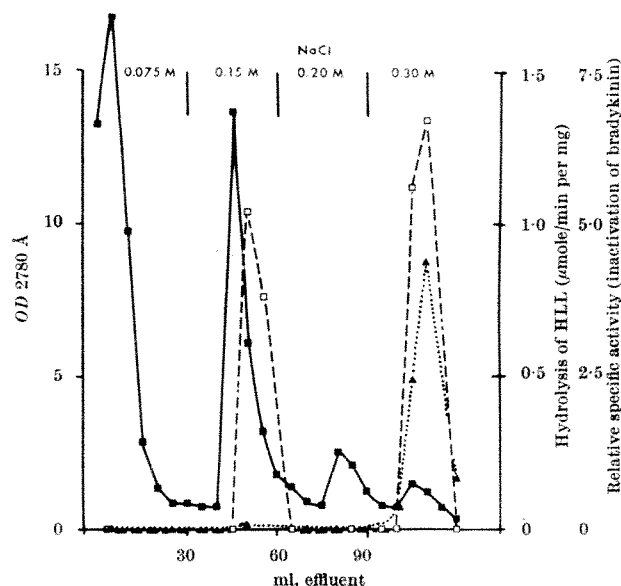


Fig. 2. Separation of the two kininases on a DEAE-'Sephadex A-50' column. 300 mg of protein obtained after gel filtration was purified on a 1 × 30 cm. column. Fractions were eluted step by step by increasing the concentration of sodium chloride in a 0.02 molar phosphate buffer, pH 6.8. 5 ml. was collected every 30 min. The activity eluted with 0.3 molar sodium chloride contains kininase I (carboxypeptidase N). Kininase II moved with the fraction that was eluted with 0.15 molar sodium chloride. HLL, Hippuryl-L-lysine. —●—, Protein; —□—, hydrolysis of bradykinin; —▲—, hydrolysis of HLL.

substrates were bradykinin and Ac-Ser-Pro-Phe-Arg-OH. The latter compound represents the carboxyl terminal tetrapeptide sequence in bradykinin. It was acetylated to avoid hydrolysis by unspecific aminopeptidases at the amino terminal end<sup>6</sup>. In the experiments using paper chromatography 0.68–1.58 mg enzyme protein was incubated with 1  $\mu$ mole of substrate in a 0.07 molar *tris* buffer, pH 7.4, for 16 h at 31° C. In the thin-layer chromatography studies 0.13–0.32 mg of enzyme protein was used with 0.2  $\mu$ mole of substrate.

Kininase II cleaved the Pro<sup>7</sup>-Phe<sup>8</sup> bond in bradykinin and in the smaller substrate; it thus removed a Phe-Arg dipeptide from the carboxyl terminal end. The identity of Phe-Arg, one of the split products, was established by comparing its  $R_F$  value with synthetic Phe-Arg, by eluting the reaction product from the thin-layer chromatography plate and hydrolysing it in 6 normal hydrochloric acid for 16 h or by reacting the split product with dansyl reagent and then hydrolysing it. The identity of the heptapeptide product of the reaction of bradykinin with kininase II was established by comparing its migration in peptide mapping with the heptapeptide obtained after digesting bradykinin with a mixture of carboxypeptidase A and B (ref. 6).

Kininase II did not act as a transferase because when Arg and Ac-Ser-Pro-Phe-OH were incubated with the enzyme, no Phe-Arg spot was observed after the incubation. Thus the enzyme did not transfer phenylalanine from the substrate to arginine to create the dipeptide. Kininase II preparations still contained a dipeptidase impurity which hydrolysed Phe-Arg on prolonged incubation.

Kininase II was further characterized in biological studies when the inactivation of bradykinin was measured on the isolated rat uterus. Here 0.1–0.3 mg protein of enzyme/ml. was incubated with 3.3  $\mu$ g of bradykinin in a 0.1 molar *tris*, pH 7.4, at room temperature. Samples were withdrawn every 5 min, diluted and assayed on the isolated organ.

Table 1. INHIBITION OF THE INACTIVATION\* OF BRADYKININ BY KININASE I AND II OF HUMAN PLASMA

Inhibitor	Concentration (moles/l.)	Relative degree of inhibition Kininase I	Kininase II
CdSO <sub>4</sub>	1 $\times 10^{-3}$	++	0
HgCl <sub>2</sub>	1 $\times 10^{-3}$	++	++
EDTA	3 $\times 10^{-3}$	++	++
1,10-Phenanthroline	1 $\times 10^{-3}$	++	++
$\epsilon$ -amino- <i>n</i> -caproic acid	3 $\times 10^{-3}$	+	0
H-Phe-Arg-OH	1 $\times 10^{-2}$	++	++
Arginine	3 $\times 10^{-3}$	++	0
Trasyolol	125 $\mu$ mol.	0/+	0

\* Inactivation of bradykinin was measured on the isolated rat uterus.

++, Complete inhibition; +, partial inhibition; 0, no inhibition.

Table 1 compares the inhibition of kininase I (carboxypeptidase N) and kininase II by various compounds. The data indicate that the pattern of inhibition for two kininases is different. Arginine,  $\epsilon$ -amino-*n*-caproic acid and cadmium sulphate inhibit kininase I only. Kininase II is remarkably resistant to cadmium sulphate, while kininase I is strongly inhibited by that compound. It was shown that the esterase activity of carboxypeptidase N is inhibited by 50 per cent by a  $2 \times 10^{-7}$  molar concentration of the metal<sup>4</sup>. Both kininases are inhibited by the dipeptide H-Phe-Arg-OH, by HgCl<sub>2</sub> and by chelating agents. Although kininase II acts as endopeptidase (peptidyl peptide hydrolase), it is inhibited by EDTA and by 1,10-phenanthroline which suggests the necessity of the presence of a metal co-factor in the enzyme. Trasyolol, the inhibitor of various proteolytic enzymes, was inactive with kininase II.

Kininase II has properties similar to those of a microsomal enzyme which we have isolated from the kidney of a pig<sup>6,7</sup>. This enzyme, named peptidase P, cleaves the same bond in bradykinin as does kininase II of blood plasma.

It has been suggested that components of the kallikrein-kinin system occur in two different forms in plasma.

Plasma of various origin contain two kininogens<sup>8,9</sup>, two kallikreins<sup>10,11</sup> and two kallikrein inhibitors<sup>12</sup>. Our findings indicate that there are also two kininases present in human plasma. Kininase I is carboxypeptidase N<sup>2,13</sup> which is inhibited differently from kininase II. Kininase I cleaves the Phe<sup>8</sup>-Arg<sup>9</sup> bond of bradykinin; kininase II inactivates the peptide by hydrolysing the Pro<sup>7</sup>-Phe<sup>8</sup> link.

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## RADIOBIOLOGY

### Distant Mutagenic Effects of Ionizing Radiation in Mammals

WHEN plant and animal tissues are irradiated with X- and  $\gamma$ -rays, humoral factors are formed which may have a mutagenic effect<sup>1-7</sup>. Mutagenic factors have also been found in irradiated organic growth media<sup>1,8-10</sup>. Here we report the findings of an investigation of the mutagenic effect of humoral toxic factors which appear in mammalian tissues after X-ray irradiation.

*The first experiments.* CC57Br male mice, 2–3 months old, were irradiated with X-rays (1,500 r. at 30 r./min) while their heads were screened by means of a thick-walled lead hemisphere. Mice of the same strain, sex and age were taken as controls and either subjected to the same manipulations without irradiation or irradiated fully screened. In all, one hundred mice were used in this experiment<sup>2</sup>. Twenty-four hours after irradiation, the animals were killed and their corneas were fixed in Carnoy liquid and stained with Behmer haematoxylin. The frequency of anaphases and telophases with bridges and fragments in the corneal cells was estimated. When mice were irradiated with their heads screened, the frequency of corneal cells with chromosome aberrations increased seven times more than those of the control animals which were completely protected by the lead shields and ten times more than the intact group (see Fig. 1).

*The second experiments.* With a radiation dose of 1,500 r., the irradiated part of the body can be considered as a source of "reflected radiation" which acts along the longitudinal axis of the body through the screened head and delivers radiation to the cornea at a dose of  $\approx 10$  r. In



Table 1. MUTAGENIC ACTION OF THE EXTRACTS FROM TISSUES OF IRRADIATED ANIMALS

Experimental animals	Series of experiments	Animals	Cornea		Per cent of aberrant mitoses	Animals	Bone marrow		Per cent of aberrant mitoses
			Normal mitoses	Aberrant mitoses			Normal mitoses	Aberrant mitoses	
Non-inbred albino rats	Intact control	14	8,847	7	0.04 ± 0.02	14	3,673	173	4.6 ± 0.32
	Injection of the extract from the tissues of intact rats	23	15,806	21	0.09 ± 0.02	22	5,891	253	4.3 ± 0.36
	Injection of the extract from the tissues of irradiated animals	24	14,638	61	0.42 ± 0.03	24	6,470	378	5.8 ± 0.88
CC57Br male mice	Intact control	25	12,130	0	0	Double dose of extract			
	Injection of extract from brain tissues of intact mice	10	2,823	0	0	9	3,751	2	0.02 ± 0.03
	Injection of extract from brain tissues of irradiated mice	57	15,533	37	0.24 ± 0.08	10	1,747	8	0.35 ± 0.09

order to rule out the possibility of reflected irradiation, the following experiment was carried out. Non-inbred albino rats and CC57Br mice were irradiated with a dose of 1,500 r. Twenty-four hours later, the animals were killed and their bodies and brains minced in cold phosphate buffer (pH 7.3) (ref. 3). Extracts were then prepared in cold aqueous saline and injected intravenously into rats and intraperitoneally into mice. Control animals received extracts from non-irradiated donors. Twenty-four hours later the eyes of the animals were enucleated, and parts of the femur and tibia near the knee were also resected from the rats. From the material obtained in this way, preparations of cornea and bone marrow were made and the number of cells with chromosomal aberrations was counted. Table 1 shows that there is a significant increase in the number of cells with chromosomal aberrations in the bone marrow and cornea of mice which have been injected with extracts from the whole bodies

Table 2. EFFECT OF DIFFERENT DOSES OF IRRADIATION ON THE MUTAGENIC PROPERTIES OF THE BRAIN EXTRACT FROM MICE

Experimental variants	Animals	Normal mitoses	Aberrant mitoses	Per cent of aberrant mitoses	Per cent of animals with negative results
Extract from intact donors	10	2,823	0	0	100.0
Extract from the brain tissues of the irradiated mice in dose of					
750 r.	11	2,334	0	0	100.0
1,500 r.	57	15,533	37	0.24 ± 0.08	42.1
3,000 r.	11	2,208	5	0.21 ± 0.09	72.7

and from the brains of irradiated mice. Extracts from the liver, spleen, testes, jejunum, fatty tissue and blood of irradiated animals did not produce any significant mutagenic effect.

The selective mutagenic activity of the irradiated brain as compared with other organs does not seem to be

related to the chemical specificity of the cells of the central nervous system because extracts from the *in vitro* irradiated mouse brain had no visible effect on the chromosomes of the cells of recipient animals. This indicates the important part played by the metabolic processes of the whole organism in the formation of "radiotoxins".

The mutagenic effect increased when the dose of the extract injected was doubled. The mutagenic activity of the extract did not change when animals were irradiated with a dose of 3,000 r., and a dose of 750 r. had no effect at all (Table 2).

There was considerable variability in the response of animals to similar amounts of mutagen. The decrease in the mutagenic effect produced when extracts from different organs of irradiated animals were used instead of whole body extracts also varied considerably (Table 3).

*The third experiments.* In the experiments reported so far, the mutagenic effect of the saline tissue extracts from irradiated animals resulted from the interaction of mutagenic agents with a complex set of factors within the organism. It was thus of interest to study this phenomenon on a less complicated model, that is, on the chromosomes of human leucocyte cultures.

In these experiments<sup>4</sup>, we used the same concentration of extract calculated for 1 ml. of culture medium. The extract was passed through a bacterial filter and then introduced into the leucocyte culture at the sixtieth hour of growth, that is, almost at the end of the premitotic stage<sup>11</sup>. We have studied 362 metaphase plates of two women 23 and 32 years old and a man 27 years old, all of them with a normal karyotype. The experiment was divided into three parts: (1) intact control; (2) leucocytes

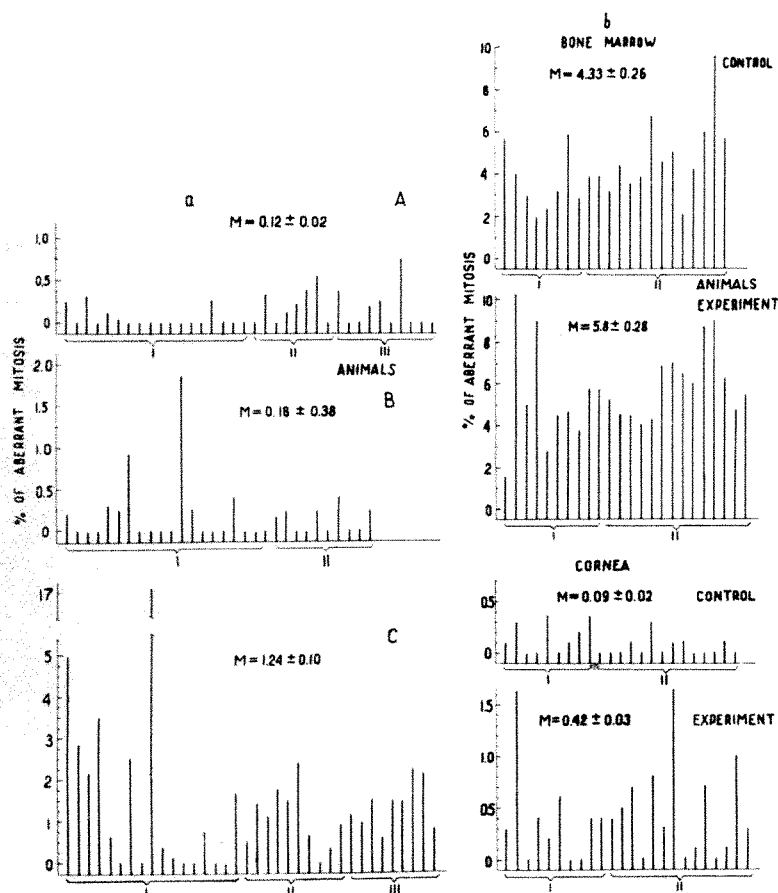


Fig. 1. Histogram of individual variability in the reaction of animals to the mutagenic action of the ionizing radiation. a, Screening of head. A, Intact control; B, screened control; C, screened head. b, Injection of salt-water extracts. I, II, III, Animal test groups.

Table 3. INCREASE IN DISAGREEMENT OF THE DATA FROM THE FIRST AND SECOND EXPERIMENTS (CORNEA)

Results/Method	Shielding of the head of mice	Extract from the whole bodies of irradiated rats	Extract from brain tissues of irradiated mice (1,500 r.)	Double dose of extract from brain tissues
Percentage of aberrant mitosis	1.24 ± 0.10	0.42 ± 0.03	0.24 ± 0.08	0.35 ± 0.09
Percentage of animals with negative results	20.0	25.0	42.1	72.8

Table 4. EFFECT OF BRAIN EXTRACT ON CHROMOSOMES OF LEUCOCYTES OF HUMAN PERIPHERAL BLOOD

Experimental variants	Number meta-phases	Chromosome aberrations					
		Aberrant metaphases		Acentric fragments		Dicentric	
		<i>M</i>	%	<i>M</i>	%	<i>M</i>	%
(1) Intact control	139	1	0.7	0	—	1	1.2
(2) Incubation of leucocytes with extract from intact mice tissue	123	7	5.7	6	4.8	1	0.8
(3) Incubation with extract from tissues of irradiated mice	100	13	13.0	12	12.0	1	1.0
$\chi^2 \geq 10.8$ ; $P = 0.999$ .							

$\chi^2 > 10.8$ ;  $P = 0.999$ .

incubated with intact mouse brain extract; (3) leucocytes incubated with the brain extract from mice which had been irradiated with 1,500 r. It can be seen from Table 4 that the frequency of spontaneous chromosome aberration in healthy donors was 0.7 per cent (out of 140 plates only one was found to have one dicentric). In experiment 2, 0.8 per cent of cells contained dicentrics and 4.8 per cent had acentric fragments. In experiment 3, the mutagenic effect of the tissue extract from irradiated animals was manifested by an increase to 13 per cent in the frequency of cells with chromosomal aberrations. These aberrations were mainly acentric fragments (12 per cent). All the aberrations in these experiments occurred in chromosomes and seemed to involve chromosomal breakages at secondary constrictions and in the region of the centromere—the heterochromatin nature of this tends to make them vulnerable to different extrinsic factors<sup>12-14</sup>.

Various hypotheses have been proposed to explain the mechanisms by which chromosomal breakages are formed after the irradiation of distant parts of the body. None of them can be considered proved, however, and the extension of this phenomenon is not yet clear.

In studying the leucocyte aneuploidy in our third experiment, we found that when the cells are incubated with extracts of irradiated tissues, hyperploid plates occur three times as frequently as in the intact control. The causes of this phenomenon are being studied.

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## Effect of Previous X-irradiation on the Response of Brain to Injury

THE normal adult mammalian central nervous system shows no early or late morphological effects after a single dose of X-irradiation up to 1,000 rads<sup>1,2</sup> and even with doses of 2,000–4,000 rads there is a delay of many months before damage appears<sup>3,4</sup>.

In spite of this absence of morphological change after moderate doses of radiation we have shown that the cerebral glial reaction to injury is diminished after both small and larger doses (200–4,000 rads) and, furthermore, that this change seems to be independent of the time between irradiation and injury.

Brains of Sprague-Dawley rats, of both sexes, 3–4 months old were irradiated, under chloral anaesthesia (300 mg/kg intraperitoneally), to single doses of X-rays of 200–4,000 rads. Irradiation was given laterally to both cerebral hemispheres through a semi-circular port of diameter 18 mm, in 2.5 mm of lead shielding. Doses were given at 90–95 r./min, 220 kV, 15 mamp, with 0.5 mm of copper and 1.0 mm of aluminium as added filtration.

At intervals of between 3 days and 6 months after the irradiation a small superficial area of the brain was injured by freezing through the calvarium. This method was used because it gave regular results without the necessity of opening the skull and thereby avoiding the risk of infection. For this a cooling device was used which consisted of a copper tube, filled with a mixture of solid carbon dioxide and alcohol, at one end of which there was an applicator tube 3 mm in diameter. This tube was placed in contact with the exposed left parietal bone of the skull for a period of 15–20 min depending on the size of the animal. A single intraperitoneal injection of 1 per cent w/v trypan blue (0.5 ml./100 g body weight) was administered at the time of injury so that the exact location of the lesion was obvious when the brains were removed 1 week later.

The animals were killed 7 days after injury with an overdose of sodium pentobarbitone ('Nembutal'). The cerebral hemispheres were removed and fixed in 1 per cent acetic acid in 10 per cent formal saline. The slice of brain containing the centre of the lesion was slowly dehydrated in alcohols and embedded in paraffin wax. Sections 5  $\mu$  thick were cut and stained with Ehrlich's haematoxylin and eosin and mounted in the usual way.

The normal response to this freezing injury was necrosis followed by cell division and infiltration commencing at the periphery of the lesion. This was clearly marked at 3 days, and 7 days after the injury the saucer shaped area was uniformly populated with cells (Fig. 1). They were approximately 2.5 mm in diameter and 0.5–1.0 mm deep.

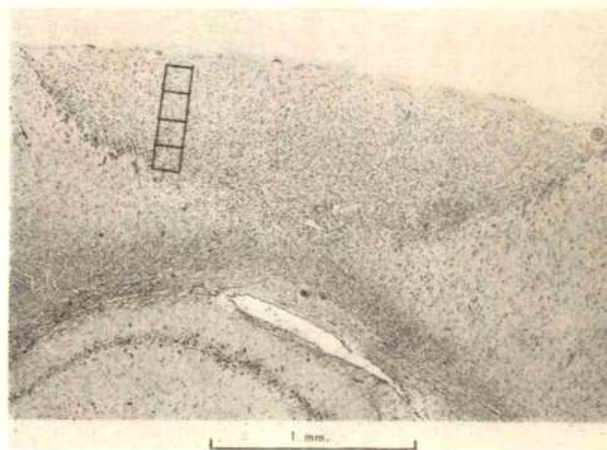


Fig. 1. Lesion on the left cerebral hemisphere of the brain of the rat, indicating the four areas (0.086 x 0.086 mm) used for cell counts, where the lesion is 0.5 mm thick.



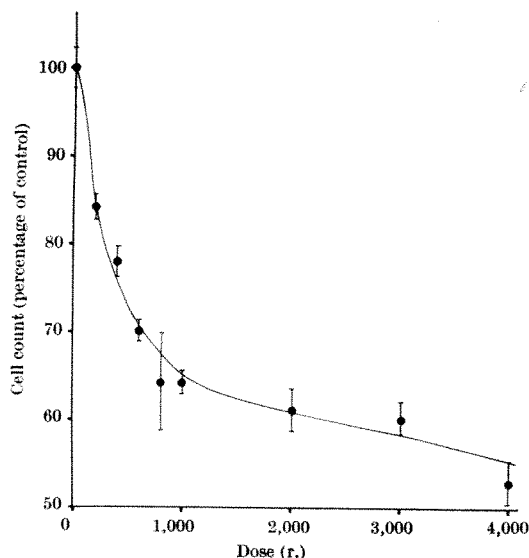


Fig. 2. Cell counts/unit area of lesion, expressed as percentage of the controls, plotted against the dose of 220 kV X-rays.

To minimize subjective errors cell counts were made to the following standard procedure. Counts were always made in an area where the lesion was 0.5 mm thick. Using a squared graticule eyepiece and an oil immersion lens (magnification 1,050) cells were counted in four consecutive areas 0.086 × 0.086 mm, perpendicular to the surface (Fig. 1). The number of cells was estimated by counting to the nearest whole nucleus found within the graticule area.

Up to five consecutive serial sections of each lesion were mounted on a slide and of these a minimum of two sections were counted. The mean number of cells counted in each section for controls was 230.

The results, for rats irradiated 3 days before injury, are shown in Fig. 2. The numbers of cells/unit area are expressed as a percentage of the non-irradiated controls.

With selected doses we varied the period between irradiation and injury and observed a similar reduction in the cellular response, although so far the numbers of animals in these groups have been small (Table 1).

Exposure dose (r.)	Cell counts/unit area of lesion as percentage of control			
	Latent period between irradiation and injury			
	3 days	3 weeks	3 months	6 months
200	84.5 ± 1.4 (5)	84.3 ± 2.5 (3)	81.9 (2)	—
400	75.5 ± 1.6	—	—	68.5 (2)
2,000	61.1 ± 3.5 (5)	62.5 ± 2.5 (4)	58.5 (2)	62.5 (2)

Results are expressed as a percentage of controls, with standard errors when three or more animals were used. Figures in brackets indicate the number of animals used in each group.

The simplest explanation of this is that glial cells do not show radiation damage until they are stimulated to divide, when they either fail to divide or die. This "storing up" of radiation damage is possibly caused by the slow turnover of glial cells in normal conditions, so that replacement does not take place to any extent. Similar examples can be cited in such organs as the liver, skin and thyroid<sup>5-7</sup> where the radiation damage can be accelerated by the introduction of factors which influence the rate of mitotic activity. The observations<sup>8</sup> that the latent period before death after irradiation of the spinal cord could be greatly reduced by hypertension could have a similar basis.

The hypothesis that previously irradiated local glial cells die on attempting cell division is dependent on the assumption that most of the cells originate endogenously. The findings that a considerable number of the macrophages in brain lesions are haematogenous<sup>9</sup> seem to dis-

agree with our results. Thus further work is in progress to determine whether our results can be completely explained by a reduction in local glial cell proliferation, or whether some more complicated mechanism may be operating.

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## BIOLOGY

### Paraquat and Sterility of Hen's Eggs

Dunachie and Fletcher's observation that paraquat at concentrations above a threshold level of 0.1–0.15 p.p.m. —about 5–7.5 µg/egg—is highly toxic to hen's eggs may seem alarming. As the second letter from ICI shows, however, in order to get 0.1 p.p.m. into an egg by feeding a fowl rather than with a syringe, it must be ingested at a concentration of 40 p.p.m., four times the concentration that ICI recommend for proper crop spraying.—Editor, *Nature*.

### Effect of Some Herbicides on the Hatching Rate of Hen's Eggs

In a previous communication<sup>1</sup> we gave hatching rates obtained after the injection, in various dosages, of a number of insecticides into hen's eggs, using the method of McLaughlin *et al.*<sup>2</sup>. The investigation has now been extended to test a number of herbicides.

Pesticides identified as contaminants in birds or their eggs in the wild have all been, so far as we are aware, organochlorine insecticides. Yet it has been found<sup>3</sup> that a number of herbicides show considerable persistence in the soil and should thus be susceptible to passage along a food chain and accumulation in animal tissues. As in our previous work, eggs have been treated in batches of twenty-five. Hatching rates, given in Table 1, have been expressed as percentages (rounded to the nearest 10 per cent) of controls, injected with solvent only. The solvent used has usually been acetone; occasionally the herbicide was brought into solution as the hydrochlorides and this has been indicated in the table. Aminotriazole and atrazine were injected in methanol; diquat, ioxynil and paraquat in water. The nomenclature of the compounds is that given in Woodford and Evans<sup>4</sup>.

In contrast with some of the insecticides, chiefly organophosphorous compounds, none of the herbicides tested produced deformities, though mecoprop, 2,4-DB, MCPA and MCPB, all chlorinated phenoxyacids, caused

Table 1

1. Dose given: 200 p.p.m. = 10 mg/egg (selected compounds only).

Percentage hatch	
100	Dalapon
90	Atrazine.HCl, chlorpropham
80	Mecoprop, dicamba, prometryne
50	2,4 D, TBA
30	Monolinuron, linuron
20	MCPA, MCPB
0	Desmetryne, dichlorprop

## 2. Dose given: 100 p.p.m. = 5 mg/egg (all compounds)

Percentage hatch	
100	Atrazine.HCl, chlorphencarb, dalapon, MABSC*, monolinuron, prometryne, simazine.HCl
90	Dicamba, TBA
80	Mecoprop, aminotriazole.HCl, chlorpropham
70	2,4 D, 2,4 DB, dichlorprop
60	Linuron
50	MCPA, MCPB, desmetryne
10	Diquat, prefix
0	Bromoxynil, DNOC, ioxynil, paraquat, pentachlorophenol

## 3. Dose given: 10 p.p.m. = 0.5 mg/egg (all compounds except prometryne and desmetryne).

Percentage hatch	
100	Aminotriazole.HCl, atrazine.HCl, chlorphencarb, chlorpropham, dalapon, ioxynil (10 per cent at 25 p.p.m.), linuron, MABSC*, MCPA, MCPB, monolinuron, prefix (50 per cent at 50 p.p.m.), simazine.HCl
90	2,4 D, dicamba, dichlorprop, mecoprop, TBA
80	2,4 D, pentachlorophenol (50 per cent at 50 p.p.m.)
70	Bromoxynil
30	DNOC (0 per cent at 20 p.p.m.; 80 per cent at 5 p.p.m.)
10	Diquat (60 per cent at 5 p.p.m.)
0	Paraquat (0 per cent at 0-25 p.p.m.; 40 per cent at 0-15 p.p.m.; 100 per cent at 0-1 p.p.m.)

\* Methyl-4-aminobenzene sulphonylcarbamate.

feather blanching. The most disturbing results are those for paraquat, which gives a heavy mortality even at 0-15 p.p.m., death occurring during the first day or two of development. By injecting a dose of 1 p.p.m. into separate batches at intervals after the commencement of incubation, we obtained a 20 per cent hatch at 2 and 4 days, but a hatch of 80 per cent and above thereafter, to the sixteenth day. When 10 p.p.m. was given on the tenth and fourteenth day, however, mortality in the later stages of development was heavy.

We thank the Agricultural Research Council for the grant it has made towards carrying out this project and the following firms who supplied chemicals: CIBA Laboratories, Ltd.; Fisons Pest Control, Ltd.; ICI, Ltd.; May and Baker, Ltd.; Mirvale Chemical Co., Ltd.; and "Shell" Research, Ltd.

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### Production and Viability of Eggs from Hens treated with Paraquat

IN the communication from Dunachie and Fletcher<sup>1</sup> it is shown that when paraquat is injected into fertile hen's eggs to give a final concentration greater than approximately 0.1 p.p.m. the subsequent rate of hatching was reduced. Because hens may eat food containing residues of paraquat from previous sprayings I made the following experiment to determine whether the amount of paraquat likely to be present would cause similar effects.

One hundred and fifty chickens, of a hybrid white leghorn strain, just past their period of maximum lay, were housed in six separate pens in one house on deep litter. Three of the pens each contained twenty-five control birds to which no treatment was given; the other three pens were similar except that the birds were given water containing 40 p.p.m. of paraquat as the dichloride salt. All the birds had free access to water and food, a record being kept of their weekly intakes. The treatment was continued for 14 days, after which time no paraquat was given to the treated group and data were collected for a further 14 days. The eggs which were laid were totalled daily, marked with the pen number and the day of laying, and stored. At the end of each week all the

eggs laid, with the exception of those cracked or misshapen, were transferred to the hatchery. A sample of three eggs per pen on alternate days was taken for analysis for paraquat by a modification of the method of Calderbank and Crowdy<sup>2</sup> and Calderbank and Yuen<sup>3</sup>. At the end of 18 days infertile eggs were rejected, their total and identification numbers being recorded. After 21 days the number of chicks hatched was counted and the unhatched eggs identified. The chicks were examined for abnormalities and then observed for a further 10 days.

Table 1. AVERAGE NUMBER OF EGGS LAID IN EACH PEN EACH WEEK

Period	Number of eggs (mean $\pm$ S.D.)	
	Control	Treated
Pre-experimental period	114 $\pm$ 12.5	106 $\pm$ 12
Week 1	96 $\pm$ 4	94 $\pm$ 15.5
Week 2	110 $\pm$ 9	93 $\pm$ 15
Week 3	107 $\pm$ 4	94 $\pm$ 13
Week 4	107 $\pm$ 4.5	110 $\pm$ 15

Table 2. ANALYSIS OF HATCHING RESULTS FROM EGGS

				No. hatched	No. abnormal eggs
				No. set (per cent)	No. set (per cent)
Week 1 and 2	Laid	Set	Fertile		
Control	619	522	483	88.4	17.2
Treated	561	465	414	83.5	24.3
Week 3 and 4					
Control	642	557	516	85.3	19.9
Treated	613	543	491	84.3	14.9

Table 1 shows the average weekly egg production of the treated and control groups. Although there was a fall in egg production during treatment the numbers of eggs produced by the treated group do not differ significantly from those of the controls. Table 2 gives the hatching results from the two groups in two periods of 14 days. The heading "abnormal eggs" includes those eggs which were infertile, did not hatch or produced an abnormal chick. While there is no difference between the hatchability of the two groups, there is a slight but significant difference ( $P = 0.02$ , Student's  $t$  test) between the numbers of abnormal eggs produced. The treatment appeared to have no significant effect on the food and water consumption of the hens nor the number or type of abnormality in the chicks. Fig. 1 shows that the amount of paraquat found in the eggs rises to about 0.1 p.p.m. and subsequently declines, being below the limits of detection 6 days after the cessation of treatment.

The maximum amounts of paraquat that have been found on food crops after proper spraying are 10 p.p.m., and normally the residues are much below this figure (ICI, internal report). Furthermore, experiments in this laboratory on other species indicate that a quantity of paraquat mixed with food is less toxic than the same amount given as a solution. Thus, although in this study with the concentrations used a small effect of paraquat on the chick embryo has been shown to occur, confirming the results of the Strathclyde workers, I feel that with the greatly reduced exposure of normal farming practice such an effect would be negligible and would be no hazard to poultry or consumers.

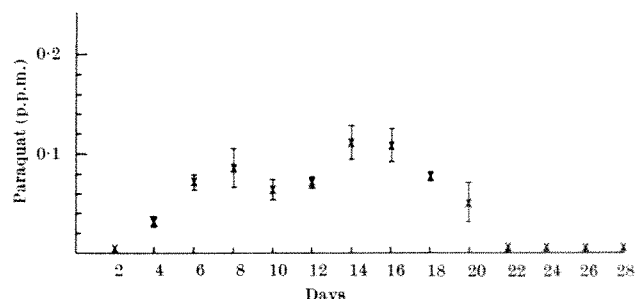


Fig. 1. The amount of paraquat found in eggs from hens given 40 p.p.m. paraquat in their drinking water (mean  $\pm$  S.E.). The treatment was stopped after the fourteenth day. The amount of paraquat in the control eggs was below the limit of detection (0.005 p.p.m.).



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### Distribution of *Biomphalaria* Species in Sudan

INTESTINAL bilharzia (caused by the trematode *Schistosoma mansoni*) is transmitted by snails of the genus *Biomphalaria*. A survey has recently been conducted to determine the distribution of these snails in the Khartoum and Blue Nile provinces of Sudan.

The occurrence of *Biomphalaria alexandrina* (Ehrenberg) has until now been recorded only from Lower Egypt<sup>1</sup> but the present survey shows that this species is frequently present in Sudan. The distribution in the White Nile appears to extend from the dam at Jebel Aulia (50 km south of Khartoum) to at least as far south as Kosti (300 km south of Khartoum). High populations were found particularly among *Eichornia* (water hyacinth) plants and to a lesser extent in pump irrigation schemes. This species was also found on the west of the Gezira irrigation scheme (taking water from the Blue Nile) but its distribution east of this is uncertain.

*Biomphalaria sudanica* (Martens) also appears to have a wider distribution than formerly recognized. High populations of this species were found at Shambat, a pump scheme 8 km north of Khartoum on the main Nile, and at various points in the White Nile between Kosti and Khartoum. Previously<sup>2</sup>, it had been found no farther north than Kosti. *Biomphalaria pfeifferi* (Krauss), while common throughout the Gezira and pump schemes from the Blue Nile, was not found in the White Nile or its associated irrigation schemes.

It can be concluded from these observations that the distribution of the snails is changing rapidly. Presumably the northwards extension of the distribution of *B. sudanica* has been influenced by a similar extension of *Eichornia* since 1958 (ref. 3) but the reasons for the southwards movement of *B. alexandrina* are obscure. Measures to control bilharzia in Sudan will need to take the present complex distribution of snails into account and recognize that snails will rapidly move back into controlled areas.

We thank Dr G. A. Mandahl-Barth for identifying specimens and the Rockefeller Foundation for financial assistance.

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### Effect of Carbon Monoxide on Metabolism of Insecticides *in vivo*

THERE have recently been several reports which indicate that the oxidative metabolism of drugs by liver microsomes involves a cytochrome-like pigment usually referred to as *P-450* (refs. 1 and 2). This pigment, which is characterized by the difference spectrum observed when microsomal suspensions reduced either with hydrosulphite or

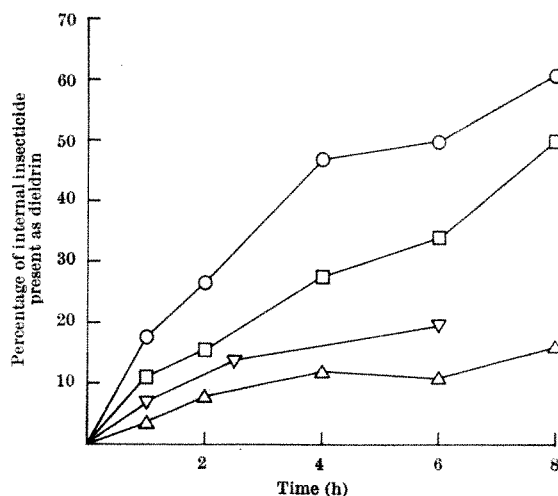


Fig. 1. Effect of carbon monoxide and oxygen tension on formation of dieldrin in houseflies treated with aldrin. ○, Flies kept in air; ▽, flies kept in 40 per cent carbon monoxide, 20 per cent oxygen, 40 per cent nitrogen; □, flies kept in 90 per cent nitrogen, 10 per cent oxygen; △, flies kept in 90 per cent carbon monoxide, 10 per cent oxygen.

with NADPH are saturated with carbon monoxide, has recently been found in microsomes from the housefly *Musca domestica* (vicina strain)<sup>3</sup>. Furthermore, the inhibition by carbon monoxide of the epoxidation of aldrin and the partial reversal of this inhibition by light supports the view that *P-450* is involved in epoxidation by fly microsomes *in vitro*. Similar evidence has been produced to support the view that this pigment is involved in the metabolism of drugs by microsomes from rat liver<sup>2</sup>. Evidence for the role of a reaction in the metabolism of insecticides *in vivo* which is sensitive to carbon monoxide has not, however, been reported.

The relative affinities of carbon monoxide and oxygen to haemoglobin, *P-450* and cytochrome oxidase are 200:1, 1:1 and 1:10, respectively<sup>4</sup>. The housefly, in common with other insects, does not rely on haemoglobin for the transport of oxygen and so it is likely to survive in a mixture of carbon monoxide and oxygen which would inhibit reactions involving *P-450*. Thus it would seem that insects may be particularly suitable animals in which to examine the effect of carbon monoxide on the metabolism of foreign compounds *in vivo*. The epoxidation of the insecticide aldrin is a reaction which is well suited for this purpose because in the housefly there is little evidence of further metabolism of the epoxide (dieldrin)<sup>5</sup>.

Four-day-old female houseflies *Musca domestica* (vicina strain), resistant to dieldrin, were treated individually with 2 µg of aldrin in 2 µl. of acetone. Groups of ten flies were confined in glass cylinders of 1 l. capacity which had previously been filled with an appropriate mixture of carbon monoxide, oxygen and nitrogen. They were supplied with sucrose and water in glass tubes plugged with cotton wool and held at a temperature of 25°C. When exposed to a gas mixture consisting of 90 per cent carbon monoxide and 10 per cent oxygen the flies were unable to fly but were not completely immobilized. Separate experiments had shown that after being exposed to this gas mixture for 16 h flies recovered completely when the gas mixture was replaced by air. In a gas mixture consisting of 20 per cent oxygen, 40 per cent carbon monoxide and 40 per cent nitrogen flies, behaved in a normal manner for at least 48 h.

At appropriate times after treatment with aldrin the flies were removed from the cylinders and immediately rinsed with two 3 ml. volumes of acetone in order to remove external aldrin. They were then homogenized and extracted twice, first with 4 ml. and then with 3 ml. of acetone. Four ml. of water and sodium sulphate was added to the acetone extract which was then extracted with two 4 ml. volumes of petroleum ether. The aldrin

and dieldrin in the petroleum ether extract was assayed by gas-liquid chromatography. Flies treated with aldrin and confined in cylinders containing either air or a mixture of 10 per cent oxygen and 90 per cent nitrogen were used as controls.

The amount of dieldrin, expressed as a percentage of the total insecticide (aldrin plus dieldrin), found inside the flies is shown in Fig. 1. In flies exposed to the gas mixture containing 40 per cent carbon monoxide, 20 per cent oxygen and 40 per cent nitrogen, the rate of formation of dieldrin is markedly lower than in flies kept in air. The rate of dieldrin formation is even lower when the carbon monoxide: oxygen ratio is increased to 9:1. This is probably due in part to lack of oxygen because the rate of dieldrin formation in the controls kept in 90 per cent nitrogen and 10 per cent oxygen is less than in those kept in air.

Similar experiments were carried out with larvae aged 4 days from the same strain of flies. The larvae were treated individually with 2  $\mu$ g of aldrin in 2  $\mu$ l. acetone before being exposed to the appropriate gas mixture and were rinsed and extracted as already described for the adult flies. The dieldrin in the larval extract, again expressed as a percentage of the internal aldrin plus dieldrin, is shown in Fig. 2. Here again the rate of dieldrin formation is reduced in insects which are exposed to gas mixtures containing carbon monoxide. This reduction cannot be attributed to a lack of aldrin, for in none of the experiments with carbon monoxide was the concentration of aldrin within the insects less than that in the controls.

Although the epoxidation of aldrin probably results in an increase in toxicity, the oxidative metabolism of many insecticides results in detoxication. The toxicity of two such compounds to the housefly *Musca domestica* has been estimated in air and in a gas mixture consisting of 40 per cent carbon monoxide, 20 per cent oxygen and 40 per cent nitrogen. The insecticides chosen were carbaryl (1-naphthyl *N*-methylcarbamate) and a cyclodiene epoxide (1,2,3,4,9,9-hexachloro-1,4,4a,5,6,7,8,8a-octahydro-5,6-epoxy-1,4-methanonaphthalene) supplied by Dr G. T. Brooks. The toxicity of both compounds to this strain of fly is known to be enhanced by the methylenedioxyphenyl synergist sesamex<sup>6,7</sup>. Furthermore, the metabolism of both compounds by microsomes is inhibited by carbon monoxide (unpublished work of S. E. Lewis and of R. J. Kuhr).

Female houseflies, 4 days old, were treated individually with the insecticide in 2  $\mu$ l. of acetone before being confined in groups of twenty in cylinders containing either air or the 40 per cent carbon monoxide gas mixture. The flies were supplied with sucrose and water as described earlier

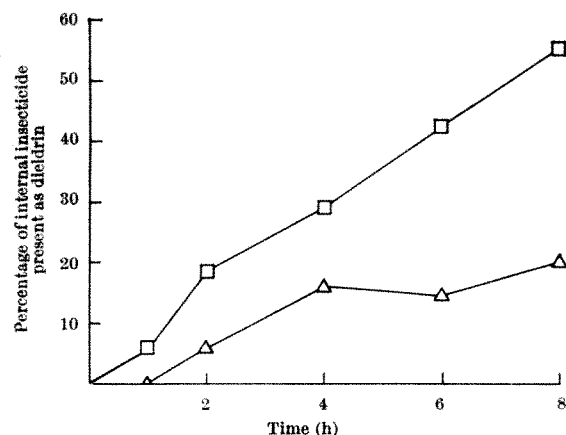


Fig. 2. The effect of carbon monoxide on formation of dieldrin in housefly larvae treated with aldrin.  $\square$ , Larvae kept in 90 per cent nitrogen, 10 per cent oxygen;  $\triangle$ , larvae kept in 90 per cent carbon monoxide, 10 per cent oxygen.

and held at a temperature of 25° C. The mortality was assessed on a count of dead flies made 24 h after treatment with various concentrations of the insecticides, and the  $LD_{50}$  estimated. When flies were treated with the cyclodiene epoxide and kept in air the  $LD_{50}$  was 2  $\mu$ g/fly—a value similar to that previously reported<sup>7</sup>; in the carbon monoxide mixture this was reduced to 0.5  $\mu$ g/fly. With carbaryl the  $LD_{50}$  for flies kept in air could not be estimated but exceeded 30  $\mu$ g/fly; in the carbon monoxide gas mixture the  $LD_{50}$  was reduced to 2  $\mu$ g/fly.

These data indicate that a component which is sensitive to carbon monoxide is involved in the metabolism of some insecticides *in vivo* and it can be argued from experiments *in vitro* (ref. 3 and unpublished results of Lewis and Kuhr) that this component is probably the microsomal cytochrome P-450.

I thank Miss C. A. Greenfield for technical assistance.  
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### Electrophoretic Polarity exhibited by Amoeboid Cells of *Naegleria gruberi*

*Naegleria gruberi* is a small ( $\sim 15\mu$  diameter) free living cell which is able to transform reversibly from the amoeboid to the flagellate state<sup>1</sup>. The amoebae move by forming one or more lobose pseudopodia, any one of which may be considered to define a functional anterior end. It was observed, quite unexpectedly, in the course of electrophoretic characterization of the cell surface, that individual freely suspended amoebae manifest a transient local difference in mobility during pseudopodal protrusions, the anterior pseudopodal end having a higher negative mobility. While this suggests that the advancing pseudopod possesses a higher negative zeta potential, and thus higher negative surface charge density than the rest of the cell, it is open to alternative interpretations.

Cells were cultured on agar slopes, together with nutrient bacteria, and collected in 0.001 molar phosphate buffer, pH 6.8. Bacteria were separated by centrifugation from 10 ml. of 0.001 molar buffer, repeated three times. Cells were resuspended in the same buffer for mobility measurements. Earlier electrophoretic measurements were made in a cylindrical cell apparatus of the type described by Bangham *et al.*<sup>2</sup>, but most were performed in a rectangular cell apparatus fitted with a return tube as described by Lukiewicz and Korohoda<sup>3</sup>. In this apparatus a single cell may be kept under continuous observation for long periods of time, and electrophoretic mobility measurements made at desired intervals. Similar values were obtained with both instruments.

From electrophoretic measurements made at 20° C in 0.001 molar phosphate buffer, the mean mobility of amoeboid cells was calculated to be  $2.11 \pm 0.13 \mu/\text{sec/V/cm}$ . At this temperature the cells are active, frequently throwing out pseudopodia without net locomotion, while freely suspended. During mobility measurements a striking pattern of cellular behaviour was repeatedly observed: on applying the electrophoretic potential, a cell in the process of pseudopodal protrusion would be seen to rotate and become aligned in the external field, and this would result in migration of the cell under the

influence of the applied potential towards the anode, pseudopod foremost. On reversing the field (6 V/cm) the amoeba would, in about 5 sec, re-orientate by rotating through  $180^\circ$  (Fig. 1). Similar behaviour was also observed in pre-aggregation cells of the slime mould *Dictyostelium discoideum* Raper. Cellular polarity has previously been described by Korohoda<sup>4</sup>, who found an identical orientation in amoeboid cells of the slime mould *Physarum nudum* MacBride, the pseudopodal tip once again showing a higher negative mobility.

The different charge densities at anterior and posterior ends of *Naegleria gruberi* amoebae, which could account for the observed differential mobility, may be estimated very roughly by measuring the separate velocities, in the direction of the field, of front and rear extremities as the cell undergoes  $180^\circ$  rotation in a field of known strength.

During re-orientation, the mean distance travelled by the centre of a cell is, from mobility data, approximately  $63\mu$ , assuming a mean cellular diameter of  $15\mu$ . Thus in the course of re-orientation the back of the cell has moved  $(63 - 15) = 48\mu$  and the front has moved  $(63 + 15) = 78\mu$ , in the direction of the field. As a first approximation therefore the difference in mobilities of the front and back of a cell putting out a pseudopod could be accounted for by a charge difference in the ratio  $78 : 48$  or  $1.6 : 1$ .

In this simple treatment, the front and back ends of the cell are considered as two completely independent bodies the mobilities of which are determined by their separate charge densities. This analogy suffers from excessive simplicity. If one imagines a model dumb-bell with joined, rather than separate, spheres, where each sphere carries a different charge density, the slower sphere of lower charge density will tend to retard the faster, and vice versa, resulting in an underestimation of the difference in mobilities calculated on the assumption that the spheres have no mechanical influence on each other.

The possibility that orientation in the direction of motion could be merely a hydrodynamic effect due to cellular asymmetry was investigated and shown to be unimportant. Cells were suitably orientated by adjustment of the field polarity in the vertical apparatus, and then allowed to sediment under gravity alone, pseudopod downwards or pseudopod upwards. It was found that the cells sedimented, regardless of their initial orientation, without change in orientation.

Another interpretation of the reversal phenomenon which is much less simple to investigate stems from the following considerations. If the rear end has an effective radius of curvature of rather less than  $1\mu$  (due to surface undulations, for example), while the anterior pseudopodal surface has an effective radius of curvature of more than  $1\mu$ , then the zeta potential and hence the mobility would be different at either end. It is possible to calculate the true surface charge taking these factors into account. It can be shown that if the radius of curvature of the rear of the cell is less than  $400 \text{ \AA}$ , while that of the front is effectively infinitely large ( $1.0\mu$ ), the ratio of mobilities at the front and back of the cell would be  $1.6 : 1.0$ . Published electron micrographs<sup>5</sup> of *Naegleria gruberi* amoebae fixed while attached and moving, however, do not show any significant local differences in surface curvature. The smallest radii of curvature are of the order of  $0.5\mu$ , easily resolved at a magnification of  $2.7 \times 10^4$ , and these are not

restricted to any particular region, so it can be concluded that there is no evidence for believing that the observed mobility differences stem from curvature differences rather than localized surface charge differences.

Increase in local negative surface charge density during pseudopodal protrusion may provide a clue to the physiological basis of behavioural polarity in moving cells. This is particularly interesting, because changes in cell membrane surface potential can lead to cytoplasmic motile responses (ref. 6 and our unpublished work).

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### Cyclic Diquaternary Salts of 1,10-Phenanthroline as One Electron Transfer Agents related to Bipyridylum Herbicides

CERTAIN diquaternary salts of 2,2'- and 4,4'-bipyridyls are herbicides<sup>1</sup>. Their mode of action is thought<sup>2</sup> to be connected with their ability to be reduced to a stable radical cation, at a potential ( $E_0$ ) of about  $-0.35$  to  $-0.45$  V, by a one electron transfer which is reversed by oxygen. Diquaternary salts of 1,10- and 3,8-phenanthroline, where the nitrogen atoms are in the same relative positions as in 2,2'- and 4,4'-bipyridyls, have not so far been investigated as herbicides although it seems reasonable to expect that they too would give stable radical cations on reduction with one electron reducing agents. This communication reports results with diquaternary salts of 1,10-phenanthroline.

Before this investigation, no diquaternary salts of 1,10-phenanthroline had been reported. 1,10-Phenanthroline reacted readily, however, with boiling ethylene dibromide to give 5,6-dihydropyrazino(1,2,3,4-*l,m,n*)-1,10-phenanthroline dibromide; (I;  $n=2$ ) (ref. 3). It reacted similarly with 1,3-dibromopropane to afford 5*H*-6,7-dihydro(1,4)-diazepino(1,2,3,4-*l,m,n*)-1,10-phenanthroline dibromide (I;  $n=3$ ), which crystallized from aqueous ethanol as pale yellow crystals, melting point  $286^\circ \text{C}$  with decomposition. The structure of (I;  $n=3$ ) was confirmed by elemental analyses (all bromine ionic) and by the nuclear magnetic resonance spectrum in deuterium oxide with sodium 3-trimethylsilyl-1-propane-sulphonate as internal reference. The nuclear magnetic resonance spectrum consisted of a multiplet at  $\delta=8.55$ - $9.85$  p.p.m. (eight aromatic protons), a triplet centred at  $\delta=5.3$  due to the four methylene protons adjacent to the quaternary nitrogen atoms and a multiplet at  $\delta=3.55$  assigned to the two central methylene protons. As expected for derivatives of the rigid planar phenanthroline

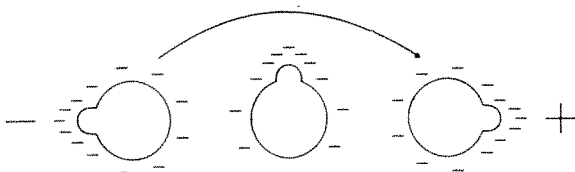
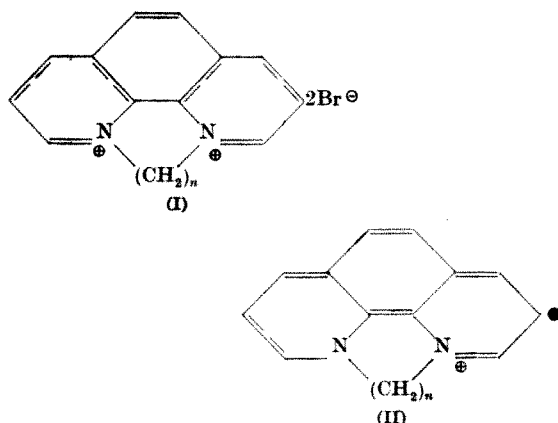


Fig. 1. Re-orientation of *Naegleria gruberi* amoeba on reversing polarity of the electrophoretic field.



ring system, the ultra-violet spectra (Table 1) of the two diquaternary salts were almost identical (compare ref. 2).

The diquaternary salts each gave a deep red solution in water on treatment with zinc dust, the coloration being attributed to the radical cation of which (II;  $n=2$  or 3) is one of the many canonical forms. The presence of a high concentration of a stable radical was confirmed in each case by the observation that the nuclear magnetic resonance spectrum of the intensely coloured solution was of low intensity and was poorly resolved. When the reducing agent was removed and the solution was shaken in air, the deep colour discharged and the nuclear magnetic resonance spectrum of the compound (I;  $n=2$  or 3) returned, thus demonstrating the reversibility of the one electron transfer. On polarographic examination in 0.067 molar Sorensen phosphate buffers of pH 5.9, 6.8 and 8.04, compounds (I;  $n=2$ ) and (I;  $n=3$ ), at concentrations between 0.001 molar and 0.0015 molar, each gave a typical symmetrical one electron reduction wave with half-wave potentials of  $-0.53$  and  $-0.52$  V respectively, against a standard calomel electrode, independent of pH and concentration. The corresponding values of  $E_0$  are  $-0.28$  and  $-0.27$  V. Diquat dibromide was included in the experiments for comparison and gave, as before<sup>4</sup>, a value of  $E_0$  of  $-0.36$  V. The polarograms of the diquaternary salts of 1,10-phenanthroline did not reveal a clear-cut second reduction step as is observed with diquat<sup>4</sup> and paraquat<sup>5</sup>, the current gradually increasing as the applied voltage decreased. It is evident from these results that the diquaternary salts (I;  $n=2$  and 3) are reduced to radical cations more easily than diquat and paraquat and that the one electron transfer is largely reversed by atmospheric oxidation.

In herbicidal tests, the diquaternary salts of 1,10-phenanthroline at a high concentration desiccated green plant tissue in a manner typical of bipyridylum herbicides<sup>1</sup> although at practical rates of application (Table 2) they were clearly much inferior to diquat.

Table 1. ULTRA-VIOLET ABSORPTION SPECTRA OF DIQUATERNARY SALTS OF 1,10-PHENANTHROLINE

Compound	$\lambda_{\max}$ (m $\mu$ )	log $\epsilon$
(I; $n=2$ )	226, 279, 295, 308, 318	4.32†, 4.61, 3.81, 3.87, 3.78
(I; $n=3$ )	226*, 281, 298, 311, 322*	4.32, 4.59, 3.76, 3.83, 3.70

\* Shoulder.

† Extinction coefficients reported previously (ref. 3) were approximate.

Table 2. GREENHOUSE POST-EMERGENT HERBICIDAL TESTS OF DIQUATERNARY SALTS OF 1,10-PHENANTHROLINE

Compound	Rate (lb./acre)	Lin-seed	Herbicide evaluation				
			Buck-wheat	Mustard	Peas	Sugar beet	Barley
(I; $n=2$ )	8	0	0	1	1	10	0
	4	0	0	0	0	5	0
(I; $n=3$ )	8	1	2	3	1	10	1
	4	1	1	2	0	9	0
Diquat dibromide	4	10	10	10	10	10	10

Compounds were applied as foliar sprays. Herbicidal effects were estimated visually after 7 days on the scale, 10 = complete kill, 0 = no effect.

The discovery of herbicidal activity, although of a relatively low order, in diquaternary salts of 1,10-phenanthroline demonstrates that the toxic action of the diquat and paraquat type is not confined to diquaternary salts of bipyridyls as has been supposed<sup>2</sup>. It also strongly supports the view that the herbicidal action of the bipyridyls is associated with their reversible one electron transfer properties<sup>1,2,6</sup>. The reasons for the low activity of the phenanthroline diquaternary salts are probably connected with their relatively large values of  $E_0$  which presumably lie just outside the potential range of the biochemical electron transfer processes with which the bipyridylum herbicides are thought to interfere.

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### Phytochrome in Red Alga, *Porphyra tenera*

THE marked stimulation of spore formation in the conchocelis phase of *Porphyra tenera* Kjellm. by short day (SD) conditions was first reported by Kurogi<sup>1</sup>. The ability of a short light interruption in the middle of a long dark period to simulate long day (LD) conditions has recently been shown, and it was therefore concluded that the response was a "genuine" photoperiodic response of the type common among flowering plants<sup>2</sup>. A rough action spectrum for the inhibition of the reproductive response by short light breaks has been determined, in an attempt to identify the pigment which mediates this photoperiodic response.

Culture methods for the conchocelis, and the techniques used for estimating growth and reproduction, have already been described<sup>2</sup>. The apparatus used for the irradiation of cultures with monochromatic light was similar to that described by Wilson and Schwabe<sup>3</sup> for similar work with *Lunularia*. A set of five interference filters was used, with peak transmissions at the wavelengths shown in Table 1 and an average waveband at 1 per cent transmission of about 70 m $\mu$ . The incident energy at each waveband was adjusted to about 90 ergs/cm<sup>2</sup>/sec.

All cultures received a main light period of 8 h of white light/day in a culture tank at 20° C. Nine different light break treatments were given at about the middle of the dark period, two cultures being used for each treatment. Five principal treatments consisted of a 60 min exposure to each of the five wavebands, and 30 min exposures to the red and far-red wavebands were also given. Two further treatments received 30 min of red followed immediately by 30 min of far-red, and 30 min of far-red followed by 30 min of red. Control cultures received no light break. The light break treatments were continued for eight

Table 1. SPORANGIA PRODUCTION IN RESPONSE TO LIGHT BREAKS OF DIFFERENT WAVEBANDS

Interference filters	Peak transmission (m $\mu$ )	Sporangial branches/0.01 ml. conchocelis			
		30 min treatments	60 min treatments	Mean	S.D.
Colour		Mean	S.D.	Mean	S.D.
Blue	446	—	—	4,753	669
Green	501	—	—	4,640	430
Yellow	564	—	—	3,900	550
Red	662.5	2,860	393	2,171	307
Far-red	737.5	4,304	930	4,496	507
Red/far-red		4,461	394	—	—
Far-red/red		2,746	357	—	—
SD Control		4,092	296	—	—

Each value represents the mean of five samples from each of two replicate cultures.



cycles (which is known to be sufficient for full induction<sup>3</sup>) and all cultures were then returned to LD conditions. Sporangia production was estimated 25 days after the beginning of SD treatment. The results are shown in Table 1.

It is evident that red was the only waveband which was effective as a light break, and that the inhibition of sporangia production caused by 60 min of red light was roughly twice that caused by 30 min of red. Far-red light was completely ineffective, even if irradiation was continued for 60 min. Far-red periods of 30 min, however, completely reversed the inhibitory effects of a preceding period of 30 min of red although it had no influence on subsequent red irradiation. Analysis of variance of the results showed that the overall variation caused by the treatments was significant at 0.1 per cent ( $F = 38.766$ , d.f. 9, 90), and that most of this variation could be attributed to the effects of red light. If the treatment mean square was partitioned between those treatments which received red, alone or after far-red, and all other treatments, the  $F$  values obtained were 103.150 (d.f. 3, 90) for the red treatments and 6.574 (d.f. 6, 90) for the others.

The photoreceptor pigment for both SD and LD photoperiodic responses in flowering plants has been shown to be the photomorphogenic pigment; phytochrome and phytochrome mediated responses have been found to exhibit a characteristic action spectrum in which the effects of red light are reversed by far-red light<sup>4</sup>. Attention was therefore concentrated on these regions of the spectrum and interference filters were selected to coincide with the absorption maxima of the two forms of phytochrome. The action spectrum which has been obtained for the SD photoperiodic response of *Porphyra* shows a peak in the red region of the spectrum, and the effects of red are completely reversed by subsequent far-red treatment. These results thus show clearly that phytochrome is involved in this response and that this pigment occurs in *Porphyra*.

There have been several previous demonstrations of phytochrome in lower plants such as mosses<sup>5</sup>, a liverwort<sup>3</sup> and two green algae<sup>6,7</sup>, but so far it has only been demonstrated in plants in which the green photosynthetic pigments predominate. This has led to the suggestion that phytochrome may be in the "evolutionary line in which chlorophylls A and B are the only light receptors in photosynthesis"<sup>8</sup>. This hypothesis must be discarded in the light of the present results. Because the presence of phytochrome can be demonstrated in an algal group the pigment composition of which differs so significantly from that of the Chlorophyta and the higher plants, it is tempting to speculate that it will also be found in other algal groups. Recent work on the structure of the chromophore of phytochrome<sup>9</sup> has revealed its similarity to the chromophores of the biliproteins, *O*-phycoerythrin and allophycoerythrin, both of which have been isolated from *Porphyra tenera*<sup>10</sup>. This similarity indicates that the Cyanophyta may well contain phytochrome, and that this pigment may therefore be basic to all photosynthetic organisms.

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## Enzymes of the Ornithine-Urea Cycle in the Chimaera *Hydrolagus coliei*

THE chimaera *Hydrolagus coliei*, commonly known as the ratfish, is one of the few remaining members of the sub-class Holocephali. The chimaeras are the only survivors of a large and diverse Palaeozoic group, the bradyodonts<sup>1</sup>. The bradyodonts and elasmobranchs appear in the late Devonian as fossils of two apparently distinct groups<sup>1</sup>. Elasmobranchs and chimaeras share the ability to maintain large tissue concentrations of urea which serves an osmoregulatory function<sup>2</sup>. All the enzymes of the ornithine-urea cycle have been found in the elasmobranchs<sup>3-7</sup>, and in this group the ornithine-urea cycle is thought to be the most important pathway for the formation of urea<sup>8</sup>. The chimaeras have not been studied for the occurrence of any of the ornithine-urea cycle enzymes except arginase, which was found in both liver and kidney of *Hydrolagus coliei*<sup>9</sup>. The presence of arginase is insufficient evidence for the occurrence of the remaining enzymes of the cycle because it occurs in groups which apparently lack a complete ornithine-urea cycle<sup>10</sup>. The present investigation was undertaken to find out whether all the enzymes of the ornithine-urea cycle are present in the liver of the ratfish *Hydrolagus coliei*.

Ratfish were obtained by otter trawl from the waters of the San Juan Archipelago, Washington, and kept in large tanks of seawater until used. The methods for assaying the enzymes were those of Ratner<sup>11</sup> for argininosuccinate synthetase, and of Brown and Cohen<sup>12</sup> for the remaining four enzymes. Modifications of these methods are necessary for the ratfish because the large tissue concentrations of urea interfere with citrulline determination<sup>13</sup>. When the assay depended on measuring either the formation or disappearance of citrulline (in those assays for carbamoyl phosphate synthetase, ornithine carbamoyltransferase and argininosuccinate synthetase), the homogenized liver was treated with urease to break down urea already present. For the remaining two enzymes, argininosuccinate lyase and arginase, urea was measured both before and after incubation, and the difference in the two values was taken as a measure of the amount of urea formed.

Liver was homogenized 1:9 with ice cold water in a glass homogenizer with a 'Teflon' pestle. The homogenate was centrifuged at 650g for 5 min at 0°-4° C. Centrifugation in the cold caused a fairly good separation of the fat present; this could then be removed by aspiration. The remaining homogenate was either diluted or used directly for the various assays.

The occurrence of the enzymes of the ornithine-urea cycle was established in several ways. The enzymes were shown to be dependent on the same substrates and co-factors known to be required for full enzyme activity in elasmobranchs and other vertebrates. There was, for example, negligible activity of carbamoyl phosphate synthetase in the absence of a metal ion activator (manganese or magnesium ions). Also, a linear increase in the activity of each of the enzymes was found with both increased protein concentration and increased incubation time within certain experimental limits. Finally, boiled homogenate used in control determinations gave little or no activity.

Comparison of the concentrations found for the ornithine-urea cycle enzymes indicates that argininosuccinate synthetase, with an activity of  $0.235 \pm 0.088$   $\mu$ moles/mg of protein/h (mean value  $\pm$  mean deviation), is the rate limiting enzyme of the ornithine-urea cycle in the ratfish. This same pattern has usually been found in other vertebrates including the elasmobranch *Squalus acanthias* (my unpublished work). Ratfish arginase activity ( $38.7 \pm 10.4$   $\mu$ moles/mg of protein/h) was less than in most other vertebrates that have been studied<sup>7,10,14</sup>. In contrast, ornithine carbamoyltransferase activity ( $161 \pm 36$   $\mu$ moles/mg of protein/h) was at about the same level that it is in

many other vertebrates including man. The levels of activity of carbamoyl phosphate synthetase and argininosuccinate lyase were  $0.82 \pm 0.11$  and  $3.77 \pm 0.44$   $\mu$ moles/mg protein/h, respectively.

The presence of the ornithine-urea cycle in both elasmobranchs and chimaeras, and the osmotic importance of urea in these two groups indicate that their ancestors lived in a marine environment at the time when they diverged. A comparative study of these lower vertebrates may give insight into the primitive characteristics of the ornithine-urea cycle.

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## PSYCHOLOGY

### Spatial Orientation in the Blind

LITTLE is known directly about the ability of congenitally blind subjects to orient themselves in an unfamiliar environment. Senden<sup>1</sup> went to considerable lengths to document the apparently inherent and unsurmountable difficulties arising from an alleged absence of a "sense of space". It is clear, however, that the cases assembled by him did not as a whole show degrees of mobility which are more commonplace today. Having worked with a wide range of blind subjects during the past 5 yr we have become convinced that whatever difficulties congenitally blind subjects may experience in problems of spatial orientation are more likely to be caused by lack of experience than by blindness as such.

One test of spatial orientation is to ask subjects to move through an unfamiliar environment by means of a map. Tactual maps in particular have been employed in clinical tests for spatial orientation during the past decade (page 270 of ref. 2 gives a summary). We have been carrying out work with a range of "maps" for blind people during the past 18 months. We have already described these maps and some earlier work<sup>3</sup>, and a full report of our recent work will be published later. Here we wish to draw attention to one finding only.

Blind boys were asked to follow a route indicated on a tactual map and were required to solve two detour problems towards the end of their route. Of six boys (aged 14-15) who were either totally blind or had perception of light only, five were effectively blind from birth. They had been trained in the use of maps by one of their masters in accordance with a syllabus suggested by us. Fig. 1

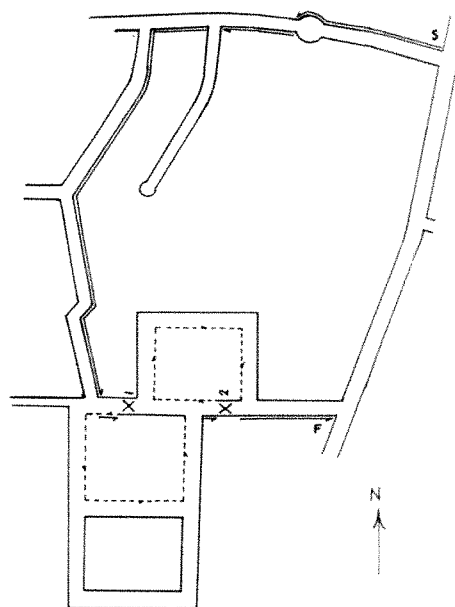


Fig. 1. The route started at S and subjects were asked to walk as shown by the double line in the direction of the half-arrows. The dotted lines mark the blocks around which detours had to be made from detour points 1 and 2 respectively. Scale, 6 in. = 1 mile.

shows the route to be followed. It was indicated on the boys' map by small pins fitted almost flush at key points. Each boy was shown the map a few minutes before setting out and asked to trace the map with his fingers to the satisfaction of the experimenter. He then set off carrying his map, until he came to detour point 1 when the experimenter explained that "the road was up" and that a detour was required. The same drill was followed at detour point 2. At each detour point subjects were first asked to solve the problem on their map before having to carry out the appropriate manoeuvre. Subjects' performance was timed over various sections of the route and errors of mobility and/or map reading were noted.

A map reading error was a deviation from the prescribed route whether this was subsequently self-corrected or not. For each detour problem subjects were given one point for solving it unaided, half a point if the experimenter had to explain the principle involved, and no point if the experimenter had to explain the specific solution.

Table 1 shows the results. It is interesting that three other boys with relatively high degrees of residual vision took between 17 and 21 min for the same task. The errors made by subjects 3 and 6 were subsequently self-corrected. All but one of the subjects managed to solve the second detour problem without help from the experimenters.

To the extent that following a route from a tactual map and solving a detour problem is accepted as evidence of the ability to orientate oneself in space, we may claim to have demonstrated this ability with congenitally blind subjects. It is hoped that use of maps for the blind will not only serve a practical purpose but also contribute to a better understanding of the manner in which those born blind handle problems of spatial orientation. It should not be too difficult to design more complex problems requiring the use of dimensional in-

Table 1. SCORES FOR SIX BLIND GRAMMAR SCHOOL BOYS

Subject	Time (min)	Map errors	Detour		Onset of blindness
			1	2	
1	47	3	0	1	Birth
2	38	3	0	1	6 yr
3	35	1	1	1	Birth
4	25	2	1	1	Birth
5	32	0	1	1	3 months
6	43	1	1	1	Birth

formation; for example, choosing the shorter of two detours.

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## SOIL SCIENCE

### Nature and Distribution in Irish Soils of a New Soil Pigment

THE isolation of a green pigment (green humic acid) probably related to perylenequinone from widely distributed soils, chiefly podzols, has been described<sup>1</sup>. A pigment of the hydroxyanthraquinone type has now been found in a large number of Irish soils. Although hydroxyanthraquinones occur widely in plant roots and fungi<sup>2</sup> we have been unable to find any previous account of their isolation from soil.

The pigment may be readily obtained from dried soil (a) by Soxhlet extraction with chloroform or (b), more specifically, by extraction into the upper (acetone) layer of a 1:1 mixture of acetone and 2 normal aqueous sodium hydroxide. Pigment was isolated from *B<sub>h</sub>* horizon of the screen (Co. Wexford) podzol (which also contains green humic acid) by method (b); it was precipitated by acidification and purified by extraction into chloroform from which it crystallized spontaneously. A sample of 13 kg of soil gave 170 mg of crystalline (by X-ray diffraction pattern) pigment, melting point  $>360^{\circ}\text{C}$ . It was soluble in alkaline acetone, concentrated sulphuric acid (purple solutions) and chloroform (yellow solution) but was insoluble in water, aqueous sodium hydroxide, dilute sulphuric acid, acetone, ethanol and other common solvents.

The pigment appeared homogeneous when eluted from columns of magnesium carbonate, silica gel or 'Sephadex LH-20'. Its colour in aqueous alkali and concentrated sulphuric acid suggested that it was a quinone; its ultra-violet and visible absorption spectra (Fig. 1) suggested that it was a hydroxyanthraquinone<sup>3</sup>. Reduction with

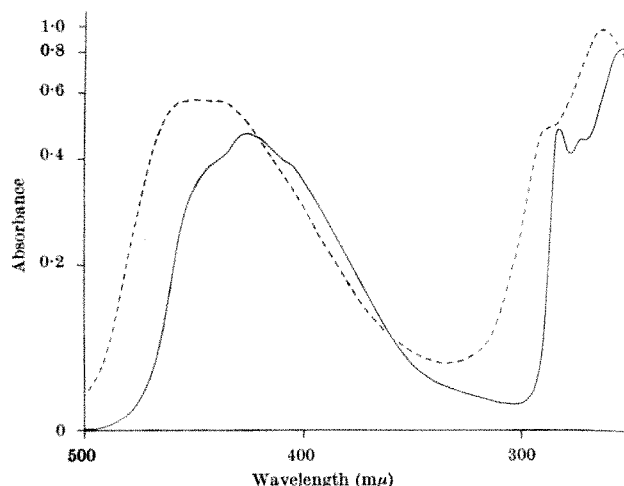


Fig. 1. Spectra in chloroform of soil pigment (—) and of 1,8-dihydroxyanthraquinone (---), the latter being almost identical with that of 1,8-dihydroxy-3-methylanthraquinone.

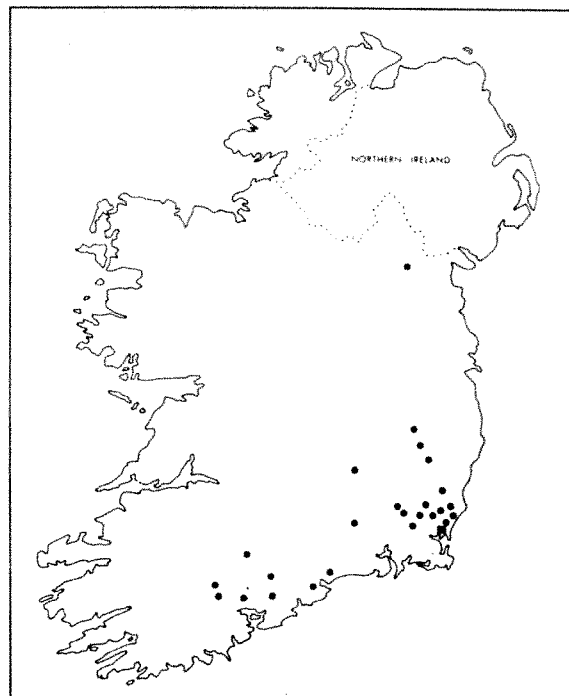


Fig. 2. Location of soils containing pigment. Approximately one in eight or twenty-four in all of the soils from the south-south-east showed pigment compared with one in more than 450 from the rest of the country. ■, Indicates screen.

zinc dust gave material with an ultra-violet spectrum similar to that shown by anthracene. Prolonged treatment in a sealed tube with hot aqueous alkaline dithionite gave, in addition to unchanged starting material, a small amount of pigment with an ultra-violet and visible spectrum identical with that of 1,8-dihydroxy-3-methylanthraquinone. The relationship of the soil pigment to 1,8-dihydroxyanthraquinone was further indicated by the similarity of the infra-red spectra in liquid paraffin of the two pigments especially in the characteristic 1,600–1,700  $\text{cm}^{-1}$  region<sup>4</sup>, both having absorption bands at 1,672 and 1,624  $\text{cm}^{-1}$ . The unknown pigment is not 1,8-dihydroxy-3-methylanthraquinone nor does it seem to be any polyhydroxyanthraquinone reported in the literature<sup>5</sup>.

Many soil samples (678) from all parts of the Republic of Ireland have been examined for pigment. These were available from a previous survey<sup>6</sup> and had been randomly selected with two sites/10  $\text{km}^2$  of the national grid. The top 4 in. of soils under permanent pasture only were taken, bog, forest and mountain areas being excluded. Soils giving a positive test were taken from the locations shown in Fig. 2. With one exception they were all in the south and south-east of the country (Fig. 2). In view of the known root growth stimulating properties of some hydroxyanthraquinones<sup>6</sup> it may be significant that the principal tillage areas are in this region.

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## APPLIED SCIENCE

## Flow of Gases in Porous Solids

ONE of us has recently developed approximate equations for the flow of gases through porous media. The model considers a binary mixture of gases *A* and *B* flowing through a porous medium.

The model is developed for a single capillary by assuming that  $n_A$ , the molar flux through the capillary of component *A* and  $n_B$ , the molar flux of *B*, are each the sum of three terms: a diffusion flux which results from the statistical movement of the molecules under the partial pressure gradient; a viscous flux which results from the general motion of the gas induced by the total pressure gradient; and a net drift flux which arises from momentum transfer requirements within the capillary tube.

The model (though developed independently) is thus similar in form to that recently reported by Wakao, Otani and Smith<sup>1</sup>. The development is, however, somewhat different (particularly with respect to the net drift term) and the method of application to porous media is by using overall constants for the media rather than by considering individual pores.

For the case of flow in a straight capillary, the equations are

$$n_A = A(p_A, p_B) \frac{dp_A}{dZ} + B(p_A, p_B) \frac{dp_B}{dZ} \quad (1)$$

$$n_B = C(p_A, p_B) \frac{dp_A}{dZ} + D(p_A, p_B) \frac{dp_B}{dZ} \quad (2)$$

where  $p_A$  and  $p_B$  are the partial pressures of *A* and *B* respectively,  $Z$  the flow path length and

$$A = - \frac{p_A}{RT} \left[ \frac{\beta}{\mu} + \frac{D_{EA}}{p_A} + \frac{\alpha \bar{V}_A \bar{V}_B - D_{EA} \Gamma_A \bar{V}_B}{\bar{V}_B p_A + \bar{V}_A p_B} \right] \quad (3)$$

$$B = - \frac{p_A}{RT} \left[ \frac{\beta}{\mu} + \frac{\alpha \bar{V}_A \bar{V}_B - D_{EB} \Gamma_B \bar{V}_A}{\bar{V}_B p_A + \bar{V}_A p_B} \right] \quad (4)$$

$$C = - \frac{p_B}{RT} \left[ \frac{\beta}{\mu} + \frac{\alpha \bar{V}_A \bar{V}_B - D_{EA} \Gamma_A \bar{V}_B}{\bar{V}_B p_A + \bar{V}_A p_B} \right] \quad (5)$$

$$D = - \frac{p_B}{RT} \left[ \frac{\beta}{\mu} + \frac{D_{EB}}{p_B} + \frac{\alpha \bar{V}_A \bar{V}_B - D_{EB} \Gamma_B \bar{V}_B}{\bar{V}_B p_A + \bar{V}_A p_B} \right] \quad (6)$$

where  $\mu$  is the gas viscosity,  $\bar{V}_A$  and  $\bar{V}_B$  the mean molecular velocities of gases *A* and *B*,  $\alpha = \pi d/16$  and  $\beta = d^2/32$ , where  $d$  is the diameter of the capillary. It is assumed that the effective diffusion coefficients for the two components are given, respectively, by the Bosanquet<sup>2</sup> interpolation relationship as

$$\frac{1}{D_{EA}} = \frac{1}{D_{AB}} + \frac{1}{D_{KA}} \quad (7)$$

$$\frac{1}{D_{EB}} = \frac{1}{D_{AB}} + \frac{1}{D_{KB}} \quad (8)$$

where  $D_{AB}$  is the binary free gas diffusion coefficient and, for a capillary,  $D_{KA} = 1/3 d \bar{V}_A$  and  $D_{KB} = 1/3 d \bar{V}_B$ . The terms  $\Gamma_A$  and  $\Gamma_B$  are introduced to allow correct calculation of the rate of momentum transfer to the wall arising from the diffusion flux. For pure intermolecular diffusion  $\Gamma = 1$ , and for pure Knudsen diffusion  $\Gamma = 3\pi/16$ . For porous media we can substitute into these equations  $B_0 \equiv \beta$  and  $4/3 K_0 \equiv \alpha$ , and  $m D_{AB} \equiv D_{AB}$ , where  $B_0$  and  $K_0$  are determined in the usual way from permeability measurements;  $m$  is also a constant for the medium. Some difficulty is found in calculating  $D_{KA}$  and  $D_{KB}$  but it seems that, to a first approximation, these could be taken as equal to  $4/3 K_0 \bar{V}_A$  and  $4/3 K_0 \bar{V}_B$ , respectively. This assumption implies that the permeability plot shows no minimum value as is found in the case of straight capillaries. For consistency with

this implication,  $\Gamma$  should be taken as unity for porous media.

It should be noted that there is now considerable experimental evidence for deviation from the straight line form for the permeability plot at low pressure, implying that, even for porous media,  $D_K > 4/3 K_0 \bar{V}$  and as  $p \rightarrow 0$ ,  $\Gamma > 1$ . For simplicity, however, it has been assumed that  $\Gamma = 1$  and  $D_K = 4/3 K_0 \bar{V}$  in the calculation described below.

In cylindrical geometries equations (1) and (2) are readily transposed into cylindrical co-ordinates as

$$n_A = A(p_A, p_B) \frac{dp_A}{dr} + B(p_A, p_B) \frac{dp_B}{dr} \quad (1a)$$

$$n_B = C(p_A, p_B) \frac{dp_A}{dr} + D(p_A, p_B) \frac{dp_B}{dr} \quad (2a)$$

where the fluxes  $n_A$  and  $n_B$  are given by

$$n_A = \frac{N_A}{2\pi r} \quad (9)$$

$$\text{and } n_B = \frac{N_B}{2\pi r} \quad (10)$$

where  $N_A$  and  $N_B$  are the molecular rates of flow of *A* and *B* per unit axial length of cylinder (moles/sec cm) and  $r$  the radius.

In the special case of zero total pressure difference across the porous sample, it is found that the flux ratio is related to the component gases' molecular weights by

$$\frac{n_A}{n_B} = \sqrt{\frac{M_B}{M_A}} \quad (11)$$

Using this relationship and noting that at zero pressure difference the viscous flow component is zero, the following result is obtained

$$N_A = - \frac{D_{EA} P}{RT} \frac{2\pi}{\ln \frac{r_2}{r_1}} \frac{1}{b^2} \left\{ (b-a) \ln \frac{1+bX_{A_2}}{1+bX_{A_1}} + ab(X_{A_2} - X_{A_1}) \right\} \quad (12)$$

where  $b = \sqrt{\frac{M_A}{M_B}} - 1$ , and  $a = \frac{D_{EB}}{D_{EA}} - 1$ ;  $X_{A_2}$  is the mole

fraction of component *A* at radius  $r_2$  and  $X_{A_1}$  the corresponding mole fraction at  $r_1$ ;  $r_1$  and  $r_2$  are the radii of the two surfaces of the hollow cylinder;  $P$  is the total pressure;  $T$  the absolute temperature of the system; and  $R$  the universal gas constant.

Equation (12) has two important special cases.

(1) Under Knudsen flow as  $P \rightarrow 0$   $D_{AB} \rightarrow \infty$  and hence  $D_{EA} \rightarrow D_{KA}$  and the relationship for  $N_A$  becomes, in cylindrical geometries

$$N_A = - \frac{D_{KA} P}{RT} \frac{2\pi}{\ln \frac{r_2}{r_1}} (X_{A_2} - X_{A_1}) \quad (13)$$

(2) Under mutual diffusion as  $D_{KA} \gg m D_{AB}$ ,  $D_{EA} \rightarrow D_{EB} \rightarrow m D_{AB}$  and  $a \rightarrow 0$ , thus

$$N_A = - \frac{m D_{AB} P}{RT} \frac{2\pi}{b \ln \frac{r_2}{r_1}} \left[ \ln \frac{(1+bX_{A_2})}{(1+bX_{A_1})} \right] \quad (14)$$

Preliminary experiments have been carried out at the AERE, Harwell, to verify this treatment using British pile grade graphite as the porous media. The specimens were cylindrical 5 in. outside diameter, 0.75 in. inside diameter and 5 in. long and they were machined from the original block of graphite with their major axis parallel



to the direction of extrusion. The gas flow occurred radially through the tube walls and was therefore perpendicular to the direction of extrusion.

Two gas pairs, nitrogen-helium and argon-helium, were studied in the counter diffusion runs. Measurements were made in the temperature range 20°–600° C at a total mean pressure of approximately 1 atm. with applied differential pressures between –1 and +12 mm of mercury. These applied pressure differences yielded flux ratios between 0.1 and 10.

Single component measurements using argon, helium and nitrogen were made over the same temperature range and at mean pressures ( $\bar{p}$ ) between 0.008 and 0.6 atm. Additional permeability measurements were made with carbon dioxide and methane at room temperature.

At zero total pressure difference the observed flux rates for binary gas diffusion for the two gas pairs were close to the theoretical values (equation 11) of 0.377 for the nitrogen-helium system and 0.317 for the argon-helium system respectively.

Values for  $D_{EA}$  were calculated from equation (12) assuming the Bosanquet relationship applies. The value of  $m$  was obtained by making the appropriate allowance for the Knudsen term. There was no significant variation of  $m$  with temperature or gas pair used and the experimental results indicated a value for  $m$  of  $0.0093 \pm 0.0005$  for the nuclear grade specimen tested. This figure is in good agreement with the figure of 0.009 found from studies of the rate of reaction between pile grade graphite and air<sup>3</sup>. The experimental measurements were repeated with a specially prepared graphite of lower porosity (18 per cent Knudsen flow at atmospheric pressure as compared with the 4 per cent of pile grade graphite). These results yielded a value for  $m$  for this graphite of  $0.0069 \pm 0.0005$ , which was also invariant with temperature.

Using the values of  $m$  found from the zero pressure difference case together with the values of the coefficients  $B_0$  and  $K_0$  measured from permeability determinations, equations (1a) and (2a) were solved numerically to yield estimates of  $N_A$  and  $N_B$  in the presence of an applied pressure gradient. The theoretical predictions and the experimentally observed flows are compared in Fig. 1. In this figure the flows are expressed as "average fluxes" defined as the flow per unit axial length divided by the log

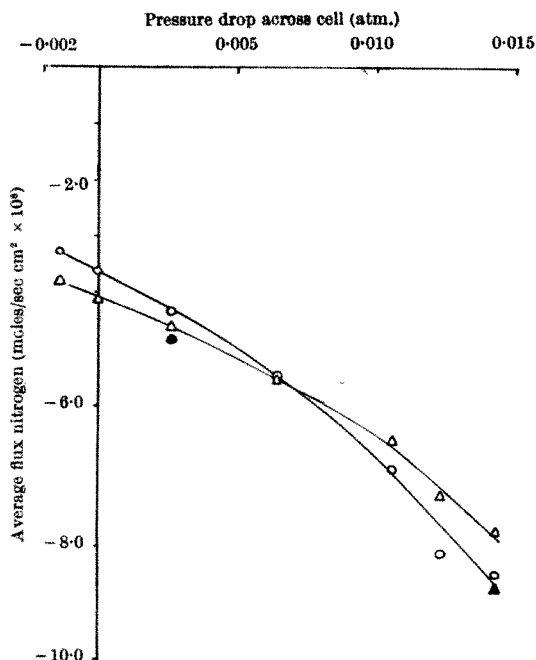


Fig. 1. Variation of gas flux with applied pressure gradient—binary gas flow.  $\Delta$ , Experimental values;  $\circ$ , predicted values.

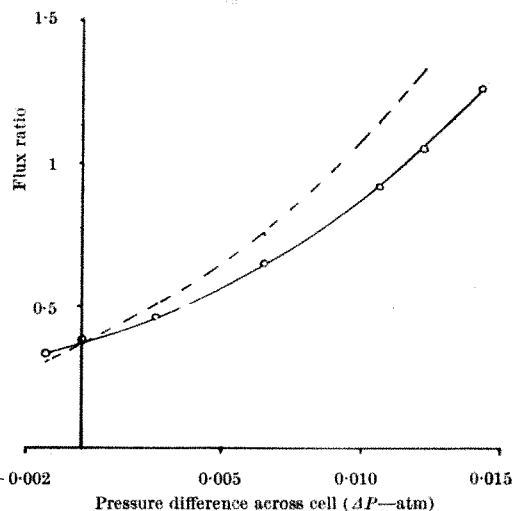


Fig. 2. Variation of flux ratio with applied pressure difference (nitrogen-helium through nuclear graphite).  $\circ$ , Observed points; —, curve calculated assuming  $m = 0.0087$ ,  $K_0 = 3.25 \times 10^{-6}$  cm,  $B_0 = 5.31 \times 10^{-10}$  cm<sup>2</sup>; ---, curve calculated assuming  $m = 0.0093$ ,  $K_0 = 2.47 \times 10^{-6}$  cm,  $B_0 = 5.10 \times 10^{-10}$  cm<sup>2</sup>.

mean area. The agreement between the two curves in this figure is reasonable. The theoretical curve was obtained using the following numerical values

$$\begin{aligned} m &= 0.0093 \\ K_0 &= 2.47 \times 10^{-6} \text{ cm} \\ B_0 &= 5.10 \times 10^{-10} \text{ cm}^2 \end{aligned}$$

As an alternative approach, inverse calculations were carried out on the actual curve drawn through the experimental points and showed that it was uniquely satisfied by the values

$$\begin{aligned} m &= 0.0087 \\ K_0 &= 3.25 \times 10^{-6} \text{ cm} \\ B_0 &= 5.31 \times 10^{-10} \text{ cm}^2 \end{aligned}$$

These derived figures are within the experimental accuracy of the directly measured values and it is concluded that the theory provides a good approximation to the actual behaviour of the system. The biggest discrepancy is in  $K_0$ , but this parameter has the largest experimental error (it is obtained by extrapolation). Also, the result is relatively insensitive to the choice of the  $K_0$  value for the diffusion is close to normal and the flow close to purely viscous.

When these best values for the parameters are used to calculate the flux ratios at various pressure differences, results as given in Fig. 2 are obtained. The dotted line was obtained using the independently determined values of  $m$ ,  $B_0$  and  $K_0$ . We therefore conclude that the theory provides a reasonable representation of the experimental observations provided that accurate values of  $m$ ,  $B_0$  and  $K_0$  can be determined.

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<sup>1</sup> Wakao, N., Otani, S., and Smith, J. M., *Amer. Inst. Chem. Eng. J.*, **11**, 435 (1965); *ibid.*, **11**, 439, (1965).

<sup>2</sup> Bosanquet, C. H., quoted by Pollard, W. G., and Present, R. D., *Phys. Rev.*, **73**, 770 (1948).

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## BOOK REVIEWS

### MASTER AND PUPIL

#### Darwin and Henslow

The Growth of an Idea. Letters 1831-1860 edited by Nora Barlow. Pp. xii+251+8 plates. (London: John Murray, 1967.) 35s. net

THE Reverend Professor John Stevens Henslow, professor first of mineralogy and then of botany at the University of Cambridge and later Rector of Hitcham, was "one chief means of giving [Darwin] a taste for Natural History", as Darwin hastened to state in the first version of the work usually known as *The Voyage of the Beagle*. Henslow was the mentor who furthered and fixed Darwin's true vocation for the natural sciences while Darwin was a Cambridge undergraduate nominally preparing for the ministry. It was also Henslow who recommended Darwin for the position of naturalist on the surveying ship *Beagle* on her voyage around the world and thus set him in the way of discovering evolution. Further, it was Henslow who counselled Darwin as to collecting and observing on that voyage and Henslow who received the collections which were sent back, who advised Darwin as to their condition and who preserved them for subsequent study. After the voyage, and as Darwin became an established scientist in his own right, the relationship between the two men changed, but friendship continued and Henslow was for Darwin "my dear old Master" until his death in 1861, twenty-one years before Darwin's. (Henslow was the elder by thirteen years.)

Those facts have been known in outline to Darwin's many biographers, and the most significant passages in Darwin's letters of 1831-1837 to Henslow were already included in the basic or, one might say, the official *Life and Letters* (1887) by Charles Darwin's son Francis. Only now, however, do we have the full text of all the known letters for that period when Henslow was a dominant factor in Darwin's life, one might almost say *in loco parentis* as concerns influence on the beginning of his career. Now, too, we can follow the less intimate but more equal friendship through Darwin's letters to Henslow from 1838 to 1860, only one previously published in full and few in part. We owe this to the continuing efforts of Darwin's granddaughter, Nora Barlow, who had already produced *Charles Darwin's Diary of the Voyage of H.M.S. Beagle* (1933), *Charles Darwin and the Voyage of the Beagle* (1945), *The Autobiography of Charles Darwin 1809-1882 with Original Omissions Restored* (1958), and *Darwin's Ornithological Notes* (1963). As in those works, Lady Barlow has again been a meticulous editor, contributing an introduction that sets the Darwin-Henslow relationship in the framework of their lives; footnotes identifying other people who are named and clarifying obscure points; a brief bibliography of the most relevant works; six useful appendices and a good index.

Even if this book contained nothing new, it would attract interest and merit thanks for extracting, uniting, and so clarifying source materials on the interaction of the two men involved. The addition of detail in the first part, until 1837, is considerable, and the whole highlights the fact that Henslow was indeed a crucial factor in Darwin's

development, indirectly and contrary to his anticipation or wishes an essential contributor to the origin of *The Origin of Species*.

Although admittedly less important, the later letters cover matters less widely familiar. Still addressed with somewhat reticent affection, Henslow here becomes one of the many colleagues from whom Darwin sought the facts he was building into the fabric of evolutionary theory. There is, for instance, an amusing sequence of letters in which Darwin is trying with evident difficulty to draw out Henslow on the closeness of alliance of species within genera without revealing that those data would bear on Darwin's still unpublished views on the origin of species. Another quite charming sequence of letters reveals that Henslow had organized a sort of posse of young girls who collected botanical specimens, especially seeds, for Darwin. Darwin, anxious to be generous but not profligate, was dubious as to whether he should reward the maidens with 3d or 6d for each packet of seeds. That particular incident was connected with an abortive attempt to determine whether Azorean species also found in the neighbourhood of Hitcham were particularly apt for overseas dispersal.

As with Lady Barlow's previous books, or indeed all that has to do with Darwin, there are innumerable nuggets of news or reminders. As just one more example, it is pointed out that part, at least, of Loren Eiseley's supposed evidence that Darwin plagiarized Edward Blyth is incorrect. Although Lady Barlow is too courteous to say so, Eiseley could have detected the fallacy before he published his claim.

In sum, this is an excellent addition to Darwiniana, and we are again indebted to its compiler and editor.

G. G. SIMPSON

### ALL THE FISH IN BRITAIN

#### British Freshwater Fishes

Factors Affecting their Distribution. By Margaret E. Varley. Pp. 148 (22 plates). (London: Fishing News (Books), Ltd., 1967.) 31s. 6d.

THIS book is one of a series providing a permanent record of annual lectures maintained by a bequest of the late Frank Buckland. Dr Varley gave the three lectures for 1963 in Glasgow, Leeds and Nottingham, and each lecture has been divided into two chapters in the book. It is concerned entirely with the factors affecting the distribution of the British freshwater fishes.

There is a short introduction which serves to inform the reader that the lectures sponsored by the Buckland Foundation are required "to pay all possible attention to the subject of Economic Fish Culture". Dr Varley then observes that the real value of most of our freshwater fishes is recreational. She indicates the number of anglers in the British Isles, and their division into "game" and "coarse" fishermen, although this division is now becoming less distinct. After a short account of the two approaches to angling, the remainder of the introduction gives a résumé of the zoological status of our freshwater fishes, while Table 1 shows their systematic positions.

In the first chapter the origin of the British freshwater fish fauna is discussed. It is noted that the present distribution of animals depends on the range of tolerance of each species and also on its past history. Thus, the relationship of the British Isles to continental Europe before and after the Ice Age is described, and related to the subsequent colonization of our fresh waters by fish. The chapter concludes with an appropriate warning about the dangers of importing species new to our fauna without very careful thought. The author states that carp introduced into South Africa and North America have done a great deal of damage to the native fish fauna because of their habit of stirring up the bottom mud.

The second chapter considers water temperature and oxygen as environmental factors affecting fishes. Here Dr Varley is in her element; she has worked on trout in experimental conditions and investigated the effect of light, crowding, food supply and water chemistry on their growth. The chapter starts by considering temperature tolerance, upper and lower lethal temperatures, and how these can to some extent be modified by the acclimatization temperature. Table 2 lists the upper lethal temperature of various species of fishes, mainly British. The basal metabolism and growth rate of fish within their thermal range are discussed, and it is noted that there is probably an optimum temperature at which they grow fastest. This leads naturally to a consideration of the temperature cycles in our natural waters, and their relationship to the distribution of fish. The amount of oxygen dissolved in the water in relation to temperature and its effect on the fish is discussed in detail. The relationship between the size of egg and the time of spawning is also examined. Dr Varley concludes that from their oxygen requirements and temperature relationships we can grade our fishes between two extreme types, the coldwater stenotherms with high oxygen consumption and the eurytherms with very low oxygen consumption.

It is a natural step from here to consider the distribution of freshwater fishes in relation to topography and this is done in the third chapter. The classification of flowing waters is first discussed, followed by a survey of still waters. Differences in temperature and oxygen content which can be associated with topographical differences between waters are examined, and are related to the species of fish occurring. Thus the distinction between, for example, the fauna of a salmonid and a cyprinid lake is related to the physiology of these fishes.

The fourth chapter considers the effect of the annual climatic cycle on freshwater fishes. Table 10 gives a summary of annual cycles in British fresh waters and Fig. 15 shows the breeding seasons. The spawning periods, size of eggs and breeding requirements of the fish are examined, and thereafter survival, growth and maturity are considered. It is stressed that we know very little about why fish die. It is noted that parasitic infection becomes more likely with increased age, although my opinion is that this is unlikely to account for more than a small percentage of deaths. Finally, the distribution of our fishes is related to their life histories.

One of the important ways in which animals affect each other is through their food supplies, and in the fifth chapter the feeding of the British freshwater fishes is discussed, and their distribution is related to diet, feeding method and possible interspecific competition. Food webs are discussed, and examples quoted. A generalized account of the feeding of many of the species of fish occurring in the British Isles is given, but the author correctly avoids excess detail which could have obscured the picture. Seasonal periodicity of feeding is noted, and the problem of overwintering is considered. It is stressed that a community of fishes with a variety of feeding habits needs an environment with a properly balanced flora and fauna and a variety of ecological niches. The dredging and canalization of rivers, and the "regulation" and fluctuation of the water level of reservoirs tend to impoverish the environment and result in poorer angling.

The final chapter discusses the commercial aspects of freshwater fishes. It is pointed out that, as a nation, we do not seriously exploit our freshwater fishes as human food. The commercial fisheries which exist for salmon, sea trout and eels are mentioned, and the potential seasonal fisheries for perch and pike indicated. Dr Varley considers that the pond rearing of rainbow trout is likely to become an increasing feature in this country. She suggests, however, that the greatest value of our fishes will always be the provision of recreation for anglers. Accordingly she concludes with a brief account of our fish fauna from this point of view.

There are twenty-two excellent plates in the book. Eleven illustrate different types of freshwater habitats, five show stages in the life cycle of the brown trout, two the egg and the alevin of perch, two illustrate food chains, and two show fish in aquaria.

A bibliography lists nine general books on fishes and fresh waters. References quoted in the text are all correctly listed, there being sixty-six in all. These give an excellent coverage of the general literature and of the work carried out in the British Isles on the aspects of the biology of our freshwater fishes considered in this book. A very adequate index is provided.

This book fills a serious gap in the literature of the British freshwater fishes. The book will be of immense value to the fishery scientist, the biologist or the angler, indeed to anyone who is interested in the origin and distribution of our fishes. Dr Varley has combined scientific thoroughness with the ability to make the book interesting and stimulating to all readers. It is to be hoped that this book will encourage further studies on our freshwater fishes, perhaps with more reference to the place of the fish in relation to the other components of the environment than has sometimes been the case in the past.

JAMES C. CHUBB

## PRESENTATION WITHOUT PURPOSE

### *Biologie et Physiologie Cellulaire*

By A. Berkaloﬀ, J. Bourguet, P. Favard et M. Guinnebault. (Collection Méthodes.) Pp. 322. (Paris: Hermann, 1967.) 48 francs.

### *Génétique et Évolution*

By Claudine Petit et Georges Prévost. (Collection Méthodes.) Pp. 392. (Paris: Hermann, 1967.) 60 francs.

THESE books are part of a series which, in British terms, would be used by undergraduates in their early years, occasionally by the bright sixth former. In both cases the text is well ordered and, for the French reader, understandable. The layout and illustrations are concise, clear and attractive. Each book consists of sections contributed by different authors and unfortunately this has produced a variable quality and a lack of unity.

The thing that strikes you about *Biologie et Physiologie Cellulaire* is the commendable way in which the interrelation of structure and function is depicted. The main cell structures are reviewed and in each case its ultrastructure, chemical constitution, physiology and, where appropriate, its origin are described economically and yet with considerable detail. The nucleus is treated in this way and a section on the morphology and physiology of cell division provides a good introduction to a topic not usually so well treated.

The last sections do not fit into this well-ordered system. One on the permeability and irritability of cells is particularly good in the way it considers research techniques in relation to the results obtained; this is an exception to a major criticism of the book. Otherwise there is a rather cursory treatment of techniques. With a subject in which research depends so delicately on valid techniques they need a much more careful scrutiny than the odd paragraph in small print. It is, for example, a fundamental error not to have included some discussion on the problem of artefacts.

*Génétique et Évolution* is really two books stuck together, the first part on genetics being vastly superior to that on evolution. The coverage of genetics is wide and supported by well-balanced accounts of the evidence for the most important findings. It commences with an elementary, but useful, résumé of the major aspects of the subject, such as variation, phenotype and genotype and mutation. The evidence for DNA being responsible for inheritance

is then reviewed and here, as with later sections, there is continual comparison linking microbial genetics with that of higher organisms. After a discussion of the role of DNA in mitosis, a very thorough account of the genetical consequences of the part meiosis plays in the life cycles of organisms leads, through a consideration of simple inheritance, crossing-over, polyploidy and so on, to a detailed account of chromosome structure and mapping.

Essentially inheritance is considered from what one may call the chromosome's point of view, not that of characteristics. This is reflected, for example, in the absence of reference to multiple alleles, polygenic inheritance, pleiotropy and the like.

Finally discussions of the nature of gene action and the genetic code precede two particularly good sections on mutation and the genetic regulation of cell metabolism.

The second part, on evolution, attempts too much in too little space. It lacks depth. There is little reference to the first part and the standard is uneven. A review of population genetics, selection mechanisms, palaeontological studies and human evolution is attempted. Particularly the latter two subjects suffer but such fundamental topics as breeding systems and polymorphism are treated very sketchily and much of the more recent work in ecological genetics is omitted. Also, in contrast with the first part, rarely is the evidence for statements suitably examined.

After reviewing these volumes I am left with contrasting impressions. They are superbly presented and yet I gain from them the realization that science really can be dull. Why, for instance, should the cool, impassive tone of the scientific paper be used in a text book? Is it really necessary—to quote a minor but symptomatic example—to utilize this sort of jargon: following Smith (1929), Brown (1932) devised an experiment before Green (1933)—particularly when no further details of the references are given?

It is true that certain aspects of scientific activity are dull and probably a spartan element in undergraduate training is necessary. It can be argued that interest and enthusiasm are best communicated through personal contact. The realism of the situation is, however, that the latter is becoming less possible in higher education except at the graduate level and that the written word has to be accepted not just as a major means of communicating information but also of attitudes. I wonder, for example, to what extent dull texts have encouraged dull students who became dull school teachers (using dull texts) communicating dull science to lively pupils who, not unreasonably in the circumstances, turned to other subjects.

What is not always realized is that the most gloriously produced book can be dull. Even good quality writing is not sufficient in itself. In fact, the most essential ingredient of a good text book is a real sense of purpose and enquiry that sparks off interest and leaves the student with the realization that it contained something significant enough to be pursued further.

With the exception of some sections of both the books being reviewed it would not seem likely that they will inspire undergraduates. Their concise exposition, however, would make them useful instructional books and, bearing in mind the general standard of the French of English science undergraduates, this is possibly all they would be used for in this country.

P. J. KELLY

## BACTERIOLOGY TEXT REVISED

### Fundamental Principles of Bacteriology

By A. J. Salle. Sixth edition. Pp. 822. (New York: McGraw-Hill Book Company; Maidenhead: McGraw-Hill Publishing Company, Ltd., 1967.) 100s.

SALLE's *Fundamental Principles of Bacteriology* has been an important student text for nearly thirty years and the

current edition continues to provide a broad introduction to bacteria with lesser contributions on yeasts, moulds and viruses. The format of this sixth edition is pleasing and well arranged and the publishers point out the extensive revision which has been achieved since its predecessor's appearance. The revision, understandably, has been uneven and chapters dealing with applied bacteriology and ecology have received little attention. This criticism is, however, partially offset by the inclusion of recent literature citations to which the reader is directed regularly throughout the text. Other chapters, notably on immunity and the immune response, have been re-written and form excellent introductions to their subjects.

Discussion of bacterial morphology, always an attractive feature of this book, has been greatly expanded and is profusely illustrated with carefully selected material. A disappointment here is the poor coverage given to membranes and membranous structures such as mesosomes and chromatophores. Such omission is made the more obvious by the strange inclusion of mitochondria in this chapter. The chapters on yeasts and moulds have remained largely unaltered and tend to be the least satisfactory in the book. The characteristics of *Mucor* and *Aspergillus* continue to be described in great detail—decidedly unnecessary for the beginning student—while mention of other important genera is omitted or curtailed. That mould cell walls are composed, in addition to cellulose and chitin, of "other substances of obscure nature" is unacceptable in a contemporary text.

The author's intention has been to expound the basic principles of bacteriology and provide explanations of the facts where possible. These aims are realized particularly well in his treatment of microscopy, for example, but departures from this objective are rather frequent. Considerable space is devoted to a catalogue of enzymes (*ex I. U. B.*) and to a Bergey style classification of bacteria. A much more critical discussion of factors affecting enzyme activities and of the principles and criteria of bacterial classification would have been desirable inclusions. The failure to introduce numerical taxonomy into the discussion is regrettable and similar criticism can be levelled at other absences such as continuous and synchronous culture. Newcomers to bacteriology should be cautious of the occasional unwarranted generalizations to be found in this book: thus, death is not invariably exponential as implied, nor are nitrogen-fixing nodules found exclusively on the roots of the Leguminosae. Despite these shortcomings, however, Salle remains a very useful and readable account of bacteriology and presents an interesting view of bacteria to new students of this and allied disciplines.

ALAN T. BULL

## CANCER PREVENTION

### The Prevention of Cancer

Edited by Ronald W. Raven and Francis J. C. Roe. Pp. xiv+397. (London: Butterworth and Co. (Publishers), Ltd., 1967.) 120s.

UNDENIABLY, cancer prevention has become an important branch of preventive medicine, and in the future its importance may be expected to increase. Any book on this topic which is edited by senior members of the staff of the Royal Marsden Hospital, London, and the Chester Beatty Research Institute, Institute of Cancer Research, will be expected to be informative and authoritative.

It is therefore disappointing to have to state that this volume, edited by R. W. Raven and F. J. C. Roe, fails to live up to this expectation, although it contains a few good chapters. One of the causes of this failure is the recruitment of too large a team of authors—"fifty eminent authorities" according to both editions of the dust jacket



—to write 376 pages of text. Another reason is a lack of co-ordination of material at the editorial level, with resulting overlap and repetition.

Apart from an introductory chapter entitled "The Principles of Cancer Prevention" and a final section of three chapters dealing with the future, the book is divided into two parts. One part, of eight chapters, deals with some environmental factors: food, cosmetics, pharmaceutical preparations, trauma, irradiation and viruses. The other part, forty-seven chapters long, deals with neoplastic diseases at thirty different sites, and also discusses methods for early diagnosis of some types of tumour, and prophylactic measures such as anti-smoking clinics.

There are particularly good chapters on "Carcinoma of the Cervix—Basic Research" by R. I. K. Elliot, "Cervix Uteri—Screening for Pre-cancerous Lesions" by O. A. N. Husain and "Industrial Aspects of Cancer of the Bladder" by H. G. Parkes. Regrettably other chapters by undoubted experts in their own field suffer from undue compression, and have become relatively uninformative précis of articles that the same authors have already published more fully elsewhere.

Many of the chapters on neoplastic diseases at particular sites throw little light on the subject—cancer prevention—which is supposed to be the theme of the book, for our ignorance of the aetiology of these conditions makes discussion fruitless. It would have been better to have combined such chapters into one, brief at that, making more space available for fuller discussion of those forms of cancer where positive recommendations for prevention can be made.

Other chapters suffer because important issues are raised and inadequately discussed, but the fact that the discussion is inadequate may not be apparent. For instance, Dr Roe states, under the heading "Detailed Consideration of Certain Food Constituents: Caffeine". "The fact that caffeine is mutagenic (Fries and Kihlman, 1948; Andrew, 1959) gives some credence (*sic*) to the possibility that it is also carcinogenic. However, Goldstein and Warren (1962) found no evidence of a correlation between caffeine consumption and cancer of the stomach or colon in a small epidemiological survey." This completes the discussion. The reader might be forgiven if he inferred that this was the only experimental or epidemiological evidence available, and because caffeine is a very widespread item in diet, for it is a constituent of tea, coffee, maté and coca-cola, among other things, and also has medicinal uses, he might be unduly alarmed. The author, surely, might have been expected to have discussed the mass of epidemiological evidence about caffeine-containing beverages that has been accumulating since the surveys published in the 1930s (refs. 1 and 2) and because he is an experimental pathologist it is surprising that animal experiments<sup>3</sup> that failed to show that coffee was carcinogenic have been ignored.

Another irritating feature of some chapters is the failure to give primary sources in the references. For example, Roe, in his chapter on "Food", says, in relation to lead, "Lead has induced kidney tumours in rats (Roe and Lancaster, 1964)", but the work cited<sup>4</sup> is only a review which refers the reader back to the people who did the original work<sup>5-8</sup>. In at least two other chapters by other authors the same experimental work is referred to (a piece of needless repetition) but the references given are those to the original articles.

Considering that much of the material in the book is in fact not about cancer prevention, and that the ancillary information which fills out the rest of it is not exhaustive and can readily be found elsewhere, the price—120s.—is high, especially because there are no illustrations of any sort. In my view the book is either too long if it meant to discuss present knowledge of cancer prevention, or too short and superficial if it is intended to give a general background to environmentally induced neoplastic disease.

For these reasons I cannot see that, in the form in which it has been published, it meets a real need.

R. A. M. CASE

<sup>1</sup> Stocks, P., and Karn, N. M., *Ann. Eugen.*, **5**, 237 (1933).

<sup>2</sup> Hoffman, F. L., *Cancer and Diet* (Williams and Wilkins Co., Baltimore, 1937).

<sup>3</sup> Peacock, P. R., in *Cancer* (edit. by Raven, R. W.), **1**, 64 (Butterworth and Co., London, 1967).

<sup>4</sup> Roe, F. J. C., and Lancaster, M. C., *Brit. Med. Bull.*, **20**, 127 (1964).

<sup>5</sup> Zollinger, H. V., *Virchows Arch. Path. Anat. Physiol.*, **323**, 694 (1958).

<sup>6</sup> Mathews, J. J., and Walpole, A. L., *Brit. J. Cancer*, **12**, 234 (1958).

<sup>7</sup> van Esch, G. J., van Genderen, H., and Vink, H. H., *Brit. J. Cancer*, **16**, 289 (1962).

<sup>8</sup> Boyland, E., Dukes, C. E., Grover, P. L., and Mitchley, B. C. V., *Brit. J. Cancer*, **16**, 283 (1962).

## WOOD FROM THE TREES

### Methods of Wood Chemistry

Vol. 1. By B. L. Browning. Pp. xxi + 384. (New York and London: Interscience Publishers, a Division of John Wiley and Sons, 1967.) 120s.

DR BROWNING describes his book as a compilation of generally employed procedures used in wood chemistry and of less common methods which would otherwise require frequent reference to a scattered periodical literature. It is, however, more than a laboratory manual for the wood chemist and contains much that is of more general interest and could be read with profit by anyone interested in wood technology. The first volume excludes methods dealing specifically with cellulose, non-cellulosic carbohydrates and lignin, these being covered in the second volume. Methods of pulp analysis are included where appropriate.

The book is in three parts; the first is an excellent introduction to the procedures commonly used in wood chemistry and would alone represent a valuable aid to the chemist working in this field. It includes a short, simple account of wood structure and covers the chemical composition of wood, general laboratory methods (drying, evaporation, filtration, extraction, chromatography and spectrophotometry), methods of sampling and the preparation of samples and the determination of water.

The second part deals with wood extractives in the wider sense of those components of the wood cell not present as structural elements in the cell wall. The various classes of extractives including tall oil and the water solubles are dealt with, the emphasis being on the analysis rather than the chemistry of these substances. However, the inclusion of more structural formulae in this section would have added to its usefulness without unduly extending it.

The third part deals with general procedures and special methods, and includes the staining and colour reactions of wood components, analysis of wood preservatives, sorption and swelling, density and specific gravity and a short but excellent chapter on the examination of bark. In the chapter on staining reactions, the benzidine test for differentiating heartwood and sapwood is described but no warning is given of the health hazard associated with benzidine. The section on preservative analysis is very short considering the importance of this subject and is the least satisfactory chapter in the book. While one can understand the author's reluctance to risk dealing at too great a length with this specialized aspect of wood chemistry, the omission of analytical methods for copper is surprising, copper salts being probably the most widely used of all inorganic substances in wood preservative formulations. Although other wet ashing methods are considered, the sulphuric acid-hydrogen peroxide digestion procedure, which is widely accepted in laboratories dealing with wood analysis, is omitted. In the chapter on density, the beta-radiation absorption method, which is especially useful with increment cores and other small samples, is not mentioned.

Most of the methods described are based on American standards but in many cases these are either already

widely accepted in Europe or differences do not invalidate the methods. A useful list of organizations which issue standards relating to wood chemistry is given in the book.

Cellulose, other cell wall polysaccharides and lignin, which together usually make up more than 90 per cent of the dry weight of the wood, are not considered in this book which may be said to deal mainly with the minor components of wood. These minor components are very diverse, however, and Dr Browning, in writing such a book, risked producing a catalogue of largely unrelated facts and procedures. By choosing relevant material rather than attempting complete coverage of the field and by a simple layout and a concise style this danger has been avoided. The result is a very readable book which should quickly establish itself as a standard reference text. N. J. KING

## HETEROCYCLIC COMPOUNDS

**The Structure and Reactions of Heterocyclic Compounds**  
By Michael H. Palmer. Pp. vii + 462. (London: Edward Arnold (Publishers), Ltd., 1967.) 75s. net.

THIS is an excellent book, and a notable addition to the chemical literature. The author has chosen to make "aromatic character" the connecting theme which runs throughout the book, and accordingly small ring systems are omitted. But although it deals only with 5 and 6-membered heterocycles the scope is still wide; it includes systems with up to four hetero-atoms, benzo-analogues and even more complex structures (for example, porphyrins and pteridines) derived from simpler units. The particular merit of this text is, however, its physically oriented approach to heterocyclic chemistry. LCAO methods are utilized in discussing structures and reactivities of heterocyclic systems, and kinetic and spectroscopic data (particularly nuclear magnetic resonance) are used liberally. There is even a chapter on mass spectroscopy fragmentation. Such a rigorous approach to this topic is welcome and long overdue. Nevertheless, the organic chemistry of the subject is not neglected. Readers will observe that little, and in some instances no, attention is paid to the biochemical aspects of the subject. For example, pteridines are discussed without mention of folic acid or butterfly-wing pigments. This attitude can be justified, however, even in terms of space.

The author's initial chapter on nomenclature and aromaticity is appropriate and valuable, and so is the selection of questions collected under chapter headings at the end of the book, but in my opinion his periodic digressions into alkaloid chemistry are somewhat out of context. They contribute nothing to an otherwise self-consistent approach, and might properly have been omitted.

The book is well written and beautifully printed—the reproduction of formulae is particularly clear. This is a "must" for all who work or teach in this field; the more so because it nicely complements existing "orthodox" texts. G. L. BUCHANAN

## WEAK INTERACTIONS

**Weak Interactions and High-Energy Neutrino Physics**

(Proceedings of the International School of Physics "Enrico Fermi", Course 32. Varenna on Lake Como, 15–27th June, 1964.) Pp. xi + 334. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1966.) 128s.

It is one of the more remarkable features of the Varenna Summer Schools, organized by the Italian Physical Society, that they manage to strike a very desirable balance between the physical and more mathematical

aspects of the subject. This certainly holds for the 1964 course on weak interactions and high-energy neutrino physics, which can be highly recommended to all physicists who want to acquire a thorough background in this field.

Professor Bernardini and Mrs C. S. Wu between them provide general introductions to beta decay and weak interactions from which the more specialized contributions can be tackled. Professors Primakoff and Dalitz contribute important expositions on "theoretical muon physics" and "properties of weak interactions" (with special reference to unitary symmetry) respectively. The experimental side to muon physics, neutrino experiments and strange-particle decays is dealt with by Professors Lederman, Schwartz and Steinberger while T. D. Lee presents a general survey of high energy neutrino reactions.

There are several briefer contributions, among them N. Cabibbo on weak interactions and unitary symmetry. The so-called octet hypothesis is of course also discussed at some length by Dalitz. Since 1964 there has been a rather stormy development initiated by Adler and Weisberger, which establishes connexions between the weak and strong interactions, but of this hardly anything was known when these lectures were given. This to some extent detracts from the usefulness of this volume, which is a great pity. S. ZIENAU

## EQUILIBRIUM STATISTICS

**Statistical Thermodynamics**

An Introduction to its Foundations. By H. J. G. Hayman. Pp. vi + 256 with 14 illustrations. (Amsterdam, London and New York: Elsevier Publishing Company, 1967.) 95s.

THIS book is a carefully reasoned introduction to the basic formalism of equilibrium statistical mechanics. The three main ensembles are extensively discussed and applied to Boltzmann, Fermi-Dirac and Bose-Einstein gases, and the only "many-body" topic being a short account of a classical imperfect gas. In spite of the term "foundations" in the sub-title the treatment in the body of the text is mathematically simple rather than rigorous although the quantum and classical forms of Liouville's theorem, the adiabatic principle and other subjects are discussed in appendices.

The argument is usually clear, although the relation between the model of a single macroscopic system and microcanonical and canonical ensembles of such models is made rather complicated. Each member of the microcanonical ensemble is assumed to be a "polysystem" consisting of a large number of weakly interacting, localized statistical elements. The latter cannot represent either the non-localized molecules of a gas or the strongly interacting ones of a condensed phase and the "polysystem" becomes physically relevant only in the fifth chapter when the elements are identified with macrosystems and the "polysystem" is a canonical ensemble. Essentially, what is treated is a microcanonical "ensemble" of canonical ensembles. The same thermodynamic functions are denoted by capital or small letters according to whether they refer to members of the microcanonical or canonical ensembles.

For an introductory text there is not quite enough emphasis on the way in which the development of the subject has resolved outstanding physical problems. The second chapter treats the quantum theory of vibrational and rotational specific heats of diatomic gases, but although it refers to the classical limiting values there is no derivation of these in the later chapters on classical theory, which contain no discussion of equipartition. There is no quantitative treatment of the specific heat of a Fermi-Dirac gas although this could be obtained without difficulty from the formalism already developed. The paragraph on number fluctuations on pages 152–3 needs justification.

Although the preface implies that the book can be used by students with no previous knowledge of the subject I would not recommend it as a student's first text. Those who already know the essentials of the theory will find the book interesting to read and discuss. G. M. BELL

## ALL ABOUT AEROSOLS

### Aerosol Science

Edited by C. N. Davies. Pp. xviii + 468. (London: Academic Press, Inc. (London), Ltd.; New York: Academic Press, Inc., 1966.) 115s.; \$10.50.

THE editor of this book, Dr C. N. Davies, of the London School of Hygiene and Tropical Medicine, is to be congratulated on organizing (and also contributing to) this account of a wide range of knowledge which has by now been accumulated about aerosols. As he says in the foreword, some of the more advanced knowledge was rather inaccessible in spite of the importance of the subject; and certainly there have been very few books which could be turned to for an adequately serviceable account, though there is now also a very recent excellent treatise by N. A. Fuchs, one of the contributors to the present work.

The contributors, distinguished researchers from various parts of Europe and the United States, are well known for their work on aerosols in universities, institutes, and other establishments, in connexion with physics (particularly health physics), medicine, engineering, or industry. They have produced quite a lengthy yet concise account of a very great amount of aerosol knowledge, both practical and theoretical. As an up to date account, well supplied with literature references, the book can be recommended as a useful, timely and convenient exposition, which will undoubtedly help in the further growth of this remarkably intricate subject, and will greatly assist many scientists, in both pure and applied fields.

The first chapter, reasonably enough, is on "Generation and Use of Monodisperse Aerosols", and is by N. A. Fuchs and A. G. Sutugin, of Moscow. It is also very satisfactory to find already on pages 1 and 2 a clear reference to the fact that the particle-size distribution in aerosols usually differs appreciably from the normal (Gaussian) distribution and approximates to the lognormal one, which is a consequence of the nature of the particle growth. The curves in Fig. 1 show the slightly different shape relative to the Gaussian form. The generation of monodisperse aerosols is considered: by condensation (adiabatic expansion of a vapour-gas mixture, or in generators of heat-exchanger type or of the mixer type, or as a result of a chemical process); and by dispersion methods (atomization of liquids or suspensions or by dispersion of a powder). Some of the items of knowledge described are of provocative interest, for example: "It has proved impossible to find condensation nuclei for all vapours. Thus mercury vapour is not condensed on the usual nuclei<sup>42</sup>. Some gases, for example NO<sub>2</sub>, promote the condensation of mercury vapour, but all attempts to produce monodisperse aerosols of mercury have failed. The vapour of indigo behaves in a similar manner<sup>43</sup>." In connexion with atomization of liquids, recent techniques for producing series of identical drops are outlined, such as Mason's method using a vibrating hypodermic needle.

The next chapter deals with "Coagulation of Aerosols", by G. Zebel (of Germany), and the basic theory of Smoluchowski (1916, 1918) is further refined. The editor notes in the foreword, however, that doubts have arisen recently about a factor of 2, in calculating the Brownian coagulation (also called "thermal coagulation") of unequal particles. It must be admitted that calculation of the grain-size distribution as a function of time involves many complications, such as the often non-spherical particle shape and unknown surface form, affecting not only the

mathematical analysis but also the assessment of the validity of the conclusions. Coagulation in external force-fields (electrical-, magnetic-, gravitational-, centrifugal-, and sonic fields) is discussed, and the effects of laminar and turbulent flow.

In the third chapter K. T. Whitby and B. Y. H. Liu from Minnesota review the electrical behaviour of aerosols—charging processes, electrical mobility of charged particles, charge decay of clouds, and so on—in connexion with coagulation of charged particles. One of the few electron micrographs in the book illustrates the chain-like aggregates of roughly spherical particles of methylene blue, a highly charged bipolar aerosol. In the fourth chapter J. Bricard and J. Pradel of Paris give a brief but detailed account of the electric charge and radioactivity of atmospheric ions and condensation nuclei.

Photophoresis, that is the motion of particles suspended in a gas when illuminated by a strong enough beam of light, is discussed theoretically by O. Preining of Vienna in the fifth chapter, and the probable importance of this effect on particles at high altitudes in the atmosphere is indicated. The effects, on particle motion, caused by gradients of concentration and temperature in the surrounding atmosphere, are dealt with in the next two chapters by L. Waldmann and K. H. Schmitt of Germany, and by P. Goldsmith and F. G. May from the AERE, Harwell—the first being on basic theories, and the second of the two on the transport in presence of water vapour (removal of radioactive particles from the atmosphere by rain, and with applications to the engineering of gas scrubbers). Here again, there is an extremely intricate problem of defining even approximately the conditions of velocity, temperature and concentration in the region of a particle.

Filtration is discussed in the eighth chapter by R. G. Dorman, largely from the point of view of theories for estimating the collection efficiency of an isolated fibre, effects of diffusion, and the filter resistance and clogging. J. Pich, of Prague, in the ninth chapter, on the other hand, develops extensively also the theories of aerosol filtration in the complementary case of membrane filters having minute pores, using recent results in fluid mechanics.

J. R. Hodgkinson, of Virginia, USA, in the tenth chapter gives a useful exposé of the optical measurement of aerosols from the extinction and scattering of light. The classical theory of Mie in 1908 led for a long time to only limited applications in this field, but the recent fuller calculations have now made this a valuable method of estimating particle sizes and concentrations.

The adhesion of fine solid particles to surfaces or each other, on contact, is reviewed by M. Corn, of Pittsburgh, USA. This feature of the behaviour of aerosols is clearly important in the aggregation or coagulation, and in collecting or filtering processes. The considerable amount of theoretical work based on molecular-force laws and various assumed particle shapes, surface roughness, and so on, is outlined, and a large body of sensitive experimental work is described briefly, covering many different materials, conditions of humidity, and states of motion of the particles. Once more, the theoretical considerations face the difficulties of knowing and defining the conditions at the surface of the contacting bodies.

Finally, the twelfth chapter, by C. N. Davies, reviews present knowledge of deposition from moving aerosols, mainly deposition of the larger particles swept along by a rapid air current, either in an enclosure or tube, or the atmosphere, under laminar flow or turbulent flow. Anyone living in a city, particularly, will appreciate the desirability of understanding the nature of the aerodynamic capture and distribution of particles such as those emitted from chimneys, industrial works, or otherwise.

Probably the main omission in this otherwise excellent book is an adequate discussion of the growth and nature of solid aerosol particles. Very few electron micrographs are

shown (see pages 83, 218, 270, 274) and do not do justice to the by now considerable evidence of this type. The extreme complexity of the conditions near the actual particles, both during their growth and subsequently, makes it all the more desirable to secure all possible definite evidence. Electron microscopy has in many cases shown directly the shapes and sizes and their distributions. Electron diffraction, also, has shown very sensitively the chemical and crystallographic nature of solid aerosols or their surface regions, and in several cases has thereby given valuable indications of the temperatures at the particle surfaces during their growth (see *Disc. Faraday Soc.*, 30, 113; 1960). Such studies have also shown the degree of surface mobility of the atoms or molecules during condensation on the growing aerosol particles and during their aggregation into chains. All such direct evidence promotes appreciably a healthy basis for considering aerosols not just as aerial intangibles or mean distributions of vague unseen units, but as defined particles of known shape and surface form.

H. WILMAN

## LEDA AND THE ASTRONAUTS

### If the Sun Dies

By Oriana Fallaci. Pp. 415. (London: William Collins, Sons and Co., Ltd., 1967.) 45s.

THIS infuriating book is probably the best thing to have been written about the American space programme. The author, an Italian journalist, is shameless in using her Florentine heritage and her femininity as yardsticks to measure the value of Project Apollo. Even more irritating is her pretence of writing what she learns back to her cultured war-scarred father in Florence. On his behalf, ostensibly, she went to Houston, Cape Kennedy and Huntsville to ask why men wanted to go to the Moon when there were neither trees nor Giotto's there. Her achievement has been in getting them to tell her—with revealing eloquence—and in recording her own conversion. The voyages to space, she decides, are right and necessary; they may be the first steps in colonizing the rest of the universe in anticipation of the day when the Sun dies.

The questions that Miss Fallaci tosses at her witnesses border on the absurd and therefore are well worth asking. Her intelligence and persistence drew out some provocative answers. To Walter Schirra, the feeling of weightlessness was "dignified freedom from everything that's dirty, sticky . . . you move well, without sweat, without difficulty, as if the biblical curse 'In the sweat of thy face and in sorrow' no longer exists." John Glenn, the freckled saint who was humbled by a bathroom cabinet, said "Don't think that people like me don't know what Shakespeare said . . . the past serves me as a guide to the future." And Dr Werner von Braun sees the lunar trip as a gymnastic exercise, a warming-up for learning how to get from one planet to another. Along with Mr Schirra, the German rocket scientist should be grateful to the author. Amid many scathing portraits, each comes through as a more complex, sensitive man than we had thought and Dr von Braun should forgive the tasteless associations by which Miss Fallaci links his smelling of lemon soap with some brutal German soldiers she saw during the war. It is the kind of nasty nuance that hovers in one's mind and the author's skill is that she has made it explicit.

The same goes for the sexual tones of the entire venture. It was time that someone spelled it all out. The rockets are obvious enough, but impatience for the docking and the reluctance to return to Earth deserve the full treatment that they get here (from the astronauts' nurse, a spinster and former midwife).

As a lucid and literate explanation of the National Aeronautics and Space Administration's plans for landing an American on the Moon, the book could hardly be

better. Both scientists and laymen will have much to learn from the voluminous details which Miss Fallaci has assembled. She did her homework well, braving the rude manners and tasteless food served in the stifling motels which border the space cities and the astronauts' personality tests in order to listen to long harangues about astrophysics and rocket fuels. She balked only at the last minute from taking a test spin in the centrifuge.

For half its considerable length, the book is spell-binding. Then the new generation of astronauts arrives; they are bald, tired before their time and, with a few exceptions, hardly distinguishable in their conformity. (One answered in astonishment, when asked if he had children: "How could I not have children at my age?") Lamentably, the story ends before the tragedy of last January gave the space race the poignancy and self-doubts that had been conspicuously absent. All in all, this was a book that needed writing; it was a stroke of brilliance that sent a woman to do it. Yeats speculated about Leda and the swan: "Did she out on his knowledge with his power, before the indifferent beak could let her drop?" The answer here seems to be yes and this book ensures that the knowledge is sharable. BRENDA MADDOX

## OBITUARIES

### Professor E. Kowalski

PROFESSOR EDWARD KOWALSKI died suddenly of coronary infarction on April 15, 1967, in Warsaw. His untimely death in the prime of life, and in his most productive period of scientific activity, has left his friends, collaborators and students with a profound sense of loss.

Professor Kowalski was best known as a haematologist and biochemist, but the range of his scientific interests extended to several branches of experimental medicine. He will be remembered especially for his contributions in the field of blood coagulation. His investigations of fibrinolysis demonstrated for the first time the significant role of the products of the degradation of fibrinogen in the various stages of blood clotting and opened up new areas of research in this very important field. His name was also closely associated with work on erythropoiesis, in connexion with his investigations on the biosynthesis of haemoglobin and the hormonal control of erythropoiesis.

At the time of his death he was not only in charge of the department of radiobiology of the Institute of Nuclear Research in Warsaw, but was also engaged in clinical work as consultant to the Institute of Rheumatology, also in Warsaw, where he simultaneously directed several research projects concerned with iron metabolism and with the physiology and biochemistry of blood platelets.

Kowalski's achievements brought him recognition from numerous scientific bodies in various countries. He was a member of editorial boards of leading haematological journals, and a member of the International Committee of Thrombosis Research.

Kowalski was not only a scientist and a clinician, but a man with a profound interest in the arts and humanities, in which he was extremely well versed. His exceptionally pleasant personality, in his day to day contacts with all those who knew him, won him many friends both in Poland and abroad who will greatly miss him.

K. ZAKRZEWSKI

### Sir John Cockcroft

SIR JOHN COCKCROFT, Master of Churchill College, Cambridge, died suddenly on Monday, September 18 (see page 1321). An obituary notice will appear in a later issue.



## University News:

Glasgow

PROFESSOR G. M. WILSON, of the Department of Pharmacology and Therapeutics, University of Sheffield, has been appointed Regius professor of the practice of medicine in succession to Sir Edward Wayne, who retires on September 30.

## Appointments

ERNST R. PARISER has been appointed chief scientist and director of engineering for the marine sciences programme of Aveco Corporation.

## Announcements

THE closing dates for the receipt of applications for travel grants from the Royal Society's parliamentary grant-in-aid have been changed to March 1, June 1 and December 1.

THE British Empire Cancer Campaign for Research and the recently founded College of Pathologists have been given £500,000 to acquire suitable premises in London as a permanent headquarters. The gift was made by Mr Michael Sobell. As well as encouraging the study by pathology of malignant disease, it is hoped that the gift will strengthen and facilitate collaboration between the two bodies. A committee will be appointed within the next few weeks to discuss how a suitable building can be acquired.

THE Lalor Foundation, Wilmington, Delaware, invites applications for its 1968 awards for postdoctorate research in the field of reproductive physiology. The awards are open to all nationalities and preference will be given to younger members of university or college faculty and staff, with an upper age limit of 41 years. The work may be carried on at the applicant's own institution or elsewhere. The awards may be for a few months or up to one year and may range from \$1,300 to \$8,500, depending on the scope and duration of projects approved. Forms of application and further information can be obtained from the Lalor Foundation, 4400 Lancaster Pike, Wilmington, Delaware 19805.

## Meetings

SEVENTEENTH Chemical Engineering Conference, October 16-18, Ontario (Programme Chairman, Dr D. R. Woods, Department of Chemical Engineering, McMaster University, Hamilton, Ontario).

CONTINENTAL Drift emphasizing the History of the South Atlantic Area, October 16-19, Montevideo, Uruguay (Dr J. T. Wilson, Director, Institute of Earth Sciences, University of Toronto, Toronto).

CLEAN Air Conference and Exhibition, October 17-20, Blackpool (National Society for Clean Air, Field House, Brems Buildings, London, EC4).

BATH Conference 1967, October 18-20, Limpley Stoke Hotel, Bath (J. Hoskin Lamble, Bath University of Technology, Upper Borough Walls, Bath, Somerset).

EXPLODING Wire Phenomenon, October 18-20, Boston (W. G. Chace, Air Force Cambridge Research Laboratories, Laurence G. Hanscom Field, Bedford, Massachusetts).

TECHNOLOGIST in the Ceramics Industry, October 20, Stoke-on-Trent (G. H. Stewart, Institute of Ceramics, Shelton House, Stoke Road, Shelton, Stoke-on-Trent).

X-RAY Techniques in Materials Science: Recent Developments, October 26-27, Institution of Electrical Engineers, London (Meetings Officer, Institute of Physics and the Physical Society, 47 Belgrave Square, London, SW1).

GLOBAL Impacts of Applied Microbiology, November 6-11, Addis Ababa (Dr Aklilu Lemma, Faculty of Science, Haile Sellassie I University, PO Box 1176, Addis Ababa, Ethiopia).

ERRATUM. In the article "Calculation of Crystal Packing: A Novel Approach to the Phase Problem" by A. Damiani, E. Giglio, A. M. Liquori and L. Mazzarella (*Nature*, 215, 1161; 1967) the following corrections should be made on p. 1161: line 29 of the left hand column should read "...  $b = 6.186 \dots$ " and line 50 of the right hand column should read "... coefficient of  $3.5 \text{ \AA}^2$ ", and not  $3.5 \text{ \AA}$  (ref. 2). On p. 1162 the volume number in reference 1 should read IV, and not 5; the second author's name in reference 11 should read Kraut and not Krant; and the name of the last author of the article should read L. Mazzarella and not L. Mazzarell.

# CORRESPONDENCE

## Vegetative Propagation

SIR,—We have in English the phrase "vegetative propagate", which is awkward. Every plant part so used and the plant that establishes from it is necessarily a member of a clone—one of its RAMETS. But this word, already established, tends to throw its emphasis on common origin.

If RAM-ET can be so formed from *ramus*, may we have the slightly more irregular PROPET, which is easy to say and to spell, and use it on those occasions where the whole catalogue: bud, bulb, corm, cutting, offset, rhizome, runner, scion, tuber, etc., is to be understood? Does anyone object?

Yours faithfully,

E. R. WALLACE

88 Hills Road,  
Cambridge.

## Head on the Block

SIR,—Whose 'Head on the Block' (*Nature*, 215, 1215; 1967)? You quote the figures of 1,947 and 1,831 for SRC Research Studentships for 1965 and 1966 as if they were significant. The footnote to Table 1 of Appendix III points out that due to the transfer of awards to NERC and SSRC "the figures for 1966 are therefore not comparable with those for 1964 and 1965". The true comparison is mentioned on p. 23 of the Report—4 per cent up in Research Studentships and 12 per cent in Advanced Course Studentships—about 7 per cent overall.

What better evidence that we are alive to the needs of the newer universities and some of the budding polytechnics could there be than the figures in Appendix III, Table 3 (105 research studentships at Sussex) and Appendix IV, Table 3. (Aston, Bath, Bradford, Brunel, City, East Anglia, Essex, Kent, Lancaster, Loughborough, Surrey, Sussex, Warwick and York—with a total value of £1.7 m out of our total of £21 m.)

I am sorry you do not like our 'Co-operative awards in pure science'—we do try. Some of us feel that to arrange for a substantial number of university research students to spend up to 3 months working on their research project in industrial laboratories is far from being an artificial link with industry.

Yours faithfully,

E. R. H. JONES

The Dyson Perrins Laboratory,  
South Parks Road,  
Oxford.

## FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

## Tuesday, September 26

SOCIETY OF CHEMICAL INDUSTRY, PLASTICS AND POLYMER GROUP (at 14 Belgrave Square, London, SW1), at 6 p.m.—Dr L. S. Moody: "Polymethylene Oxide".

## Tuesday, September 26—Thursday, September 28

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2)—Conference on "Magnetic Materials and their Applications".

## Wednesday, September 27

SOCIETY FOR ANALYTICAL CHEMISTRY, THIN-LAYER CHROMATOGRAPHY GROUP (in the Lecture Theatre of Ipswich Civic College, Rope Walk, Ipswich), at 2.30 p.m.—Meeting on "Thin-Layer Chromatography Applied to Foods and Related Compounds".

## Thursday, September 28

INSTITUTION OF MECHANICAL ENGINEERS, APPLIED MECHANICS GROUP (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p.m.—Discussion Meeting on "Steels with a High Yield to Tensile Strength Ratio".

## Thursday, September 28—Friday, September 29

EUGENICS SOCIETY (in the Meeting Rooms of the Zoological Society of London, Regent's Park, London, NW1), at 10.15 a.m. on Thursday and 10.30 a.m. on Friday—Symposium on "Genetic and Environmental Influences on Behaviour".

INSTITUTE OF BIOLOGY (at the Royal Geographical Society, Kensington Gore, London, SW7), at 10 a.m. daily—Symposium on "The Problems of Birds as Pests".

## Monday, October 2

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 9.30 a.m.—Colloquium on "Definition, Realization and Use of Time and Frequency".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 5.30 p.m.—Mr D. K. S. Bain: "Power-System Model".

INSTITUTION OF MECHANICAL ENGINEERS, STEAM PLANT GROUP (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p.m.—Discussion Meeting on "The Combination of the Gas Turbine and Industrial Steam Production".

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (at Shell Centre Theatre, Shell Centre, London, SE1), at 6.30 p.m.—Scientific Films.

## APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

LECTURERS or ASSISTANT LECTURERS (with research experience in either some aspect of computing science, such as system programming, or in numerical analysis) in the COMPUTING DEPARTMENT—The Secretary of the University Court, The University, Glasgow (September 29).

ASSISTANT LIBRARIAN (Grade I) (with a good honours degree) with the title of Sub-Librarian in the Faculties of Medicine and Veterinary Science—The Registrar, The University, Liverpool, quoting Ref. RV/168/N (September 30).

ASSISTANT LIBRARIAN to supervise ordering and accessioning and for other administrative duties—The Librarian, School of Oriental and African Studies (University of London), London, WC1 (September 30).

EXPERIMENTAL OFFICER (with experience in instrument design or construction involving electronics or optics, and preferably some experience in spectroscopy) in the DEPARTMENT OF CHEMISTRY—The Registrar, The University, Manchester 13, quoting Ref. 144/67/Na (September 30).

RESEARCH ASSISTANT in the DEPARTMENT OF BOTANY to assist in the study by electron microscopy of the correlations between ultra-structural features and physiology in fungi—The Registrar and Secretary, University of Durham, Old Shire Hall, Durham (September 30).

LECTURER/ASSISTANT LECTURER (with an honours degree in botany, a research degree in a relevant field, and some teaching experience) in the SCHOOL OF BIOLOGICAL SCIENCES, University of Malaya—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (Kuala Lumpur and London, October 6).

SCIENTIFIC or SENIOR SCIENTIFIC OFFICER (with a good honours degree and postgraduate research experience) for comparative studies on the nitrogen, potassium, and phosphate nutrition of vegetable crops—The Secretary, National Vegetable Research Station, Wellesbourne, Warwick (October 6).

LECTURER in BIOCHEMISTRY—The Secretary, The University, Dundee, Scotland (October 7).

SENIOR LECTURER in the DEPARTMENT OF PHYSIOLOGY, The Medical School—The Registrar, The University, Nottingham (October 8).

LECTURER and an ASSISTANT LECTURER in COMPUTER SCIENCE—The Secretary, Birkbeck College (University of London), Malet Street, London, WC1 (October 9).

LECTURER (with a good honours degree and/or a higher degree in sociology or a cognate discipline) in SOCIOLOGY in the DEPARTMENT OF EDUCATION—The Registrar, University College of North Wales, Bangor, North Wales (October 9).

LECTURER (preferably with teaching and research interests in physical geography and particularly in the field of climatology) in GEOGRAPHY at the University of Newcastle, New South Wales, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (Australia and London, October 13).

LECTURER in INORGANIC or ANALYTICAL CHEMISTRY at the University of Natal, Durban—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (South Africa and London, October 15).

LECTURER in PHYSIOLOGY, and a LECTURER in HISTOLOGY (medical or science graduates) at the University of Cape Town—The Secretary-General, Association of Commonwealth Universities (Branch Office), Marlborough

House, Pall Mall, London, SW1; and The Registrar, University of Cape Town, Private Bag, Rondebosch, Cape Town, South Africa (October 15).

DEMONSTRATOR/SENIOR DEMONSTRATOR in the DEPARTMENT OF INORGANIC CHEMISTRY—The Registrar, The University, Newcastle upon Tyne 2 (October 20).

SENIOR LECTURER (with a degree in geology with research experience, preferably to Ph.D. level) in GEOLOGY at the University of Queensland—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (London and Brisbane, October 27).

ASSISTANT EXPERIMENTAL OFFICER or EXPERIMENTAL OFFICER (with H.N.C. or equivalent qualification in chemistry, biochemistry or biology) in the DEPARTMENT OF RADIOBIOCHEMISTRY to assist in a programme of research involving the application of isotopic tracer techniques to biochemical problems—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, Reading, Berkshire, quoting Ref. 67/83.

BIOCHEMIST or CHEMIST (honours graduate or Ph.D.) to investigate the chemical composition of normal and osteoporotic human bone—The Director, Medical Research Council, Mineral Metabolism Unit, The General Infirmary, Leeds.

LECTURER (with teaching and research experience in pharmacology and/or related aspects of physiology) in PHARMACOLOGY—The Secretary and Registrar, Bath University of Technology, Claverton Down, Bath, Somerset.

PALYNOLOGIST (with experience in Palaeozoic and early Mesozoic biostratigraphy, preferably of the Southern Hemisphere) for research and applied palynology—The Director, Bernard Price Institute for Palaeontological Research, University of the Witwatersrand, Johannesburg, South Africa.

PHYSICIST (honours graduate or Ph.D. preferably with previous experience in the operation and maintenance of isotope-counting equipment and in the preparation of biological samples) to collaborate in a biological research programme involving the measurement of radioactive isotopes (calcium, iodine, carbon) and the development of techniques for the measurement of calcium absorption and bone turnover in man—The Director, Medical Research Council, Mineral Metabolism Unit, The General Infirmary, Leeds.

PHYSIOLOGICAL PSYCHOLOGIST/ERGONOMIST to work on the essential qualities and performance characteristics of powered artificial hands—The Director, Powered Limbs Research Unit, West Hendon Hospital, Goldsmith Avenue, London, NW9.

POSTDOCTORAL FELLOW in the DEPARTMENT OF CHEMISTRY to work in the field of the kinetics of atomic and free radical reactions in the gas phase, isomerization and exchange reactions of free radicals and fundamental processes in photosensitization—Professor D. J. Le Roy, Department of Chemistry, University of Toronto, Toronto 5, Ontario, Canada.

PROFESSOR and HEAD of the DEPARTMENT OF MICROBIOLOGY—The Dean, Ontario Agricultural College, University of Guelph, Guelph, Ontario, Canada.

SENIOR TECHNICIAN I in the BIOCHEMISTRY SECTION of the GROUP PATHOLOGY DEPARTMENT based at the North Lonsdale Hospital, Barrow-in-Furness—The Group Secretary, Barrow and Furness Hospital Management Committee, 105 Abbey Road, Barrow-in-Furness.

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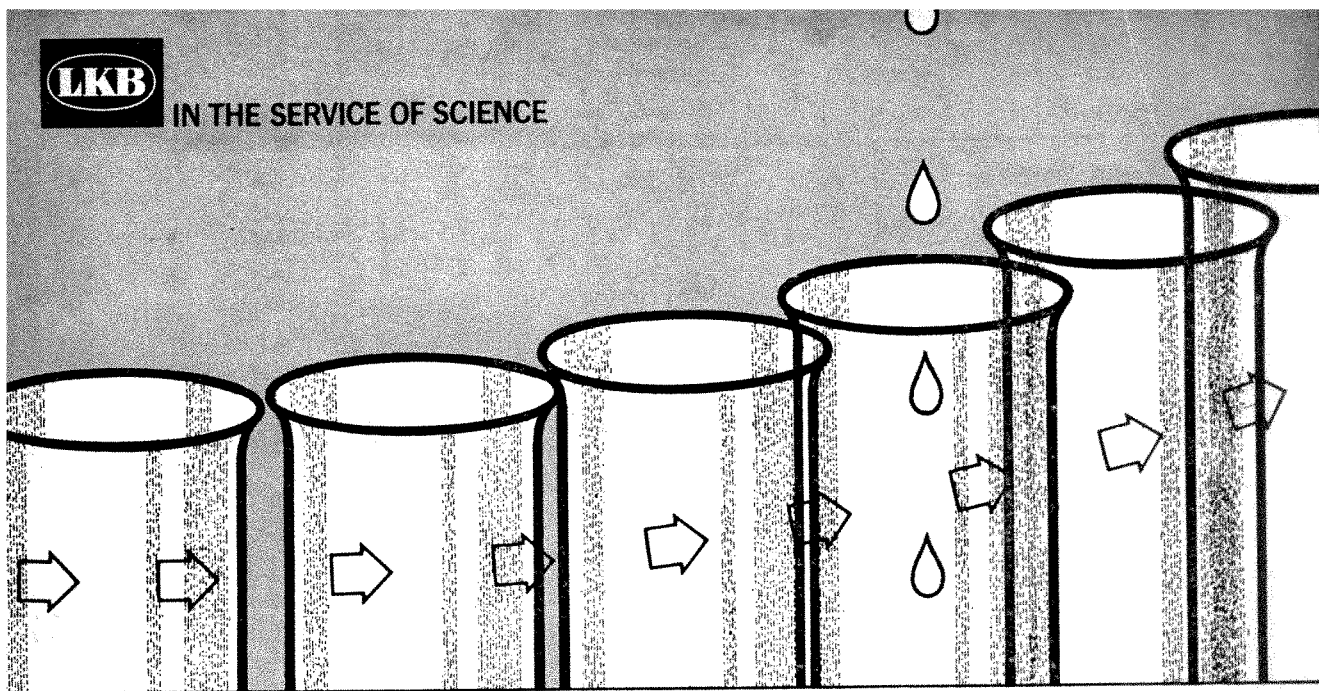
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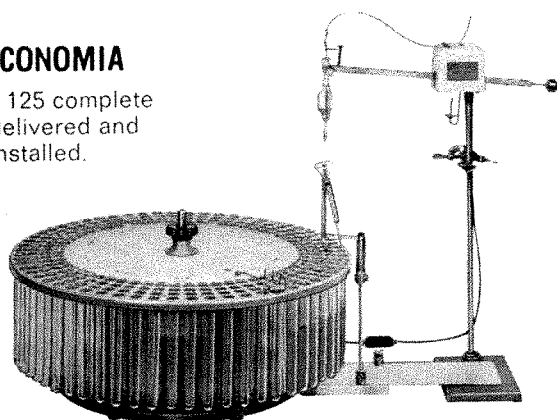


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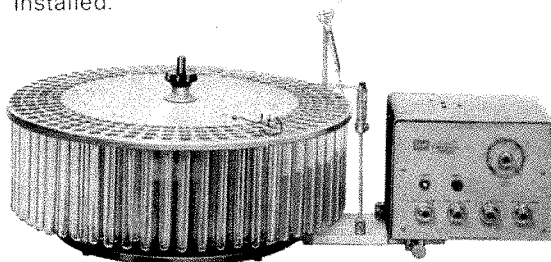
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DEPARTMENT

Applications are invited for a number of positions in the biological science departments in the University College of Townsville. In conjunction with the existing disciplines of Botany and Zoology strong emphasis is being given to Marine Biology and a professorial appointment has been made.

**Senior Lecturer:** Applicants should have a Ph.D. degree and several years' research and postgraduate training. An interest in the physiology of tropical marine organisms would be an advantage but candidates with other interests will be considered.

**Lecturer:** Applicants should have a higher degree and preference will be given to those whose research interests include experimental taxonomy, animal behaviour, terrestrial ecology or some aspect of vertebrate or invertebrate embryology.

**Senior Demonstrator/Demonstrator:** Applicants should have an honours degree in botany or zoology.

Opportunities for research are available with each position.

**Salaries** (at present under review)

Senior Lecturer: \$A6,400 by \$A200 (6) to \$A7,600.

Lecturer: \$A4,800 by \$A200 (7) to \$A6,200.

Senior Demonstrator: \$A3,800 by \$A100 (5) to \$A4,300.

Demonstrator: \$A3,000 by \$A100 (4) to \$A3,400.

A northern allowance of \$A60 is payable in each case.

The College provides superannuation similar to the F.S.S.U. Scheme, Housing Assistance, Study Leave and Travel Grants. Reasonable travel and removal expenses are payable.

Additional information and application forms may be obtained from the Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1.

Applications close in Australia and London on October 23, 1967. (984)

## UNIVERSITY OF AUCKLAND NEW ZEALAND

### SCHOOL OF MEDICINE

Applications are invited from suitably qualified persons for the following positions in the new School of Medicine:

### CHAIR IN ANATOMY

### CHAIR IN PHYSIOLOGY

In conjunction with colleagues in other disciplines the Professors will be responsible for the development of a co-ordinated course in Human Biology, a new Bachelor of Science degree course for medical students commencing in 1968. They will be responsible for organizing clinical and postgraduate studies in their respective subjects.

The Professors will also be expected to advise on academic and technical staff required for their Departments.

Medical salaries are at present under review. Further information about salaries is included in the conditions of appointment, copies of which are obtainable from the Secretary-General, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1.

In the case of new members of staff coming from overseas the practice is to pay salaries from an approved departure date.

Travel and other removal expenses are allowed within specified limits. Upon the completion of five years' satisfactory service, return fares on an approved basis to country of origin may be granted to the person appointed and his dependent family, provided that such fares are not being met from some other source.

Improved conditions are now in force for candidates holding F.S.S.U. policies.

Applications close in New Zealand and London on October 31, 1967. (986)

## THE LONDON HOSPITAL WHITECHAPEL, E.1

### RESEARCH ASSISTANT

required for one year from mid-September to direct a Research Unit working on human colonic physiology in conjunction with surgical staff. Facilities at The London Hospital and St. Mark's Hospital.

Applications, naming two referees, to the House Governor by September 30. (948)

## SHIPBUILDING AT SEA

It must be galling indeed for other shipping lines to see the effortless ease with which the Cunard Steamship Company has persuaded the British Government to part with £24 million to help with the cost of building another transatlantic liner. As even Cunard readily admits, transporting people across the Atlantic in enormous liners is no longer a growth industry. Traffic has shrunk from nearly one million passengers a year immediately after the war to a mere 600,000 in recent years. Most of these have been crossing in the summer months. The Atlantic is not a cheerful place in the winter and the Queen Mary, the last great Cunarder, used sometimes to arrive at Southampton with only 50 passengers on board, waited on hand and foot by the crew of 1,000. This is no way to make money.

This is one reason why the new Cunarder, named with an entirely predictable sense of conservatism "Queen Elizabeth II", will try to recoup Atlantic losses by cruises in warmer climates during the winter. Whether this will be possible is still uncertain, but it certainly took some of the gloss off the launching ceremony on September 20. When ships were ships, they were at once the quickest and the most pleasant way of reaching America. Now, it seems, they must potter about the southern oceans for half the year in order to maintain an outworn tradition in the Atlantic for the other half. It was hard to feel that the launching of the Queen Elizabeth II was anything but an extension of the nostalgic scenes at Southampton the week before when the Queen Mary left for America for the last time.

It is likely to prove an expensive nostalgia. Cunard will be paying off the loan from the British Government at the rate of at least £2 million a year for the first twelve years of the liner's life. This is likely to ensure that the passenger side of Cunard's activities will continue to show a loss, and the unpalatable prospect emerges of the Government being forced to take over from Cunard the most expensive pleasure steamer in the world. The truth is that passenger ships are a glut on the market. Cunard was fortunate to be able to sell the Queen Mary to the city of Long Beach, California, for £1,230,000—well above the market price. Union Castle recently sold the Capetown Castle, a ship several years younger than the Queen Mary, for a sum it refuses to reveal—it may well have been no more than £100,000. Cunard hopes that in future its profitability will be ensured by its heavy investment in the container cargo market, but the benefits of this will take time to be felt.

What effects will all this have on shipbuilding? The launching of the Queen Mary in the thirties was a signal for new optimism in the industry; the Queen

Elizabeth II, unfortunately, is more widely interpreted as a sign of hardships to come. Shipbuilding in Britain is going through yet another crisis. At the end of June, only 1.9 million gross tons of shipping were on order in British yards, representing barely 18 months work—less in some areas. There has been a modicum of jubilation now that the Esso Petroleum group has placed orders for four giant tankers of 240,000 tons with Swan Hunter on the Tyne and Harland and Wolff in Belfast, but these will not keep the wolf from the door indefinitely. In the long run, the shipbuilders need encouragement in the form of a reduction in the price of steel plate. The cost of steel is an important item, making up between 20 and 25 per cent of the total cost of a ship. It is no wonder that the shipbuilders, as a way of forcing prices down, are threatening to buy British steel on the Swedish market, where it sells for £35 a ton—much less than the home price of £42 a ton.

Clearly this is no more than a ploy to make Lord Melchett, Chairman of the British Steel Corporation, think hard about his determination not to cut prices for a single industry, but there is a precedent for it. Until 1949, all shipbuilding steel in Britain was sold at a discount—in 1931 the discount was 22.5 per cent, but by 1939 it had fallen to 6.5 per cent. After the war, shipbuilders with full order books had no need of the subsidy, and it came to an end. But the Geddes Committee on the shipbuilding industry, which reported 18 months ago, suggested that the price differential should be reinstated as a matter of urgency. The committee said that the costs of steel to the shipbuilders should be reduced by 10 per cent, leading to a 2 per cent reduction in the cost of building merchant ships. On a very large tanker of 200,000 tons, this would produce a cost reduction of about £120,000, and bring British costs more nearly into line with Continental and Japanese yards.

There are, however, some solid arguments against the idea. Steel company profits have themselves been under strong pressure, and a subsidy to shipbuilders, the companies say, would force up the price of steel to other users. Should car manufacturers, or manufacturers of structural steel or pressure vessels, be handicapped for the sake of the shipbuilders? On economic grounds, the answer must be no. While the shipbuilding industry cannot be ignored, it is nevertheless surprisingly small. From the 27 yards covered by the Geddes inquiry, sales in 1963 amounted to £159 million. In the same year, the turnover of the British Motor Corporation was £444 million and the Distillers Company £303 million. Even taken as a whole, the shipbuilding industry is comparatively small. It is also diffuse, a consideration which led the



Geddes Committee to recommend amalgamations on a wide scale. In fact, these mergers have been happening, first on the Tyne, and more recently on the Clyde, but mergers are no substitute for orders. The trend towards larger and larger ships is also acting against the multiplicity of shipyards in Britain—for each ton produced, the average amount of shipyard work has fallen.

Given the need for rationalization—which is admitted inside as well as outside the industry—it is hard to argue in favour of measures designed to enable the industry to stagger on in its present form. Things may well get worse before they get better, but to relax the pressure on shipbuilding now would be to lose the benefits of rationalization later. One way out would be an overall reduction in steel prices but, although this will probably be one of Lord Melchett's first objectives, it would be imprudent to count on anything substantial. There is another argument the Government would be wise to ignore. As it happens, shipbuilding is concentrated where unemployment is already high—the north-east, Belfast and the Clyde. The Ministry of Power seems to have been persuaded to abandon its cheap fuel policy in the interests of keeping miners employed, and there is a danger that pressure will be brought to bear on the BSC to abandon its policy of uniform prices to do the same for the shipyards. It is a pressure that should be resisted.

## ANTI-WHO MISSILES

ALTHOUGH the announcement on September 19 from Washington that the United States is to deploy a "thin" defence against ballistic missiles has caused a great stir, there is still no means of knowing just why the decision has been made at just this time. It is true that the pressures in favour of anti-ballistic missiles have been apparent for months or even years, but the US Secretary of Defense, Mr Robert McNamara, should long since have grown used to them. There is nothing in the history of the past few months to suggest that he has now surrendered to what is, after all, only a small and familiar part of the public opinion with which he has to live.

So is it the case, as Mr McNamara said, that the immediate provocation is the likelihood that mainland China will be equipped with inter-continental missiles by the early seventies? And is that a sufficient argument? Mr McNamara is of course in a position to know better than most people outside China just what the Chinese are about, but it is hard to think that the Chinese will have anything like an effective long-range missile system before 1975 or thereabouts. After all, a few missiles do not make a striking force but only, sometimes, a provocation. So is it possible that the Department of Defense has its eyes on the Soviet Union instead, and that the continuing deployment of anti-ballistic missiles there has tipped the balance of the argument in favour of a

similar policy in the United States? Here again, there is something in the argument but not enough to make a convincing case. In his announcement on September 19, Mr McNamara went out of his way to claim a "three to one" superiority in long-range missiles, which implies that the Russian anti-missile defences, whatever they are, cannot decisively alter the strategic balance. In other words, if the United States has waited this long before building an anti-missile system for itself, it is hard to see why it could not have waited yet another year.

Even within the United States there are powerful arguments in favour of delay. For one thing, there is always the possibility that the system soon to be installed will turn out to be a much less durable investment than some of its supporters expect. The plan is that there shall be two separate kinds of anti-missile missiles—one called Spartan with long range which is intended to make interceptions at a considerable height above the Earth, and another called Sprint designed for rapid acceleration towards hostile missiles nearing their targets. The awkward facts of missile defence by themselves compel some kind of dependence on a mixed system like this. Long-range interception is preferable for all kinds of reasons, only some of them economic, but it is also necessarily a chancy business, at least in the present state of rocket technology. It follows that there must also be some kind of point defence of the kind that Sprint is intended to provide. The snag, from the point of view of American taxpayers anxious to know what they will get for \$5,000 million spent on anti-missile rockets, is the likelihood that the system now to be installed will soon be superseded by more effective systems. Much simpler radar systems than those around which the new defences will be built are notoriously prone to all kinds of uncertainties when first introduced to active service. From a strictly technical point of view, delay should save money and bring greater effectiveness, although performance is bound always to fall a long way short of perfection.

These, however, are parochial arguments. The decision to build an anti-missile system in the United States is more worrying because of what its consequences will be elsewhere. The simple argument that this is yet another twist in the costly spiral which has led to the development of increasingly sophisticated and dangerous weapons since the Second World War is probably, as it stands, too simple by half. For one thing, the resources that the United States and the Soviet Union will devote to these activities are probably only small fractions of what they spend already on defence. (They are also unlikely at this stage to undertake to build fallout shelters on the scale which logic demands should accompany anti-missile systems, more or less on the principle that a defensive weapon which kills its own people is no defence at all.) It is also probable that, in the years immediately ahead, anti-missile rockets will occupy a comparatively undignified place in the military budgets of both countries. There will be a

temptation to spend enough to stay in the business without taking resources away from more urgent projects. Moreover, there is no prospect in the measurable future that countries other than the United States and the Soviet Union will be tempted to follow suit. Yet, even if there is no arms race in the classical meaning of the term, it is an important setback that the two credible nuclear powers have now abandoned their tacit agreement that plausible mutual deterrence is some kind of assurance of stability. The American Government has creditably taken the initiative, in the past few months, in trying to negotiate an agreement not to deploy anti-missile rockets, and the report that it has been rebuffed is not by itself a proof that the time has come to abandon the attempt. A few months more might have made a difference.

Where relations with other nations are in question, the timing of this development is particularly unfortunate. It is tactless, to say the best of it, for the two nuclear powers to have embarked on the development of another generation of nuclear weapons at a time when they are both apparently doing everything they can to win the agreement of lesser fry to the non-proliferation treaty being negotiated at Geneva. Will this help the Indians or the Italians to sign away their rights, however theoretical, to manufacture nuclear weapons for themselves? The decision in the United States is likely to have more particular consequences in Europe, where anti-missile systems are likely indefinitely to remain too expensive and unworkable. One result will be that the French will be confirmed in their official view that the United States cannot be trusted with the defence of Europe. Those nations in Western Europe who consider that the North Atlantic Treaty Organization has a useful job to do will find it necessary to whistle harder to keep the spirits up. This is a lot to lose for such a little gain. Yet nowhere in Mr McNamara's statement is there any sign of a move to help create in mainland China—by discussion, although public speeches can do a lot of good—the kind of awareness of the damage which nuclear weapons can do which is, in the long run, the only assurance that nuclear powers will make a rational appraisal of their responsibilities. One way and another, this is a lot to lose for such a little gain.

## ANOTHER CROCK OF GOLD?

THE new academic year seems to be starting quietly enough in Britain, and this may be just as well for all those with responsibilities for the smooth running of the forty-four universities now in business. There is, after all, plenty of hard work to be done. The anxieties of the late summer, when some people proclaimed that they would not have all the equipment necessary to operate laboratories efficiently, seem not to have materialized in their full rigour. And it will be some

weeks before it is known just what kind of settlement the University Grants Committee has reached with the Department of Education and Science on the financing of the next quinquennium which begins a year from now. In the circumstances it is tempting to forget that the year ahead is likely to be critical for British universities. In the circumstances, it is a public service that Professor Max Beloff has been sounding off (*Minerva*, 5, 520; 1967) about the relationship between British universities and the public purse. He will at least succeed in reminding several people of the battles that lie almost immediately ahead.

Professor Beloff has also raised the unfashionable cry of academic freedom. He is principally concerned with the intrusion of public administration into the running of the universities. To him, the prospect that the accounts of the universities shall be open to inspection by a civil servant—now chosen to be the permanent secretary at the Department of Education and Science—is a red herring. The real threat is the way in which the University Grants Committee and the research councils are able, independently or in concert, to influence the way in which universities conduct their business. Innovation is usually dependent on the public purse—that is how the argument goes. The result is that autonomy has almost disappeared. But since it is unthinkable that the one who has paid the piper will refrain indefinitely from calling the tune, the universities must win back their independence by finding some substantial source of private funds. By this test, as Professor Beloff is quick to point out, it was mistaken of the Franks Commission to recommend a further centralization of the University of Oxford. Robbing the colleges of their independence will only put the university as a whole in pawn.

Nobody will deny the daring of this sally, for ever since the Franks Commission eighteen months ago, Oxford has been lying low for fear of attracting too much attention. And there is, of course, a great deal in what Professor Beloff has to say. The universities have been through a bad spell. The UGC has by its tactlessness provided half a dozen reminders of who is master now. The Secretary of State for Education and Science provided a nasty slap in the face in December 1966 by deciding without consulting the universities that students from overseas should pay bigger fees. At the same time it has become all too obvious that the universities are badly equipped to defend themselves. It will be a long time before the universities can answer back persuasively. It is, however, wrong and dangerous to think it possible to build a shield against intrusion by collecting private funds. In simple money terms, it is unlikely that anything less than £20 million a year—say, 10 per cent of university expenditure—would suffice to keep the universities independent. But where is such a fortune to come from in a country which is hard pressed to raise much smaller sums for equally deserving causes? And how could private endowments safeguard the independence of the universities as a whole, not just that of the best favoured? These questions answer

themselves. In the long run it would be a great advantage if British universities had more money to call their own (and they could do a lot to help themselves by playing on the affections of their alumni), but there is no realistic prospect that the universities will be able to buy their way out of trouble.

In any case, the weakness of the universities is partly their own creation. They are too vulnerable to sharp questioning about cost-effectiveness. Although some of the newer universities have taken it on themselves to carry out deliberate studies of the most efficient ways of teaching, many institutions are unreasonably sensitive to suggestions that different ways might bring greater benefits. Ironically enough, teachers as well as students often suffer from the same mismanagement. In Oxford, for example, the Franks Report—as much a defence of the present arrangements for undergraduate education as a proposal for reform—showed quite clearly how strictly administrative changes could bring great benefits in the working of the university. Sir Hans Krebs, in his article in this issue (see page 1441), shows how the same muddles impede the conduct of research at Oxford. So is it surprising that the custodians of the public purse should have become uncomfortably suspicious of the way the universities conduct their business? And because there is no prospect that British universities will ever be entirely free from dependence on public funds, is it realistic of them to seek autonomy without first taking scrupulous care to see that their own operations are above criticism of all kinds? In the long run, the universities will only be able to defend themselves against intrusion when they are better able than their would-be critics to decide what part universities should play in modern society and then to decide how best to attain desirable objectives. This means in practice that the universities must organize themselves for change. Somehow they must design a means by which they can arrive, by common consent, at workable decisions.

Strengthening the Committee of Vice-Chancellors is an obvious step to take, but means will also have to be found to give the people who work in universities a better sense of being represented and a clearer conviction that they must be bound by collective decisions. Inevitably, universities will have to become more attentive to problems of cost-effectiveness. They need to be so enterprising in this respect that they can forestall the criticisms of those outside. When they have done everything that can be done in ways like these, it will be time enough to pass the hat around.

## TOO MUCH IRON?

FOR several decades it has been plain that there are serious problems to be solved before the abundance of iron in the parts of the universe accessible to observation can be properly accounted for. The most conspicuous difficulty to be overcome is that iron seems

to be relatively much less abundant in the atmosphere of the Sun than in the solid objects of the Solar System—the metallic parts of the chondrites which reach the Earth as meteorites and the Earth itself. The article by P. G. Harris and D. C. Tozer on page 1449 of this issue is an ingenious attempt to account for what seems to be a segregation of iron into the substance of the inner planets. It is, of course, improbable that doubts will now be resolved—the authors would probably be the first to admit as much. But there is at least a chance that interest will be re-awakened in this awkward problem, which can only be a benefit.

The first thing to be said is that the problem of iron in the Solar System is almost the personal creation of Professor Harold Urey, who with his collaborators over several decades has compiled the evidence which shows the disconcerting variation of the abundance of iron from one object to another. The ratio of iron to silicon in the atmosphere of the Sun is less than a third of that in the chondrites, and only a fifth of the ratio of iron to silicon which must be assumed if the Earth has a molten core of iron and nickel of much the same composition as the metallic parts of meteorites. It is true that the relative abundance of iron on the Earth is comparable with that in the carbonaceous chondrites, which by this test are the most likely representatives of primeval matter, but this coincidence only makes it more necessary to explain why the Sun appears to be deficient in iron. Are the measurements in error? Or was there some process of fractionation during the formation of the Solar System which concentrated segregated iron in the inner planets?

Harris and Tozer have suggested a mechanism of the second kind. They suggest that iron may have accumulated preferentially in the inner planets as a result of magnetic attractions between particles of iron dust in the material left over after the formation of the Sun. The point is that particles of iron held magnetically together are less likely than other kinds of dust to be swept away by the outward radiation pressure of the Sun. One difficulty, of course, is that it will only be possible to test this argument when much more is known about the details of the formation of the planets and about the mechanisms by means of which their composition has been altered in the past five thousand million years or so. Another is that of accepting such a comparatively simple explanation for what has seemed, in recent years, to be an extremely complicated problem. In the long run it is probable that room will have to be found in any comprehensive theory for at least some of the explanations which Urey has devised to explain, for example, how some kinds of meteorites may have lost some kinds of elements preferentially. Indeed, the complications of these arguments have at least the virtue that they bring together several different kinds of observations—the compositions of meteorites, the geophysics of the planets and the theoretical calculations of the natural abundances of the elements, for example. But none of this implies that there is no room for new ideas. Here, as elsewhere, every innovation helps.

## NEWS AND VIEWS

### Engineers in Profile

As part of their efforts to stimulate engineering, and to encourage more children to become engineers, the Ministry of Technology and the Council of Engineering Institutions decided some time ago that it would be useful to know exactly what an engineer is. The result of their investigation, a booklet entitled *The Survey of Professional Engineers 1966*, was published this week. The ministry claims that it is the most comprehensive study yet made of a profession in Britain; questionnaires were sent to no fewer than 25,000 engineers, of whom 86 per cent replied.

The typical engineer, it seems, is young (two-thirds of the sample were under 45) and works in industry. More than half of the sample work in private industry, while nationalized industries took another 14.2 per cent. Universities and colleges of technology employed only 455 engineers, 2.4 per cent of the sample. The study also provides some useful information about engineers' salaries. The median salary for 30 year old engineers, for instance, is about £1,500 a year, and by the age of 40 has climbed to £2,100. Those in the upper decile earn £2,000 a year at 30, but have gone up sharply to £3,300 by the age of 40. The top salary recorded was £17,400, and 10 per cent of the whole sample earned at least £3,500.

There does seem to be an advantage in being a graduate in engineering; 55 per cent of graduate engineers earned more than £2,000, while just over 40 per cent of non-graduate engineers did so. Graduates are twice as likely to earn £3,000 as non-graduates. Of different categories of engineers, chemical engineers are still the best paid, although other branches have narrowed the gap since 1959. Over the period of six years, electrical engineers have improved their incomes by 7.2 per cent per year, while chemical engineers have increased by only 3.9 per cent per year. Chemical engineers still manage to earn more, however, for which their large graduate entry must take some of the credit; the median salary for chemical engineers is £2,729, while that for mechanical engineers is £2,249, electrical engineers £2,404 and civil engineers £2,268.

The engineers returning the questionnaire were also asked to make a judgment of their levels of responsibility. Despite the human tendency to exaggerate, which is likely to exaggerate the degree to which engineers shoulder industry's burdens, only 11.4 per cent of the sample considered themselves top management. A further 22.3 thought they were middle management, and 25.8 favoured junior management. The rest, 40.4 per cent, described themselves as performing technical and engineering functions.

### No Coals to Newcastle

CONFUSION increases about the policy of the British Government towards fuel in general and coal in particular. The policy of cheap—or at least moderately cheap—fuel put forward by the Minister of Power, Mr Richard Marsh, as recently as July has for practical

purposes been abandoned by the Prime Minister's decision that there should be a halt to the closing of collieries. But for how long will the Government be able to resist economic pressures on the British coal industry which have their parallels in most other countries in Europe? For a month, perhaps, until the Ministry of Power has time to produce its new White Paper on fuel policy—by which time the annual conferences of the Labour Party and the National Union of Mineworkers will conveniently be over? Or until the spring of 1968, when miners thrown out of work would be less conspicuous? Or will there be a more or less permanent postponement of the full rigours of cheap fuel?

Uncertainty about the Government's intention has now been given an awkward twist by Mr Dan Smith, a government stalwart in Newcastle upon Tyne, and chairman of the North-east Regional Development Council, who says that he will resign if the National Coal Board closes down one large colliery in his district. This is not so much an attempt to give the policies of the National Coal Board a parochial flavour as a protest against the way in which a public body appointed only two years ago to guide planning and development in the North-east has been made to feel that it is but a piece of window-dressing. (The chairman of the corresponding council for the north-west of England, Dr C. L. Carter, disclosed a similar sense of grievance only a week or so ago.) Mr Smith's protest has been given some extra piquancy by conflicting reports about the Coal Board's intentions towards the colliery in question, but it is hard to think that all those now in dispute with one another will be able to keep both their dignity and their jobs.

Altogether, 19 collieries would have been closed in the ordinary course of events before the end of 1967 and six are likely to shut in any case because reserves are worked out. Precisely what it will cost to keep the others going is hard to calculate, partly because exact figures are not available and partly because there are no simple standards of comparison. On the assumption that the National Coal Board counts the most costly pits among its potential closures, however, the total cost of the unwanted coal produced in the coming winter will be measured in tens of millions of pounds. Moreover, it is plain that the British mining industry has a long way to go before it has dispensed with its long uneconomic tail. The cost of producing heat energy from British collieries ranges from less than 3d. a therm (=10<sup>6</sup> British thermal units) to more than twice as much, as shown in the following table.

Cost (pence per therm)	Production (millions of tons)
Less than 3	10.4
3-4	50.8
4-5	59.7
5-6	27.3
More than 6	9.5

In a world in which the Ministry of Power has vowed not to agree to a price for North Sea gas anything like 5d. a therm—the price agreed two years ago for the first supplies from the well sunk by British Petroleum—even the target of an annual production of 155 million tons of coal by the end of the sixties may well, by then, seem an inexplicable extravagance.



## Message from Kapitza

PHYSICISTS in Britain and particularly in Cambridge have been heartened by the following telegram of sympathy about the death of Sir John Cockcroft sent last week by Dr Peter Kapitza:

Dear Vice-Master,

Please convey to the fellows and members of Churchill College my deep sympathy and condolences at the sad event of the death of your master. I am deeply shocked by the news of the death of my friend Sir John Cockcroft, as we knew each other for more than 40 years. He was a physicist of great insight, masterly technological gifts and a brilliant organizer. With his quiet and charming ways Sir John was a stubborn fighter for progressive views not only in science but also in international affairs. We know Sir John as a promoter of peace and international collaboration. It was my privilege together with Sir John to bring closer the scientists of our two countries. I sincerely hope that this fine tradition will be followed by Churchill College. Churchill College was the last great achievement to which Sir John brought so much of his personality, thought and touching love and where he and Lady Cockcroft with such tact lived up to their high position as first dwellers in the Master's Lodge of Churchill College.

Yours very sincerely, PETER KAPITZA

Dr Kenneth McQuillen, Vice-Master of the College, replied the same day in the following terms:

We are deeply touched by your stirring message of sympathy on the tragic death of our master, Sir John Cockcroft. It will help us to follow the tradition of bringing closer the scientists of our two countries which he and you have done so much to establish and which was so close to his heart.

This friendly exchange is much valued not merely as a mark of the respect in which Sir John Cockcroft was held but also because it has helped to take some of the sting out of the sudden departure of Dr V. Kachenko from London. It is known that Dr Kapitza took a personal interest in Dr Kachenko's stay in Britain. It is therefore hoped that his telegram may fairly be taken as a sign that scientists in the Soviet Union will not be alienated from the programme of scientific exchanges by the mismanagement of Dr Kachenko's departure.

## Successor for Kerr

THE eight months search for a new president for the University of California ended last week with the appointment of Mr Charles Hitch as a successor to Dr Clark Kerr. Mr Hitch is at present with the Rand Corporation, but his reputation since the war stems from his work with the Department of Defense and the Bureau of the Budget, where he has had an important influence in the development of techniques for the introduction of cost-effectiveness criteria into military and economic planning. Given that Dr Kerr's departure from the presidency took place at a time when the Governor of California, Mr Ronald Reagan, was pressing for a reduction of the cost of running the university, it is ironical and possibly significant as well that Mr Hitch has in the past few years been paying

close attention to the cost-effectiveness of running universities.

Mr Hitch has an outstanding reputation as an administrator, although his career began before the Second World War in academic life. He was a Rhodes Scholar in the early thirties and taught economics at Oxford until the outbreak of the war. After military service he returned to the United States and the Rand Corporation. His spell in Washington began in 1961, when he was appointed Comptroller at the Department of Defense. His work with the Bureau of the Budget began in 1964. He returned to the Rand Corporation a year ago.

It remains to be seen how the appointment of Mr Hitch to the presidency will affect relations between the Governor of California and his university. Since the departure of Dr Kerr, the Board of Regents seems to have reasserted some at least of its traditional independence; theoretically it should function in relations between the university and the state much as the University Grants Committee used to function in the public dealings of British universities. One sign of this is that the Board of Regents is planning to submit a budget for the academic year beginning in 1968 which represents an increase of roughly 25 per cent on what it has had to spend this year. In part, of course, the size of this increase reflects the way in which the budget for 1967 was reduced immediately after Mr Reagan came to power, but obviously it includes a considerable element of growth as well. But it is unlikely that Mr Hitch will be able to pare down the budget intelligently, which implies that he will find himself defending a budget which is larger than the governor can welcome. The University of California may find that Mr Hitch must show his mettle before the year is out.

## Money for Students

A SCHEME for helping American students to pay for higher education has been devised by a committee of the Science Advisory Committee in Washington under Professor J. R. Zacharias of the Massachusetts Institute of Technology. In a report now published, the committee urges that there should be established what is called an "educational opportunity bank" which would be able to lend money to a student in return for an agreement to pay back a percentage of his income for the thirty or forty years he is likely to be at work after graduation. According to the plan, the bank would be an agency of the federal government. It would borrow money from the government or from private sources. It is estimated that the scheme would be self-financing if borrowers paid 1 per cent of their gross income for a period of thirty years.

The scheme is likely to excite interest elsewhere than in the United States. In Britain, for example, there has recently been talk of providing some part of the cost of higher education by loans to students. Similar schemes already operate in Denmark.

## New Look for Welding

IN order to encourage the teaching of welding, Dr Richard Weck, director of the British Welding Research Association, has been appointed Visiting

Industrial Professor in the Departments of Mechanical Engineering and Metallurgy at the Imperial College of Science and Technology. This is the most obvious part of what will be an important scheme for collaboration in teaching and research between the Imperial College of Science and Technology and the British Welding Research Association.

The resources of the research association and the college are to be combined to expand the teaching of welding technology at all levels. Undergraduates at the college will be able to spend short periods of instruction at the association's laboratories, and the association's staff will help the college to provide courses of lectures for postgraduate students. A number of short refresher courses, of three to four weeks in specific fields of the technology, will be held for staff from industry by the college and association jointly. These will be held partly at the college and partly at the association's conference centre at Abington, near Cambridge. The scheme also envisages close collaboration in research; already work of interest to the association is being carried out at Imperial College. The facilities of the two departments of the college will be available to the association staff and the specialist equipment of the association will be available to the college. Younger staff members in the association will also be able to qualify for higher degrees from the college.

## Watching the Sun

A DISH aerial one mile in diameter which can adjust to the position of the Sun at one second intervals is clearly not a practical proposition, but a new radio heliograph two miles in diameter which was opened on September 22 at Calgoora, Australia, is designed to observe the Sun with equivalent resolution. Complete pictures of the solar disk showing great detail will be taken at one second intervals by the telescope, so that close observation of the rapid changes that occur during solar flares and sunspot activity will be possible. With an operating frequency of 80 megacycles, the resolution is thought to be about 2 minutes of arc. The telescope consists of 96 aerials set up in fixed positions round a circle two miles in diameter. Each aerial is a dish 45 ft. in diameter and is automatically driven to follow the Sun for 4 hours a day while the pictures are taken.

Signals from each aerial are amplified 100 times before being transferred by cable to the central point. The synthesis technique is the basis of the complicated calculations which are then made by computers, correlating the output of all possible pairs of aerials. The heliograph is part of the Australian Commonwealth Scientific and Industrial Research Organization's Solar Observatory, and was designed by Dr Paul Wild. It has taken five years to build and has been financed by donations of £230,000 from the Ford Foundation.

Within the circle of the radio heliograph at Calgoora the Physics Division of the CSIRO has set up a 12 in. optical telescope. It incorporates a specially developed filter which cuts down scanning time to only a few seconds, and should pick out details of areas only 250 miles in diameter on the Sun's surface 93 million miles away. The cine-magnetograph is under the direction of Dr R. G. Giovanelli, chief of the division, and is supported by the United States National

Aeronautics and Space Administration. A small telescope, which watches for solar flares as part of the world-wide patrol network, completes this array of tools for the Sun specialist.

## Water Games

THE Hydraulics Research Station of the Ministry of Technology at Wallingford (Berkshire) still finds that most problems in open channel flow must be solved by building models and running water through them. One result is that the station's programme of investigations at any time has a pleasing diversity. At present, for example, the station is studying the effects of very long waves in a model of the proposed Brighton yacht marina. The model is built beneath a two-dimensional array of lights, and the reflexions in the water with an exposure time equal to one wave period are photographed. This provides information about the maximum slope of the water surface. Opponents of the marina at Brighton will be distressed to hear that so far the work at Wallingford has shown no technical objections to the design.

The station is also intending to build a large model of Morecambe Bay to test the feasibility of a barrage and to determine the best position for one. The chief concern will be to determine the effect of the barrage on siltation and erosion in Morecambe Bay, particularly at the ports of Heysham, Fleetwood and Barrow in Furness. The investigation is part of a larger study being carried out by Sir Alexander Gibb and Partners for the Water Resources Board.

Civil disturbances at Hong Kong have not prevented the station setting out to study the building of a tunnel across the harbour by sinking prefabricated concrete sections, each 424 ft. long and weighing 30,000 tons. The sections would be floated into place and then sunk, a delicate operation with such enormous sections. If the hydrodynamic forces acting on the tunnel sections are not precisely known, there is a danger that the tunnel will either sink too fast or bob up again to the surface. The necessary information is being supplied by the use of a 'Perspex' model at Wallingford, built to a scale of 1 in 100. There is also a feasibility study of the disposal of London sewage by pipeline rather than by boat, and an attempt to devise a way of removing a sand bar at the mouth of the Baram River in Sarawak so as to make the river navigable for at least 60 miles.

All these projects are done on direct contract, and represent about half the work of the station. The other half is divided equally between research work with no specific client in mind and more basic studies of hydraulic science. Theoretical work at the station has shown, for example, that it is possible to obtain perfect dynamic similarity when modelling the process of sediment transport, and two channels of the same shape but different sizes have been built to demonstrate this. This work provides a theoretical basis for the large models. The station costs about £500,000 per year to run and last year earned £308,000. Recently, as part of a review by the Ministry of Technology of research establishments, the station was asked to justify its existence to British industry by calculating how much money was saved as a result of work at Wallingford. By conducting a survey of the small number of projects where accurate costings are possible,

the director, Mr R. H. C. Russell, claims to have shown a £7 million saving to industry for an expenditure of about £700,000.

## Linneans Wanted

A LITTLE public relations can often do a lot of good, as the Linnean Society seems to have decided. The society, formed in 1788 by a splinter group from the Royal Society, concentrates on the biological sciences. It has now decided, in co-operation with the Department of Extramural Studies of London University, to run a series of lectures on the "Historical Background to Modern Botany" in an attempt to become better known and to attract more members, particularly keen amateurs. It is also running a natural history education programme with a course of lectures for 120 promising sixth form girls and boys, and has started a student associateship of the society to attract young members between the ages of 18 and 24.

The society also intends to launch an appeal for £100,000. With the money it hopes to rehouse the valuable Linnaeus Collections and its library of 90,000 books, and to adapt the rooms in Burlington House which it acquired when the Royal Society moved out.

## More Nuclear Safeguards

THE US Atomic Energy Commission has provided further evidence of its concern for the safeguarding of nuclear materials of various kinds by its decision to establish a committee to provide technical advice on the subject. The chairman will be Mr J. Palfrey, a lawyer at Columbia University, who was a member of the AEC for four years until 1966. The fourteen ordinary members of the committee include scientists, industrialists and lawyers. The AEC says that it will rely on the committee for advice about the application of safeguards to particular problems as these occur, as well as for the development of general policy. Although the setting up of such a committee was not explicitly suggested by the Lumb panel on safeguards procedures which reported earlier this year, it is part and parcel of what seems to be a thorough review of safeguards procedures by the AEC.

At least three separate lines of argument have given impetus to this tendency. The growth of the nuclear power industry in the United States is spectacular enough in itself to account for a renewal of interest in the subject. The amendment of the Atomic Energy Act which allows private companies to own nuclear fuels and to process them has emphasized the need for explicit criteria for a sharp line between the permissible and the illicit, and for means of ensuring that these criteria are observed. (Already one plutonium reprocessing plant and a number of fuel fabrication plants are operating under private ownership in the United States.) But it is also plain that international pre-occupation with the treaty to limit the spread of nuclear weapons has made necessary a more explicit policy on safeguards, although the AEC as such can do very little in that respect but stand on the sidelines and show willing.

This is why the chief interest of the work of the new committee will be the way in which it helps to provide national precedents for what may eventually

become international agreements on safeguards. In this sense it may already be significant that the AEC has decided to assign permanent inspectors to some at least of the privately owned processing plants—the plant operated by Nuclear Services Incorporated for extracting plutonium from spent fuel, for example. Developments like these could well nudge the IAEA into the conviction that itinerant inspectors like those now based at Vienna may not always be able to exercise detailed control. There will also be great interest in the criteria for deciding what imprecisions in the safeguards system can be safely tolerated, which it will presumably be a part of the new committee's job to develop. It is less certain whether the committee will also pick up one of the more controversial recommendations of the Lumb panel—the notion that there should be stiff fines and prison sentences for people participating in breaches of the safeguards system. At first sight, there may be a little too much of James Bond in the suggestion that infringement of the regulations should expose people to the risk of five years in prison or fines of \$10,000, but if there are not criminal penalties of this kind, it may occasionally be extremely hard to know how conspirators should be dealt with under existing laws.

## New Reactors

THE United Kingdom Atomic Energy Authority's steam generating heavy water reactor at Winfrith, Dorset (*Nature*, **214**, 867; 1967), has been loaded with fuel and heavy water and the first stage of operation, at low power, has been reached. Construction started in May 1963, and the reactor is expected to be operating at full power by the end of this year, when it will deliver 100 MW of electricity into the national grid. The AEA has high hopes of the reactor in the export markets. In the size range 300–500 MW, the economics and fuelling of this reactor make it particularly attractive. New Zealand, which hopes to have about eight reactors by the late seventies, has already sent engineers to look at it.

The reactor has a pressure tube construction with heavy water as the moderator and natural water as coolant. Although the prototype at Winfrith uses slightly enriched uranium oxide fuel, the actual production reactor will use natural uranium. This may be an attraction in countries where people fear dependence on a small number of sources of fuel supply. The steam generated in the core passes directly to a turbo-alternator to generate electricity and not through a secondary cooling system.

The authority's VIPER reactor, intended for research, is also now in operation at Aldermaston, where it was designed and built. The reactor produces much more intense pulses of neutron and gamma radiation levels than those available from present steady state research reactors. At full power, there are  $3 \cdot 10^{17}$  fissions producing, in 500  $\mu$ sec,  $10^{15}$  neutrons/cm<sup>2</sup> and a gamma-ray dose of 30,000 rads. The reactor power rises to a peak of 20,000 MW. Because the reactor does not have forced cooling, it must be allowed to cool at its own speed, and can generate only two pulses a day.

Pulses are produced by the rapid movement of a uranium fuel rod into and out of the core. This increases the reactivity of the reactor into the prompt

critical region. The power in this region increases rapidly—doubling every 70  $\mu$ sec. The neutron pulse is limited by the inherent characteristics of the reactor. First, as the temperature rises the fuel expands, thus increasing the loss of neutrons by leakage. At the same time the effective width of the resonance capture peaks of uranium-238 increases, which implies that more neutrons are captured and the probability of fission is reduced.

The VIPER reactor differs from earlier fast pulsed reactors, such as the American Godiva, because the broadening of the resonance lines at higher temperatures, itself a Doppler effect caused by thermal motion, provides a large part of the temperature dependence of the reaction. This same effect is an important inherent safety factor in fast breeder reactors of the type now being considered for power stations.

## Britain's Chemist

THE annual report of the Laboratory of the Government Chemist for 1966, just published (HMSO, 15s.), describes the work which is being done by the staff of 426. Customs and excise work accounts for 42 per cent of everything, and covers a wide field, from the detection of prohibited substances in food and tobacco to decisions on the nature of goods for duty purposes. The laboratory also produces evidence in cases of drug smuggling. Research is at present going on into methods of analysis of such spirits as whisky, largely because of the need for certificates of authenticity for export cargoes.

The division dealing with environmental health and safety investigates a large number of possible sources of hazard and carried out twenty-four investigations during 1966, mostly on such topics as pesticides, fluoridation and drug samples. The laboratory endeavours to publish the results of its analyses if, in doing so, any doubts about public safety will be allayed.

Because so much of the work of the laboratory is analytical, it is hardly surprising that a substantial part of the research carried out in the new Research Division concerns analysis techniques and the possibilities of mechanization in this field. There are, however, other topics under consideration. Dental fillings and cement have come under scrutiny, and £14,000 has already been spent in trying to make improvements.

The laboratory received 240,000 different enquiries during the year, on subjects ranging from oysters and sheep dip to preservatives and effluent. To answer these questions, and to keep in touch with the public and maintain laboratories in Cyprus, Singapore and Antwerp, a total of £950,000 was spent.

## Keeping Flukes at Bay

SCHISTOSOMIASIS, caused by infection of blood and internal organs with parasitic flukes, has received much attention in the laboratory, while control measures have been progressing slowly. In a report on the epidemiology and control of the disease, a World Health Organization Expert Committee summarizes the knowledge available in the hope that it will help to co-ordinate the efforts of laboratory and field workers. (*WHO Technical Report Series*, No. 372.)

Flukes of the family Schistosomatidae spend part of their life cycle, the miracidium stage, as parasites of aquatic snails. The free swimming cercaria stage passes into the water and the life cycle continues when the cercaria enters the blood stream of a second host, which may be man. The eggs pass out in the faeces and from them come the miracidia. In tropical regions agricultural schemes which involve irrigation are increasing the prevalence of schistosomiasis; when water is supplied to an area it becomes capable of supporting larger populations of snails and humans, and the possibility of transmission of parasites is greatly increased. In Brazil the disease caused by *Schistosoma mansoni* has spread into the southern states after agricultural development involving irrigation. In Africa there has been an increase in the occurrence of new foci of *S. mansoni* and *S. haematobium* in areas where there are many small systems for improving water supply. In the past schistosomiasis has been referred to as a rural disease, but transmission can now occur in urban areas if conditions are favourable.

Community surveys designed to assess the effect of schistosomiasis on health have shown the situation to be more serious than was realized. Radiological studies have revealed a high prevalence of urological lesions in young people, and the occurrence of the lesions shows good correlation with data concerning egg production. This has necessitated new thinking about infection with *S. haematobium* which was previously thought to be benign.

Control programmes have varying degrees of success according to ecology, budget and available manpower. In some areas the objective may be the modest one of limiting the spread of the infection. In favourable circumstances transmission may be reduced, or the disease may be eradicated. The committee recommends that one of these three objectives be chosen before a control programme is launched. Snails can be controlled most effectively by chemical means. There are several molluscicides available, for example niclosamide and copper sulphate, and the choice depends on the conditions of the habitat. It is essential to distribute the chemical evenly throughout the water so that all snails and their eggs are killed. Some molluscicides have harmful effects on other organisms, and the committee considers that the biocidal aspects of widespread use of molluscicides must be investigated.

Biological control has been successful in Puerto Rico, where the snail *Marisa cornuarietis* has been introduced; it competes for food with the schistosome host *Biomphalaria glabrata* and consumes the eggs of the second snail. Combination of chemical control with this means of biological control has almost eliminated *B. glabrata* in some areas, which suggests that biological control may be very useful if used together with other measures.

## Rubber Magnets

ONE of the most intriguing exhibits at a symposium organized this week by the Institution of Electrical Engineers on magnetic materials and their applications were magnets made of rubber. They were described by Mr A. Edwards, Deputy Managing Director of James Neill and Company (Magnets and Steel), the company which manufactures them.



The rubber magnets consist of ferrite particles compounded with rubber or plastic—efforts to bond crushed magnet alloys have always failed. The rubber base can be loaded with as much as 90 per cent of ferrite by weight without losing its flexibility, and the ferrite retains most of its magnetic properties if the loading is above 80 per cent. At a loading of 92 per cent, for example, the rubber magnet has a remanence of 1,628 gauss, and a maximum attractive power of 25.3 oz./sq. in., quite adequate for a number of purposes. The rubber magnet is produced in the form of a sheet 0.04 in. thick, 18 in. wide and 50 ft. long, and is magnetized on one surface by a pattern of stripes of alternate north and south polarity. In the technique used by James Neill and Company, the alternate stripes of magnetization are each twice as wide as the thickness of the sheet. In this way it is possible to produce a thin sheet of unlimited area which is magnetized all over. Mr Edwards suggested a variety of uses for the magnetic material. It can be used to hold the doors of refrigerators shut, or, more unusually, as a means of attaching symbols to a steel display board. Although the rubber magnets are brown, their appearance can be improved by bonding a layer of polished PVC to the non-magnetized surface. Since the PVC surface accepts screen printing or writing, Mr Edwards hopes that a vast new market in inexpensive magnetic charting or recording boards may be opened up. The sheet is also used in the sumps of engines or gearboxes, to pick up wear particles, and could be used as the stator in small electric motors.

## Handbook for Desalinators

AN investigation of integrated power stations and desalination plants has just been completed by Applied Research and Engineering Limited (AREL)—a subsidiary of the Capper-Neill Group. The report was commissioned by the International Atomic Energy Agency and is due to be published early in 1968. It is a step by step guide to costs which no would-be builder of desalination plants should be without. The costs of the various processes involved in a plant of this kind have been worked out separately, so that the feasibility of a particular scheme can be calculated before an expensive full scale enquiry is begun. Throughout the report, however, it is made clear that individual circumstances can markedly influence costs. The cost of labour, for example, varies from place to place.

Dual plants make economic use of low grade waste heat from electricity generating stations to purify sea water, but their chief advantage is that it becomes economic to build larger power stations than could be justified by the demand for electricity by itself. But scale brings economies. At present the multi-stage flash evaporator is considered to be the only economic process for purifying sea water, and in the report it is studied in combination with three types of nuclear power reactors—light water, gas cooled and fast breeder reactors. The costs of steam in small desalination plants are high, so that for comparison fossil sources of heat are included in these circumstances. A variety of conditions is considered, up to the limiting case in which water is the important product and the electricity produced is just enough to keep the plant running.

When the overall costs have been calculated, they must be allocated between the products. The IAEA has given several possible but purely arbitrary methods in its Technical Report Series (No. 69) and of these AREL has chosen a system the effect of which is to use whatever savings there may be because of economies of scale to reduce the cost of electricity rather than the cost of water. As a result, the cost of water is higher than in some other methods of cost allocation, but the somewhat arbitrary nature of this apportionment of costs is emphasized throughout the report. This, no doubt, is why it is suggested that one authority operating both parts of the plant may be the best solution.

There seems to be very little to choose between different types of reactors. The report includes detailed calculations of the costs of using gas cooled and light water reactors in conjunction with back pressure turbo alternators. For an output of 100 million US gallons of water a day and a power output of 300 megawatts, the costs of water and power produced by the two kinds of reactors are almost identical. The power is reckoned to cost 3.4 mills per kilowatt hour (1 mill, \$0.001) from the light water reactor (exhaust temperature 230° F.) and 3.6 mills per kilowatt hour for the gas cooled type (exhaust temperature 270° F.). The cost of water is 33 cents per 1,000 US gallons for the light water reactor and 35.2 cents per 1,000 US gallons for the gas cooled version. The report also sets out to predict what advantages there will be when fast breeder reactors are in service—both electricity and water may be reduced in cost by 30 per cent.

## A Role for Melanoproteins

from our Microbiology Correspondent

WITHIN the past few years some attention has been devoted to that intractable and so-called secondary group of microbial products, the melanins. Attempts to describe these polyphenolics chemically have been largely unrewarding, while the definitions of their physiological role in micro-organisms have scarcely progressed beyond the stage of intelligent speculation. Now, however, several functions of microbial melanins or melanin-protein conjugates are being discovered. The presence of melanin in the walls of certain fungi appears to be an important factor in resistance to lysis by soil bacteria—a thesis developed most notably by Alexander's group at Cornell University (for example, Bloomfield and Alexander, *J. Bact.*, **93**, 1276; 1967). Again, cell and spore walls impregnated with melanin confer protection against ultra-violet irradiation and there is a frequent correlation between ecological distribution, especially of fungi, and presence of the pigment.

The current issue of the *Journal of General Microbiology* (**48**, 269; 1967) includes a report from R. C. Hignett and D. S. Kirkham on yet another function of microbial melanin—an extracellularly produced melanin or, more precisely, a melanoprotein. These workers have studied the relationship between pathogenicity and culture characteristics of *Venturia inaequalis*, the causal fungus of apple scab. Several fungi, both saprophytes and pathogens included, produce dark extracellular pigments—usually termed "presumed melanins"—when grown in submerged culture. It has been impossible to say whether or not such synthesis is a

cultural artefact, but the results of Hignett and Kirkham, argue against such a conclusion at least in the case of *Venturia*.

The *Venturia* melanoprotein was fractionated on the basis of ethanol solubility and the fraction insoluble in 75 per cent ethanol (N75) was further resolved by gel filtration. The effects of these melanoproteins were studied by injecting them into the petioles of young apple plants and by using non-toxic indicator substances such as glucose labelled with carbon-14. Two important effects were observed. First, there was a marked accumulation of the glucose in the vascular system of the inoculated leaf, whereas the intervenal tissue was unpermeated. The second effect was a strong inhibition of  $^{14}\text{C}$  glucose uptake accompanied by an even permeation throughout the leaf. These effects seem to be specific, for a variety of proteins similarly injected did not elicit these responses.

Later experiments have tested whether metabolite transport in plants infected with fungus is disturbed in the same way as plants treated with melanoprotein. Twelve days after fungal inoculation the labelled glucose was accumulating at the sites of presumptive lesions and two days later the distribution of the glucose and the sporulating lesions were identical. Further evidence for the involvement of melanoprotein in lesion development was provided by its co-inoculation with *Venturia* spores into test plants. A marked stimulation of lesion development occurred when certain fractions of N75 were applied in this way. The melanoprotein fraction responsible for the second effect also has directional properties and prevents movement of metabolites towards the midrib from where they might be transported away from the leaf. Although an accumulation of plant metabolites at lesion sites has been reported in several host-parasite systems, it was not known whether such activity was under the control of host or pathogen. Hignett and Kirkham's observations are therefore an important contribution to the understanding of phytopathogenicity and illustrate a highly sensitive mechanism by which at least one pathogen redirects the metabolism of its host to its own advantage.

## Kinds of Ribosomes

from our Molecular Biology Correspondent

WITH the isolation of mitochondrial ribosomes by Küntzel and Noll, reported last week in *Nature* (215, 1340; 1967), a clearer pattern of the evolution of the ribosome is beginning to emerge. The mitochondrial ribosomes turn out to have a sedimentation coefficient of 73S, and to contain RNA components of 20.5S and 16.4S. They therefore resemble chloroplast ribosomes (67S, with RNA components of 20.8S and 15.7S under the same conditions) and bacterial ribosomes, of which the most familiar example, from *E. coli*, sediment at 70S, with RNA of 21S and 16S. The possibility that chloroplasts and mitochondria enjoy an independent existence within the cell, and that they originated as some type of symbiotic bacteria, has aroused wide interest.

Apart from size, various other systematic differences between the different classes of ribosomes may, however, be noted. Thus bacterial ribosomes contain about 50 per cent of protein, whereas in plant and animal ribosomes this figure seems always to be near 60 per

cent. In terms of base composition of the RNA, animal ribosomes show considerable differences from all others, most noticeably in a consistently higher content of cytosine. It is also relevant that the molecular weight ranges within the above mentioned classes are in fact quite broad—the spread of 66S to 73S in bacterial ribosomes on going from the photosynthetic bacterium *R. rubrum* to *B. licheniformis*, corresponds to a difference of about 25 per cent in molecular weight. (The sedimentation coefficient varies roughly as the square-root of the molecular weight.) Although many of the older data stand in need of revision, and the conditions under which different authors have worked are not always sufficiently alike to permit good comparisons, there is a general tendency, in particular in micro-organisms, of increasing molecular weight with evolutionary status. (Some interesting speculation along these lines is to be found in the article by Küntzel and Noll.)

How these ribosomes differ functionally is a subject of which the surface has so far hardly been scratched, but some interesting experiments on the functioning of heterologous protein synthesizing systems have recently been reported. A revealing study comes from Parisi *et al.* (*J. Mol. Biol.*, 28, 295; 1967), who have examined poly-U directed cell-free systems with components from two disparate species of bacteria, *E. coli* and *B. subtilis* (70S ribosomes), and from a micro-organism (yeast), a higher plant (bean seedlings) and an animal tissue (rat liver), all having ribosomes of the 80S type. Although the combination of ribosomes of one category with the supernatant enzymes from cells of the other kind gave no significant incorporation, there was substantial incorporation whenever components were mixed within one group. The effect was consistent and striking. To show that the limiting factor was not the failure of synthesis of the aminoacyl-tRNA in the heterologous systems, the experiments were also performed with pre-formed phenylalanyl-tRNA. The source of the latter (whether from the one type of cell or the other) had little effect on the efficiency of incorporation of the phenylalanine. It was also demonstrated that the incompatible systems did not degrade the heterologous aminoacyl-tRNA, neither were any specific inhibitors present in the heterologous supernatants, since these did not interfere when added to functional homologous systems. It appears most probable, therefore, that ribosomes recognize only the relevant protein synthesizing enzymes from sufficiently closely related cells. The similarities between ribosomes within the 70S and 80S groups therefore go beyond their common physical characteristics, and well into the realm of function.

## Peptides without Ribosomes

from our Cell Biology Correspondent

ANTIBIOTICS like the gramicidins, tyrocidine and licheniformin are short peptide chains of between 10 and about 40 amino-acids synthesized by spore forming bacteria at certain stages in their life cycle. Apart from their antibiotic activity, these peptides are of very great interest because synthesis of them does not involve ribosomes, mRNA and all the other machinery of protein synthesis. (A group working at the University of Hawaii had claimed that gramicidin is synthesized on ribosomes, but they have recently retracted



this view, having failed to repeat their earlier experiments (Bhagavan *et al.*, *Biochemistry*, **5**, 3844; 1966.) Furthermore, these antibiotic peptides contain either D isomeric forms of amino-acids or amino-acids, such as ornithine, not present in proteins.

If the information for the sequence of the amino-acids in these peptides is not coded in an mRNA molecule, where does it reside? One obvious possibility, suggested by Mach *et al.* (1963), is that the enzymes, or multi-enzyme complexes which synthesize the peptides, have sufficient structural specificity to recognize the unfinished peptide chain and the next amino-acid that has to be incorporated. This hypothesis cannot be tested until an enzyme system has been isolated and purified, and in *Biochemistry* (**6**, 2552; 1967) Tomino *et al.* report some progress in this direction. They have isolated and partially purified an enzyme system from *B. brevis* which, when supplied with the necessary amino-acids, ATP,  $Mg^{++}$  ions and reducing agent, synthesizes the cyclic decapeptide gramicidin S. The system is devoid of ribosomes and is not inhibited by puromycin, chloramphenicol or RNase, which inhibit protein synthesis. Column chromatography resolves the enzyme system into two fractions, I and II, neither of which alone will synthesize gramicidin S or any intermediary peptide. Fraction II appears to be a racemase which in the presence of ATP converts L-phenylalanine into activated D-phenylalanine, which in the presence of both fractions is incorporated into gramicidin S. Added D-phenylalanine is incorporated directly by the complete system, but surprisingly when both D- and L-phenylalanine are present in the incubation mixture it is the L-phenylalanine that is preferentially incorporated after ATP dependent racemization.

Obviously this work is only a beginning. It is not yet known even if splitting of ATP is necessary for the formation of peptide bonds in gramicidin S, but further purification of these enzyme fractions should answer this question and may well show whether or not the sequence specificity resides in the structure of the enzyme complex.

## Planetary Nebulae

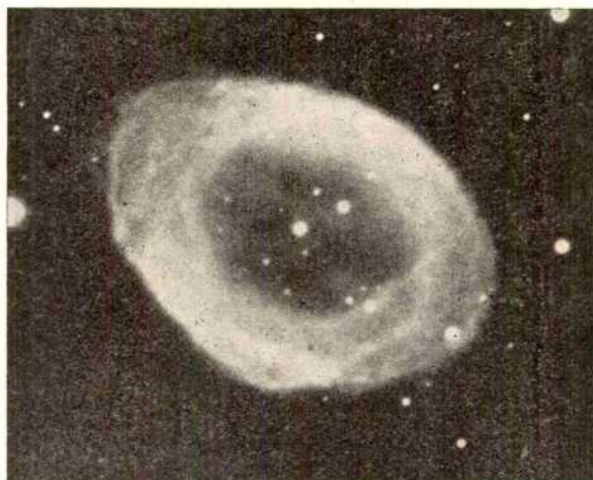
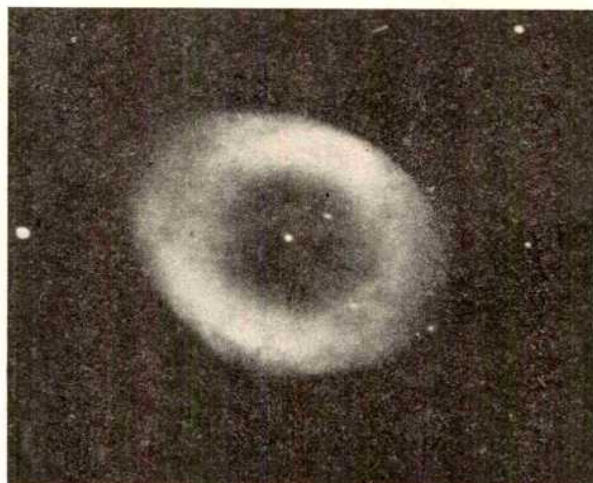
ABOUT 2,000 planetary nebulae have so far been identified in the galaxy. They emit light in various characteristic spectral lines, and when resolved are often seen to have a ring-like or butterfly form. The typical planetary nebula has a mass between 0.1 and 0.5 of the mass of the Sun ( $M_{\odot}$ ) and linear dimensions of order 0.1 parsec. Planetary nebulae are therefore quite different from ordinary HII regions, which are much larger and more massive and which are always associated with a group of O or B stars. A planetary nebula usually has just one exciting star with a luminosity between  $10^2$  and  $10^4$  times that of the Sun and a surface temperature between  $5 \times 10^4$  and  $10^5$  degrees Kelvin.

This kind of star is quite different from an unevolved O or B star. Its mass is somewhat less than a solar mass instead of 20 or 30  $M_{\odot}$ , its radius is much smaller than that of the Sun and its energy source is the transmutation of  $^4\text{He}$  into  $^{12}\text{C}$  rather than the conversion of H into  $^4\text{He}$ . In other words, the central star of a planetary nebula is a highly evolved star, now moving towards the left and near the end of the horizontal branch of the Hertzsprung-Russell diagram. Even

together with its central star, a planetary nebula has insufficient mass to have condensed by gravitation out of the interstellar medium at large; it follows that the star must have formed at some much earlier epoch as part of an association which has now dissolved, and that the material of the nebula must have been ejected at some stage in the history of the central star, perhaps during the evolution from the red giant stage.

Simple observations can lead to a remarkable amount of information about the physical state of a planetary nebula. The first step is to find the typical electron density  $n_e$  by measuring the intensity ratio of the emission lines of  $O^+$  at  $\lambda\lambda$  3726, 3729 Å. The ratio is different at low and at high values of  $n_e$  and is particularly sensitive to change near  $n_e \approx 10^4 \text{ cm}^{-3}$ . Many nebulae are found to have electron densities of about this order.

Next, it is possible to determine the emission measure ( $EM$ ) by observing the surface brightness of the nebula, either in the radio continuum or in the Balmer lines of hydrogen. The  $EM$  is proportional to  $n_e^2 l$ , and so one can deduce a typical linear scale  $l$ ; knowing also the angular size  $\theta$  of the object, its distance  $R (=l/\theta)$  may be deduced. The distance in turn fixes a value for the absolute visual magnitude  $M_v$  of the central star in terms of its apparent magnitude  $m_v$ .



Photographs from the Mount Wilson and Palomar Observatories.

NGC 6720. Planetary nebula ("Ring") in Lyra. Green (top) and yellow (bottom) light. 200 in. Hale.



The nebula may be used as a detector of photons in the far ultra-violet by observing and interpreting the ionization balance of various constituents such as He, He<sup>+</sup>, He<sup>++</sup>, O, O<sup>+</sup>, O<sup>++</sup> and so on. The balance is determined by the luminosity of the central star in the far ultra-violet; having found this luminosity one can estimate the effective temperature of the star.

In another connexion, the distances  $R$  fix the positions of the various nebulae with respect to the galaxy, and it appears that they are distributed like typical members of Population I stars. Further, there may be disagreement between the emission measures as given by radio and optical methods; if so the discrepancy must be ascribed to extinction in the visual range of wavelengths by interstellar dust grains, and observations for different objects lead to information about the distribution of dust in the galactic disk.

The central stars of planetary nebulae follow a well defined sequence of stellar evolution in the Hertzsprung-Russell diagram, and take about  $10^4$  years to move through the range in which they are associated

with planetary nebulae. Theoretical models agree tolerably well with the observed data. At the end of the sequence, the central stars approach the region of the white dwarfs. Exact numbers are not yet agreed, but at least a substantial minority of white dwarfs must have evolved in this way.

In summary, the overall properties of planetary nebulae are now well understood, but this understanding is based on rather primitive theoretical models. For example, it is often assumed that planetary nebulae are spherical in shape, and this is obviously not so. Work should now be started which is more realistic and allows for dynamical effects. For example, it seems that Lyman- $\alpha$  radiation is produced freely in a nebula and cannot easily escape. Does the resulting build-up of radiation pressure have an important mechanical effect? Can one understand the ring-like or butterfly-shaped appearance of many planetary nebulae in terms of some symmetry property of the central star, or of the original nebula? What is the origin of the features with a very fine structure which are seen in so many nebulae?

## Computerized Library of Deep Sea Soundings

by

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More than a million deep sea depth soundings have been assembled in a computerized library which is to be used to make a new average ocean depth model.

THE work reported here has been prompted by the need for an average model of ocean depth for numerical computations on a world-wide basis. Such a model is necessary, for example, in dynamic problems of sea motion such as the study of ocean tides and the propagation of tsunamis, in geodesy in general and in the spherical harmonic analysis of earth topography. A second and more direct use is to provide a more efficient method of compiling and plotting soundings recorded in a variety of units, scales and formats. Once the sounding profiles are digitized, the variety of charts and vertical profiles required in physiographic studies<sup>1</sup> can be prepared by machine. This work, until recently, was all done by hand. A third use relates to quantitative textural analysis of topography (slopes, wavelengths, amplitudes) and to detailed hypsometric studies—subjects now of increasing interest to oceanographers<sup>2</sup>.

We now have access by computer to a world-wide coverage of more than 1 million deep sea soundings. It is the purpose of this article to provide a general account of the methods used in digitizing, processing and evaluating these data, and to indicate some of the applications for this library.

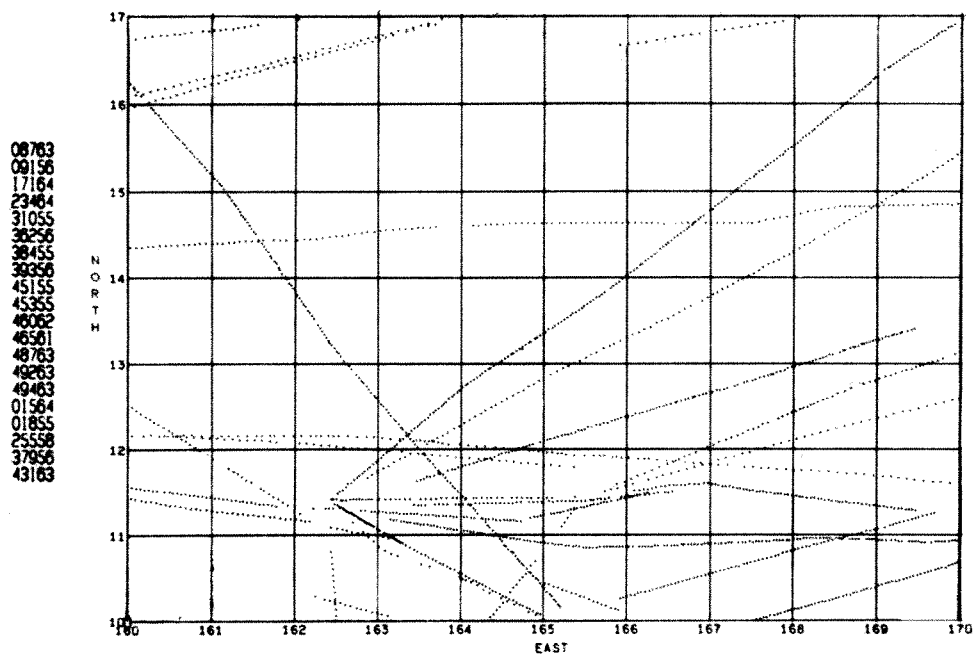
In 1961, one of us<sup>3,4</sup> devised a method to use irregularly spaced soundings to compute average ocean depth values for the areas prescribed by any regular grid. To test the method, the sounding nearest the intersection of each 1° grid line (where available) was selected from hydrographic

charts and bathymetric maps and used to compute the average depth for each 1° square on a Mercator grid. This provided a tentative model ocean for numerical computations of global, geophysical character. The results were so encouraging that we decided to launch a large scale project for computing improved depth averages. After considering the number of soundings available for the world ocean, we decided that a model based on 1 million points was a realistic initial goal.

Since the 1920s virtually all deep-sea soundings have been obtained with echo sounding methods. The echo soundings collected in the data libraries of the US Naval Oceanographic Office, the Woods Hole Oceanographic Institution, the Scripps Institution of Oceanography, the Lamont Geological Observatory and other oceanographic institutions are recorded in units of 1/400 sec travel time; this unit is known as the standard unit ( $t_s$ ) or the nominal fathom. To obtain a value approaching true depth, a correction for the geographic variation in vertical sound velocity is made according to a standard table, and these corrections may be as large as 600 m. Such velocity corrected depths are not completely accurate because of imperfections in the tables, seasonal variations and other factors. Soundings recorded on charts by the British Commonwealth hydrographic departments usually include such corrections. In addition to these general procedures, individual institutions or individual workers throughout the world have introduced other units equal to 1/420, 1/750, 1/725 sec and have adopted tables other than the standard one to obtain "corrected depths". Because all

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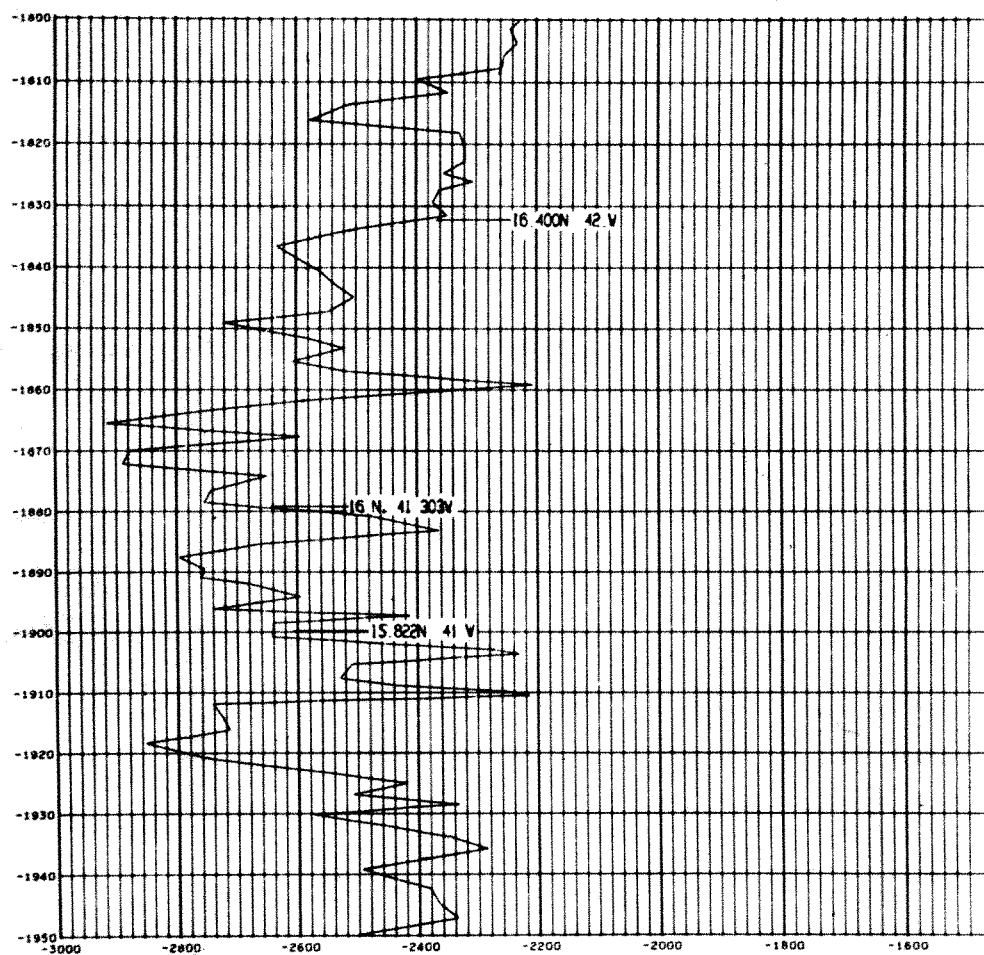
0469 2003N

05/13/67

COMPOSITE MINIATURE MAPS

UNCORRECTED FATHOMS

Fig. 1. Computer plotted track index for a single source sheet. The track identification numbers are listed on the upper left. The library number (0469) of the sheet and area designation (2003N) are shown in the lower left. Such plots are used in checking for position errors and omissions in the verification process.



0143 V- 9 0503N 9 F 04/13/66 FRAME 13

Fig. 2. Representative computer plotted vertical profile. Useful in checking for depth errors. Such profiles are a convenient way for presenting the essential information required in physiographic studies of the ocean floor. Depth (horizontal co-ordinate) in fathoms, distance (vertical co-ordinate) in nautical miles. Vertical exaggeration is 100 : 1.

echo soundings are measurements of time, accurate information concerning the units and corrections applied and the type, scale and ultimate accuracy of the recorder used is essential if soundings obtained from various sources are to be combined.

We used as the basic source information for our digital library the soundings on the master plotting sheets for deep sea soundings maintained by the US Naval Oceanographic Office, the Lamont Geological Observatory, the hydrographic departments of the United Kingdom, South Africa, Australia, New Zealand, the Netherlands and Germany.

Each sounding line was inspected for completeness and accuracy of position and depth, and assigned a rating based on general quality. Precision depth measurements accurate to 1 standard unit ( $t_e$ ), located by methods accurate to better than 1 nautical mile and recorded on charts with a spacing between soundings of no more than 2 miles, were given the highest rank. Soundings of unknown origin, scattered soundings, and soundings of known origin where accuracy was less than 100  $t_e$  or spacing more than 15 miles were in general not included in the library.

Soundings were recorded on punched cards with semi-automatic instruments known as  $x$ - $y$  co-ordinate digitizers with a precision for recording co-ordinates of 0.001 in. In this procedure, the recording head was set over the sounding to be read from the plotting sheet. Its depth value was punched by operating a keyboard and its co-ordinates were recorded automatically. The whole process is subject to human error, so verification is imperative. To do this all digitizations were done twice, preferably by two different operators. The two sets of digitized data were then checked one against the other for agreement within predetermined limits of tolerance. This procedure has the advantage that the verification can be done completely automatically on an electronic computer. Initially we used an IBM 7094 computer and later an IBM System 360 Model 75.

For a quick check on position errors, the tracks were plotted either at a reduced scale or at the original scale, and inspected for errors (Fig. 1). By examining computer-plotted vertical profiles gross discrepancies and inconsistencies were eliminated. Position plots and profiles of the digitized tracks were generated with the aid of a cathode ray tube plotter ( $s-c$  4020). This device, peripheral to the computer, reads computer-generated magnetic tape from which it produces the plots and depth profiles on 7.5 × 7.5 in. frames. Position plots and vertical profiles of all tracks are incorporated in the bathymetric library.

The results of the recording, after verification and corrections, are stored on magnetic tape. For each sounding, the following data are recorded: (1) Co-ordinates—latitude and longitude. (2) Sounding (as

recorded on source sheet) and units ( $t_e$ ,  $fm$ ,  $m$ , etc.). (3) Source sheet number. (4) Source track documentation number. (5) Source country. (6) Reliability rating.

We use an auxiliary programme for applying or removing corrections for the velocity of sound according to Matthews's tables<sup>5</sup>.

The library now includes over 1 million points from more than 8,000 track segments originally plotted on more than 2,000 source sheets. For every individual sounding-track segment a location plot is included in the library, as well as lists of co-ordinates, depth values, and pertinent source and technical data for each sounding. Vertical profiles have been prepared at an exaggeration of 100:1 for more than 2 million miles of sounding tracks (Fig. 2).

Such a large library necessitates an extensive index. Sounding tracks have been compiled for more than 700 plotting areas according to the US Naval Oceanographic Office numbering system. The library includes lists of all tracks for each area as well as complete listings of source information, including original scales, serial numbers and dates entered. Data from the expeditions of the Lamont Geological Observatory and the US National Science Foundation's research vessel USNS Eltanin are continuously fed into the library. Since 1966, all soundings data acquired by the US Naval Oceanographic Office have been routinely digitized with a system compatible with our library, and survey ships have recently begun collecting bathymetric data in digital form. This new information can be quickly incorporated by machine with data already in the library.

The data library will now be used to construct a new average ocean depth model for use in tidal studies and other global applications. It has already provided plots and profiles for deep sea physiographic studies and will be employed in further quantitative studies of submarine topography.

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We thank Troy Holcombe for valuable assistance.

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## The Making of a Scientist

by

H. A. KREBS

Scientists are not so much born as made by those who teach them research, which argues for the perpetuation of centres of excellence. This was the theme of this address by Sir Hans Krebs at the inauguration of the Department of Biochemistry at the University of Newcastle upon Tyne earlier this year.

I BECAME interested in my subject because students have asked me from time to time: "How does one become a Nobel laureate?" I have never before attempted to answer this question because I felt unable to offer an

impromptu comment, but when the same question repeated itself I began to reflect on possible answers.

First, I must criticize the question as not being quite appropriate. What is appropriate is the related question:

"How can distinction, or excellence, be attained in science?". Nobel awards are to some measure a matter of good luck, because their number is too small to do justice to all who would merit an award. A methodical way of finding an answer to the modified question is to study the history and characteristics of scientists of distinction. For this purpose I need a convenient criterion of distinction and, despite what I have just said (and despite some personal embarrassment), I will use the Nobel award as a mark of distinction, for want of a better criterion.

If I ask myself how it came about that one day I found myself in Stockholm, I have not the slightest doubt that I owe this good fortune to the circumstance that I had an outstanding teacher at the critical stage of my scientific career, when from my twenty-fifth to my twenty-ninth year I was associated with Otto Warburg in Berlin. He set an example in the methods and quality of first-rate research. Without him I am sure I would never have reached those standards which are a prerequisite for being considered by the Nobel Committees. I will say a few words later on what in particular I feel I learned from him, but before doing this I would like to examine to what extent the importance of an outstanding teacher applies to other Nobel laureates.

Warburg himself was a Nobel laureate in 1931. He received the prize for his work on the chemical nature of a key enzyme in the reactions between molecular oxygen and foodstuffs in cellular respiration. I was lucky to witness this work from the closest quarters and to take a subsidiary part in it. What were the origins of Warburg's standards? In an autobiographical note<sup>1</sup> which he wrote in 1964, he remarked that: "the most important event in the career of a young scientist is the personal contact with the great scientists of his time. Such an event happened to me in my life when Emil Fischer accepted me in 1903 as a co-worker in protein chemistry. During the following three years I met Fischer almost daily and prepared, under his guidance, the first optically active peptides". So Warburg's experience and views are very much the same as my own. Let me follow up the story further.

Emil Fischer, Warburg's teacher, was one of the most outstanding chemists of his time. He was awarded a Nobel Prize in 1902 for his work on the chemical structure of sugars, the first of his long series of great achievements. Fischer in turn was a pupil and prolonged associate of another Nobel laureate, Adolf von Baeyer, who received the Nobel Prize after Fischer in 1905, for his discoveries in the field of the chemistry of dyestuffs, in particular for the synthesis of indigo.

## Teachers

Since Nobel awards began only in 1901 this criterion of excellence cannot be used for the assessment of excellence in the nineteenth century, but the scientific "genealogy" of earlier teachers and pupils in Fig. 1 shows that von Baeyer was a pupil of Kekulé (famous for his contributions to the structure of organic compounds, especially the ring structure of benzene), and that Kekulé was a pupil of Liebig (who laid the foundation of organic chemistry). Evidently there was also an association with very distinguished teachers in the earlier generations of scientists; had Nobel awards existed in their time, Liebig and Kekulé would certainly have been laureates.

Liebig has provided his own testimony on the importance of a great teacher. He was a pupil of the French chemist Gay-Lussac, the discoverer of some of the fundamental laws of the behaviour of gases. At the time of Gay-Lussac and the young Liebig, Paris was the centre of Continental science and of Continental chemistry in particular. Liebig worked under him in Paris and referred to this experience<sup>2</sup> in the following terms. "The course of my whole life was determined by the fact that Gay-Lussac accepted me in his laboratory as a collaborator

and pupil." This is almost the same wording as that of Warburg, written 100 years later. Gay-Lussac was in turn a product of the great French school of chemists, including in particular Berthollet, who pioneered in the concepts of combustion (abandoning the phlogiston theory in favour of the role of oxygen) and elucidated the chemistry of chlorine, ammonia and hydrocyanic acid. One of Berthollet's teachers was Lavoisier.

In every case the association between teacher and pupil was close and prolonged, extending to the mature stage of the pupil, to what we would now call post-graduate and postdoctoral levels. It was not merely a matter of attending a course of lectures but of researching together over a period of years.

## Genealogy

So my scientific "genealogy" as summarized in Fig. 1 drives home the point that, in many instances, distinction breeds distinction or, in other words, distinction develops if nurtured by distinction. This is further borne out very forcibly by a consideration of a more extended family tree of scientists. Fig. 2, derived from a chart exhibited in the Munich Museum of Science and Technology (Deutsches Museum), summarizes the genealogy of the Nobel laureates descended from von Baeyer, the pupil of Liebig, and this includes seventeen names. Outstanding discoveries can be associated with all the names. A fuller chart<sup>3</sup>, beginning two generations earlier with Liebig, contains more than 60 exceptionally distinguished names and includes more than 30 Nobel laureates.

Seeing this kind of agglomeration of laureates within a scientific family, the sceptic might well suspect a bias in favour of giving prizes to pupils of laureates. In short, does nepotism play a part in the awards? I hope everybody will agree that the answer to this question is an emphatic "No". The high standing, in the eyes of the world, of Nobel awards is derived from the general recognition of the absolute integrity of the Nobel Committees, and from the knowledge that these committees take a tremendous amount of trouble in finding the most worthy persons.

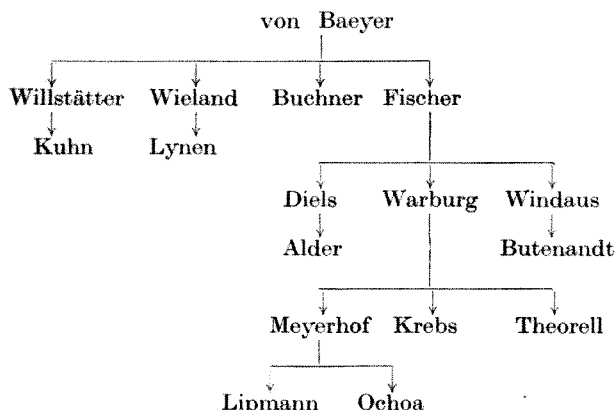
What, then, is it in particular that can be learned from teachers of special distinction? Above all, what they teach is a high standard of research. We measure everything, including ourselves, by comparisons; and in the absence of someone with outstanding ability there is a risk that we easily come to believe that we are excellent and much better than the next man. Mediocre people may appear big to themselves (and to others) if they are surrounded by small circumstances. By the same token, big people feel dwarfed in the company of giants, and this is a most useful feeling. So what the giants of science teach us is to see ourselves modestly and not to overrate ourselves. This is a general point.

Let me now try to be more specific and quote what individuals have themselves thought about the influence of their teachers. Warburg<sup>1</sup> in his autobiographical

Fig. 1. SCIENTIFIC GENEALOGY

Berthollet	1748-1822
↓	
Gay-Lussac	1778-1850
↓	
Liebig	1803-1873
↓	
Kekulé	1829-1896
↓	
von Baeyer	1835-1917
↓	
E. Fischer	1852-1919
↓	
Warburg	1883 (born)
↓	
Krebs	1900 (born)

Fig. 2. GENEALOGY OF THE VON BAEYER "FAMILY"  
The arrows indicate the teacher-pupil link. All members  
of this "family" are Nobel laureates



note summarized this with reference to his association with Emil Fischer: "I learned that the scientist must have the courage to attack the great unsolved problems of his time and that solutions can usually be forced by carrying out innumerable experiments without much critical hesitation." If I try to summarize what I learned in particular from Warburg I would say he was to me an example of asking the right kind of question, of forging new tools for tackling the chosen problems, of being ruthless in self-criticism and of taking pains in verifying facts, of expressing results and ideas clearly and concisely and of altogether focusing his life on true values. An earlier witness on this question of what one learns from an outstanding teacher was Kekulé who, in 1890, when he was 61, remarked that above all he learned from his teacher Liebig the habit of hard work. He related<sup>4</sup> that Liebig had told him, "If you wish to be a chemist you must be willing to work so hard as to ruin your health. He who is not prepared to do this will not get far in chemistry nowadays". Kekulé added, "For many years four, or sometimes even three, hours of sleep were enough for me". Kekulé, of course, went a bit too far, quite a lot too far, but I do think there is a great deal of truth in attaching importance to the capacity for very hard work.

### Opportunities

A recent witness on this question of what distinguished teaching can convey is Jacques Monod<sup>5</sup>, who received a Nobel Prize in 1965. In his Nobel Lecture he commented on the importance to him of a Rockefeller Fellowship which gave him the opportunity to work at the California Institute of Technology in the laboratory of Morgan. He describes the influence which the contact with the distinguished people meant to his development as a scientist: "This was a revelation to me—a revelation of what a group of scientists could be like when engaged in creative activity, and sharing it in constant exchange of ideas, bold speculations and strong criticisms: it was a revelation of personalities of great stature such as George Beadle, Sterling Emerson, Bridges, Sturtevant, Jack Schultz and Ephrussi, all of whom were then working in Morgan's Department." Morgan was at that time a Nobel laureate and Beadle became one later.

There is one more witness I want to quote in connexion with the special qualities of what leaders in a subject can teach. This is Otto Loewi, who was a Nobel laureate in 1936, a pharmacologist and physiologist. He said this about the leading physiologists of the nineteenth century and their influence on their pupils<sup>6</sup>: "They shared to the highest degree the qualities of contagious enthusiasm, broadmindedness and imagination, humility and deep devotion to their pupils. These are qualities which in themselves suffice to attract outstanding students. . . .

Besides the art of experimenting and observing, the pupils learned the ways of thinking required by science. They learned how to select the object to be explored, how to interpret and evaluate the results obtained, and how to integrate them into the whole body of knowledge. In this way students were not only made familiar with methods and facts, but were imbued with the general scientific spirit which shapes the pattern of the true scholar and investigator."

So, above all, attitudes rather than knowledge are conveyed by the distinguished teacher. Technical skills can be learned from many teachers and, like a modicum of intelligence, are, of course, prerequisites for successful research. What is critical is the use of skills, how to assess their potentialities and their limitations; how to improve, to rejuvenate, to supplement them. But perhaps the most important element of attitude is humility, because from it flows a self-critical mind and the continuous effort to learn and to improve. Also of great importance is the enthusiasm conveyed from teacher to pupil: it is the root of a large capacity for work; it makes the research worker look on research not as work but as a hobby and it also induces him to say "No" when he is faced with tempting diversions leading him to the "corridors of power" or travel on innumerable trips abroad.

### Question

I have referred to the importance of asking the right kind of question in choosing a research problem, avoiding those which may give a quick result and concentrating on those which are really worth while tackling. Paul Weiss<sup>7</sup> remarked: "The primary aim of research must not just be more facts and more facts, but more facts of strategic value". By strategic value he meant that an observation or an experiment should lead to the clarification of a problem or deeper insight into a phenomenon, or to the linking of previously unrelated facts and ideas. Goethe<sup>8</sup> expressed the same idea much earlier: "Progress in research is much hindered because people concern themselves with that which is not worth knowing, and that which cannot be known". Medawar<sup>9</sup> has recently stated very succinctly: "If politics is the art of the possible, science is the art of the soluble". How to select worthwhile soluble problems and how to create the tools required to achieve a solution is something that scientists learn from the great figures in science rather than from books.

I would like to underline, on the basis of my own experience, what Monod said about the importance of belonging to a group of scientists such as he found in the California Institute of Technology. Association with a leading teacher almost automatically brings about close association with outstanding contemporaries of the pupil because great teachers tend to attract good people. Students at all levels learn as much from their fellow students as from their seniors and this was certainly true in my own case. Warburg's laboratory at Dahlem, where I served my apprenticeship, was surrounded by other centres of distinction. It was in the same building as Meyerhof's laboratory and the contacts between the two biochemical groups were very close. My own contemporaries included many young people who later became outstanding scientists. There were Ochoa and Lipmann, who became Nobel laureates. There was Lohmann, who discovered ATP and the structure of co-carboxylase; there was Karl Meyer, who discovered hyaluronic acid; there were Hans Gaffron, David Nachmansohn, Dean Burk, Frank Schmitt, Ralph Gerard and Hermann Blaschko. Among the numerous other outstanding scientists working within a few hundred yards, and getting together regularly at the weekly colloquia, were Neuberg, Hahn, Meitner, Haber, Polanyi and Bonhoeffer.

There are many other examples of such centres of excellence and breeding grounds of scientists. Cambridge, for example, was a centre of excellence in physiology and



biochemistry in the early decades of this century because Foster, Langley, Hopkins, Barcroft and Adrian were each surrounded by a group of enthusiastic young people of great ability. Cambridge, of course, at the same time was also a centre of excellence in physics, thanks to J. J. Thomson and Rutherford.

No doubt Cambridge and Oxford owe some of their special standing to their size, which made it possible to assemble broadly based groups in a single subject at a time when provincial universities were usually restricted to very small departments with little scope for the cross-fertilization which occurs in the larger groups. It is gratifying to see the recent developments in the provincial universities which have removed this restriction and go a long way in providing a first rate environment.

What I have said so far is not merely a matter of historical reflexions. There are lessons to be learned, in particular by policy makers in the universities who aim at making universities into centres of excellence. As excellence in research is one of the main ultimate roots of all academic excellence, including that of undergraduate teaching, universities ought to do everything in their power to create opportunities for first rate research work by their staff. But do they? Or, being willing, are they given the means, in terms of facilities and cash, to do so?

### Leadership

In the course of this century there have been only two *really* fundamental advances in the sciences: the first was in the field of atomic physics, leading to the creation of quantum mechanics and the release of atomic energy. The second was in biology where the fusion of biochemistry, biophysics and genetics to form molecular biology has led to an understanding of basic biological phenomena which, only a generation ago, seemed beyond the reach of science altogether. When we compare the circumstances which led to these two great advances we find, as Max Delbrück<sup>10</sup> has pointed out, remarkable differences in the manner in which they have been achieved. Atomic physics was created almost exclusively within the framework of traditional university institutions, whereas in biology the modern developments have not come from the traditional departments of biology. They are largely the results of the efforts of chemists, physicists and biologists, who frequently worked in non-biology departments, and outside the universities. In Britain, decisive advances associated with the names of Wilkins, Crick, Watson, Perutz and Kendrew were made in the Medical Research Council units in London at King's College and at Cambridge, and both these units, financed by the Medical Research Council, were placed in physics and not in biology laboratories. In France, the decisive contributions associated with the names of Lwoff, Monod and Jacob came from the Pasteur Institute, an institution not controlled by a university. In the United States the Rockefeller Institute was a major contributor, through the work of Avery, MacLeod and McCarty, to the new developments. It is indeed most remarkable that universities allowed the initiative in advancing the frontiers of knowledge to slip out of their hands in this way.

The loss of leadership in science by the universities is also borne out by statistics of the Nobel awards to British scientists, which are shown in Table 1. Out of 18 British awards since 1950, only 10 laureates have earned their awards when holding university appointments—and at least one of them, myself, had a privileged appointment with very light teaching and administrative duties at the critical time. The statistics become even more telling when they are limited to the more recent times. Since 1960 only three Nobel awards went to the universities in Britain and five to non-university scientists (and this includes the physical sciences). In this table "other centres" means, in every case except one, the Medical Research Council's units. The exception is A. L. Hodgkin at Cambridge who holds a full-time research professorship of the Royal

Society. In comparing these figures one has to bear in mind that the financial resources of the universities are very much greater, as a whole, than those of the Medical Research Council or the Royal Society. The funds at the disposal of the Medical Research Council were rather less than 5 per cent of those available to the universities, and universities employ probably more than 10 times as many scientists as the Medical Research Council. In spite of this handicap the Medical Research Council has a much larger share in the number of Nobel laureates.

Table 1. BRITISH NOBEL AWARDS SINCE 1950

Universities (10)		Other centres (8)	
C. F. Powell	(1950)	A. J. P. Martin	(1952)
J. D. Cockcroft	(1951)	R. L. M. Synge	(1952)
E. T. S. Walton	(1951)	F. Sanger	(1958)
H. A. Krebs	(1953)	F. M. Perutz	(1962)
M. Born	(1954)	J. C. Kendrew	(1962)
C. N. Hinshelwood	(1956)	F. H. C. Crick	(1962)
A. R. Todd	(1957)	M. H. F. Wilkins	(1962)
P. B. Medawar	(1960)	A. L. Hodgkin	(1963)
A. F. Huxley	(1963)		
D. C. Hodgkin	(1964)		

Another illustration of this trend is provided by the statistics of the Fellowship of the Royal Society. Of 32 Fellows elected in March 1967, only 13 did their decisive work in the universities and some of these 13 were again in privileged positions within the university, occupying research posts without teaching commitments.

Why then have the universities lost their leading position in research? I believe the answer is simple. There is plenty of potential talent in British universities to achieve distinction in science; what is lacking is simply time. Real research of a fundamental character requires a tremendous amount of time. It cannot be done at odd spare moments, nor can it be delegated to technicians or PhD students. The trouble is that senior and junior academic staff tend to be grossly overloaded with teaching, administration and college administration—in particular at Oxford. This overloading often begins at a very early stage of the academic career and leaves junior people insufficient time to mature during the postdoctoral stage. What scientists need for maturing are, I think, several postdoctoral years of essentially full-time research before they embark on teaching on a major scale.

### Policies

Another illustration of the importance of time for establishing academic standing is the relatively large number of university professors supplied by Medical Research Council establishments. Between 1961 and 1966, no fewer than 42 Medical Research Council staff went to universities to take up professorial appointments. This was possible because the Medical Research Council provides opportunities that universities cannot provide, giving scientists, above all, enough time. Thus Medical Research Council establishments have proved to be very effective breeding grounds for scientists suitable for senior university posts. I ought to emphasize that it is quite wrong to blame the Medical Research Council (as has been done) for keeping some excellent people away from the universities, when these people, after maturing, return to the universities well prepared for senior appointments.

Research, unlike routine jobs such as teaching or doctoring or administration, needs a minimum critical effort to be effective, and this minimum is very demanding in time. I have often heard it said by those university people who do not know what scientific research means, "Well, if you only have half the amount of time you feel you ought to have, cut down your research by half. What does it matter?"

This reasoning is false. It is like the idea that in order to cut down the noise of an aeroplane engine the speed of the engine should be reduced. Up to a limited point, of

course, this works and the aircraft just travels more slowly. But soon there comes a point when it will no longer remain airborne. At low engine speed it can still taxi along the ground, but that is all.

Scientific research requires a high minimum critical momentum. Effectiveness in research is not just proportional to the effort. The scientist who has insufficient time may manage to taxi along over well ploughed grounds but he will have the greatest difficulty in becoming airborne—doing something really new and original. On the other hand, once he has gathered momentum he will soon find himself in new and unknown territory. One of the most effective ways of attaining a powerful momentum is belonging to a team. Contrary to what some may feel, membership of a team does not at all imply loss of individual scope, of individual initiative, of individual achievement, of individual recognition. What the team provides is a background of aggregate skill, experience and help. This background forms the starting point for individual enterprise.

In the last resort, then, the reason for failing to obtain excellence, in spite of great potentialities, is in many cases the circumstance that those responsible for the organization of the lives of scientists rob them of time.

All this leads to the large question of whether our universities today do as much as they ought to in providing centres of excellence in science, a matter taken for granted a generation ago. In many American universities this is a frequent subject for discussion, and it is perhaps significant that the present United States Secretary for Health, Education and Welfare, John Gardner<sup>11</sup> (formerly President of the Carnegie Foundation for the Advancement of Teaching), has written a provocative book called *Excellence* with the sub-title "Can we be equal and excellent too?". I am not at all sure whether our main financial sponsors, the University Grants Committee and in particular the Treasury, give sufficient thought and

money to the importance of cultivating excellence in the universities; to the fact that in science, teaching and research always go together and that in this age of science the cultivation of excellence in science is not an academic exercise but a source of economic and political strength.

My own apprehensions are naturally influenced by my personal experience at Oxford where, under the banner of equality and democracy, circumstances operate powerfully against the development of excellence in science. In quite a few spheres of the life of this country I fear we have too much equality and too little promotion of excellence. At Oxford very few of the excellent young scientists are given a chance to develop their potentialities in scientific research, merely because they are deprived of the time. A large number of promising and distinguished scientists have for these reasons left Oxford or refused appointments there. This might benefit other British universities if they can show themselves more sympathetic or able to help them, but lack of opportunities, especially in terms of time, has also contributed towards the "brain drain".

Unless we in the universities are aware of these problems and continuously strive for the maintenance of high standards, we are bound to deteriorate. This is a matter of general concern to university people.

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## Structure of N-terminal Fragments of Fibrinogen and Specificity of Thrombin

by

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Determination of the amino-acid sequences of N-terminal fragments from human fibrinogen A and B chains shows that this region of the molecule is highly cross-linked with disulphide bridges and suggests why the proteolytic action of thrombin is so highly specific.

THE fibrinogen molecule is built up from three peptide chains, A, B and C (refs. 1-9). The molecular weight of 340,000 determined for the protein<sup>10</sup> is for a dimeric form of the molecule. The formation of fibrin threads is preceded by a limited proteolysis of the fibrinogen molecule, resulting in the release of fibrinopeptides A and B, respectively, from the N-terminal end of the A- and B-chain of fibrinogen<sup>11-14</sup>. The enzyme causing this limited proteolysis is thrombin. In its proteolytic action on fibrinogen thrombin has a narrow specificity of action. On synthetic substrates of low molecular weight, on the other hand, its action closely resembles trypsin; for example, it cleaves compounds like tosylarginine methyl ester<sup>15</sup>. When acting on fibrinogen only a few bonds are rapidly hydrolysed. These are the arginyl-glycine bonds linking the

fibrinopeptides to the rest of the fibrinogen molecule. Some other arginyl and possibly also lysyl bonds in fibrinogen may be split by the enzyme, but apparently at a much slower rate (compare ref. 16).

It has been suggested that the fibrinopeptides might contain structural features which are partially responsible for the narrow specificity of the enzyme<sup>8,16-20</sup>. Structures which might favour a rapid association between enzyme or substrate (or possibly activate the enzyme) are present in the C-terminal part of fibrinopeptide A (refs. 16, 17 and 20). This idea has mainly arisen from the fact that the C-terminal part of fibrinopeptide A has been essentially unchanged during mammalian evolution. Furthermore, it has been shown that fibrinopeptides inhibit thrombin action<sup>13,17</sup>. It should also be mentioned that when cross-

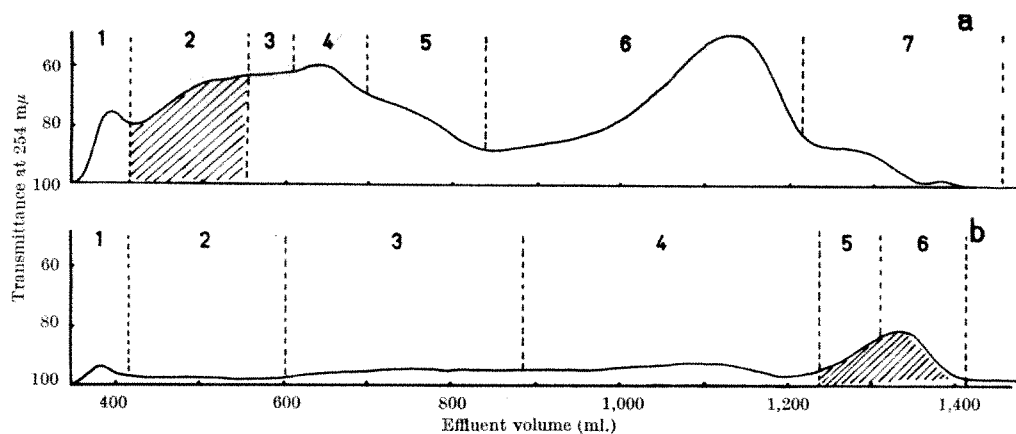


Fig. 1. Elution pattern of fibrinogen treated with cyanogenbromide on 'Sephadex' before (a) and after reduction and alkylation (b). Column: 'Sephadex G-100', 20-28 cm<sup>2</sup> × 66-70 cm. Flow rate: 60 ml./h. Equilibration and elution with 10 per cent acetic acid in a and 10 per cent acetic acid containing 5 per cent ethanol in b. The dashed part of the chromatogram shows the main fraction which on incubation with thrombin released fibrinopeptide A. Small amounts of this peptide were also released from fractions 1 and 3. (The conditions for digestion were: 1 per cent peptide in 0.2 molar ammonium bicarbonate, pH 8.5, 17-35 NIH units/ml., 37° C, 1 h.)

reacting thrombin and fibrinogen from several mammalian species, thrombins from different species show remarkably small differences in their clotting action on fibrinogen from one species (ref. 21 and unpublished observations of A.-C. Teger-Nilsson).

The release of fibrinopeptide A from fibrinogen seems to be a *sine qua non* for the polymerization of fibrinogen units. In all the species so far investigated the release of the B-peptide is slower than that of the A-peptide and its release appears to be of no importance at least in the initial polymerization (compare ref. 16). Comparative studies show that the B-peptide has been subjected to more changes than the A-peptide during evolution<sup>18,20</sup>.

Thus there is some evidence that in the clotting process the interaction of thrombin is determined mainly by structures in the fibrinopeptides; however, in any discussion of thrombin action it would be injudicious not to take into consideration the adjacent structures on the other side of the bonds split, let alone the complex conformation of the whole fibrinogen molecule. In the present report the amino-acid sequences around the bonds split by thrombin in human fibrinogen and their implications are discussed.

A preliminary step in the study was the isolation of N-terminal fragments of the A-chain and B-chain of fibrinogen containing the covalently linked fibrinopeptides A and B.

A fragment containing fibrinopeptide A can be isolated, after digestion of sulphitolyzed fibrinogen with plasmin, by gel-filtration, column chromatography and electrophoresis<sup>22</sup>. The fragment is composed of 43 amino-acid residues. Thus it contains 27 amino-acid residues in addition to the 16 in the A-peptide. This plasminic fragment does not contain methionine. Thus it should be possible to obtain a still longer N-terminal fragment of the A-chain by treating fibrinogen with cyanogen bromide (CNBr)<sup>23</sup>. Such a fragment was in fact produced by cleavage of native fibrinogen dissolved in 70 per cent formic acid<sup>24</sup> with cyanogen bromide. The reaction products were partially separated on a 'Sephadex G-100' column equilibrated with 10 per cent acetic acid (Fig. 1a). Fraction 2, which on treatment with thrombin released both fibrinopeptide A and B, was reduced with mercaptoethanol and afterwards alkylated with iodoacetic acid<sup>25</sup>. Excess reagent was removed by gel filtration ('Sephadex G-10' equilibrated with 10 per cent acetic acid). The S-carboxymethyl peptides were again fractionated on 'Sephadex G-100' equilibrated with 10 per cent acetic acid containing 5 per cent ethanol (Fig. 1b). Fractions 5 and 6 (Fig. 1b) were the only fractions which released appreciable amounts of fibrinopeptide A on digestion with thrombin. These fractions could be further purified by re-cycling gel

filtration<sup>26</sup> on 'Sephadex G-100', or by partition chromatography on propoxy-'Sephadex'. Amino-acid analyses indicate that the purified peptide preparation, 'mother peptide A', contains 50 amino-acid residues (Table 1). It is seven amino-acids longer than the fragment obtained from a plasminic digest. The small amounts of threonine and isoleucine (see Table 1) are most likely derived from a contaminating peptide which has so far proved extremely difficult to separate from the main peptide.

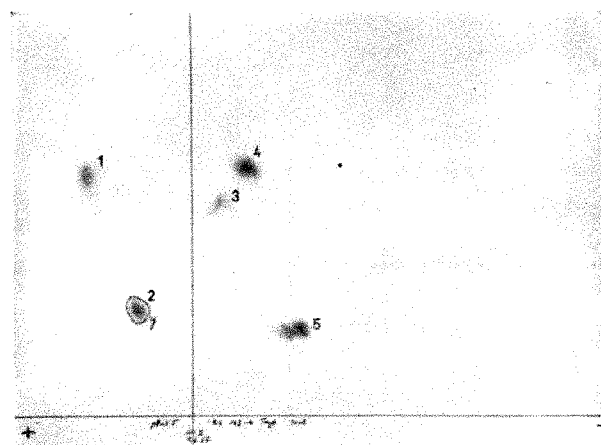


Fig. 2. Two-dimensional electrophoresis chromatography<sup>27</sup> of the valyl-peptide, T2 (see Table 1). High voltage electrophoresis was run in pyridine : glacial acetic acid : water (100 : 35 : 4,865, v/v), pH 5.5 at 2,000 V, 55 m.amp, for 2.5 h in a horizontal apparatus (AB Analysteknik, Valentuna, Sweden) cooled with tap water. Descending chromatography in n-butanol : pyridine : glacial acetic acid : water (150 : 100 : 30 : 120, v/v) at 20° C for about 12 h. The peptides were stained with ninhydrin. y, Yellow spot.

Alanine was found to be the predominant N-terminal amino-acid of mother peptide A, but there were also traces of aspartic acid and asparagine. The amino-acid sequence of the first nine residues from the N-terminal end of this material, determined by the Edman (compare ref. 8) procedure, was identical to that of human fibrinopeptide A. Hence we concluded that the mother peptide A was mainly derived from the N-terminal end of the fibrinogen-A chain.

The peptide preparation was susceptible to the action of thrombin; fibrinopeptide A (ref. 8) was released on incubation with the enzyme (0.4 per cent peptide in 0.15 molar ammonium bicarbonate, adjusted to pH 8.5 with solid CO<sub>2</sub>, was digested with thrombin (350 NIH\* units/

\* National Institutes of Health.

mg) at a concentration of 78 NIH units/ml., for 3–4 h at 37°C. In addition to fibrinopeptide A its analogues fibrinopeptide AP and Y (ref. 8) were released. The presence of small amounts of fibrinopeptide Y in the material explains the occurrence of traces of N-terminal aspartic acid found in addition to the main N-terminal alanine. From these results we concluded that the peptide preparation essentially consists of the N-terminal ends of the A-, AP- and Y-chains of human fibrinogen.

Electrophoresis and N-terminal analysis of the thrombin digest of mother peptide A revealed that thrombin had produced two other fragments in addition to fibrinopeptide A and its analogues. One was a basic tripeptide (T1) (Table 1) with the sequence Gly-Pro-Arg and the other a peptide (T2) containing 31 amino-acid residues (Table 1) having the following partial sequence: Val-Val-Glu-Arg-His-GIN-Ser-Ala-Cys-Lys-Asp-Ser-Asp-. These peptides were isolated from the digest by means of gel filtration on 'Sephadex G-50'. The tripeptide (T1) was separated from the fibrinopeptides by high voltage electrophoresis at pH 4.1.

Table 1. AMINO-ACID COMPOSITION OF N-TERMINAL PEPTIDE ("MOTHER PEPTIDE A") OF A-CHAIN OF FIBRINOGEN AND DIFFERENT FRAGMENTS OF IT

Amino-acid	"Mother peptide A"	T1	T2	Try 1	Try 2
Ala	2.71 (3)	—	1.07 (1)	Trace	—
Arg	3.72 (4)	0.96 (1)	2.00 (2)	Trace	0.99 (1)
Asp	5.97 (6)	—	3.91 (4)	3.83 (4)	Trace
Cys/2*	3.44† (4)	—	4.08† (4)	0.91 (1)	1.72 (2)
Glu	4.64† (5)	—	2.86 (3)	0.83 (1)	0.19
Gly	6.60 (7)	1.04 (1)	1.33 (1)	Trace	1.01 (1)
His	1.00 (1)	—	1.00 (1)	Trace	—
Ile	Trace	—	Trace	—	—
Leu	1.01 (1)	—	0.13	—	—
Lys	2.23 (2)	—	2.14 (2)	1.07 (1)	—
Met‡	(0.3) (1)	—	— (1)	—	—
Phe	1.81 (2)	—	1.01 (1)	0.96 (1)	Trace
Pro	2.97 (3)	1.00 (1)	2.10 (2)	0.85 (1)	0.95 (1)
Ser	5.26† (5)	—	3.79† (4)	1.76 (2)	0.95 (1)
Thr	0.26	—	Trace	Trace	Trace
Trp	2.22 (2)	—	— (2)	— (2)	—
Tyr	1.03† (1)	—	0.98 (1)	0.98 (1)	Trace
Val	3.02 (3)	—	1.88 (2)	Trace	Trace
Unknown peaks§	0.80	—	0.41	0.24	—
Total residues	50	3	31	14	6

The amino-acid analysis was performed with a Technicon 'Auto Analyser'. The hydrolysis was performed in 5.7 normal HCl at 110°C. The mother peptide and T-2 were hydrolysed for 22 and 48 h; the other peptides only for 22 h. The most probable number of residues is indicated within parentheses.

\* Determined as sum of S-carboxymethyl cysteine and cystine.

† Extrapolated value.

‡ Methionine was recovered as homoserine. This peak appeared in front of glutamic acid and was sometimes not completely separated from this amino-acid.

§ This peak, apparently derived from glutamic acid, was observed in front of glycine. In some runs, it could not be separated from glycine. The colour yield has been assumed to be the same as for norleucine.

In order to align the two fragments, T1 and T2, we treated fibrinogen with thrombin, thus releasing fibrinopeptide A and B, yielding fibrin. We then isolated the N-terminal fragments of the A-chain of the fibrin. To do this the fibrin was dissolved in 70 per cent formic acid, treated with CNBr and the reaction products separated on 'Sephadex G-100'. An elution pattern similar to that shown in Fig. 1a was obtained. The fraction with the same elution volume as fraction 2 (Fig. 1a) was pooled, mercaptolysed and alkylated. After S-carboxymethylation the fractionation on 'Sephadex G-100' was repeated. The elution pattern was essentially the same as that shown in Fig. 1b. The fraction corresponding to fractions 5 and 6 (Fig. 1b) was pooled. This peptide preparation had glycine as N-terminal amino-acid and the following partial sequence: Gly-Pro-Arg-Val-Val-. These results show that the tripeptide Gly-Pro-Arg (T1) succeeds fibrinopeptide A (or analogues) and precedes fragment T2 in mother peptide A.

To obtain more information on the amino-acid sequence of the C-terminal part of the mother peptide A, the so-called valyl peptide (T2) was digested with trypsin<sup>27</sup> (weight ratio enzyme: substrate, 1:100) at pH 8.5 (0.2 molar ammonium bicarbonate adjusted with solid CO<sub>2</sub>) for

3–4 h at 37°C. The five principal tryptic peptides (Try 1–5), revealed on a "finger-print" (Fig. 2), were isolated by gel filtration ('Sephadex G-25' in 10 per cent acetic acid) and paper electrophoresis at pH 3.5–5.5. Try 3 was shown to be identical with homoserine and must thus be C-terminal in the valyl peptide (T2). Try 4 was a tetrapeptide with the sequence: Val-Val-Glu-Arg. Try 5 was a hexapeptide with the sequence: His\*-GIN-Ser-Ala-Cys†-Lys. Try 2 was a hexapeptide (Table 1) with the sequence: Cys-Pro-Ser-Gly-Cys-Arg. Try 1 was a peptide containing 14 amino-acid residues (Table 1) with the following partial sequence: Asp-Ser-Asp-Trp-Pro-Phe-. It was the only peptide on the finger-print that gave a positive reaction with reagents that stain tryptophan. Analysis<sup>28</sup> of the whole mother peptide A indicated that it contains two tryptophan residues. Both of these must be in Try 1 since neither the fibrinopeptide A nor the other tryptic peptides (2–5) from mother peptide A contain tryptophan. From all the above analyses the partial sequence for the mother peptide A could be deduced (Fig. 3). The sequence seems the same for 43 amino-acid residues from the N-terminal end for both the plasmic fragment<sup>22</sup> and that produced by CNBr.

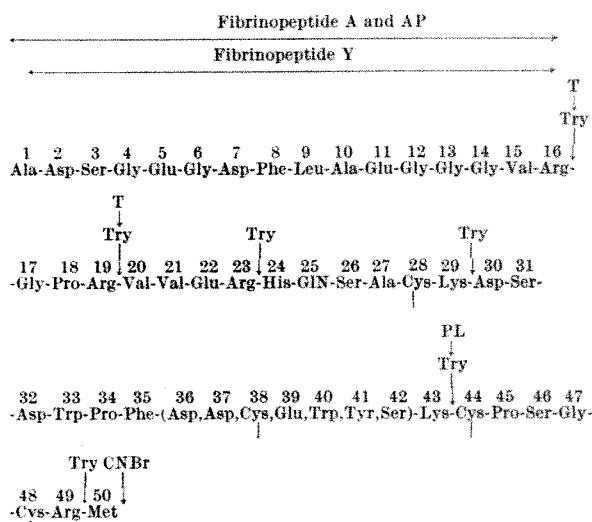


Fig. 3. Amino-acid sequence of the N-terminal part of the A-chain of human fibrinogen. The cleavage site by thrombin (T), trypsin (Try), plasmin (PL) and cyanogen bromide (CNBr) is indicated by arrows. Amino-acid residues within brackets are deduced from the amino-acid analysis of the acid hydrolysate of Try 1 (compare Table 1). The AP peptide is phosphorylated at residue No. 3.

The amino-acid sequence of the N-terminal fragment of the A-chain presents some interesting features. The first half-cystine residue is at position 28 (Fig. 3). From there on to the C-terminal end of the "mother peptide A" there are three more half-cystine residues. Altogether 14 per cent of the half-cystine residues of the whole fibrinogen molecule<sup>29</sup> are located in the N-terminal peptide which constitutes only about 3 per cent of the fibrinogen molecule. Because no free SH-groups have been demonstrated in fibrinogen<sup>30</sup> these half-cystine residues must take part in the formation of disulphide bridges. If all the disulphide bridges are interchain it would mean that about 30 per cent of the half-cystine residues of fibrinogen are linked together with the N-terminal part of the A-chain. This area of the molecule in any case forms a dense "disulphide knot". We have so far no definite information on which parts of

\* In the first cycle of the Edman procedure PTH-histidine together with small amounts of PTH-glutamine were found. The appearance of a succeeding amino-acid residue in a sequence during a degradation cycle where histidine is involved has also been noted for some other peptides (unpublished observations of Blomback). The cleavage of the chain, under the conditions used, apparently takes place during the coupling with phenylisocyanate at pH 9–9.5.

† Identified as the PTH-derivative of S-carboxymethyl cysteine.



the molecule are joined by disulphide bridges. However, the action of CNBr on fibrinogen suggests that the N-terminal part of the B- and C-chains might be joined to the N-terminal region of the A-chain. After splitting the molecule with CNBr and fractionating the mixture on 'Sephadex G-100', the disulphide containing fraction 2 (Fig. 1a) contains the N-terminal parts of both the A-chain and the B-chain. It also contains the N-terminal part of the C-chain. (Note added in proof. This fragment has the following partial N-terminal sequence: Tyr-Val-Ala-Thr.) The fibrinopeptide A portion of the molecule is probably not involved in the "disulphide knot". Its overall hydrophilic nature makes it likely that it is on the surface of the molecule.

We have also isolated the N-terminal fragments of the B-chain containing covalently linked fibrinopeptide B from both plasmic digests of sulphitylated fibrinogen<sup>22</sup> and cyanogen bromide treated native fibrinogen. These were referred to as plasmic mother peptide B and CNBr mother peptide B. The CNBr mother peptide B, fraction 3 (Fig. 1b), released fibrinopeptide B on incubation with thrombin. After alkylation this fraction was partially purified by gel filtration on 'Sephadex G-75' in 10 per cent acetic acid and subsequently on 'Sephadex G-100' in the same medium. The purified S-carboxymethylated CNBr mother peptide B is much longer than the corresponding fragment obtained from a plasmic digest<sup>22</sup> which contains only 21 amino-acid residues. Phenylisothiocyanate fails to react with the CNBr mother peptide B and after digestion with thrombin and low voltage electrophoresis at pH 6.4 it yields two main and four minor fractions. One of the main fractions has the same electrophoretic mobility at pH 6.4 as fibrinopeptide B (ref. 8) and is furthermore ninhydrin negative but Sakaguchi positive. The other main fragment has the following partial amino-acid sequence: Gly-His-Arg-Pro-Leu-Asp-. This sequence is identical with the sequence of six amino-acid residues, from the N-terminal end, of the C-terminal heptapeptide obtained after digestion of the plasmic mother peptide B with thrombin<sup>22</sup>. From these results the N-terminal sequence of the first 20 amino-acid residues at the N-terminal end of the B-chain of fibrinogen was deduced (Fig. 4). The minor thrombinic fractions have not been analysed further.

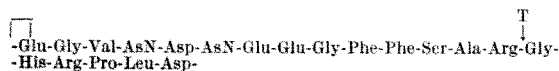


Fig. 4. Amino-acid sequence of the N-terminal part of the B-chain of human fibrinogen. The arrow indicates the cleavage site by thrombin.

On treatment of the CNBr mother peptide B with trypsin about ten fragments could be demonstrated on a finger-print<sup>27</sup> but, except for fibrinopeptide B, they have not yet been characterized.

These results give some clues regarding the requirements of thrombin action. The difference in rate of cleavage of the A-fibrinopeptide arginyl bond in fibrinogen and of other arginyl or lysyl bonds in fibrinogen or other proteins is to some extent a matter of "accessibility". One would expect that unfolding the substrate would increase the number of bonds split. When the N-terminal fragment produced by cleavage of the molecule with CNBr is digested with thrombin the tripeptide, Gly-Pro-Arg, is released in addition to fibrinopeptide A. If native fibrin, which is resistant to the further action of thrombin, is treated with CNBr and then digested with thrombin the same tripeptide is released, which means that the No. 19 arginyl bond (Fig. 3) in the A-chain of fibrinogen and the corresponding bond in fibrin have both become available for the acylation reaction with thrombin. Thrombin also seems to split additional bonds in the CNBr mother peptide B. These experiments certainly show that the cleavage by the enzyme can be influenced by modifying the substrate. The number of arginyl or lysyl bonds

that are hydrolysed in the protein treated with CNBr, however, is still very low. Reduction and alkylation of the treated protein do not change the situation appreciably. This means that, although thrombin has a trypsin like specificity of action on acylated arginyl and lysyl esters (compare ref. 16), only a few of the about 100-150 trypsin susceptible bonds in fibrinogen and fibrin (per 170,000 unit molecular weight) are accessible to the enzyme even after cleavage of all methionyl and S-S bonds in the molecule.

We are apparently dealing with an enzyme with an extraordinary hidden active centre which for steric reasons can react easily only with the N-terminal parts of the A- and B-chain of fibrinogen and fibrin, even when the protein presumably has been unfolded to a high degree. One might speculate that the location of a proline residue in close vicinity to the vulnerable arginyl bond in both the A and B N-terminal fragments (Figs. 3 and 4) creates a sterically more suitable structure for the active centre of the enzyme.

In order to explain the differences in susceptibility of the different arginyl and lysyl bonds in fibrinogen and fibrin towards thrombin we have to consider not only steric factors but also specific interaction. The extremely rapid release of the A-fibrinopeptide during the clotting process, its great stability during mammalian evolution and the facts presented in this report reinforce our previous suggestion (compare refs. 16 and 20) that specific structures in the A-fibrinopeptide or its immediate surroundings favour an "opening up" of the active centre of the enzyme or a firm association between enzyme and substrate. Thorough kinetic analysis of thrombin action on the N-terminal fragments of the A- and B-chain of fibrinogen or fibrin and on derivatives of them would certainly shed more light on this question.

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# Fractionation of Iron in the Solar System

by

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In the preplanetary dust cloud, particle adhesion and aggregation would have been most effective for magnetic grains. Enrichment of iron in the region of the inner planets could have occurred by a particle-size fractionation, the outward dispersal of small, metal-poor aggregates leaving behind only the larger aggregates enriched in magnetic particles of iron.

CURRENT theories on the formation of the solar planetary system<sup>1-3</sup> have met with some success in accounting for its present dynamical properties, but there is still great uncertainty about the processes that have led to the measured densities of the planets<sup>4-6</sup> (Table 1). Different degrees of phase transformation seem inadequate to account for the density differences, which are consequently attributed to chemical differences. Any unitary theory of formation of the solar system must therefore account for chemical fractionation either in the preplanetary solar nebula or at some subsequent stage. Adequate theories exist as to why some of the lightest elements, which are gaseous or have gaseous compounds, are depleted in the inner planets relative to their solar abundances, but, as Urey<sup>7</sup> emphasizes, it is much more difficult to explain any differences in the relative abundances of the non-volatile elements of medium or heavy atomic weight, either among the inner planets or between these planets and the Sun.

Table 1. DENSITIES OF THE INNER PLANETS

Planet	Mass*	Density g/cm <sup>3</sup>	Wt. per cent of metal phase†
Mercury	0.054	5.33	63
Venus	0.81	5.15	26
Earth	1.00	5.52	32
(Moon)	0.012	3.33	5
Mars	0.11	4.00	19
Asteroids ‡ per cent	10 <sup>-4</sup>	3.5	0-30

\* Expressed relative to the Earth.

† Calculated by assuming that the various bodies are effectively two phase systems of densities 3.3 g/cm<sup>3</sup> and 7.9 g/cm<sup>3</sup> (recalculated to mean density of 10 kbars).

‡ Density and metal content of asteroids are based on the values for chondrites.

All values taken from Ringwood<sup>6</sup>. The values of Urey<sup>7</sup> differ slightly.

Perhaps the most significant evidence comes from analyses of meteoritic material and the solar atmosphere. In a general discussion of the chemical analyses of chondritic meteorites (in particular, Type 1 carbonaceous chondrites) and the spectral analysis of the solar atmosphere, Urey<sup>7,8</sup> suggests that abundance differences are quite well established at least for iron and some other elements of the iron group. If carbonaceous chondrites (or any other chondrite) are assumed to be average samples of primitive asteroidal material, some chemical fractionation process has increased by a factor of 3 or 4 the iron content relative to an average for non-volatile light elements, of planetary material at that general solar distance.

The evidence for important chemical differences among the inner planets is based on an interpretation of their mean densities. A unique solution is impossible with present data, but one treatment of the problem is to assume that inner planets are composed of different proportions of metal and silicate corresponding to terrestrial core and mantle material (Table 1, after Ringwood<sup>6</sup>). The comparison of the iron content or of the iron to

silicon ratio of inner planets such as the Earth, with solar or meteoritic material, then rests on assumptions about the composition of the terrestrial core and mantle. For example, Urey<sup>7,8</sup> assumes that the core material in each planet is essentially of the same composition as the metal phase in most meteorites, that is an iron-nickel alloy, and because the planets differ from one another in density and metal content, they differ also in overall chemistry, as shown by the iron to silicon ratios of Table 2. On the other hand, Ringwood<sup>6</sup> considers that the inner planets have the same content of involatile elements (and the same iron to silicon ratio) as chondritic and solar material, and originally consisted of material of the composition of carbonaceous chondrites. During and after formation of the planets, they suffered varying degrees of auto reduction to give rise to their present ratios of free metal to silicate.

Table 2. ATOMIC IRON-SILICON RATIOS

Body	Iron-silicon ratio
Sun	0.2
Mercury	~3.0
Venus	~1.0
Earth	1.0
(Moon)	0.3
Mars	0.6
Chondrites*	0.7
Carb. chondrites (type 1)	0.9

\* Mean of low and high iron chondrites.

All values taken from Urey<sup>8</sup>.

The purpose of the present article is to describe qualitatively how a degree of the chemical inhomogeneity inferred by Urey may have been achieved at the preplanetary stage during cooling of the solar nebula. An examination of Table 2 shows that the degree of iron enrichment found by Urey<sup>7,8</sup> decreases fairly regularly with increasing distance from the Sun, and that all the terrestrial planets and chondrites have a greater iron enrichment than the solar atmosphere. Accepting that planets have been created from material left behind at the equator of a contracting, rotating solar nebula<sup>1-3</sup>, either non-ferrous material has been preferentially lost outward from orbits near the Sun, or iron has been brought inward from greater distance to these orbits. This article suggests the preferential loss of iron-poor material and the retention of iron-enriched material in the region of the inner planets by a process of particle-size fractionation.

We shall try to trace the chemical history of iron in the nebular material. We shall assume that the discarded material had a chemical composition closely similar to the present Sun. During rapid cooling from an initial ejection temperature of 1,000-2,000° K, there would have been a continuous adjustment of chemical equilibrium between involatile compounds present as a dust and the residual gas. At temperatures above 1,000° K and in the presence

of hydrogen and water in cosmic molecular proportions (500:1) the reaction;



would have been well to the right. From the calculations of Urey<sup>10</sup> and Latimer<sup>11</sup>, modified by Mueller<sup>12</sup> to allow for the activity of  $\text{Fe}^{2+}$  in solid solution, the iron would have been virtually all in the metal phase, with  $\text{Fe}^{2+}$  in only 1 per cent of the cation positions in ferromagnesian silicates at 750° C. At about 300° C, enough iron would have become oxidized to occupy 20 per cent of the cation positions, and at 100–200° C oxidation would have been virtually complete.

The size of particles formed by direct condensation from the gas phase is very uncertain. Arguing by analogy, chemical smokes on formation often have particle size of  $10^{-4}$  to  $10^{-5}$  cm;  $10^{-5}$  cm is also quoted for the size of cosmic dust, and, according to Wood<sup>13</sup>, the groundmass of carbonaceous chondrites appears to contain particles of  $10^{-4}$  to  $10^{-6}$  cm. Dynamical arguments suggest that there must have been growth in the particle size of the matter which is now the inner planets, to some minimum size, while its mean free path was much smaller than the cloud, and the electromagnetic radiation in the cloud approximately isotropic. Otherwise it would have been rapidly dispersed by radiation pressure and perhaps a solar wind. For the present solar luminosity this critical size is about  $10^{-4}$  cm and we shall at first assume this as the primary particle size. It can be shown that if the present mass of the inner planets was distributed as such particles within the present Martian orbit, the mean free path based on interparticle collisions is at most only  $10^8$  cm. With such a small mean free path, the collision velocity distribution of the dust particles will be determined by the residual particle velocities, after the local streaming velocity needed to balance solar gravitational attraction by centrifugal force is subtracted. Turbulent motions will probably not significantly affect the calculations given later. If allowance is made for the presence in the cloud of hydrogen in solar proportions, it may be demonstrated that it is reasonable to regard the dust particles as being in complete thermal equilibrium with the gas. In this case, the mean dust-particle velocity is about 0.1 cm/sec for  $10^{-4}$  cm particles at 1,000° K, and the mean collision velocity is twice this for a Maxwell distributed system.

In the earliest stages before self-gravitation becomes significant, the aggregation of the primary dust particles will be by mechanisms of adhesion. In the presence of hydrogen in solar proportions the gas pressure could be as high as  $10^{-1}$  atmospheres so that the adhesion by chemical bonding observed under ultra-high vacuum will not occur<sup>14</sup>. A much weaker load-independent adhesion is found in the presence of gases, which is attributed to dispersion forces. Surface films and electrostatic forces can also contribute to adhesion, but with the exception of electrostatic forces they are all of extremely short range and could never give capture or "sticking" cross sections significantly greater than the optical cross section—probably much less. Ambient conditions do not favour electrostatic charging and it is a moot point whether an electrostatically enhanced collision cross section would lead to a correspondingly enhanced capture cross section; charge sharing would occur on contact and the resulting electrostatic repulsion might reduce the chance of adhesion by an even larger factor. This seems a point worth experimental check.

We would like to suggest that an interesting long range interaction that can lead to enhanced capture or "sticking" cross sections is the magnetostatic attraction of ferromagnetic dust particles. It will be seen that with a Curie point for iron or iron-nickel alloy of more than 700° C and an average time interval between dust particle collisions based purely on the optical cross section, of the order of one year, there would have been time for a very large number of such collisions before oxidation of the

iron occurred to any significant degree. The calculation of capture cross sections for this interaction is somewhat involved and we give an outline of our method of estimation.

Despite the fact that one can adequately treat the dust collisions as two body interactions, ignoring damping effects introduced by residual gas, the general problem of computing orbital motion resulting from magnetic interaction is extremely difficult. The magnetization of the primary particles, even if they were as large as  $10^{-3}$  cm, would be of uniform single domain type and, in general, effectively fixed by shape and crystalline anisotropy with respect to the axes of the inertia tensor. A full discussion of the motion in this case of non-central forces is impractical and one must be content with a demonstration of the enhancement of capture, accurate to within perhaps a factor of five, based on a central force approximation.

Because we are interested only in the possibility of the interaction leading to greatly enhanced capture it is permissible to neglect all except the dipole moments  $M_1$  and  $M_2$  of the particles. The use of the central force approximation with reduced mass  $\mu$  in this situation is equivalent to assuming that the relative orientation of the dipoles is fixed in the collinear position and we shall assume that this is such as to minimize the potential energy of the system. A necessary condition for this to be valid is that the period of oscillations of one particle in the magnetic field of the other is smaller than the time for the radius vector to describe an angle of  $2\pi$ . The absence of any consideration of the process by which the requisite collinearity of the moments is attained at great distances may be responsible for an overestimation of the capture cross section by a factor of two or three. The potential energy is

$$-\frac{2 M_1 M_2}{r^3}$$

and it is well known that with such rapidly varying potentials, orbits are either open or involve spiralling to the centre of force if the impact parameter is less than a certain value<sup>15</sup>. It may be shown that the cross section for spiralling, which we identify with the capture cross section, is given by

$$\sigma = 3\pi \left( \frac{2 M_1 M_2}{\mu V_\infty^2} \right)^{\frac{2}{3}}$$

where  $V_\infty$  is the velocity at infinite separation. Putting the particle volumes both equal to  $V$  and an intensity of magnetization  $I$ , we have

$$\mu = m/2$$

$$V_\infty^2 = 2 V^2 = \frac{6 kT}{m}$$

and substitution gives

$$\sigma = 3\pi \left( \frac{2 V^2 I^2}{3 kT} \right)^{\frac{2}{3}}$$

For the thermalized dust particles envisaged here with the magnetization  $I$  of about 200 E.M.U. (a value that would be attained about 50° C below the Curie point of about 1,000° K) we obtain  $\sigma = 1.2 \times 10^{-4}$  cm<sup>2</sup>. This is about  $2 \cdot 10^4$  times the optical cross section. At a separation of 100 particle diameters, the magnetic field resulting from each moment is about  $10^{-3}$  gauss and a general ambient field large enough to affect the capture probabilities seems unlikely. Further investigation indicates that for these particles the ratio of the instantaneous orbital period at the critical impact parameter distance of order  $\sqrt{\sigma}$ , to the period of small oscillations of a particle in the field of the other is approximately 70 and decreases to about 10 at the instant of contact. This seems to justify the use of the central force approximation in this case.

More general consideration indicates that this ratio of periods is proportional to  $(V I^2)^{\frac{1}{3}}$  while the ratio of capture

to optical cross sections for a thermalized dust cloud varies as  $(V^2)^{-1/2}$ . From this we may conclude that because the intensity of magnetization is limited to less than about five times the value chosen previously, enhancement of aggregation by magnetic attraction is negligible for particles much smaller than  $10^{-5}$  cm.

The efficiency of magnetic capture for the resulting aggregates probably declines as the number of primary particles forming them increases. Only if the particles were added with random moments and the bodies retained thermal velocities would the ratio of capture and optical cross sections remain unchanged. In practice there will be definite cancellation of moments that will lead to a decline in this ratio as  $N^{-1/2}$ , where  $N$  is the number of particles per body; if the particles do not remain thermalized and collision velocities are fixed by the terminal velocity in the gas, it falls as roughly  $N^{-2}$ . For a thermalized cloud of  $10^{-4}$  cm particles, the aggregations would grow most rapidly to about  $10^3$  particles and thereafter optical and capture cross sections would be virtually identical. This could still be a much greater capture cross section than for silicate dust of comparable optical size.

Let us now examine how these results might be used to explain the large scale chemical inhomogeneity referred to previously. At high temperatures in a thermalized dust cloud, the cross sections for adhesion are probably extremely small compared with the optical cross section and all primary dust particles might well survive as independent bodies down to the Curie temperature. Thereafter the cross section for mutual capture of the metallic particles grows by an enormous factor for only a further fall in temperature of a few tens of degrees Celsius. The magnetic metallic dust would rapidly be collected into aggregates of at least several thousand particles in a time of the order of years. The further evolution of the system will depend on the relative timing of the cooling and the escape of residual gas. The degree of oxidation of the aggregates, which might also act as a cementing process, will be dependent on the pressure of residual gas left below  $300^\circ\text{C}$ .

The outward removal of the gas as from the region of the inner planets would be accompanied by a particle size sorting<sup>16</sup>, the finer particles being carried away with the gases. Also when the residual gas was removed, the remaining particles would have been subjected to the full

effect of radiation pressure and solar wind. All these factors would have resulted in the outward removal of the smaller particles and aggregates, and a preferential retention of the larger aggregates. It is reasonable to suppose that aggregates large enough to avoid expulsion from the vicinity of the Sun were iron-enriched, and hence the iron to silicon ratio in the inner planets became higher than the solar value. There would be a progressive decrease outwards, in iron content, among the inner planets and asteroids. Each planet would have a distinctive chemical composition, so that solar and meteorite compositions could not be quantitatively applied to the Earth, in the comparison of elements that are likely to have undergone pre-planetary fractionation.

This mechanism could have caused some fractionation of nickel and cobalt together with iron; comparison of chondritic and solar abundances indicates that this did not occur. An explanation may lie in the possible volatility of these elements in a reducing atmosphere containing carbonaceous and nitrogenous compounds, and in the role of supersaturation during condensation of planetary material<sup>17</sup>. In any case, the recognition of a very specific aggregation mechanism may help to narrow the range of plausible physical conditions during the pre-planetary stage of the solar system.

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## Geochronology of the St Helena Volcanoes

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Recent theories of crustal spreading propose that the volcanic islands of the Atlantic have drifted away from the mid-ocean ridge. Do these theories stand up to a close geochronological scrutiny of some of the islands in question?

THERE has been considerable recent interest in the idea that there is a correlation between the ages of oceanic volcanic islands and their nearest distance from a mid-ocean ridge. It has been suggested that such a correlation lends support to the hypothesis of the spreading apart of ocean floors, and thus to the hypothesis of continental drift<sup>1-3</sup>. There has also been recent interest in the possi-

bility of using radiometric ages to investigate the life history of volcanic islands<sup>4-11</sup>. In both cases there are at present only rather scanty quantitative data available<sup>4-12</sup>. It is our object to add to the data by presenting the results of a stratigraphically controlled geochronological study of the island of St Helena using the potassium/argon method.



The volcanic island of St Helena lies in the South Atlantic ocean at longitude  $5^{\circ} 40' \text{ W.}$  and latitude  $16^{\circ} 00' \text{ S.}$ , more than 800 km east of the crest of the Mid-Atlantic Ridge. The island is elongated in a north-east/south-west direction, with maximum linear dimensions of approximately 16.1 km and 9.7 km and an area of about 120 km<sup>2</sup>. St Helena rises through 4,400 m of water to an overall height of about 5,220 m above the ocean floor. The relief of the island is very rugged, and all levels of the sub-aerial volcanoes are exposed as a result of the severe erosion. All the rocks exposed on the island belong to the alkali olivine basalt-trachyte-phonolite assemblage.

The island is formed by the coalescence of two broad basaltic shield volcanoes (Fig. 1). The smaller north-eastern volcano is in part buried beneath the flanks of the main volcano. Eruptions from a third, minor, centre have resulted in extensive flooding of both volcanoes in the east and north-east of the island. The eruptions have occurred predominantly through fissures and pronounced dyke concentrations occur in the central areas of both volcanoes. A number of highly alkaline dykes and parasitic masses intrude the central regions and upper flanks of the south-western shield.

Because the exposures are good in the arid low central regions of the main volcano, it has been possible to establish an accurate stratigraphic sequence. The

younger lavas frequently occur as erosional relics in the thickly vegetated high parts of the island, however, and it has proved very difficult in several cases to determine their relative ages by their field relationships. In the deeply eroded central areas it is impossible stratigraphically to correlate the alkaline intrusive rocks directly with each other or with younger flow groups.

Twenty-four rocks were eventually selected for radiometric dating; of these about half were from stratigraphic levels which could be accurately correlated relative to each other. The others were mainly late intrusive rocks and relic flows of less certain relative ages.

The structure of the smaller volcano is less complex than that of the south-western shield. The oldest rocks—very highly altered breccias of uncertain origin—are exposed as screens between swarms of dykes in the central region. In the dominant sub-aerial flows and pyroclastics of the shield only one major erosional break is seen, separating a very late minor sequence of basaltic agglomerates and thin flows from the main sequence. The western and southern flanks of this volcano are buried beneath flows from the south-west, but one small erosional "window" is exposed in the valley at Jamestown (Fig. 1). Only three rocks from this volcano have been dated: two flows from stratigraphically low (679) and high (145) levels in the sequence, and a dyke (678) known to cut the lower flows (679) in the central region (Fig. 2).

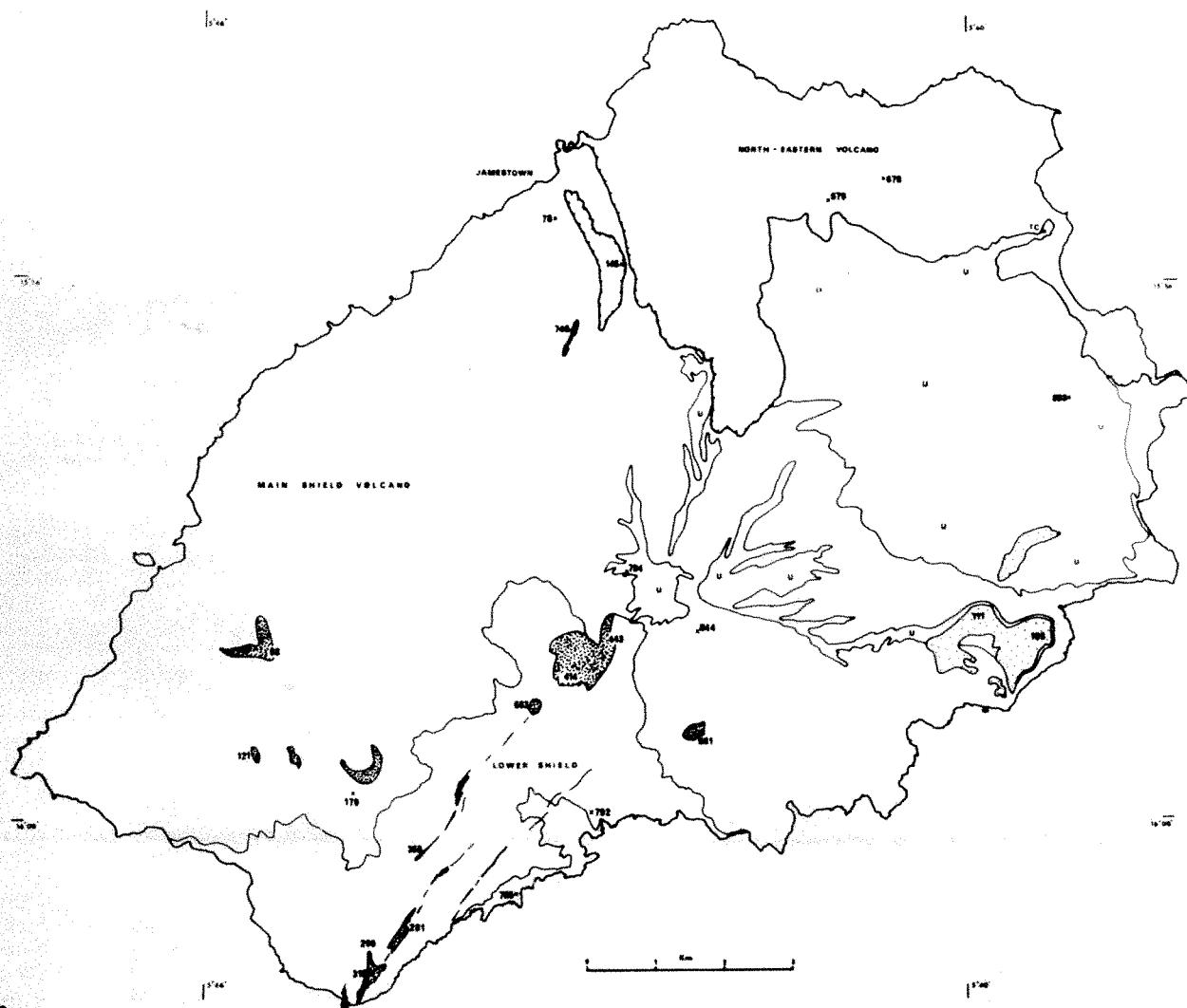


Fig. 1. Major stratigraphic divisions of the St Helena volcanoes. U, Late lavas of the third centre; dotted areas, late trachytic flows and equivalent extrusives; heavy stippled areas and black, alkaline intrusive rocks.

The structure of the south-western volcano is very complex. The centre of volcanic activity has moved at frequent intervals, and at low levels in the volcano extensive pyroclastic deposits, frequently from local cinder cone concentrations, are interstratified with well defined groups of lavas. The positions of the dated rocks are shown in Fig. 2, which is based on the relative ages of the various units as determined from field evidence.

Only one group of altered flows underlies the oldest dated thick group of lavas (785). Overlying these are largely altered pyroclastics and flows, up to 500 m thick locally and divisible into several distinct groups. The sequence forming the lower shield is highly complex, and late activity resulted in the extrusion of a number of thick trachybasaltic flows (792) over an erosional surface cut into several groups of the sequence.

Above these early groups extensive lava flows become dominant and form the main part of the shield. Several distinct groups of flows are apparent, with minor unconformities throughout the pile. Specimens 179 and 78 are from two of these flow groups separated by about 500 m of basaltic and trachybasaltic flows. It is these major shield forming flows which overlap and bury the western and southern flanks of the north-eastern volcano.

The flanks of the volcanoes were extensively eroded during a prolonged period of quiescence. In the east of the island a broad, deep depression was eroded through flows of both volcanoes in the area of their overlap (Fig. 1). Activity subsequently recommenced at a third, minor centre on the flanks of the main shield and lava infilled the erosional depression and other smaller valleys. These late flows may be divided into two groups, an older thick sequence of basaltic lavas (803, and probably 794),

and a younger group of intermediate lavas (TC). The two groups are separated by another marked erosional surface. The trachytic flow domes (105, 111) in the east of the island (Fig. 1) are known from field evidence to be younger than the 803 group of flows and may also be younger than the later TC group.

In the central high ground of the island relic patches of a thick porphyritic olivine basalt (844) are preserved. Although apparently associated with the two late flow groups of the third centre, the isolated nature of the exposures makes it impossible to assess its true stratigraphic position.

The main basaltic shield is intruded by a large number of highly alkaline dykes and parasitic masses. Several of the individual bodies (88, 121, 661) cut flows younger than 179, but the majority of the intrusions are not seen in direct relation to flows as young as 179 (281, 290, 313, 358, 414, 443, 683). Specimen 746 is from an irregular parasitic intrusion which cuts through the upper levels of the thick pile of late basaltic flows of the main shield.

From the field evidence, therefore, it is possible to establish a fairly accurate time sequence for the older extrusive, and some later intrusive, rocks. The younger extrusives and intrusives, however, were both difficult to correlate with any certainty.

A thin section of each rock was examined; in general any specimen that showed evidence of alteration other than minor alteration of olivine<sup>4</sup> was rejected, as were any amygdaloidal lavas. In a few cases (samples TC, 105, 111, 121, 746) it was necessary to relax the selection criteria slightly because the samples were the only ones available from certain important stratigraphic positions. Petrographic descriptions and the locations of the specimens are given in Table 2.

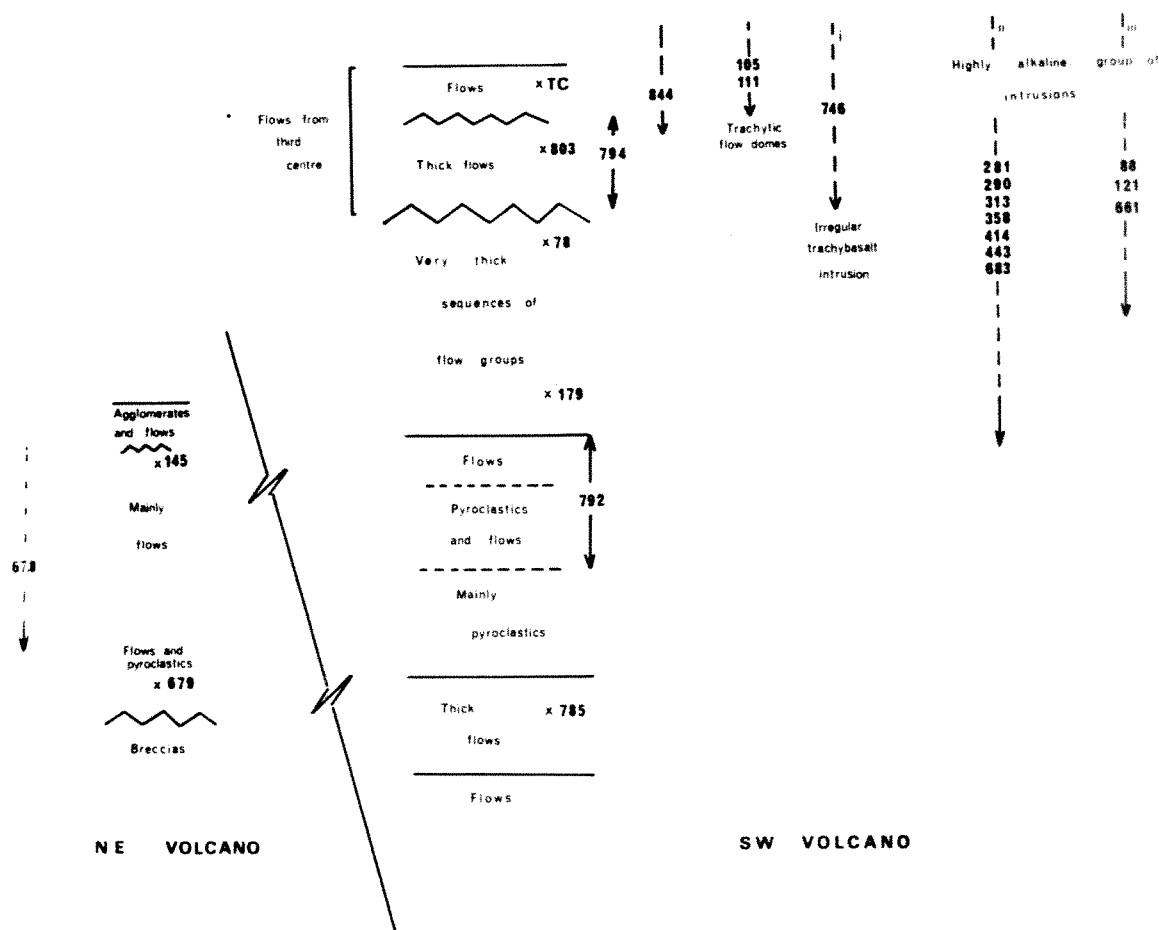


Fig. 2. Positions of dated specimens in simplified stratigraphic chart; arrow heads denote minimum and maximum ages based on field evidence.

In order to avoid sampling errors the rocks were crushed and sieved to 40–80 mesh size. All the ages were measured on whole rock samples.

Argon extractions were made in a 'Pyrex' high vacuum fusion system which was baked overnight at 200° C in order to drive off the majority of adsorbed atmospheric argon from the samples and glassware. Each sample (usually of about 10 g mass) was fused in a molybdenum crucible within the fusion system at about 1,400° C for 20 min by radio frequency induction heating. A tracer of argon-38 ( $\approx 1 \times 10^{-5}$  c.c. S.T.P.) was added before fusion, and purification of the gases was made over hot copper oxide (CuO) and titanium, with a hot tungsten filament to dissociate hydrocarbons (chiefly methane).

The purified argon was led through a Granville-Phillips leak valve directly to a Reynolds all glass bakable mass spectrometer operated in the dynamic mode. The argon was determined in the usual way by isotope dilution. A bulb to dispense argon-38 tracer was used, similar in design to that recently described by Lanphere and Dalrymple<sup>11</sup> but in use at Oxford for many years. Calibration of the argon-38 tracer was made against known volumes of air, and secondary standards including the Massachusetts Institute of Technology biotite B3230.

Three important samples were also analysed for argon using an 'MS10' mass spectrometer. This instrument is free from the mass 36 background (and from memory effects) which can sometimes vitiate results obtained with the Reynolds machine.

Potassium was determined in duplicate by flame photometry, using an 'EEL' flame photometer burning a mixture of air and coal gas and modified by the incorporation of an interference filter peaked at 7666 Å and with a pass band of 96 Å. The sensitivity in this region of the spectrum was improved by replacing the standard photocell by a 'Megatron' infra-red photocell and the precision of observation was greatly enhanced by using a potentiometric chart recorder with the instrument.

A very careful investigation has been made of the interfering effects on the determination of potassium of the presence of other cations and anions. The interference effects found are similar to, but different in detail from, those reported by Cooper for the Perkin-Elmer instrument<sup>12</sup>. A buffering technique has been developed to overcome the interference effects; the buffering solution contains 1,000 p.p.m. of each of the elements sodium, aluminium, magnesium, calcium and iron in chloride form, and is made weakly acid (1,000 p.p.m.) with hydrochloric acid (about 36 per cent HCl) to prevent hydrolysis. The technique now used for potassium analysis makes use of no chemical separation, but involves the addition to both unknown and potassium standard solutions of the buffer solution on a 1:1 basis by volume. Determination of the concentration of potassium in the buffered unknown solution is made by bracketing it between two buffered standard potassium solutions 1 p.p.m. apart in concentration, in the range 1–10 p.p.m. of potassium.

The technique has been calibrated against interlaboratory standards (G1, W1, B3203) and against the neutron activation technique in the range 0.1 per cent–10 per cent potassium, and has been proved capable of an accuracy of  $\pm 1.5$  per cent throughout this range.

For each of the twenty-four specimens dated, potassium analyses were made in duplicate and argon analyses were duplicated on six of the samples. In all cases replicate potassium determinations agree to within 1 per cent, and analysis of inter-laboratory standards leads to an estimate of an absolute accuracy of  $\pm 2$  per cent. The principal factor influencing the errors in the ages is the error in measuring the argon content, which in its turn was principally in error because of the rather large atmospheric argon contamination (ranging from 20 to 70 per cent) associated with these rocks.

In order to be able properly to compute the effect on the age of the errors in the various measured parameters

Table 1

Sample No.	Potassium analysis (wt. per cent K)	Radiogenic <sup>40</sup> A (10 <sup>-8</sup> c.c./g)	Per cent radiogenic argon	Age (× 10 <sup>8</sup> yr)
TC	2.37 ± 0.04	80.4	65	8.5 ± 0.4
78	1.84 ± 0.04	54.1	51	7.3 ± 0.4
	1.83 ± 0.04	54.0*	41	7.3 ± 0.5
88	2.97 ± 0.06	86.7	57	7.3 ± 0.4
105	2.85 ± 0.06	99.4	32	8.7 ± 1.0
111	2.73 ± 0.06	88.1	28	8.1 ± 1.0
121	4.13 ± 0.08	126.0	70	7.5 ± 0.4
145	0.60 ± 0.01	27.4	35	11.4 ± 1.0
179	0.56 ± 0.01	22.6	39	10.1 ± 0.8
281	4.23 ± 0.08	115.6	73	6.8 ± 0.4
290	4.22 ± 0.08	131.0	25	7.7 ± 1.2
313	3.90 ± 0.08	127.6	31	8.2 ± 1.0
358	4.10 ± 0.08	125.3	70	7.6 ± 0.4
414	3.52 ± 0.07	114.6	70	8.1 ± 0.4
443	4.07 ± 0.08	123.7	78	7.5 ± 0.4
661	3.57 ± 0.07	104.5	77	7.3 ± 0.4
678	0.79 ± 0.01	35.9	51	11.4 ± 1.0
679	1.10 ± 0.02	64.3	50	14.6 ± 1.0
	1.11 ± 0.02	61.91*	60	14.0 ± 1.0
683	3.88 ± 0.08	118.4	80	7.7 ± 0.4
746	1.55 ± 0.02	52.1	48	8.4 ± 0.6
785	0.44 ± 0.01	20.1	39	11.3 ± 1.0
792	1.25 ± 0.02	54.4	45	10.9 ± 0.8
794	1.05 ± 0.02	40.4	41	9.6 ± 0.8
803	1.09 ± 0.02	40.2	32	9.2 ± 1.0
844	0.88 ± 0.01	32.9	50	9.3 ± 0.6

Adopted constants:  $\lambda_\beta = 4.72 \times 10^{-10}$  yr<sup>-1</sup>;  $\lambda_e = 0.584 \times 10^{-10}$  yr<sup>-1</sup>;  $^{40}\text{K} = 1.19 \times 10^{-2}$  atom per cent.

\* Argon determined on 'MS10' mass spectrometer.

a straightforward statistical analysis was carried out. The resulting expression for the standard deviation of the age is

$$\sigma(\text{age}) = \frac{1.712 \times 10^4}{(1 + 9.082R)} \cdot \sigma(R) \times 10^6 \text{ yr} \quad (1)$$

where

$$R = 1463 \times [^{40}\text{A}] / [K] \quad (2)$$

where

$$[^{40}\text{A}] = (\text{c.c. S.T.P.}) / \text{g of radiogenic } ^{40}\text{A}.$$

[K] is the weight per cent of potassium and the values adopted for various constants are  $\lambda_\beta = 4.72 \times 10^{-10}$  yr<sup>-1</sup>;  $\lambda_e = 0.584 \times 10^{-10}$  yr<sup>-1</sup>;  $^{40}\text{K} = 1.19 \times 10^{-2}$  atom per cent.

Assuming that the errors propagate independently we may write for  $\sigma(R)$  the expression

$$\sigma(R) = \frac{1463}{[K]} \sqrt{\sigma^2([^{40}\text{A}]) + \frac{[^{40}\text{A}]^2}{[K]^2} \cdot \sigma^2([K])} \quad (3)$$

which, if we assume that  $\sigma([K])/[K] = 1.5 \times 10^{-2}$ —a 1.5 per cent error at the 68 per cent confidence level—reduces to

$$\sigma(R) = \frac{1463}{[K]} \cdot \sqrt{\sigma^2([^{40}\text{A}]) + 2.25 \times 10^{-4} [^{40}\text{A}]^2} \quad (4)$$

It is fairly easy to show, from the expressions used in the determination of argon by isotope dilution, that the standard deviation of the radiogenic argon-40/g,  $\sigma([^{40}\text{A}])$ , depends on two terms according to the relation

$$\sigma^2([^{40}\text{A}]) = \{\sigma^2(T) + \sigma^2(a)\} / m \quad (5)$$

where  $m$  is the mass of sample taken in grams,  $T$  is the total volume of argon (radiogenic plus atmospheric) evolved from the sample and  $a$  is the volume of atmospheric argon evolved from the sample. The standard deviations  $\sigma(T)$  and  $\sigma(a)$  may be computed from the standard errors associated with the measurement of the isotopic ratios of the argon evolved from the sample, atmospheric argon and the tracer argon. The explicit formulae will not be given here. An alternative is to establish  $\sigma(T)$  and  $\sigma(a)$  (in the range of values of  $T$  and  $a$  appropriate to

the study in hand) by replicate analysis; for the St Helena samples  $T$  could be determined with a reproducibility of  $\pm 1$  per cent while  $a$  could be determined to  $\pm 2$  per cent. It then follows that the standard deviation of the quantity  $[^{40}\text{A}]$  is given by

$$100 \cdot \frac{\sigma([^{40}\text{A}])}{[^{40}\text{A}]} = \frac{\sqrt{(4(a/T)^2 + 1)}}{[1 - (a/T)]} \quad (6)$$

The errors quoted in this work are twice the standard deviation calculated from expression (1), using expressions (4) and (6), and are thus calculated at the 95 per cent confidence level.

The potassium/argon ages from Table 1, shown graphically in Fig. 3, may be compared with Fig. 2, which shows the stratigraphic position of the samples as determined in the field. The radiometric ages correlate well with the stratigraphic ordering for those specimens which could accurately be placed stratigraphically. The age for lava flow 78 is believed to be anomalous. Because its stratigraphic position is well determined, and specifically because it is in the flow group cut by the 746 intrusion (dated at  $8.4 \pm 0.6 \times 10^6$  yr, which is in full agreement with the other dates), it is believed that argon has been lost as a result of re-heating by the overlying flow. The ages obtained for the late relic flows (794, 844) suggest

their contemporaneity with the activity of the third centre.

The overlap of the radiometric ages for the two volcanoes still does not solve the problem of the time relations between the centres of activity. Specimen 785 ( $11.3 \pm 1.0 \times 10^6$  yr) is from the lowest levels of the south-western volcano; specimen 145 ( $11.4 \pm 1.0 \times 10^6$  yr) is from the top part of the north-eastern shield, a stratigraphically low specimen from which gives an age of  $14.6 \pm 1.0 \times 10^6$  yr (679). The lack of late intrusives and restricted development of late extrusives in the north-east perhaps strengthen the possibility, suggested by the radiometric ages, that the shift of the eruptive centres, 12 km to the south-west, marked a complete transfer of major extrusive activity.

The radiometric ages establish that the south-western basaltic shield volcano was built up in approximately  $3 \times 10^6$  yr ( $8.5\text{--}11.3 \times 10^6$  yr). The late flows above the unconformity (Fig. 2) appear to be markedly later ( $8.5\text{--}9.6 \times 10^6$  yr) than the bulk of the shield forming extrusives ( $10.1\text{--}11.3 \times 10^6$  yr), and form less than a quarter of the volume of the volcano. The highly alkaline intrusives, volumetrically less than 5 per cent of the total volcanic products, belong to a group significantly younger than the shield as a whole (the average age is approximately  $7.6 \times 10^6$  yr). These late intrusives have been interpreted

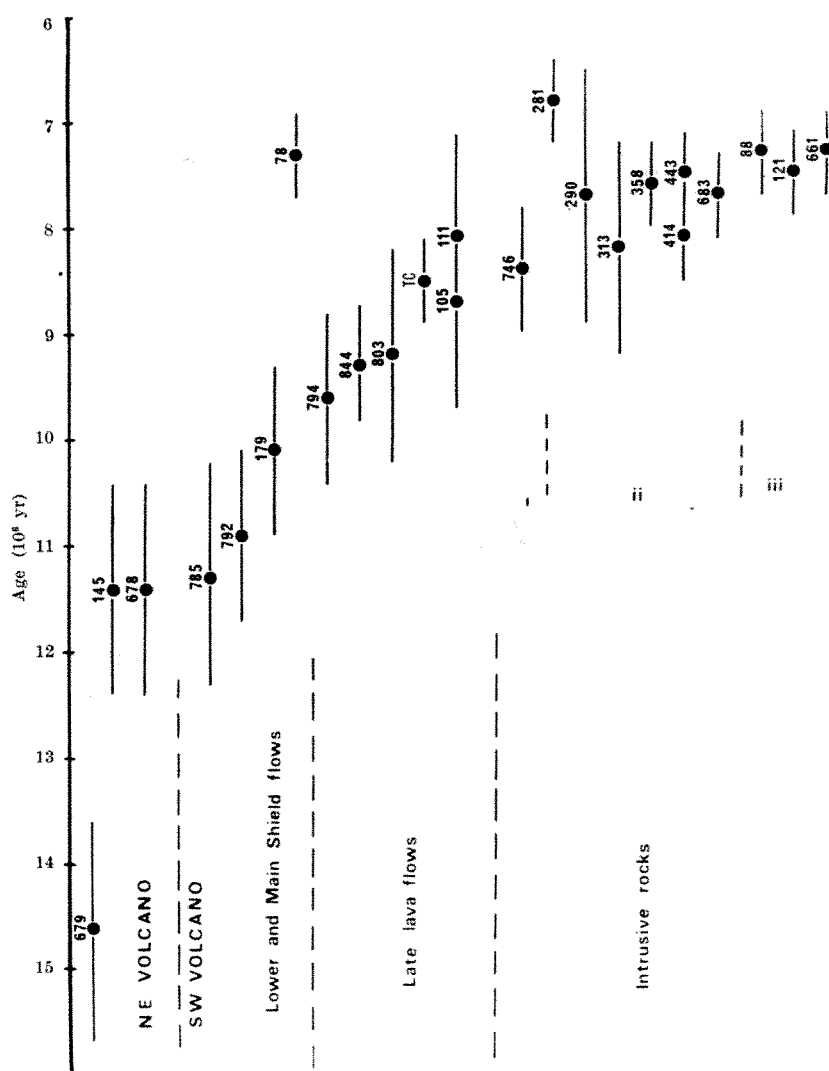


Fig. 3. Radiometric ages; all but intrusive rocks are shown in stratigraphic sequence left to right. Intrusives (groups i, ii and iii of Fig. 2) are arranged for simplicity in order of specimen numbers.



as products of a late, highly differentiated magma chamber at a high level in the volcanic pile<sup>14</sup>. It is conceivable from these dates that the magma may have had considerable time in which to differentiate before the intrusion of these late rocks.

The age/volume relationships demonstrate a slowing down in activity throughout the formation of the main volcano. The late flows and minor flank activity appear to have occupied a period of time approximately equal to that taken to build the bulk of the main shield. The volumetrically almost insignificant late alkaline intrusives represent the volcanic history over a comparable period of time. This succession of events presents a close analogy with Hawaiian activity of the Kohala type, where late differentiates are separated by a marked unconformity from alkali basaltic and intermediate flows which form the late parts of the shield<sup>15</sup>; which is tholeiitic in the Hawaiian case.

The total sub-aerial active life of the island is a minimum of  $7.5 \times 10^6$  yr—even the single main volcano presents a minimum active life of  $4 \times 10^6$  yr. This time scale appears comparable with preliminary age determinations on the Canary Islands<sup>7</sup>. In view of the scarcity of data from other islands in the Atlantic, the geochronological results for St Helena and the Canaries strongly suggest that the general conclusions on the active life of volcanic oceanic islands drawn by Gass<sup>8</sup> from geochronological studies of the Tristan group are not tenable. It is clear that the results of studies of the rate of formation of one volcanic pile cannot necessarily be extended as generalizations to others. Furthermore, the conclusions on the Tristan group are themselves based on much less evidence than that available for either St Helena or the Canaries. There is direct evidence only for the rate of formation of Tristan itself, and none for Inaccessible or Nightingale—indeed, the direct geochronological evidence for Nightingale would appear, by itself, to support equally well the hypothesis that that island was built up over a period of approximately  $18 \times 10^6$  yr.

In considerations of how far the results on St Helena support theories of ocean floor spread<sup>1-3,16</sup> two main points should be noted. The rocks forming the island of St Helena above sea level (even allowing for erosion) form approximately 5 per cent of the total volume of the volcanic pile, and represent up to  $7.5 \times 10^6$  yr of its history. These ages cannot be extrapolated down into the pile because of the unknown rate of submarine extrusion. Wilson<sup>1-3</sup> has stressed that the ages which he quoted for oceanic islands were minima, and thus that his calculations of rates of ocean floor spread were maxima. In view of the possible age range from a single island, and the impossibility of extrapolation through the volcanic pile, it would appear that these dates can strictly only be used as minimum dates for the commencement of sub-aerial volcanic activity. It should be emphasized that the date required for correlation with theories of ocean floor spreading is that of the commencement of volcanic activity at the particular centre, and that the rocks recording this lie at the base of the volcanic pile. It is certainly true, however, that the radiometric ages for St Helena reported here support in a general way the concept of ocean floor spreading. It is clear that the formation of St Helena took place more than  $14 \times 10^6$  yr ago, which is certainly much older than the age of volcanism on the North Atlantic Ridge and does in fact accord quite well with the linear correlation between age and distance from the ridge given by Wilson<sup>1</sup>.

Evidence from the Canary Islands, Cape Verde Islands and Tristan da Cunha demonstrates that islands may still be volcanically active when at considerable distances from the crest of the Mid-Atlantic Ridge. Individual islands in the Atlantic have acted as point sources for the prolonged extrusion of magma, and theories of ocean floor spreading must account for this continuity of activity over considerable periods of time (and thus over con-

Table 2. PETROGRAPHIC DESCRIPTIONS OF SPECIMENS

Specimen No.	Description
TC:	Slightly altered, fine-grained trachyandesite. Fayalitic olivine microphenocrysts in good trachytic texture of feldspars and very poikilitic aegirine-augite. Partial alteration of the olivine and groundmass to turbid phyllosilicates.
78:	Very fine grained trachybasalt. "Iddingsitized" groundmass olivines. Phenocrystal titanomagnetite and apatite.
88:	Medium-grained trachyte. Rare fayalitic olivine, and titanomagnetite in a mass of alkali feldspar laths and ragged aegirine-augite.
105:	Highly oxidized, slightly altered trachyte. Altered fayalitic olivine and groundmass. Perfectly unaltered alkali feldspars with trachytic texture.
111:	Slightly altered trachyte with perfect fluidal texture. Fayalitic olivines partially altered to green phyllosilicates. Microphenocrysts of titanomagnetite and sub-poikilitic or anhedral aegirine-augite.
121:	Fine-grained (phonolitic) trachyte. Microphenocrysts of alkali feldspar in a finer grained mass of alkali feldspars and late poikilitic aegirine-augite; rare euhedra of ? hydronepheline. Perfect trachytic texture.
145:	Porphyritic olivine-pyroxene basalt. Slight "iddingsitization" of all olivines. Groundmass very fine-grained: pyroxene, olivine, plagioclase and titanomagnetite.
179:	Porphyritic pyroxene-olivine basalt. Groundmass of fine-grained olivine, pyroxene, plagioclase and titanomagnetite. Slightest trace of "iddingsitization" of phenocrystal olivine.
281:	Phonolite. Phenocrystal alkali feldspar, and small euhedral nephelins, ragged anhedral to sub-poikilitic aegirine/aegirine-augite, in a very fine-grained groundmass of nepheline and alkali feldspar.
290:	Medium-grained phonolite. Alkali feldspar laths in little matrix of smaller laths, anhedral nepheline and very poikilitic aegirine with rare cossyrite.
313:	Medium-grained phonolite. Alkali feldspar prisms in finer mass of feldspar laths, minute nepheline euhedra, titanomagnetites and ragged to sub-poikilitic aegirine.
358:	Fine-grained phonolitic-trachyte. Rare phenocrysts of alkali feldspar in very fine groundmass of feldspar laths, rare nephelins, titanomagnetite, and mossy aegirine-augite.
414:	Medium to fine-grained phonolitic-trachyte. Subhedral alkali feldspar prisms in fine-grained mass of feldspars, minute nephelins, titanomagnetite, and subhedral to sub-poikilitic aegirine-augite.
443:	Medium to fine-grained phonolitic-trachyte with perfect trachytic texture. Minute nepheline euhedra, and ragged sub-poikilitic dark green aegirine-augite.
661:	Phonolitic-trachyte. Microphenocrysts of euhedral alkali feldspar and nepheline in groundmass of alkali feldspar laths, sub-poikilitic aegirine-augite, and very rare fayalitic olivine.
678:	Porphyritic pyroxene-olivine basalt. Groundmass of fine-grained olivine, pyroxene, plagioclase and titanomagnetite. Slight phyllosilicate alteration of the olivine along cracks.
679:	Porphyritic pyroxene, feldspar, olivine, titanomagnetite basalt. Fine-grained groundmass of similar minerals. Slight alteration of olivine along cracks to phyllosilicates.
683:	Medium-grained phonolitic-trachyte. Alkali feldspar prisms in fine-grained mass of alkali feldspar, nepheline, and anhedral to sub-poikilitic aegirine-augite/aegirine.
746:	Highly oxidized trachybasalt, with olivine, plagioclase laths with weak fluidal texture.
785:	Porphyritic olivine-pyroxene basalt. Fine-grained groundmass. Very slight traces of "iddingsitization" of olivines.
792:	Medium-grained basalt; slightly "iddingsitized" olivine microphenocrysts in mass of plagioclase, titanomagnetite, and granular pyroxene. Late interstitial biotite; very slight traces of green phyllosilicates in patches in the groundmass.
794:	Basalt. Microphenocrysts of olivine and plagioclase in finer groundmass of pyroxene, titanomagnetite, plagioclase and olivine. Slight phyllosilicate alteration of olivine microphenocrysts.
803:	Fine-grained basalt. Microphenocrysts of olivine and plagioclase in a groundmass of olivine, titanite, plagioclase and titanomagnetite. Slight alteration of the olivine along cracks to phyllosilicates.
844:	Porphyritic olivine-pyroxene basalt. Fine-grained groundmass of plagioclase, pyroxene, olivine and titanomagnetite.

siderable distances), perhaps by the parallel movement of the magma source with each volcanic island.

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# An Evolutionary Sequence for Strong Radio Sources

by

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The authors attempt to show that the results of Heeschen on extra-galactic radio sources can be simply interpreted in terms of a consistent evolutionary sequence. They suggest that only three stages of evolution are required: particle injection into a jet-like structure; adiabatic expansion of a relativistic gas; and, finally, pressure equilibrium with the inter-galactic gas. This suggests that the quasars are at cosmological distances.

HEESCHEN<sup>1</sup> has recently compared the 1,400 Mc/s absolute radio luminosity,  $L$ , and surface brightness,  $B$ , of many extra-galactic radio sources, and found a remarkable, well defined correlation. It is the purpose of this article to suggest that Heeschen's results may be interpreted as evidence of a possible radio source evolutionary sequence which is very similar to that suggested by Gold<sup>2</sup>. We shall find that the location of a source in the sequence is determined by the evolutionary stage extant: particle injection, adiabatic expansion of a relativistic gas, or pressure equilibrium with the inter-galactic medium. The location is also correlated with spectral index, which reflects the age of the radiating material.

To facilitate the discussion we have reproduced Heeschen's results in Fig. 1. Heeschen found that the quasi-stellar objects fall on a zero slope sequence in the  $\log L$ - $\log B$  plane at the absolute luminosity  $L \approx 10^{28}$  W/c/s (on the assumption that they are at cosmological distances) and that the radio galaxies form a well defined sequence adjoining that of the quasi-stellar objects with a mean slope of about 1.5. Spiral and irregular galaxies are clustered at the base of the radio galaxy sequence. Several sources are outside these sequences, particularly the cores of three core-halo objects: M87, 3C 218, and Perseus A. Their halo components are found in the radio galaxy sequence. Outside the sequences, and in the same region as the cores, are also one Seyfert galaxy, NGC 1068, and one peculiar galaxy, M82. It is significant, as pointed out by Heeschen, that each anomalous object is known to be associated with a violent event in its nucleus.

The quasi-stellar object sequence indicates that the quasi-stellar objects have similar intrinsic luminosities at 1,400 Mc/s. There is other evidence in support of this observation. If the quasi-stellar objects do have a fixed intrinsic luminosity at a particular frequency then there should be a relation between their apparent radio magnitude at that frequency and their red-shift. Hoyle and Burbidge<sup>3</sup> have explored this possibility by plotting the apparent radio magnitude at 178 Mc/s against  $(1 + \log z)$ , where  $z$  is the usual red-shift, and showed that the result was simply a scatter diagram. Bolton<sup>4</sup>, however, has constructed a similar diagram for the apparent radio magnitudes at the frequency 1,400 Mc/s where the effects of self-absorption tend to be less, and has shown that if only the quasi-stellar objects with spectral indices less than 0.5 are considered then the scatter is considerably reduced, indicating that such quasi-stellar objects may be better characterized by a fixed intrinsic luminosity. Further, Kafka<sup>5</sup> has calculated the intrinsic spectra for 31 quasi-stellar objects in the 3C revised catalogue by

applying to the observed spectra the cosmological corrections appropriate to Friedmann models with several values of  $q$  and zero cosmological constant. Inspection of his results for  $q = -1$  (steady state universe) indicates that while the scatter in luminosities at the frequency 1,500 Mc/s for all the 31 quasi-stellar objects is about a factor of 250, if only the five in his sample with strikingly flat spectra are considered (3C 273, 3C 345, 3C 138, 3C 309.1, 3C 286), the scatter in their luminosities is reduced to a factor of about 20. For other values of  $q$  the scatter in both groups is less. Even with such limited statistics, the indications are that the quasi-stellar objects with flat spectra may have a sharply bounded intrinsic luminosity. Using the table of radio source parameters compiled by Howard *et al.*<sup>6</sup>, we find that half the quasi-stellar objects plotted by Heeschen<sup>1</sup> have spectral indices less than 0.6 so that we may perhaps expect those to be of the same intrinsic luminosity.

There is evidence that the spectra of radio galaxies steepen with time. Kellermann<sup>7</sup> has compared the spectral index with the projected linear separation of the two components in double radio sources and has shown that there is an increase in the spectral index with increase in the separation of the components. Cohen, Gundersmann and Harris<sup>8</sup> have demonstrated that quasi-stellar objects

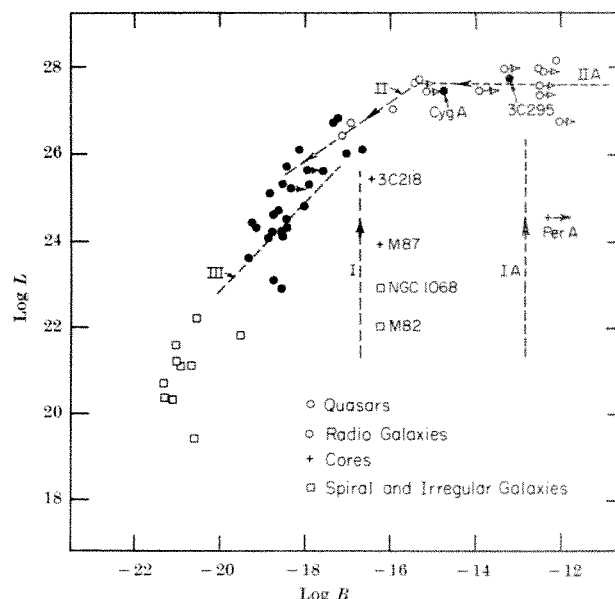


Fig. 1. The absolute radio luminosity,  $L$ , and surface brightness,  $B$ , at 1,400 Mc/s for various radio sources<sup>1</sup>. We have added the possible evolutionary paths I, IA, II, IIA, and III.

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with flat spectra are particularly strong scintillators and are thus sources of exceptionally small angular size. Thus the radio emission of quasi-stellar objects with flat spectra seems to originate from a small volume and they are likely to be young.

Setting aside discussion of the quasi-stellar object sequence for the moment, we shall show that Heesch's results suggest the following evolutionary path of a typical radio source. In the first phase a violent event occurs in a small region inside a galaxy and the radio source makes its initial appearance in the  $\log L$ - $\log B$  plane as an object of high brightness but small luminosity. It is likely, as noted by Gold<sup>2</sup>, that while the relativistic gas produced by the violent event is in the galaxy, it is not free to expand isotropically, but rather is guided by the galactic magnetic fields into a jet such as is observed in M87. In its early stages, then, the radio source will in general have a core-halo structure, and as we shall show, its core will evolve along one of the vertical lines in Fig. 1, say, path I, with the direction of increasing time indicated by the arrow.

The second phase occurs when the relativistic gas has reached the inter-galactic medium and is thus able to expand more nearly isotropically as a "cloud". The slope of the evolutionary path in this phase will be shown to be

$$\frac{d \log L}{d \log B} \approx 0.7$$

The final phase is reached when the cloud of relativistic particles has stopped expanding because it has come into pressure equilibrium with the inter-galactic medium. The external pressure on the cloud may be caused by the ambient magnetic pressure of the inter-galactic medium<sup>2</sup>, or may be of dynamical origin as a result of the motion of the cloud through the inter-galactic medium (unpublished work of D. S. DeYoung). Even though the cloud has stopped increasing its size, it will may have sufficient transverse momentum to continue to separate from its parent galaxy. The relativistic particles continue to lose energy by synchrotron radiation so that their energy spectrum steepens, but the size of the cloud remains fixed. Thus this phase will be shown to be characterized by a slope of 1 in the  $\log L$ - $\log B$  plane.

We now discuss each of these evolutionary stages in greater detail. For an optically thin source of average emissivity  $\epsilon$ , the absolute luminosity,  $L$ , and surface brightness,  $B$ , are

$$L = \epsilon V \quad B = \epsilon V / \Omega \quad (1)$$

where  $V$  is the volume of the source and  $\Omega$  the solid angle it subtends. In the case that the energy spectrum of the relativistic electrons is

$$n(r, E) dE = K(r) E^{-m} dE \quad (2)$$

the volume emissivity resulting from synchrotron radiation is

$$\epsilon_v \propto K H^{\frac{m+1}{2}} v^{\frac{1-m}{2}} \quad (3)$$

where  $H$  is the average magnetic field strength and  $v$  is the frequency of the radiation. It will be useful to replace  $K$  in (3) by its dependence on the total relativistic electron number density  $N$  which is the integral of (2) between the upper and lower energy cut-off,  $E_2$  and  $E_1$ , respectively. As  $(E_2/E_1) \gg 1$ , we may write (3) as

$$\epsilon_v \propto \begin{cases} N E_1^{m-1} H^{\frac{m+1}{2}} v^{\frac{1-m}{2}} & m > 1 \\ N \log \frac{E_2}{E_1} H v^0 & m = 0 \\ N E_2^{m-1} H^{\frac{m+1}{2}} v^{\frac{1-m}{2}} & m < 1 \end{cases} \quad (4)$$

We now consider the evolutionary path during the first phase. For guidance, we take the core of M87 as representative of the cores of core-halo objects. We can characterize these sources by a jet inside the body of a galaxy. We take the jet to be continuously supplied with relativistic particles by the explosions inside the galaxy, and as forcing its way out in such a way that it increases its length  $l$  while maintaining the same diameter. Then we may take  $N \approx$  constant,  $H \approx$  constant, so that  $L \propto l$ , and  $B \approx$  constant. Thus, as the jet lengthens, the source evolves in the direction of increasing luminosity along a line of constant brightness. The paucity of core-halo objects suggests that this stage of evolution is particularly rapid.

The evidence clearly suggests that the jets eventually reach the inter-galactic medium where they evolve into the common double-lobed radio structure. During this second phase we consider the cloud of relativistic particles to be roughly spherical (of radius  $r$ ) and to have its full complement of relativistic particles. It expands until it comes to pressure equilibrium with the inter-galactic medium. During the expansion, by continuity and flux conservation,  $N$  is proportional to  $r^{-3}$ ,  $H$  is proportional to  $r^{-2}$ , and if in addition, the expansion is adiabatic (with ratio of specific heats  $\gamma = 4/3$  as appropriate to a relativistic gas) then  $E$  is proportional to  $r^{-1}$ . From (4) we have that (provided that  $m \neq 1$ )  $L$  is proportional to  $r^{-2m}$  and  $B$  is proportional to  $r^{-2m-2}$ . Hence

$$L \propto B^{m/(m+1)} \quad (5)$$

In the expansion in the second phase therefore the radio sources should evolve with a slope of  $m/m+1$  in the  $\log L$ - $\log B$  plane. Taking  $m \approx 2.4$  which is a mean value for double radio sources, the slope is  $\approx 0.7$  which we have drawn in Fig. 1 as path II. As  $m$  increases with time the slope steepens to 1. The increase in slope towards the value is evident at the lower luminosity end of path II.

We observed in Fig. 1 that in the radio galaxy portion of the diagram beyond path II there is a steepening in the radio galaxy sequence. It is unlikely that this is caused by the steepening of the slope  $m/m+1$ , as calculated for the expansion phase, because the radio galaxies in this portion of the  $\log L$ - $\log B$  diagram have about the same spectral indices as those lying near path II. We suggest rather that in the final stage of the evolutionary sequence the cloud of relativistic particles has come to pressure equilibrium with the inter-galactic medium and no longer expands. The dimensions of the cloud are fixed, so that in this stage of evolution the luminosity and brightness remain directly proportional to each other and the evolutionary path has slope 1 in the  $\log L$ - $\log B$  diagram (path III). The evolution is of course in the direction of decreasing luminosity since the emissivity decreases with time as a result of the synchrotron losses of the relativistic particles.

Looking now at the surprisingly flat sequence described by the quasi-stellar objects, we note that the nearly zero slope is likely a consequence of the flat intrinsic source spectra. It is natural to suggest that we are dealing here with young sources just beginning their expansion, in the second phase, into the inter-galactic medium. They have commenced their evolution with a rapid particle injection into a smaller volume than the radio galaxies, so that the first phase has followed path IA. The energy spectrum of the relativistic electrons has not yet been steepened by losses. It is not known what should be assumed for the original unsteepened energy spectrum, but the criterion of frequency spectrum flatness used above indicates that we should take  $m < 2$ . For the special case  $m = 1$  the slope in the  $\log L$ - $\log B$  plane is  $1/2$  and for smaller  $m$ s it decreases as  $m/m+1$  to 0 when  $m = 0$ ; that is when we have a flat energy spectrum. This range of values for the slope seems to be compatible with Heesch's<sup>1</sup> observed quasi-stellar object sequence. As these sources continue

to evolve and steepen their energy spectrum, their evolutionary path IIA steepens its slope into that of the "normal" expansion phase, path II.

In summary we have attempted to show that Heesch's observations may be interpreted in terms of a consistent evolutionary sequence. We must emphasize that at any one time different radio components of the same object may be found in different phases of the evolution. For example, while the intense double radio sources are in the second or final phase, the emission of the parent galaxy may be located among that of the normal spirals and irregulars.

Throughout the discussion we have implicitly assumed the cosmological nature of the quasi-stellar objects. We suggest that the radio emission properties of the quasi-

stellar objects fit so naturally into the proposed sequence that it argues that they are at cosmological distances.

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## Specific Adsorption of Anions

by

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The specific adsorption of anions depends on the  $pK$  values of the anion acids. The adsorbed anions confer a negative charge on the surface displacing the zero point of charge to lower  $pH$  values.

ADSORPTION of anions at mineral surfaces is important in soils because of the limit this process imposes on the availability of plant nutrients which occur naturally as anions or are added in anionic form in fertilizers. The nature of an oxide solution interface is determined both by the hydrogen ion concentration and the presence of specifically adsorbed anions within the co-ordination shell of, for instance, an iron atom.

Protons and hydroxyl ions are the potential determining ions<sup>1</sup> in the non-specific adsorption of such anions as chlorine and nitrate on kaolinite<sup>2</sup>, ferric<sup>3</sup> and other oxides. When adsorption of protons occurs in excess of that for hydroxyl ions the surfaces become positively charged, the magnitude of the charge increasing with ionic strength at any given  $pH$  below the zero point of charge (ZPC). In these circumstances the positive charge is located on the inner Helmholtz layer with the balancing anion located either in the outer Helmholtz layer<sup>4</sup> or the diffuse double layer. Such adsorption is termed non-specific.

Changes in bulk concentration of the anions, orthophosphate, pyrophosphate, tripolyphosphate, selenite, silicate and fluoride after adsorption show that these anions are bound to a far greater extent than would be predicted from their concentrations when compared to a non-specifically adsorbed anion. At any  $pH$  there is a maximum adsorption<sup>5</sup> of such anions and when these maxima are plotted against  $pH$ , curves of the type shown in Fig. 1 are obtained (adsorption envelope). These curves are insensitive to changes in the ionic strength so that the adsorption is not determined by the properties of the diffuse double layer or the outer Helmholtz layer.

For the silicate and fluoride ions there is an adsorption maximum at the  $pK$  value, while for orthophosphate

there is a discontinuity at each  $pK$  value, the one at the last  $pK$  value being the most marked. The good linear correlation (Fig. 2) between the  $pK$  values of a number

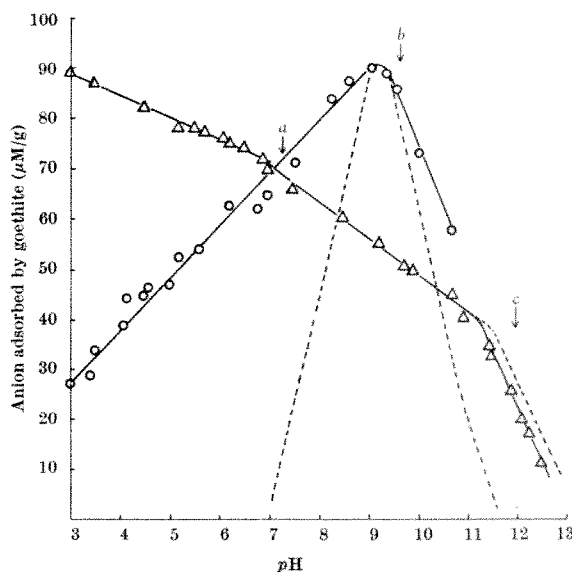


Fig. 1. Adsorption envelope. Variation in the maximum adsorption of silicate and orthophosphate on goethite with  $pH$  at  $20^\circ C$  in the presence of 0.1 molar sodium chloride as supporting electrolyte:  $\circ$  silicate;  $\Delta$ , orthophosphate. Broken curves indicate the relationship obtained from the equation. Amount of anion adsorbed =  $2V\sqrt{\alpha(1-\alpha)}$  where  $V$  is the amount of anion adsorbed at  $pH$  9.2 for  $H_4SiO_4$  and  $pH$  11.3 for  $H_3PO_4$ ,  $\alpha$  is the degree of dissociation of  $H_4SiO_4$  and  $HPO_4^{2-}$ . a,  $H_3PO_4$ ,  $pK_2$ ; b,  $H_4SiO_4$ ,  $pK_1$ ; c,  $H_3PO_4$ ,  $pK_1$ .

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of weak acids and the  $pH$  values at the discontinuities on the adsorption envelopes indicates the wide applicability of this relationship. The results indicate that the undissociated free acid and the most highly charged form of the anion are not adsorbed when present alone, that is, at low and high  $pH$  with respect to the  $pK$ , but that the presence of both forms of the anion is necessary before adsorption can take place. Thus the requirement for adsorption of an anion of a weak monobasic acid is the presence of both a proton donor (acid) and acceptor (salt).

It can be shown that the amount of adsorption should be proportional to  $2\sqrt{\alpha(1-\alpha)}$  where  $\alpha$  is the proportion of the most highly charged form of the anion and the adsorption envelope should pass through a maxima at the  $pK$  value and be described by a relationship,

$$A = 2V\sqrt{\alpha(1-\alpha)} = 2V\sqrt{\frac{K[H^+]}{(K+[H^+])^2}} \quad (1)$$

where  $A$  is the amount of the ion adsorbed per unit weight of adsorbent,  $\alpha$  the degree of dissociation of the acid or acid anion in solution,  $V$  the amount of ion adsorbed at the maximum, and  $K$  is the dissociation constant of the most highly charged anion that is adsorbed.

If the acid is polybasic (for example, phosphoric acid), then the acid anions (for example,  $HPO_4^-$ ) produced with decreasing  $pH$  past the last  $pK$  value can themselves accept and donate protons without requiring the presence of two types of anion. Instead of passing through a maximum the envelope shows a marked decrease in slope as it passes through the last  $pK$  value (Fig. 1, phosphate).

Fig. 1 shows that equation (1) applies reasonably well to the results for the rise to the last  $pK$  of phosphoric acid. The deviations for silicic acid at the extremes of  $pH$  must be caused by an interaction between the ions and the surface and/or the ions already adsorbed which is not taken into account.

For a polybasic acid after the "envelope" passes through the last  $pK$  it follows a Temkin isotherm (Fig. 1, phosphate), probably because of a linearly increasing interaction energy as the surface coverage increases. As further  $pK$  values are negotiated the adsorbing species

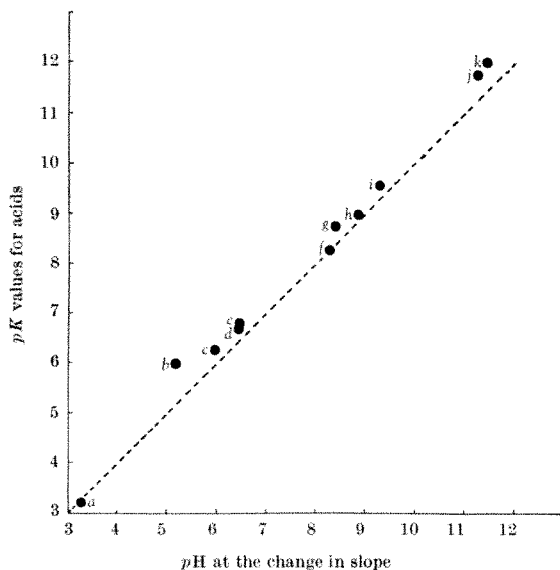


Fig. 2. The relationship between the  $pK$  values for weak acids and the  $pH$  for breaks in slope of the curve for maximum adsorption against  $pH$ . All acid dissociation constants, except  $pK_3$  for  $H_3PO_4$  and  $H_3AsO_4$ , were determined in 0.1 molar sodium chloride. Approximate values for the two exceptions were obtained from values given in the literature. The broken line indicates the ideal relationship. a, HF; b,  $H_2P_2O_7$ ; c,  $H_2P_2O_7$ ; d,  $H_2P_2O_7$ ; e,  $H_2P_2O_7$ ; f,  $H_2P_2O_7$ ; g,  $H_2P_2O_7$ ; h,  $H_2P_2O_7$ ; i,  $H_2P_2O_7$ ; j,  $H_2P_2O_7$ ; k,  $H_2P_2O_7$ .

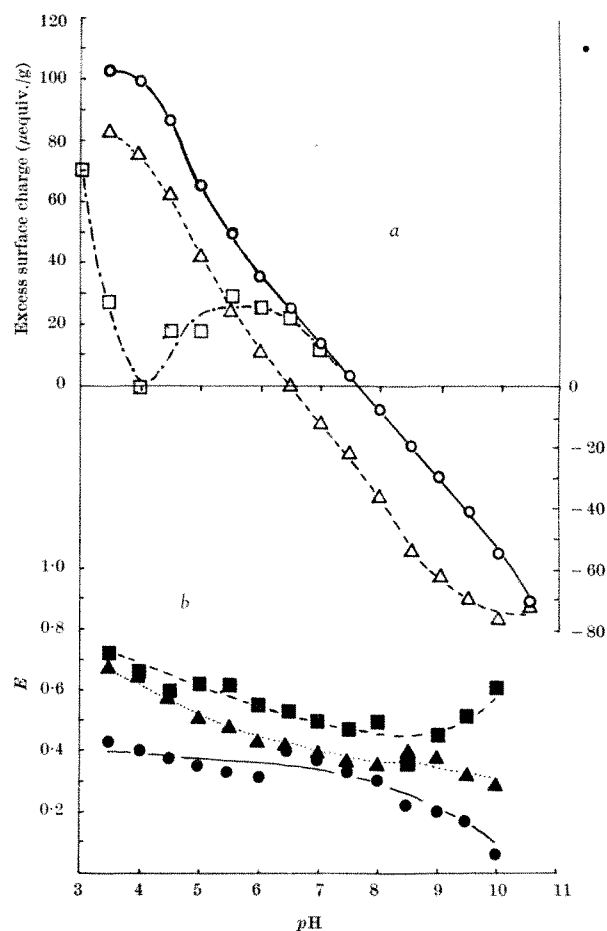


Fig. 3. a, The effect of adsorption of silicate and fluoride on the curve relating excess surface charge to solution  $pH$ .  $\circ$ , 0.1 molar sodium chloride;  $\square$ , 0.1 molar sodium chloride + 60 p.p.m. fluoride as NaF;  $\triangle$ , 0.1 molar sodium chloride + 69 p.p.m. silicon as  $H_2SiO_4$ . Note that fluoride is not adsorbed at  $pH$  values higher than the natural zero point of charge of the oxide. b, The negative charge contributed to the surface/mole of silicon adsorbed ( $E$ ), as a function of  $pH$  and ionic strength of the supporting electrolyte.  $\bullet$ , 0.01 molar sodium chloride;  $\blacktriangle$ , 0.1 molar sodium chloride;  $\blacksquare$ , 1.0 molar sodium chloride.

undergoes an abrupt diminution in charge resulting in a change in slope of the Temkin regions of the "envelope" at that  $pH$ . The direction of change is determined by the net effect of the interactions between ions on the surface and the ion/surface interaction. When the undissociated acid species becomes the dominant form the envelope should fall following equation (1).

From acid/base titration curves in the presence and absence of specifically adsorbed anions and the adsorption measurements the effect of the specifically adsorbed anions on the surface charge has been determined and some results presented in Fig. 3. The zero point of charge (ZPC) is shifted to more acid values and thus specific adsorption of an anion renders the surface more negative. This it must do by entering the inner Helmholtz layer<sup>4</sup>, that is the first co-ordination sphere of the metal ions on the oxide surface, through displacement of a co-ordinated hydroxyl ion. The displacement is a ligand exchange reaction. A negative charge is developed only if a proportion of the hydroxyl ions liberated from the inner layer is in effect retained by the surface through the formation of water as shown in Fig. 4, i-iii. This may be contrasted with the formation of a positive charge balanced by non-specific adsorption and non-specific exchange (Fig. 4, iv and v).

These ideas explain why weak acid anions show maximum adsorption at about the  $pK$  value, since at this  $pH$  the displaced hydroxyl can be retained by adsorption and ionization of the acid form of the anion.

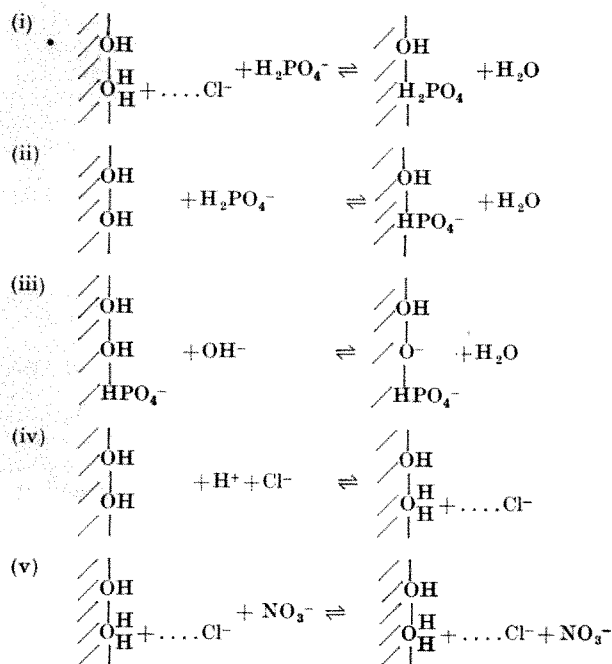


Fig. 4. Anion reactions at the oxide/solution interface. (i) Neutralization of positive charge at pH values below the ZPC; (ii) ionization of a proton of an adsorbed acid anion; (iii) enhancement of the tendency of the surface itself to acquire a negative charge; (iv) non-specific adsorption; (v) non-specific exchange.

The charge ( $\sigma$ ) per unit weight of adsorbent in the presence of the adsorbed anion is given by the expression,

$$\sigma = \sigma_0 + AE$$

where  $\sigma_0$  is the charge per unit weight of adsorbent in the absence of specific adsorption and  $E$  the charge contribu-

tion per adsorbed ion. An example of the variation in  $E$  is given in Fig. 3b.  $E$  is the resultant of the actual exchange of the anion for the hydroxyl ion and the uptake of this ion by the surface in the presence of the anion, that is, of processes (i), (ii) and (iii).

$A$  and  $E$  may be varied independently of one another by change of anion, ionic strength and anion concentration and the same charge can be made up in any number of ways. Thus, with varying pH,  $\sigma$  could have several maxima and minima and more than one zero point. For example, if all the adsorbed anion is removed through pH change before the natural ZPC of the surface is reached, then two ZPC could be obtained (Fig. 3).

The concepts discussed here also explain the observation that anions specifically adsorbed at a given pH could not be desorbed<sup>5</sup> by washing with a solution of non-specifically adsorbed anions (for example, chlorine ions) at the same pH and ionic strength. It is essential for desorption of specifically adsorbed anions to make the charge on the surface more negative than the equilibrium value at which adsorption took place. Non-specifically adsorbed anions cannot make the charge more negative at constant ionic strength and thus are ineffective. Anions capable of specific adsorption to a greater extent than the anion on the surface can, however, make the surface more negative, liberating hydroxyl ions which result in desorption. In essence the same effect, that is, an increase in the net negative charge, is accomplished by raising the pH, thus increasing the adsorption of hydroxyl ions.

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## Immunological Reactivity of Lymphoid Cells after Treatment with Anti-lymphocytic Serum

by

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Immunological reactivity of lymphoid cells after treatment with anti-lymphocytic serum has been measured by their ability to mount a graft-versus-host reaction. This technique makes it possible to test the "blindfolding" hypothesis of ALS action.

It is now well established that anti-lymphocytic serum (ALS) prepared in animals of another species can prolong the life of homografts very significantly when injected into the recipients at or soon after transplantation<sup>1-11</sup>. Several hypotheses have been proposed to explain its mode of action, among them Medawar and Levey's suggestion of lymphocyte "blindfolding"<sup>8</sup>, that is, that coating of lymphocytes with xenogeneic protein in the form of antibody prevents the receptor sites of the cells from recognizing the allogeneic antigens of the graft.

The experiments described here combine the more conventional assays of ALS, such as determination of antibody titres and effect on skin homograft survival,

with an assay based on the graft-versus-host reaction (runt disease) which is aroused when allogeneic lymph node cells are injected into newborn mice. This assay has the advantage of measuring the reactivity of serum-treated cells in an antibody-free environment.

Five New Zealand white rabbits were injected with  $300 \times 10^6$  CBA mouse lymph node cells on days 0, 7 and 15. Immediately before the third injection 50 ml. of blood was taken from each animal, and they were all bled out on day 29. All sera were pooled, heated at 56° C for 30 min and absorbed three times with CBA red blood cells freed from white cells by repeated filtration through glass wool. Absorptions were carried out overnight at 5° C and the ratio of packed red cells and serum was approximately 1 : 10. The serum (ALS 2266) was sterilized

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by Seitz filtration and stored at  $-20^{\circ}\text{C}$ ; it was non-lethal to mice injected intravenously with 0.5 ml. Normal rabbit serum (NRS) was handled in the same way.

Agglutinins to nucleated cells were estimated in the following way. Two-fold dilutions of serum were prepared in Ringer-phosphate buffer (pH 7.4) containing 5 per cent NRS. To each dilution an equal volume of lymphoid cells suspended in the same medium was added, the final concentration of the cells being  $6 \times 10^6/\text{ml}$ . The tubes were gently shaken at room temperature for 1 h and scored microscopically.

Red cell agglutinins and lysins were measured as follows. For agglutinins, two-fold dilutions (0.5 ml.) of serum in 0.1 per cent polyvinylpyrrolidone-saline received 0.05 ml. of a 2.5 per cent suspension of mouse red blood cells. The tubes were shaken gently for 2 min and left at  $4^{\circ}\text{C}$  overnight. The degree of agglutination was ascertained by viewing the bottom of the tubes in a concave mirror. For lysins, tubes were set up as described here except that dilutions were made in normal saline. After shaking, the tubes were incubated at  $37^{\circ}\text{C}$  for 30 min; 0.25 ml. of a 1:3 dilution of fresh guinea-pig serum was then added to each tube, and, after shaking, the tubes were incubated for a further 30 min. The degree of lysis was estimated by the colour of the supernatant after centrifugation.

To detect antibody on the surface of peripheral blood cells, smears were prepared from the blood of ALS-treated mice and fixed in 95 per cent ethanol for 10 min. The presence of rabbit gamma globulin on white blood cells was then demonstrated using a fluorescein-labelled goat anti-rabbit globulin (Burroughs Wellcome) and the standard sandwich technique described by Nairn<sup>12</sup>.

Skin was grafted by the method of Billingham and Medawar<sup>13</sup>. Recipients were injected with 0.5 ml. of serum 2 and 5 days after grafting, and in this we followed Medawar and Levey<sup>8</sup>, although in our experiments the serum was given intraperitoneally. Epidermal cell suspension was prepared from tails of *CBA* mice essentially as described by Medawar<sup>14</sup>.

The graft-versus-host assay was carried out in the following way. One ml. of ALS was injected intraperitoneally into each female *C57Bl* recipient and cervical, axillary and inguinal lymph nodes were removed at appropriate intervals. Cell suspensions were prepared in Ringer-phosphate solution (pH 7.4)<sup>15</sup> containing 5 per cent heated *C57Bl* serum; the final cell concentration was usually  $20 \times 10^6/\text{ml}$ . Newborn *A* strain mice were injected intravenously within 20 h of birth with 0.05 ml. of the suspension, that is, with  $1 \times 10^6$  cells. These experiments were controlled by using cell donors treated with normal rabbit serum (NRS).

*In vitro* treatment of lymph node cells with ALS was carried out at room temperature at a cell concentration of  $25 \times 10^6/\text{ml}$ . Serum concentrations varied from 1:20 to 1:500 (final concentrations). The preparation of the suspensions was as here, except that the medium consisted of ninety-five volumes of Ringer-phosphate solution and five volumes of 4 per cent dextran ('Intradex', Glaxo) and clumps were removed by filtration through cotton wool. After exposure to ALS the cell suspension was diluted by the addition of two volumes of Ringer-phosphate without dextran and spun at 1,500*g* for 5 min. They were washed again in Ringer-phosphate solution and finally resuspended in medium. *In vitro* treatment with ALS caused clumping that was proportional to the concentration of the serum, and for most experiments a final concentration of 1:100 was accepted as optimal. Cell clumps were removed by filtration through cotton wool, and a considerable loss of cells occurred at this stage.

Cells were trypsinized at concentrations of  $40 \times 10^6/\text{ml}$ . at room temperature and at a pH of about 7.4. The concentration of trypsin (Difco, 1:250) was 0.05 per cent, and the duration of exposure 45 min. At the end of the incubation period the suspension was cooled to  $5^{\circ}\text{C}$  and

diluted with two volumes of medium; it was then spun at 1,300*g* for 5 min and resuspended in fresh medium.

Incubation with and handling in trypsin damaged many lymph node cells and the number of cells was markedly reduced, but after the final resuspension the number of non-viable cells was negligible. Viability was determined by dye exclusion tests with nigrosin, the final concentration of the dye being 0.01 per cent. All counts were made 3 min after addition of the dye, which had previously been dialysed.

Table 1 summarizes the ability of whole serum to agglutinate lymph node cells, thymocytes and epidermal cells from several strains of mice. Although there was no sign of strain specificity and titres were as strong against thymocytes as they were against lymph node cells, *CBA* epidermal cells registered very low titres. On the face of it this suggests that lymph node and thymus cells have antigens that are absent from the surface of epidermal cells, and that this may be so is underlined by our finding that five sera produced by the injection of rabbits with mouse epidermal cells had high titres against epidermal cells (1,024, 512, 1,280, 640, 1,280) but low titres against lymph node cells (64, 8, 8, 8, 16) (unpublished data). Furthermore, when tested *in vivo* these sera prolonged the life of *A* strain skin grafts on *CBA* mice by only 3 days, an observation which could, however, mean that the timing of our immunization schedule was unsuitable (compare ref. 8; ALS can be successfully absorbed with a variety of tissues).

Table 1. AGGLUTININ TITRES OF RABBIT ANTI-*CBA* LYMPH NODE CELL SERUM TESTED AGAINST VARIOUS CELL TYPES FROM SEVERAL MOUSE STRAINS

Serum fraction	Cell type	Donor strain	Titre
Whole	Red cells	<i>CBA</i>	128*
Whole	Epidermal cells	<i>CBA</i>	8
Whole	Lymph node cells	<i>CBA</i>	1,024
Whole	Thymocytes	<i>CBA</i>	1,024
7S†	Lymph node cells	<i>CBA</i>	1,024
7S+2ME‡	Lymph node cells	<i>CBA</i>	1,024
19S†	Lymph node cells	<i>CBA</i>	32
19S+2ME‡	Lymph node cells	<i>CBA</i>	4
Whole	Lymph node cells	<i>C3H</i>	1,024
Whole	Thymocytes	<i>C3H</i>	1,024
Whole	Lymph node cells	<i>A</i>	1,024
Whole	Thymocytes	<i>A</i>	1,024
Whole	Lymph node cells	<i>C57</i>	2,048
Whole	Thymocytes	<i>C57</i>	2,048

The cytotoxic titre of ALS 2266 was 256-512.

\* The haemolytic titre (guinea-pig complement added) was identical.

† 7S and 19S fractions prepared on a  $44 \times 3$  cm 'Sephadex G-200' column.

‡ 2-Mercaptoethanol.

The suggestion that there may be some degree of tissue specificity certainly deserves further investigation. Meanwhile, from a clinical point of view it would seem prudent to use only lymphoid tissues in the preparation of ALS.

It will also be seen from Table 1 that the lymph node cell agglutinins reside mainly in the 7S fraction, an observation in agreement with the data of James and Medawar<sup>16</sup>, who find that 19S fractions are virtually inactive when tested *in vivo*.

Notwithstanding the lack of strain specificity so far as agglutinating antibodies are concerned, a clear-cut difference was found in the ability of the serum to prolong the life of *A* strain skin homografts on *CBA* and *C57Bl* recipients (Table 2). The implications of this are not at all clear; it might suggest that agglutinating antibodies are not a sound guide to the *in vivo* reactivity of ALS. At any rate, there can be little doubt that a measure of

Table 2. MEDIAN SURVIVAL TIMES (DAYS) OF *A* STRAIN TAIL SKIN GRAFTS TRANSPLANTED TO ALS AND NRS-TREATED *CBA* AND *C57Bl* MALE RECIPIENTS

No. of mice	Strain	Treatment	MST
20	<i>CBA</i>	ALS	30
20	<i>CBA</i>	NRS	11
20	<i>C57</i>	ALS	12
18	<i>C57</i>	NRS	9½

Serum (0.5 ml.) was injected intraperitoneally 2 and 5 days after skin-grafting.

strain specificity exists, for our observation is confirmed and extended by experiments on another serum (unpublished data).

A four-fold drop in the number of circulating white cells occurred within 4 h when 1 ml. of ALS was injected intraperitoneally into ten adult *C57Bl* males, which were bled alternately in groups of five, and this low level was maintained for 24 h (Fig. 1a). The proportion of lymphocytes also declined (Fig. 1b), and the absolute number of circulating lymphocytes reached its nadir by 12 h, when it was about one-eighth of its normal value. Recovery occurred remarkably quickly and by the fourth day the number of cells was back to normal. The rapidity with which both the drop and recovery of lymphocytes came about was more extreme than that observed by Levey

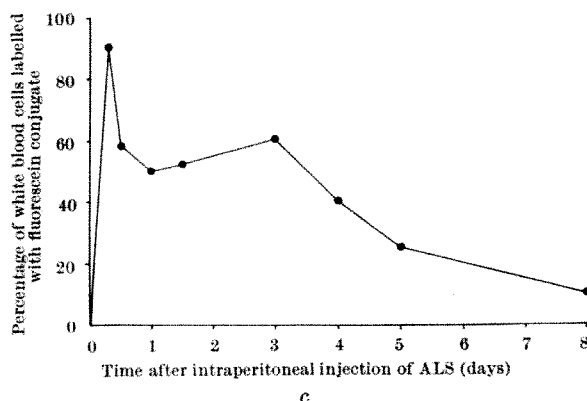
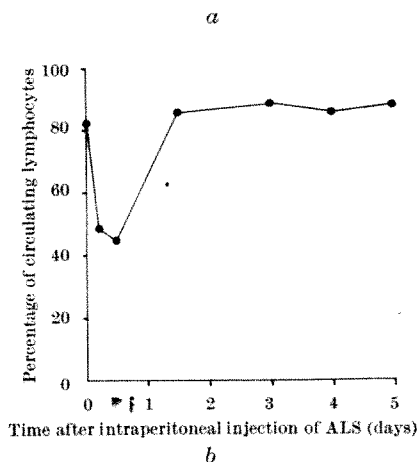
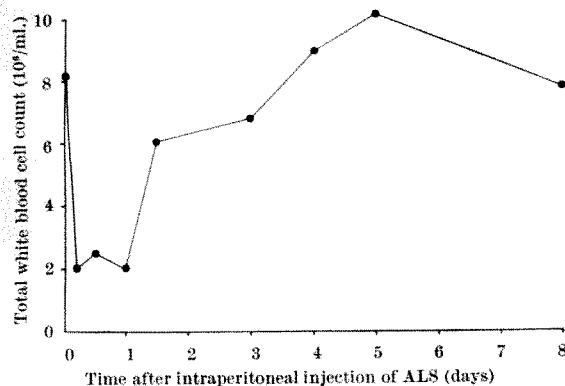


Fig. 1. (a) Effect of intraperitoneal injection of 1.0 ml. of ALS on the total white blood cell count of *C57Bl* male mice. (b) Percentage of circulating lymphocytes (compared with total white cells) in *C57Bl* male mice after intraperitoneal injection of 1.0 ml. of ALS. (c) Percentage of circulating white blood cells from *C57Bl* male mice which were labelled with fluorescein conjugated goat-anti-rabbit gamma globulin after intraperitoneal injection of 1.0 ml. of ALS.

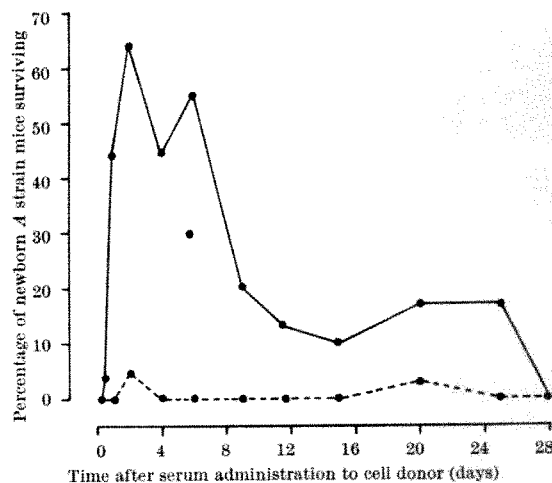


Fig. 2. Percentage of newborn A strain mice surviving 50 days after intravenous injection of  $10^6$  *C57Bl* female lymph node cells removed at various times after serum pretreatment. —●—, Donors injected intraperitoneally with 1.0 ml. of ALS. - - -○- - -, Donors injected intraperitoneally with 1.0 ml. of normal rabbit serum.

and Medawar<sup>8</sup> in *CBA* mice, and the rapid rate of recovery argues the more strongly against the possibility that non-reactivity of mice treated with ALS is primarily caused by depletion of circulating lymphocytes<sup>9</sup>.

The data on the graft-versus-host assay in newborn mice are summarized in Table 3 and Fig. 2. Although lymph node cells from donors treated with NRS almost invariably caused the recipients to die by the sixteenth day, cells from donors taken 2 days after intraperitoneal injection of a single dose of 1 ml. of ALS allowed 64 per cent of the recipients to survive into adult life. This protective effect was not significant until 24 h after serum injection; it was at its peak on days 1-6; the decline observed thereafter was first rapid and then relatively slow, until on day 28 it reached the control value of 0.

Table 3. SURVIVAL OF A STRAIN MICE INJECTED INTRAVENOUSLY, WITHIN 20 H OF BIRTH, WITH  $1 \times 10^6$  LYMPH NODE CELLS REMOVED AT VARIOUS TIMES FROM ALS-TREATED AND NRS-TREATED ADULT *C57Bl* FEMALES

Interval between serum injection* and experiment	ALS-treated donors		NRS-treated donors	
	No. of new-borns injected	Per cent surviving†	No. of new-borns injected	Per cent surviving†
4 h	18	0	17	6
8 h	22	0	23	0
12 h	22	4	21	0
24 h	16	44	15	0
2 days	22	64	21	5
4 days	23	44	26	0
6 days	20	55	16	0
9 days	26	20	26	0
12 days	23	13	21	5
15 days	30	10	31	0
20 days	30	17	30	3
25 days	23	17	15	0
28 days	30	0	15	0
40 days	17	0	10	0

\* Serum treatment = 1.0 ml. of ALS or NRS intraperitoneally at time 0.

† Animals grafted when 2 months old (see Table 5).

That the effect of the serum had a "latent period" of about 24 h suggests that its action during this period was principally on circulating cells rather than on the cells sequestered in the lymphoid organs. This suggestion has some appeal in view of the fact that at 4 h about 90 per cent of circulating white cells were found to be coated with fluorescein-conjugated antibody (Fig. 1c). Our attempts to collect blood lymphocytes 4 or 12 h after injection to test their reactivity were frustrated because there were so few lymphocytes and they tended to agglutinate badly during extraction.

The percentage of circulating cells coated with antibody was maintained between 12 h and 3 days, and then it went into a fairly rapid decline which slowed down after day 5. Except in the first 12 h the percentage of coated



Table 4. SURVIVAL TIMES OF *C57Bl* TAIL SKIN GRAFTS ON A STRAIN MICE WHICH HAD SURVIVED FOR 50 DAYS AFTER INTRAVENOUS INJECTION OF *C57Bl* LYMPH NODE CELLS FROM SERUM-TREATED DONORS (SEE TABLE 3)

Intraperitoneal treatment of original <i>C57Bl</i> donor	No. of survivors grafted	No. of grafts surviving on day									
		7	8	9	10	11	13	14	15	45	50 90
1 ml. of ALS											
12 h	1	1	0								
24 h	6	3	1	0							
2 days	12	5	4	2	2	2	2	2	1	1	1*
4 days	7	3	1	0							
6 days	10	5	2	2	2	0					
9 days	5	1	1	1	1	0					
12 days	3	2	2	1	1	1	0				
15 days	3	1	0								
20 days	4	4	3	1	1	1	1	1	1	1	0
25 days	2	1	0								
1 ml. of NRS											
All times	4	4	2	2	2	1	1	0			
No serum	18	18	17	12	0	0					
5 × 0.2 ml. of ALS											
4 days	11	9	5	1	0						
18 days	5	5	5	1	0						
31 days	3	1	1	1	0						

\*Animal killed with graft in excellent condition; spleen was chimaeric for *C57Bl* antigens.

cells therefore roughly followed the reduction in *in vivo* responsiveness of lymph node cells. As unresponsiveness to skin homografts is in force at a time when relatively few cells can be expected to show visible signs of coating, it must be inferred that (a) antibody is present on the surface but in amounts that would be difficult to trace by the fluorescent antibody technique, or (b) "blindfolding" or some other consequence of surface-attached protein plays a part in the early phase, but retention of antibody on the surface is no longer necessary once the cells have gone through one or more cell divisions, or (c) "blindfolding" is not involved at any stage, coating of cells being merely incidental to the induction of unresponsiveness through other phenomena such as "sterile activation".

Table 5. SURVIVAL OF A STRAIN MICE INJECTED INTRAVENOUSLY, WITHIN 20 H OF BIRTH, WITH  $1 \times 10^6$  *C57Bl* LYMPH NODE CELLS TREATED *in vivo* WITH 1 ML. OF ALS FOLLOWED 2 DAYS LATER BY *in vitro* EXPOSURE TO TRYPSIN

Experiment No.	Serum	Trypsin treatment	No. of mice	Survivors on day									
				12	14	16	18	20	22	24	26	28	30
50	ALS 2266	0.05 per cent for 45 min	7	6	3	1	0						
	ALS 2266	None	6	6	4	3	3	3	3	2	2	2	2*
	Normal rabbit serum	0.05 per cent for 45 min	7	4	2	1	0						
	Normal rabbit serum	None	7	5	1	1	0						

\* Grafted with *C57Bl* skin at 2 months; normal survival time.

Some of the survivors were grafted with *C57Bl* skin when they were 2 months old. One would expect few of them to be tolerant, for they had received only  $1 \times 10^6$  cells neonatally<sup>17</sup>. It is therefore interesting that 2/53 were tolerant (Table 4), suggesting that in these animals the cells had remained viable. The question of whether the cells treated with serum survive after injection into newborn mice is being carefully investigated, and will be discussed elsewhere<sup>18</sup>.

Table 5 gives the results of an experiment in which an attempt was made to restore reactivity to cells treated with ALS by exposing them to the action of trypsin. Although this is so far the only experiment of its kind, we give the results because it is internally well controlled (the experiment has since been repeated with very similar

results<sup>18</sup>). A cell suspension was prepared from the lymph nodes of *C57Bl* mice 2 days after intraperitoneal injection of 1 ml. of ALS—that is, at a time when suppression of responsiveness is near its peak. The experiment included a group of recipients that had received cells untouched by trypsin as well as recipients of cells treated with NRS: tentative as any conclusion must be at this stage, the data (Table 5) suggest that it may indeed be possible to restore reactivity, for the cells exposed to trypsin had the same level of reactivity as the controls. This result cannot be caused by any non-specific action of trypsin unless it is postulated that trypsin enhances immunological reactivity—a possibility that can be dismissed because NRS-treated, trypsinized cells behaved normally in this and in other experiments.

*In vitro* exposure of lymph node cells to ALS for as little as 30 min at room temperature effectively prevented mortality (Table 6), and it was shown that almost all the cells had been coated; even exposure for 1 min, followed immediately by washing, had a slight effect. Concentrations of ALS down to 1:100 gave good protection to neonatal cell recipients, but there was a rapid fall-off in efficacy with lower dilutions, 1:500 being indistinguishable from NRS controls. When higher doses of cells ( $5$  or  $10 \times 10^6$ ) exposed to 1:100 ALS were injected there was some delay in mortality, but only one mouse survived to be grafted with *C57Bl* skin: it was immune. The absence of tolerance in this one animal does not necessarily prove that cells treated *in vitro* with ALS do not survive in their hosts, for the animal may have been merely one of those in which the host-versus-graft reaction had gained the upper hand. While it is true that, judged by dye exclusion tests, about 95 per cent of the cells were viable at the time of injection, it may nevertheless be argued that cytotoxic effects may have been produced by

the presence of complement in the recipients. Experiments are in progress to test the validity of this objection, and preliminary results indeed suggest that cells treated *in vitro* do not always survive in their hosts<sup>18</sup>.

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Table 6. SURVIVAL OF A STRAIN MICE INJECTED INTRAVENOUSLY, WITHIN 20 H OF BIRTH, WITH  $1 \times 10^6$  FEMALE *C57Bl* LYMPH NODE CELLS INCUBATED *in vitro* AT ROOM TEMPERATURE WITH ALS

Experiment No.	No. of mice	Concentration of ALS	Incubation time (min)	Survivors on day									
				12	14	16	18	20	22	24	26	28	
57	12	1:20	30	12	12	12	12	12	12	12	12	12*	
57	17	1:100	30	17	17	17	16	16	16	16	16	16*	
59	11	1:250	30	9	6	2	1	0					
59	11	1:500	30	7	2	1	0						
58	14	NRS	30	6	2	0							
64, 72	18	—	—	9	0								
63, 64	15	1:100	1	14	7	5	2	1	1	1	1	1*	
63‡	15	1:100	30	12	10	8	7	5	3	3	3	3†	
63§	13	1:100	30	12	9	3	2	0					

\* Grafted with female *C57Bl* skin at 2 months; weak immunity.

† One survivor at 2 months; grafting revealed immunity.

‡ Injected with  $5 \times 10^6$  cells.

§ Injected with  $10 \times 10^6$  cells.

# Classically Conditioned Discrimination in the Planarian, *Dugesia dorotocephala*

by

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Planarians have been trained in a Pavlovian way with one conditioning stimulus followed by another. Extinction trials followed, and the results indicate that the animals can differentiate between the two conditioning stimuli and respond appropriately.

SINCE Van Oye's classic study in 1920<sup>1</sup> of planarian behaviour, considerable experimental evidence<sup>2,3</sup> has suggested that platyhelminths are capable of learning even rather complex tasks, including some types of conditioned discriminations. For example, in a study by Griffard and Peirce<sup>4</sup>, planarians were trained to turn in one direction when one conditioning stimulus (light) appeared and in the opposite direction at the appearance of a second conditioning stimulus (vibration); polarized shock was the unconditioned stimulus. Jensen<sup>5</sup>, however, expressed dissatisfaction with the Griffard and Peirce experiment because their procedure did not involve reversal learning or extinction training. Our investigation was designed to overcome Jensen's objections; it consisted of four different stages of training, two involving acquisition of a conditioned response and two involving extinction.

Sixteen laboratory-hatched planarians (*Dugesia dorotocephala*) about 1 cm long were taken randomly from the stock supply of the Planarian Research Group at the Mental Health Research Institute at the University of Michigan. The parent stock had come from Buckhorn Springs, Oklahoma. The planarians were maintained at approximately 22° C throughout the experiment. They were housed individually in small glass custard cups filled with artificial pond water<sup>6</sup> and were fed brine shrimp for 1 h/week. The water was changed immediately after each feeding. The animals were maintained on a normal diurnal lighting cycle.

The training apparatus<sup>2</sup> was a block of opaque white plastic in which had been machined a square-shaped trough measuring 11 cm along each side. The trough had a semi-circular cross-sectional area of approximately 0.5 cm<sup>2</sup>. A glass tube at one corner of the trough delivered a weak jet of air which kept the water in the trough moving slowly in one direction.

The unconditioned stimulus was a pulsed direct current produced by a 6 V storage battery and delivered through a Harvard inductorium to four electrodes, one of which was mounted in each corner of the trough so as to be flush with the bottom surface. Electrodes diagonal to each other were of the same polarity, so that a planarian in the trough was always between an anode and a cathode. Conditional stimulus 2 was weak vibration produced by a 60 cycles/sec oscillator and delivered through an amplifier to a 2.5 in. speaker mounted under the plastic trough. Background illumination was dim and diffuse, being about 75 ft.-candles measured at the level of the trough. When conditioned stimulus 1 was turned on, the illumination increased to about 1,000 ft.-candles.

The intensity of the unconditioned stimulus was adjusted so that the shock presented was the minimal intensity that would always evoke a vigorous unconditioned response in the animals, which was a longitudinal contraction of the body that typically involved a turning of the anterior end of the animal towards the cathode. The intensities of the two conditioned stimuli were also adjusted so that in eight previously untrained pilot animals the response rate was less than 20 per cent to either stimulus. In this pilot study, the animals were also given exposure to the unconditioned stimulus (shock)

presented randomly to prevent any pairing of the unconditioned stimulus with either conditioned stimulus.

Two troughs mounted side by side but carefully shielded from each other were used during the experiment.

Before each day of training, six non-experimental planarians were placed in each trough for about 30 min so that there was a fresh layer of mucus on all trough surfaces. The slime-providing planarians were then removed from the troughs and the water was carefully changed. An experimental subject was then gently transported to each trough from its home aquarium by means of a large camel hair brush and allowed about 5 min to adapt to the trough before the day's trials began. A trial was begun only after the planarian had been gliding smoothly for more than 10 sec in a straight line in the direction of one of the electrodes; the planarian additionally had to be at least 2 cm from the nearest electrode when a trial began. (The speed of locomotion of planarians in these conditions is about 12 cm/min.)

During stages 1 and 3 of the experiment, each planarian was given twenty-five random presentations daily of both conditioning stimuli (1 and 2), each stimulus appearing for 4 sec. The unconditioned stimulus (shock) was presented during the final second of all twenty-five presentations of one of the two conditioned stimuli, but was never paired with the other conditioned stimulus. The minimum elapsed time between unconditioned stimuli was 60 sec. During stages 2 and 4, twenty-five daily presentations of each of the two conditioned stimuli were given as usual, but no shock was presented. A "trial" consisted of the presentation, at least 30 sec apart, of both conditioned stimulus 1 and conditioned stimulus 2, one of which might be paired with the unconditioned stimulus. Thus each animal was given twenty-five "trials"/day. The training procedure used was one found to optimize learning in several previous studies<sup>2</sup>.

For eight planarians (group LV), the light (conditioned stimulus 1) was paired with the shock during stage 1, while the vibration (conditioned stimulus 2) was paired with the shock during stage 3. For the other eight animals (group VL) the order was reversed. During stages 1 and 3, the animals were trained until they reached the criterion level of responding, then were put on extinction training. They were deemed to have reached the criterion of conditioning when, during any block of twenty-five trials, their response level to the "paired" (conditioning) stimulus exceeded 50 per cent and their response level to the "unpaired" ("neutral") stimulus was less than 15 per cent. The criterion for extinction during stages 2 and 4 was reached when the animals showed a response rate of less than 15 per cent to both stimuli during a block of twenty-five trials.

Responses made by the planarians during the initial 3 sec of the presentation of any stimulus were recorded. A response was defined as either a vigorous longitudinal contraction of the body, a turn of the anterior end of the animal through at least a 90° angle, or both. During most of the experiment, observations were made by only one experienced observer. During the first twenty-five extinction trials, however, during stages 2 and 4, the responses of four animals (two from each group) were recorded in-

dependently by two trained observers. This part of the experiment was run "blind", in that neither experimenter knew the previous history of the animals he was observing. Inter-judge reliabilities averaged more than 94 per cent.

Two animals, one from each group, were run simultaneously in the two adjacent troughs. The experiment was replicated four times; in each replication four animals (two from each group) were put through the complete experimental paradigm. No animal was discarded for any reason.

Figs. 1 and 2 present the mean frequency of responding during all four stages of training for group LV and group VL, respectively. In stage 1, using the *t* test for difference between means of matched groups (one-tailed), the mean difference between the number of responses to the "paired" as compared with the "unpaired" stimuli was statistically significant ( $P=0.01$ ) for both groups after an average of only seventy-five trials.

During stage 2 (extinction), the difference between the means was significant ( $P=0.01$ ) for the first two blocks of trials for both groups. During stage 3 (reversal), the difference between the means was significant ( $P=0.05$ ) from the very first block of trials onwards for both groups. During stage 4 (extinction), the difference between the means was significant ( $P=0.01$ ) for both groups during the first block of trials but not during the second block.

The mean number of trials to criterion in stage 1 was 213 for group LV and 173 for group VL. The mean number of trials to criterion in stage 3 was 113 for group LV and 125 for group VL. Both groups learned faster during stage 3 than during stage 1 ( $P=0.01$  for group LV;  $P=0.5$  for group VL).

Fig. 3 shows the mean frequency of responding during all stages of training for the twelve animals tested by one observer and the four animals tested "blind" during stages 2 and 4 (extinction) by two independent observers. In Fig. 3, data from groups LV and VL are thus combined. There were no significant differences between the animals scored by one observer and the animals scored blind by two independent judges.

The results of the present study support the previous findings<sup>4,6-9</sup> that planarians are capable of making a conditioned discrimination. Because, during the present experiment, each animal served as its own control, it does not seem likely that these results can be explained in terms of "sensitization" or "pseudo-conditioning". It should also be noted that significant differences were found not only during stages 1 and 3 (acquisition) but also during stages 2 and 4 (extinction), in contradistinction to earlier reports by Halas and his associates<sup>10,11</sup>. Possible explanations for the failure of these latter experimenters to find evidence for conditioning have been discussed elsewhere<sup>12</sup>.

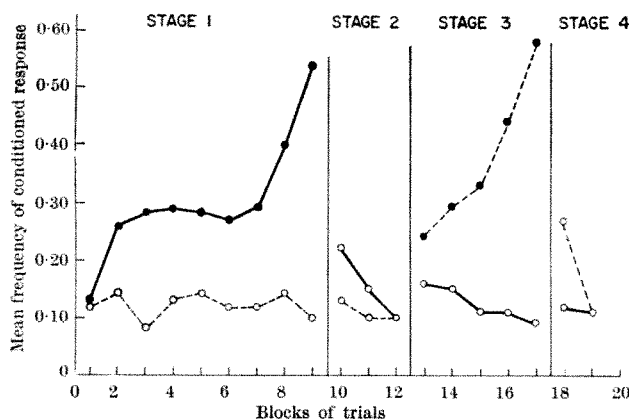


Fig. 1. Response curves for group LV. There were twenty-five trials in each block.  $N=8$ . —●—●, Conditioning stimulus 1 (light) paired with shock; —●—●, conditioning stimulus 2 (vibration) paired with shock; —○—○, conditioning stimulus 1 (light) not paired with shock; —○—○, conditioning stimulus 2 (vibration) not paired with shock.

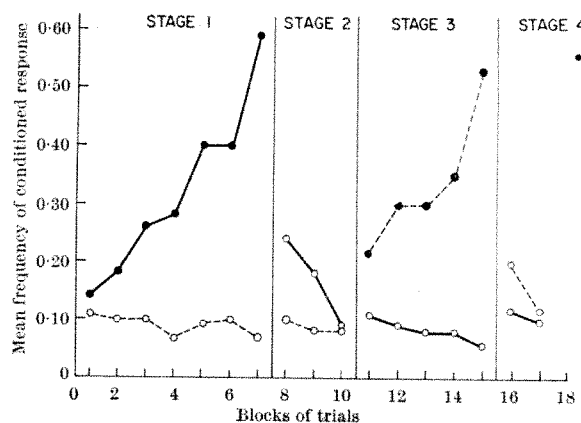


Fig. 2. Response curves for group VL. There were twenty-five trials in each block.  $N=8$ . —●—●, Conditioning stimulus 2 (vibration) paired with shock; —●—●, conditioning stimulus 1 (light) paired with shock; —○—○, conditioning stimulus 2 (vibration) not paired with shock; —○—○, conditioning stimulus 1 (light) not paired with shock.

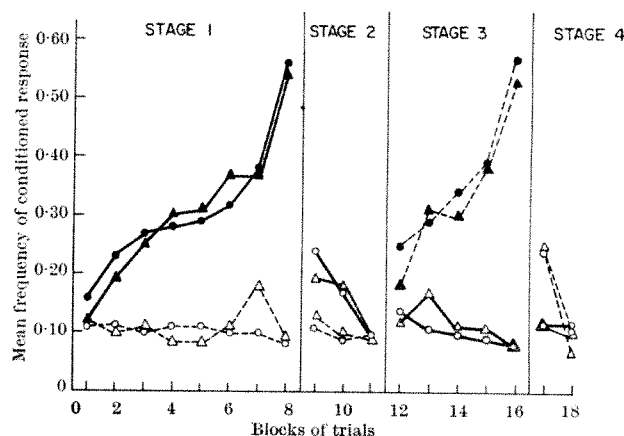


Fig. 3. Response curves (circles) for a group of twelve animals judged by one observer compared with the curves (triangles) for a group of four animals judged "blind" by two independent observers during the first blocks of trials in stages 2 and 4. Solid figures denote pairing of conditioned with unconditioned stimulus. Open figures denote conditioned stimulus presented without unconditioned stimulus.

Both Kimmel and Harrell<sup>6,7</sup> and Jacobson, Horowitz and Fried<sup>9</sup> report that vibration is a more effective conditioned stimulus than is light. Because, in our experiment, the LV animals conditioned more slowly than the VL animals during stage 1, but faster than the VL animals in stage 3, our results tend to confirm these previous results.

The findings of the present study, combined with the recent report<sup>9</sup> that learning occurs in planarians given "forward" conditioning trials (in which the pairing of conditioned with unconditioned stimulus was the same as in our study) but did not occur in planarians given "backwards", "simultaneous" or "pseudo-conditioning" training, should go a long way towards answering in the affirmative the question, "Can planarians be conditioned reliably?"

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## LETTERS TO THE EDITOR

## PLANETARY SCIENCE

## Dependence of Decametric Radio Emission from Jupiter on the Positions of the First Two Galilean Satellites

JOVIAN decametric radio emission is controlled by Io, the first Galilean satellite of the planet, and it has been assumed that the determining factor is the position of Io in its orbit as measured from superior geocentric conjunction<sup>1</sup> or from superior heliocentric conjunction<sup>2</sup>. It has also been shown that the probability of emission is dependent on the position of Io with respect to the plane which contains the magnetic axis of the planet<sup>2,3</sup>.

I have now analysed statistically the observations at Boulder, Colorado, of the decametric radio emission between 1961 and 1965 (refs. 4 and 5). I have sought correlations between the emission in various ranges of frequency and the position of the first three Galilean satellites of Jupiter with respect to the Sun, the Earth and the plane which contains the Jovian magnetic axis.

The radio events used in the analysis were divided into three groups according to the centre frequency. The centre frequency is the arithmetic mean of the lower and upper limits of the observed frequency range. Table 1 shows the classification chosen and the numbers of events in each class. The total number of events was 1,151. The data were stored on magnetic tapes and the search for the effects of the satellites was made by suitable computer

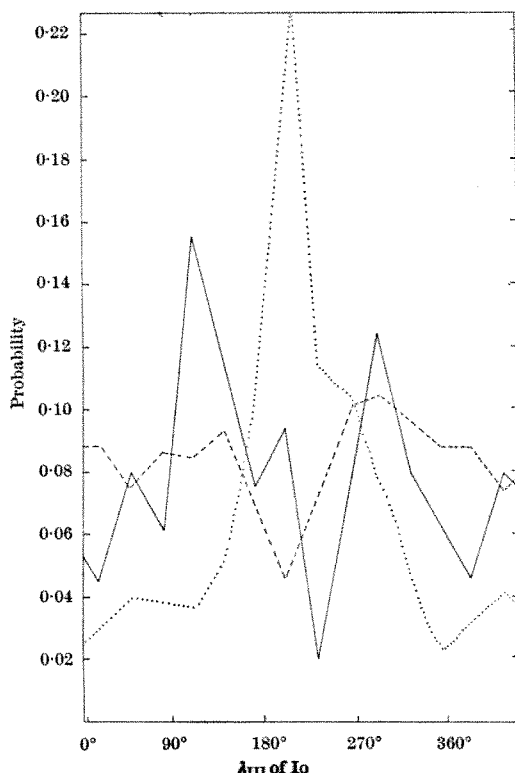


Fig. 1. The probability of emission as a function of the Jovicentric longitude of Io on different frequencies. —, < 15 Mc/s; ---, 15-20 Mc/s; . . . , > 20 Mc/s.

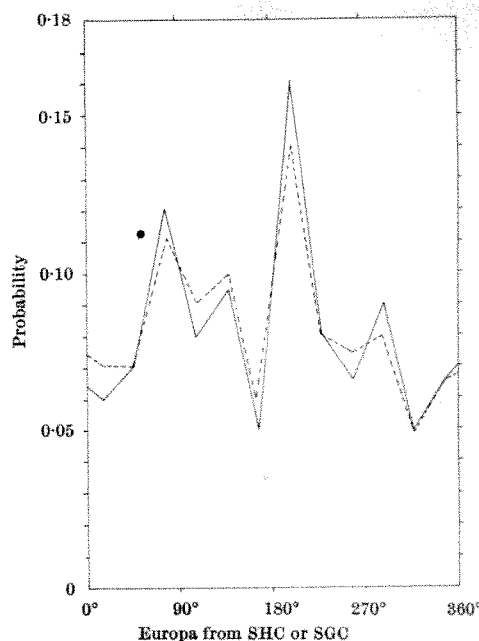


Fig. 2. Probability of emission on frequencies below 15 Mc/s as a function of the departure of Europa from SHC (—) and SGC (---).

programmes. The positions of the satellites were computed for the mid-point in time of each event.

Table 1. CLASSIFICATION OF JOVIAN RADIO EVENTS AND THE NUMBER OF EVENTS IN EACH GROUP

Group	Centre frequency	No. of events
1	< 15 Mc/s	198
2	15-20 Mc/s	579
3	21-30 Mc/s	374

Fig. 1 represents the probability of emission in three frequency ranges as a function of the Jovicentric longitude of Io. The probability of emission is increased at frequencies below 15 Mc/s when the angular departure of Io from the plane containing the magnetic axis of Jupiter is about 90 degrees. At frequencies greater than 20 Mc/s, the probability of emission reaches its maximum when Io is above the Jovian magnetic pole ( $\lambda_{III} = 200$  degrees). In fact, roughly 60 per cent of the events occur when the position of Io is between 165 and 225 degrees. The other magnetic pole at  $\lambda_{III} = 20$  degrees seems to have no influence, which may be explained by the fact that the magnetic dipole is shifted towards the northern rotational pole of Jupiter<sup>6</sup>.

Fig. 2 shows the probability of emission as a function of the angular departure of the satellite Europa from superior heliocentric conjunction (SHC) and from superior geocentric conjunction (SGC). It indicates that the probability of emission is increased at frequencies below 15 Mc/s when Europa is about 195 degrees from SHC. Statistical chi-square test shows that this peak is significant ( $P \approx 0.99$ ). The corresponding peak in the SGC-curve (about 195 degrees from SGC) has a probability of about 0.95. For Io no difference was found between the geocentric and heliocentric maxima.

The forty-one events which occurred when the angular departure of Europa from SHC was from 176 to 225 degrees were investigated in detail. Fig. 3 shows the probability of emission as a function of the longitude of Io from SHC. Evidently the influence of Io at about 240 degrees from the SHC is enhanced. When Io is about 15 degrees from the SHC, the probability of receiving radio emission on frequencies below 15 Mc/s also increases when Europa and Io are situated on opposite sides of Jupiter. Fig. 3 also shows the probability of emission as a function of the Jovicentric longitude of Europa when



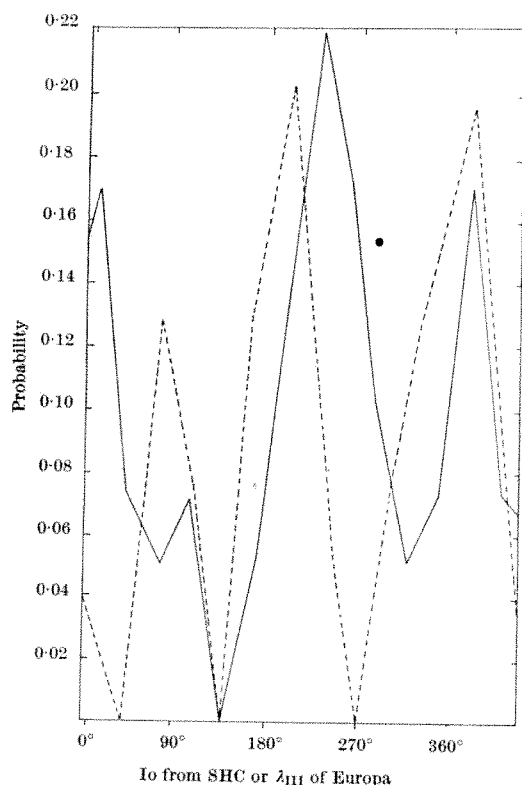


Fig. 3. The probability of emission as a function of the position of Io from SHC (—) and of the Jovicentric longitude of Europa (---). Only events during which Europa was from 176 to 225 degrees from SHC on frequencies below 15 Mc/s are considered.

Europa is from 176 to 225 degrees from SHC. The probability is highest when Europa is close to the plane containing the magnetic axis of Jupiter (Jovicentric longitudes of Europa about 200 and 20 degrees).

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### Rates of Secular Variation in the North-east Atlantic

TOTAL field magnetic data have been collected by proton magnetometers towed behind ships along tracks in the north-east Atlantic during the 11 years between 1956 and 1966, and have been plotted on 1:1,000,000 plotting sheets covering Admiralty plotting sheet areas 29, 30, 41, 42, 43, 59 and 60 (Fig. 1).

In order to reduce these data to magnetic anomalies I have subtracted from the observed values a predicted regional field taken from a chart of magnetic fields in the north-eastern Atlantic drawn by Bullard, Hill and Mason<sup>1</sup>. To reduce all the data to the same epoch (1958.0) it was necessary to make a correction for secular variation.

Before choosing the rates of secular variation two assumptions were made: (a) that the rate was constant within each plotting sheet area; and (b) that the rate of

secular change remained constant over the 11 yr period. The rates of secular variation chosen were extracted from a chart of secular change for epochs 1955.0 and 1960.0 prepared by Leaton<sup>2,3</sup>. The chart was based on results from magnetic observatories on land. In the course of this work sufficient data were accumulated to make an independent check on the rate of secular variation predicted from the land observations.

A simple analysis of the collected data was made: the observed values of total field intensity, without correction for daily variation or the magnetic effect of the ship, were plotted at intervals of 10 min along tracks of the ship. Where two or more tracks crossed, the position, time, date and total field value were listed. The difference in observed values between tracks at the cross-over was divided by the time difference in years between the observations. The mean was then taken for each plotting sheet area and the probable error calculated using Peters's formula

$$r = \pm 0.8453 \frac{\sum |v|}{n\sqrt{n-1}}$$

where  $v$  is the sum of the residuals and  $n$  is the number of observations. The result should then be the mean annual rate of secular change.

In the course of this some obviously inaccurate cross-overs were found. Some errors could result from the fact that no correction was made for daily variation in the geomagnetic field which might account for  $\pm 25\gamma$  and possibly more in times of magnetic storms. In general no data on daily variation were available other than from distant land observatories and so no corrections were applied. Nor was any correction made for the magnetic effect of the ship at the magnetometer; this is dependent on course and could produce errors of  $\pm 10\gamma$ .

Errors and inaccuracies in navigation, particularly in areas of steep magnetic gradient, caused some of the bad cross-overs. Much of the area has been within the range of the Decca and Loran radio navigation station for several years, however, and later tracks should be accurate to  $\pm 1$  mile. Tracks farther offshore and out of the range of Decca and Loran, as in area 41, depended on clear skies for astrofixes and some of the tracks are probably only accurate to  $\pm 5$  miles.

Means were calculated only for the plotting sheet areas which had a sufficient number of reliable cross-overs.

The results are shown in Table 1.

Area	Lat. and long. covered	No. of cross-overs	Mean annual rate of change	Error	Previously assumed values
29	48°-54° N. 6°-21° W.	26	+20.2 $\gamma$	$\pm 5.78 \gamma$	+20 $\gamma$
42	42°-48° N. 12°-24° W.	36	+15.6 $\gamma$	$\pm 4.23 \gamma$	+15 $\gamma$
43	42°-48° N. 0°-12° W.	137	+17.1 $\gamma$	$\pm 1.34 \gamma$	+15 $\gamma$
60	36°-42° N. 6°-18° W.	38	+8.7 $\gamma$	$\pm 3.44 \gamma$	+10 $\gamma$

This is the first independent check on rates of secular variation for an oceanic area using off-shore data and the generalized values which we obtained for each plotting

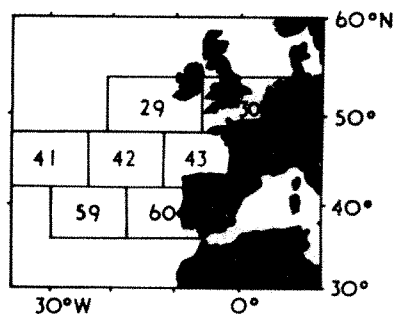


Fig. 1. Map of plotting sheet areas.

sheet area agree remarkably well with those calculated from land observatory data.

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### Natural Infrasonic of Five Seconds Period

BOTH infrasonic air pressure waves with periods of 4–6 sec, known as microbaroms<sup>1</sup>, and seismic ground waves or microseisms, are generated within marine cyclonic storms. This communication considers briefly the origin of microbaroms and their relationship to microseisms and describes and interprets the recently observed semi-diurnal variation of microbaroms. The general characteristics of microbaroms have been described elsewhere—for example, as summarized by Donn and Posmentier<sup>2</sup>.

Cook<sup>3</sup> suggested a line source origin in which ocean wave energy is transformed to acoustic energy at the shore when a semi-infinite train of waves is stopped abruptly. To test this hypothesis we set up a portable capacitor-microphone system at points ranging from the breaker zone on the south shore of Long Island to this laboratory, 65 km to the north. Although higher than average swell was present from an offshore hurricane, there was no significant variation in microbarom amplitude over the range involved. The observations tend to negate the surf mechanism and indicate that the infrasonic waves were coming from a region, distant or large in relation to the 65 km range.

On the basis of a case study, Donn and Posmentier<sup>2</sup> concluded that both microseisms and microbaroms were generated in the ocean water-bottom system and in the atmosphere, respectively, by the same mechanism. This conclusion is supported by the comparison of microseism and microbarom spectra in Fig. 1a which was developed from a simultaneous 45 min magnetic tape recording to both sets of signals, a playback of which is shown in Fig. 1b. The double cyclone system in Fig. 2 was the source of both microseisms and microbaroms.

The air and ground waves have remarkably similar power spectra—including relatively fine details. This was also observed for a different case by Donn and Posmentier<sup>2</sup>. Despite their simultaneous arrival, it was shown by both Cook<sup>3</sup> and Donn and Posmentier<sup>2</sup> that coupling between the ground and air waves during propagation is not possible. Ocean waves appear to be the only mechanism within the storm source capable of exciting these different phenomena with such similar characteristics. My own earlier observations of ocean waves and microseisms seemed to negate this mechanism of microseism origin and so the conclusions based on these new, cogent observations are a reversal of opinion. Posmentier<sup>4</sup> has extended the microseism theory of Longuet-Higgins<sup>5</sup> to include the oscillations of the centre of gravity of the air above standing ocean waves and has obtained amplitudes and periods of infrasonic waves close to those observed at distances of hundreds of kilometres.

In addition to the well known seasonal variation of microbaroms (low intensity in summer) from a variation in storm intensity and in upper level winds, prominent diurnal and semidiurnal changes in intensity also occur.

Existing literature (for example, Saxer<sup>6</sup>) refers only to the diurnal variations of microbaroms, but those observed here usually show a pronounced semidiurnal change when an intense marine storm exists and when the signal is not obscured by local pressure noise induced by wind. Peaks of activity are characteristically centered just after local noon and local midnight (Fig. 3). Although our tripartite

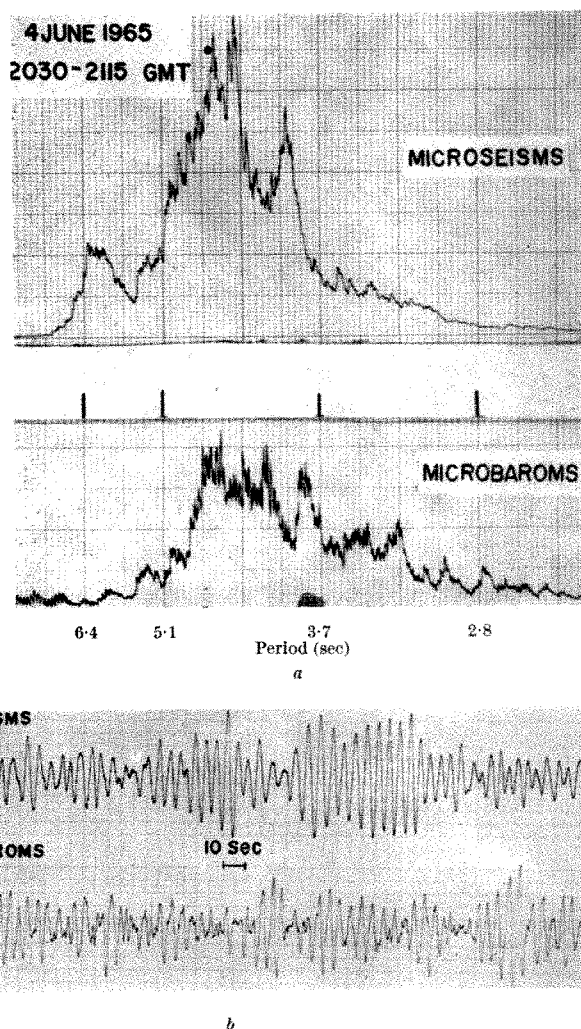


Fig. 1. a, Power spectra of microseisms and microbaroms recorded simultaneously on analogue magnetic tape and processed through a statistical analogue computer (Noratom ISAC). The scales of power on the Y axes are not equivalent. b, Analogue record of a small portion of the 45 min of signal the analyses of which are shown in Fig. 1a. Microseisms were recorded by an electronic, vertical component seismograph having a pendulum period of 15 sec; microbaroms by a capacitor microphone with the output filtered through a 1–10 sec pass band to suppress wind noise.

station for infrasonic wave detection has been in operation less than a year, it now seems that the semidiurnal oscillation variation is weak or absent from late spring to early autumn—the time when signal intensity is generally low.

The cause of the strong semidiurnal oscillation is not yet fully explained, but the 12 h periodicity with peaks close to local noon and midnight suggests an origin related to solar thermal or solar tidal effects for the following reasons. The storm sources of the microbaroms are between hundreds and one to three thousand kilometres away. Infrasonic waves from such storms must therefore follow paths that curve gently into the upper layers of the atmosphere where the temperature and wind structure may be quite different from conditions near the surface. Layers with high sound velocities because of higher temperatures and higher wind speed (in the direction of wave travel) tend to refract rays originating at the surface back to the surface. Layers of lower velocities tend to refract rays

away from the surface. The temperature increase in the upper stratosphere and lower mesosphere produces an increase in sound speed of about 30 to 35 m/sec. Although

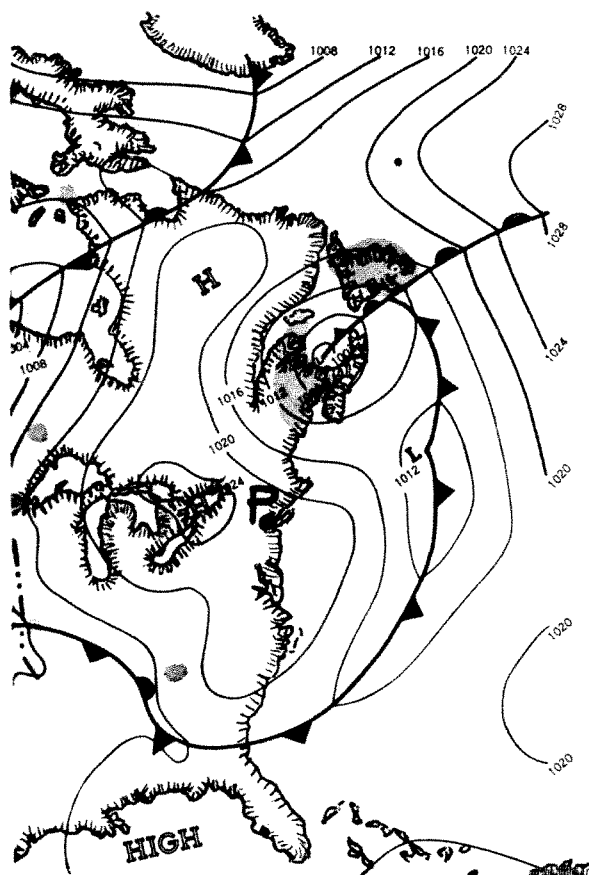


Fig. 2. Weather chart of the western North Atlantic Ocean showing the storm pattern related to the microseisms and microbaroms of Fig. 1. Palisades (Lamont Geological Observatory location) is shown at "P".

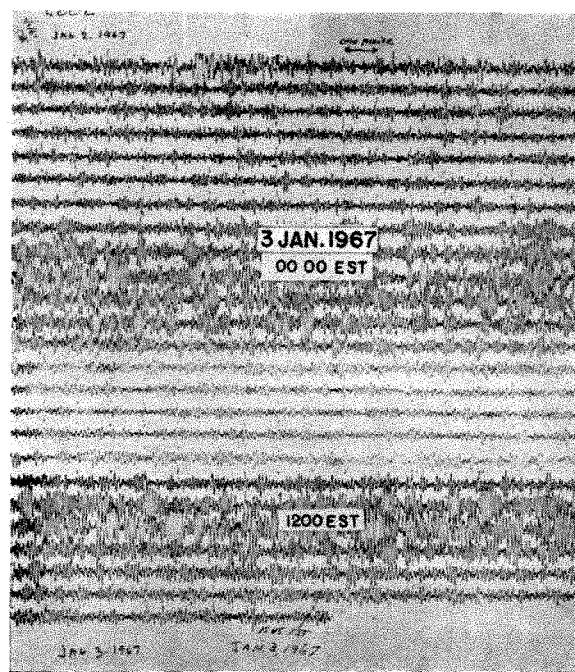


Fig. 3. A portion of the 24 h record of infrasonic waves recorded on January 2-3, 1967, at the Lamont Geological Observatory (Palisades, NY) with the use of Globe microphonic pressure transducers. About 17 min of each line, which contain 60 min, are shown in the copy. Timing marks are made at intervals of 1 min. (EST is 75° meridian time.)

this effect produces a "sound channel" between the surface and about 50 km, no semidiurnal or diurnal variability of the type required is known. Conceivably a rising and falling of the reflecting layer may occur from solar and auroral heating, and this may vary the positions of reflection and produce the observed effect. Preliminary results indicate that wind variations are the more important factor.

Tidal wind components show a pronounced semidiurnal clockwise rotation in the northern hemisphere<sup>7</sup>. At an elevation of about 100 km the tidal component is reported to have a speed of about 30 m/sec. The normal track and development of cyclones are such that the most common infrasonic signal of storm origin arrives from a direction north-east to east-north-east of Palisades. According to Stolov<sup>7</sup>, winds at about 100 km come from a north-easterly direction at about 0100-0200 and 1300-1400 local solar time. The 6 h differential in the ambient wind speed is thus 60 m/sec. The mean zonal winds above the tropopause increase from about 20 to about 70 m/sec, and so the tidal wind effect would seem, in a preliminary examination, to be of significance in producing the semidiurnal oscillation.

On at least two occasions of strong semidiurnal oscillations the signal, at times of wave amplitude maxima, arrived from well developed storms to the north-east of Palisades as determined by our tripartite station. But at the times of minima, the signals arrived from the north-west. At these times, intense storms were present off the west coast of Canada some 3,000 or more kilometres away. The tidal wind component would also be from the west at these times. The semidiurnal variation is under continuing investigation. It should be noted that this discussion refers to storm generating areas at least hundreds of kilometres from the recording station. When they are quite close to the station, microbarom activity is continuously high with no obvious time variations.

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### Palaeomagnetism of Atlantic Islands: Fernando Noroña

BECAUSE of their role in testing the crustal spreading hypothesis<sup>1</sup> the ages of Atlantic islands have assumed a prime importance<sup>2</sup>. Conventional geological methods are not usually applicable to studies of oceanic islands, because of the general lack of stratigraphically useful fossils and the dominantly volcanic environment. Palaeomagnetic methods, as pioneered by Einarsson<sup>3</sup>, however, can provide valuable stratigraphic indices in volcanic regions. In addition, if defined from a time span sufficient to average out secular variation<sup>4</sup>, palaeomagnetic poles can provide age estimates, because with increasing age they have occupied positions at increasing angular distances from the present geographic pole. Palaeomagnetic surveys are forming the bases of diverse studies of Atlantic islands. This is the third in a series of reports<sup>5,6</sup>, in which the palaeomagnetic poles resulting from these surveys are reported.

Fernando Noroña (Fig. 1) is an island of 18.4 km<sup>2</sup> area on the geomagnetic equator, at latitude 3° 50' S. and longitude 32° 26' W., approximately 345 km from the

north-east coast of Brazil. Almeida<sup>7</sup> has carried out a detailed geological survey, including extensive chemical and petrological analyses, to define the volcanic history of the island. Two principal stratigraphic units are defined (Fig. 1). The older Remédios formation contains no effusive rocks, but is highly diversified, consisting of various pyroclastic rocks cut by plugs of alkali basalt, essexite porphyry, trachyte, large phonolite domes, and dykes of very variable petrological type. Above an irregular unconformity, the younger Quixaba formation consists of ankaramite lavas and intercalated pyroclastics. The physiography is dominated by the phonolite domes, with "Pico" being regarded as "the most striking landmark in the South Atlantic Ocean"<sup>8</sup>. In common with outcrops on most oceanic islands, no sound age estimates are possible. Almeida limits speculation to the following: "The age is certainly pre-glacial, and considerations about the age of volcanism in the Atlantic Ocean seem to indicate that it ought not to be older than Senonian (Upper Cretaceous)"<sup>9</sup>.

Wilson<sup>10</sup> noted that a pre-continental drift configuration would involve virtual superimposition of the magmatic sources of the islands of the Gulf of Guinea (Fernando Po, Principe, Annabon) and Fernando Noroña, and that the

available geochemical data are consistent with such a similarity.

At least four cores were drilled from each of twenty-eight separate bodies which represent most of the outcrops on the island. Sample locations are shown in Fig. 1. The cores, of 2.5 cm diameter and average length 12 cm, were oriented in geographic co-ordinates while still attached to outcrop. An astatic magnetometer and alternating field demagnetizing unit were used to determine the natural remanent magnetism (NRM) of each specimen. The results for each body are given in Table 1. Polished thin sections from all specimens have been examined using a Reichert 'Zetopan-pol' reflexion microscope at  $\times 1,200$  under oil, and Curie points have been measured in each specimen. Full results will be reported elsewhere, but it can be stated here that there is no reason to believe that the NRM measured is not caused dominantly by the ambient magnetic field during the initial cooling of the bodies sampled, although the NRM in four bodies is insufficiently well defined<sup>11</sup> to be included in group statistical analyses<sup>12</sup>.

The virtual geomagnetic poles (or surface expressions of the hypothetical geocentric dipoles which would give rise to the observed NRM) for each body are shown in

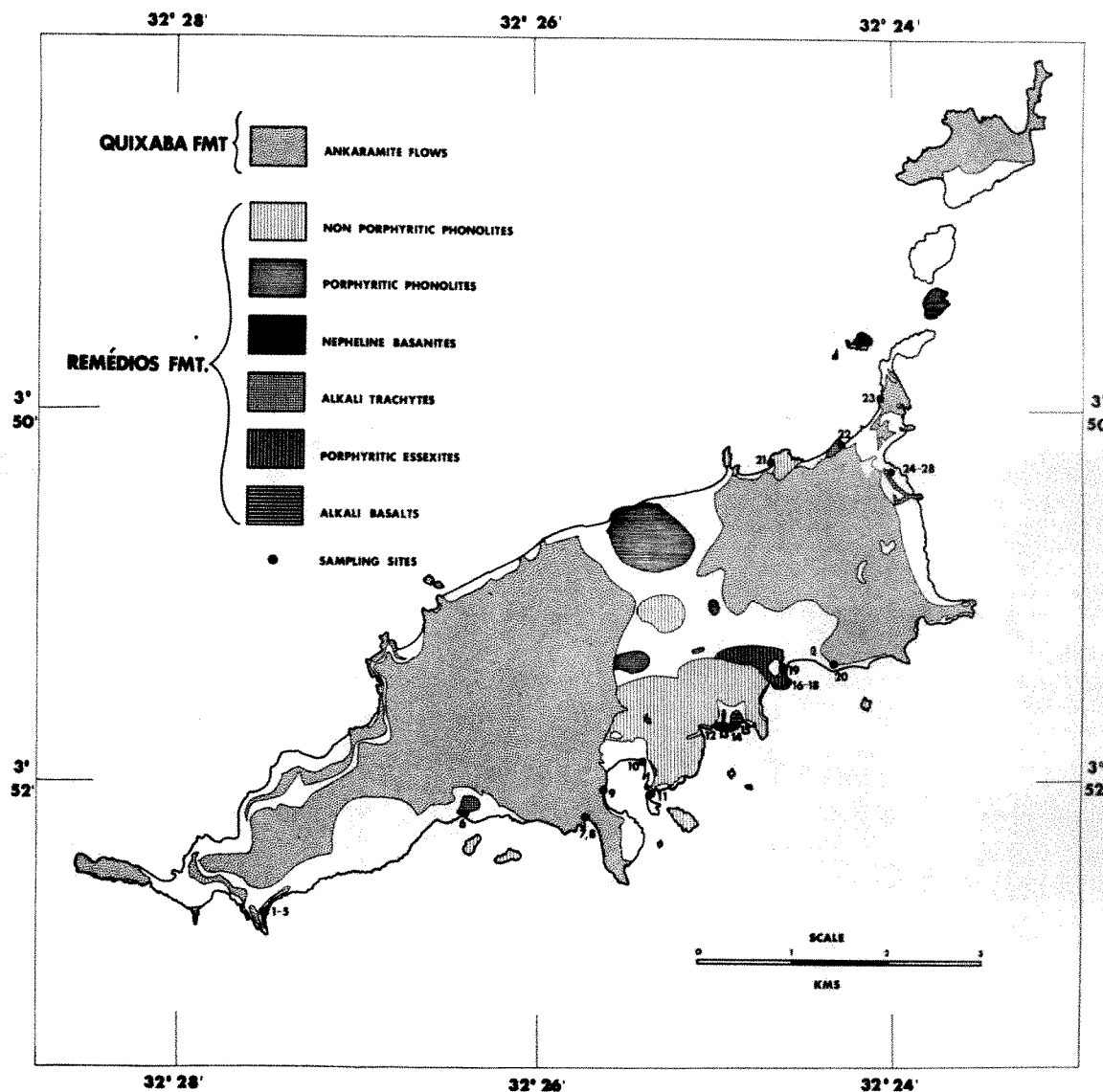


Fig. 1. Geological map of Fernando Noroña, after Almeida<sup>7</sup>. Sample sites are shown as solid circles, with numbers corresponding to the data presented in Table 1.



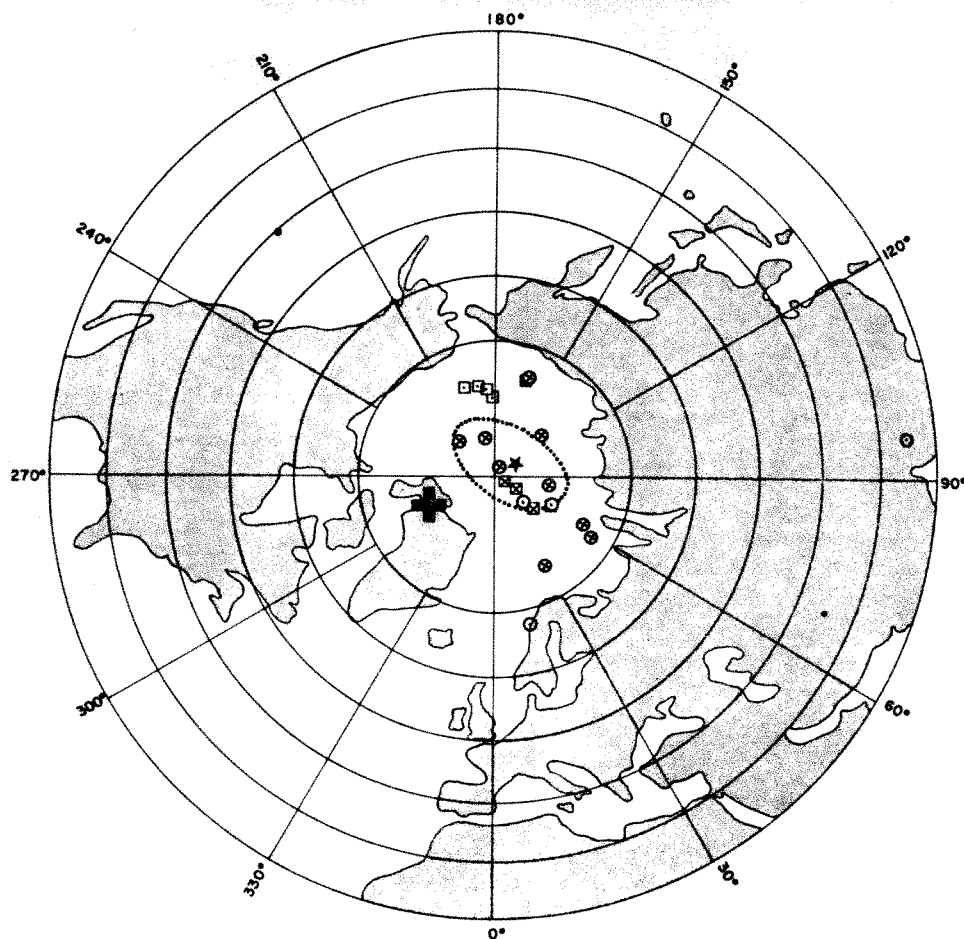


Fig. 2. Virtual geomagnetic poles for each of the twenty-four units which provide data of sufficient precision. Results from the older Remédios formation are shown as circles; those from the younger Quixaba formation are shown as squares. A cross within the circle or square signifies normal polarity; a dot within the circle or square signifies reversed polarity; (reversed or southern hemisphere poles are transposed to the northern hemisphere). The mean of all the virtual geomagnetic poles (or palaeomagnetic pole) is shown as a star. The dotted oval is the 95 per cent confidence oval of the palaeomagnetic pole. The position of the present geomagnetic pole is shown by a heavy cross.

Table 1. FERNANDO NOROÏHA PALAEOMAGNETIC DATA  
(Each unit)

Body number	N	$k_0$	$k_{100}$	$k_{200}$	$k_{max}$	D	I	$a_{95}$	$\theta'$	$\theta''$
Quixaba formation										
1	4	514.6	303.5	250.3	413.7	170.5	+26.7	4.5	9.5	-76.2
2	4	364.8	491.5	393.8	857.3	171.9	+27.0	3.1	4.7	-76.9
3	4	948.2	352.8	252.0	259.1	172.6	+26.5	4.3	2.9	-77.6
4	4	89.8	258.7	166.2	417.9	173.3	+30.6	4.5	354.6	-75.8
5	4	1,033.7	436.6	581.2	876.5	169.0	+23.8	3.1	18.9	-76.2
7	4	35.5	195.1	30.0	195.1	358.7	-06.8	6.6	78.7	88.8
8	4	42.5	29.4	19.5	30.7	358.5	-06.8	16.8	65.2	86.5
9	4	101.7	128.6	149.6	151.4	350.6	-04.8	7.5	66.3	80.5
20*	4	0.9	0.9	0.9	1.0					
23	4	3.9	133.3	154.3	214.9	352.8	-08.1	6.3	55.9	82.9
Remédios formation										
6	4	4.3	6.0	2.0	6.1	355.7	-33.3	40.7	163.8	75.1
10	4	15.4	33.3	24.8	36.3	14.2	-09.5	15.4	61.7	75.9
11	4	11.4	3.7	22.8	25.8	53.0	+77.2	18.4	347.3	10.8
12	4	80.7	122.3	236.3	239.7	189.1	+10.2	5.9	246.6	-80.8
13*	4	1.9	2.1	2.4	2.8					
14	4	20.5	70.5	72.0	83.6	3.8	-00.1	10.1	192.1	84.6
15	4	1.9	3.2	7.1	38.0	196.5	-23.0	15.1	193.3	-67.2
16*	4	1.4	1.4	1.6	1.6					
17	4	139.2	431.0	320.7	604.4	355.9	-33.9	3.7	162.5	74.8
18	4	50.6	693.6	582.3	779.2	185.3	+05.0	3.3	223.0	-84.6
19	4	51.4	173.1	13.9	173.1	359.7	-09.9	7.0	165.3	88.9
21	5	2.1	7.8	1.0	9.7	240.3	+56.0	25.9	276.6	-25.9
22	4	17.5	60.5	39.6	60.5	353.6	-01.3	11.9	81.4	81.9
24	4	44.9	116.9	288.9	304.4	13.0	-05.9	5.3	30.0	75.3
25	4	23.5	33.3	10.5	33.3	7.6	-05.0	16.1	227.3	82.2
26*	4	3.6	3.0	2.5	3.1					
27	4	5.6	27.6	9.2	47.0	343.9	-06.8	13.5	58.9	73.9
28	4	9.1	46.7	1.7	46.7	1.8	-24.5	13.6	136.5	80.7

N is the number of cores.  $k_0$ ,  $k_{100}$ ,  $k_{200}$  is the Fisher<sup>12</sup> precision parameter of original NRM, and following demagnetization in alternating magnetic fields of 100 and 200 oersteds respectively.  $k_{max}$  is the maximum precision parameter obtained by combining either 100 or 200 oersted NRM directions to obtain minimum scatter. D and I are the declination and inclination of NRM, east of geographic north, and with respect to horizontal (+ is below, - is above).  $a_{95}$  is the semi-vertical angle of 95 per cent confidence cone.  $\theta'$  and  $\theta''$  are longitude and latitude of virtual geomagnetic poles, in degrees east, with negative sign indicating southern latitudes. D, I,  $a_{95}$ ,  $\theta'$ , and  $\theta''$  are all based on  $k_{max}$  NRM, with unit vector per core.

\* Indicates that the data do not provide an NRM direction with sufficient precision<sup>11</sup>, and hence data are rejected.

Table 2. FERNANDO NOROÏHA PALAEOMAGNETIC DATA

(Group analysis)											
Group	<i>B</i>	<i>N</i>	<i>k</i> <sub>0</sub>	<i>k</i> <sub>100</sub>	<i>k</i> <sub>200</sub>	<i>k</i> <sub>max</sub>	<i>D</i>	<i>I</i>	<i>a</i> <sub>95</sub>	<i>Q'</i>	<i>Q</i>
Quixaba fmt.	9	36	29.6	57.0	49.7	52.1	353.0	-17.9	7.2	20.1	81.2
Remédios fmt.	15	61	6.8	6.6	5.2	6.5	6.0	-07.6	16.2	237.2	84.0
All units	24	97	9.6	8.9	7.5	9.1	0.8	-11.9	10.4	126.5	87.7

Symbols as in Table 1, except here unit vector per body is applied. In addition *B* is the number of bodies sampled. Note that in some of the above cases *k*<sub>max</sub> < *k*<sub>100</sub>. This is not a computational error: *k*<sub>max</sub> results from unit vector being applied to each 'least scatter' (*k*<sub>max</sub>) body as in Table 1, and there is no reason why this must be > *k*<sub>100</sub> or *k*<sub>200</sub>.

Fig. 2. Table 2 shows the results of combining the data to obtain the palaeomagnetic poles for both formations, and for the entire survey. The palaeomagnetic pole for the entire survey is shown in Fig. 2, together with the 95 per cent confidence oval. Polarity variations in both formations suggest a sufficient time span for probable cancellation of short period or secular variations<sup>13</sup>. Fig. 2 clearly shows that the palaeomagnetic pole for Fernando Noroïha is not significantly different from that caused by the present axial dipole. Comparison of this fact with much independent data strongly suggests that Fernando Noroïha does not exceed Miocene age in outcrop. For example, Doell and Cox<sup>14</sup> suggest that post-Miocene geomagnetic poles are not on average divergent for the present geographic pole; and Irving<sup>15</sup> shows a clear difference between Lower Tertiary palaeomagnetic poles and the present geographic pole.

This suggested age limitation for Fernando Noroïha would clearly invalidate any comparison of the island surface with the islands of the Gulf of Guinea in terms of any pre-drift configuration, for even if continental drift is considered proved the Gulf of Guinea and north-eastern Brazil could not be envisaged as being sufficiently close to yield the same magmatic sources for outcrops of the respective islands during the Upper Tertiary.

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### Additional Theory of Origin of Fiamme in Ignimbrites

THE pantelleritic Quaternary volcano Fantale (lat. 8° 58' N., long. 39° 54' E.) is situated at the northern end of the main Ethiopian rift valley. During a field excursion to this volcano, we made a brief examination of a recent ignimbrite. This pale green ash flow, which was probably erupted in association with the formation

of the Fantale Caldera<sup>1</sup>, is particularly rich in fiamme. Fiamme are flattened, black, glassy inclusions with flame-like cross sections. These are usually considered to have formed by the collapse of included molten pumice under the load of the overlying portions of the pyroclastic flow<sup>2</sup>. As we shall show, it seems unlikely that the fiamme in the Fantale ignimbrite originated in this way; the purpose of this communication is to suggest an alternative origin.

The Fantale ignimbrite covers much of the plain of Matahara surrounding the volcano to the south and west, and is also present in places on the slopes of the mountain. As the unit is traced uphill towards the caldera, however, the ignimbrite becomes much less extensive. The thickness varies considerably because of the irregular topography of the volcano before the eruption. Nevertheless, it can be shown that the unit is thinner (0.3–2 m) on the sloping flanks of the volcano, and very much thicker on the surrounding Matahara Plain (about 20 m). A striking feature is the absence of an air-fall pumice deposit either below or above the welded tuff. Wherever erosion or faulting allows examination of its whole thickness, the ignimbrite is revealed as being completely welded from top to bottom, in the more extreme cases to a hard flinty rock.

Fiamme are present throughout the flow, and although not regularly distributed they are definitely not localized in the lower parts of the unit. Even when the ignimbrite is only 30 cm thick, well developed, severely flattened fiamme are present. Under the microscope these show no evidence of a collapsed pumice origin, but instead many are filled with spherical bubbles. It is striking that the highly flattened inclusions contain generally undeformed vesicles. Similar fiamme are also present in the topmost part of the flow on the Matahara Plain. In the absence of any thick overlying tuff therefore it is unlikely that the fiamme in many parts of this unit could have originated by the collapse of included molten pumice. This is particularly obvious because the presence of euhedral uncorroded anorthoclase phenocrysts suggests that unusually high eruptive temperatures were not involved.

It is suggested that on eruption the Fantale ignimbrite was composed of the usual gas phase loaded with shards, crystals and lumps of molten pumice. In addition it contained abundant inclusions of non-vesiculated, gas-rich molten glass. These glassy inclusions must have been very fluid on eruption, probably because of their particularly high gas content<sup>3</sup>. Thus on deposition they became very flattened, even when the weight of the overlying ignimbrite was very small. After deposition and flattening, the inclusions at last began to vesiculate<sup>4</sup>, forming the spherical bubbles.

It should be stressed that we do not suggest that fiamme in "normal" ignimbrites originated in this way. The mode of origin outlined here, however, must be considered when fiamme are found in extremely thin ignimbritic units.

Further work on this striking unit is in progress. Studies of present day ignimbrites in Alaska<sup>5</sup> indicate that "normal" ash flow deposits do not show the extreme welding and flattening in the absence of any overload, as is visible at Fantale. Particular efforts are being made to understand this feature. Other workers interested in this problem are invited to write to us; we shall be pleased

to supply typical thin sections and other information in exchange for comparable material.

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## Origin of the Keuper Salt in Britain

EXPLORATION for hydrocarbons in the southern North Sea has shown that over a large part of it the Mesozoic rocks are underlain by Upper Permian (Zechstein), containing thick salt layers which have reacted to the overburden load by developing a wide range of halokinetic structures, from salt pillows to diapiric salt plugs<sup>1,2</sup>. Some of the plugs penetrate the overlying beds to levels high in the Mesozoic and Tertiary rocks, and in some cases approach the level of the sea floor. Seismic profiles show sharp variations in stratal thickness associated with the plugs, commonly indicating a history of movement from at least Lower Jurassic times onwards.

It has been suggested that certain anomalous sedimentary features of eastern England may be related to the erosion of Triassic rocks in North Sea salt plugs<sup>1</sup>. Thus the Albian Red Chalk development requires a source of fine-grained ferruginous detritus which could well be from this source, and the two Cretaceous ironstones of east Lincolnshire could reflect erosion of ferruginous Trias. To these may perhaps be added the unique red-bed facies of the Upper Rhaetic of north Lincolnshire and south Yorkshire<sup>3</sup>.

The salt movements can, however, be shown in some cases to have affected beds as early as the Middle Triassic Muschelkalk, and this has suggested an explanation for the problem of the Keuper salt beds in Britain. It has long seemed to us that the great bulk of sodium chloride in the Keuper associated with minimal quantities of calcium sulphate and other salts precludes direct origin by evaporation of sea water; it must have been derived from pre-existing salt beds. The suggestion that the Keuper salt was derived from normal sea waters, which precipitated dolomite and gypsum in crossing "a broad shelf far to the south-east" (unpublished work of W. B. Evans) is negated by the evidence of Keuper development available to us. No such massive—or even significant—quantities of Keuper dolomite or gypsum are known in the relevant area.

Certain of the north-west German Zechstein salt plugs are known to have reached the diapiric stage in Keuper times<sup>4</sup>, and the same may be true of some of the plugs on the western side of the North Sea. On reaching the surface they would be leached by the rivers or lakes of the Triassic landscape, just as the desert rivers of South Persia are now leaching the emergent plugs of Cambrian salt. A supply of sodium chloride brine would thus become available, almost free of sulphates and carbonates, for subsequent redeposition by evaporation in the deeper parts of an inland basin. Such may be the source for the Keuper salt beds in Britain.

The main British Keuper salt beds are located in the deep Triassic basins of western England, in Lancashire, Cheshire, Worcestershire, Somerset and the Isle of Man.

This limitation may be a function of the basin type, involving rapid subsidence, with consequent heavy deposition and with relatively steep flanks which may be in part fault controlled. Evidence has recently been adduced to show that much of this western area was topographically low in Triassic times<sup>5</sup>. Alternatively, but in our view less likely, the westerly location of the thick salt beds might indicate that the source was more local, and that the Irish Sea basin is not only an important Triassic feature<sup>6,7</sup> but includes Permian evaporites thick enough to have developed piercement salt plugs which supplied separated halite for the Keuper basins.

Salt beds with only very small quantities of associated gypsum or anhydrite which occur in the Keuper of the North Sea and north-west Germany may also, it is suggested, owe their origin to the process described here.

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## PHYSICS

### Vacuum Arc Voltage

IN an investigation of the cathode mechanism of the vacuum arc, in a vacuum of  $10^{-5}$  torr, arc voltages were recorded for forty-one different cathode metals. Oscillographic measurements were made at the minimum stable arcing current over a period of 20 sec; values varied from 5 to 25 V. Each arcing voltage consisted of a broad band of noise 2–3 V in width with a well defined lower edge  $V_L$ . The quantity  $V_L$  was measured for all forty-one elements and used as a parameter for correlation with the solid state properties of the cathode.

It is suggested that a connexion exists between arcing voltage and the plasma frequency  $\omega_p$  of the electrons in the cathode metal. Should there be a direct connexion, such that

$$eV_L = \frac{h\omega_p}{2\pi} = \frac{h}{2\pi} \sqrt{\frac{sN\rho e^2}{Wm\epsilon_0}} \text{ joules} \quad (1)$$

using M.K.S. units, so

$$V_L = \frac{h}{2\pi} \sqrt{\frac{sN\rho}{Wm\epsilon_0}} \text{ volts} \quad (2)$$

where  $\frac{h\omega_p}{2\pi}$  is the plasma oscillation energy of the electrons

in the solid ( $h$  is Planck's constant);  $\omega_p^2 = \frac{ne^2}{m\epsilon_0}$  gives the

plasma frequency of the electrons in the solid metal cathode (M.K.S.);  $n$  = effective free electron density in the metal (per  $m^3$ );  $m$  = mass of electron in kg;  $e$  = charge of electron;  $N$  = Avogadro's number;  $\rho$  = density of metal (solid state),  $kg/m^3$ ;  $W$  = atomic weight;  $s$  = number of electrons/atom contributing towards  $n$ ;  $\epsilon_0$  = permittivity of free space.

Then the experimental results should give information on  $s$ —the number of electrons/atom contributing towards the plasma oscillations in the metal.

Element	$V_L$ (V)	$s$	Element	$V_L$ (V)	$s$	Element	$V_L$ (V)	$s$
Li	9.0	1.27	La	10.5	2.98	Sb	8.8	1.71
Na	7.0	1.41	Nd	11.5	3.3	Bi	7.5	1.45
Cu	15.5	2.05	Gd	11-11.5	3.0	Cr	15.5	2.11
Ag	12.5	1.93	Dy	11.5	3.02	Mo	23.0	6.0
Au	12.9	2.05	Er	11.5	2.9	W	23.2	6.17
Mg	11.0	2.04	Yb	6.5-7.1	1.26-1.5	Te	11.0	2.99
Ca	8.0	1.98	Ti	15.5	3.04	Mn	12.0	1.34
Sr	7.0	1.99	Zr	18.5	5.88	Fe	15.5	2.05
Ba	6.3-6.5	1.88	Hf	15.0-20.0	3.64-6.4	Co	18.9	2.83
Zn	9.1	0.91	C	12.0	0.93	Ni	16.5	2.17
Cd	8.1	1.02	Sn	10.5	2.14	Pd	16.0	2.41
Hg	7.5	1.00	Pb	8.0	1.41	Pt	13.5	2.01
Al	15.5	2.89	Nb	19-24.0	4.7-7.5			
Ga	10.0	1.43	Ta	20-23.0	5.2-6.9			
In	9.5	1.71						

The analysis was carried out using equations (1) and (2) and results are tabulated in Table 1.

If the connexion between  $s$  and the valence is not a coincidence, one would expect the best fit for elements which react chemically with a unique valency, valency electrons being clearly differentiated in their energy from those in inner shells. On the whole, the best fit between  $s$  and valence is found for the alkaline and rare earths and aluminium, in which this requirement is fulfilled.

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## THE SOLID STATE

### Radiophotoluminescence of Biphenyl in Methylcyclohexane Glass

It is known that the recombination of positive and negative species trapped in rigid matrices during gamma-irradiation at 77° K leads to the emission of light. This recombination, and the subsequent luminescence, can be induced either by warming the sample to certain characteristic temperatures (radiothermoluminescence, RTL) or by illuminating it at 77° K with light of a suitable wavelength (radiophotoluminescence, RPL). In previous work<sup>1</sup> it was shown that in methylcyclohexane glass containing aromatic compounds two peaks of RTL are observed, one of which could be ascribed to the recombination of cations with electrons trapped in the glass and the other to the recombination of cations with negative ions. The emission spectra observed correspond to the fluorescence and phosphorescence spectra of the dissolved aromatic compounds, the phosphorescence spectrum being enhanced in comparison with photoluminescence. We have found that identical emission spectra could be obtained by illuminating the sample with infra-red light (1.2-1.8 $\mu$ ), though our results have not yet been published.

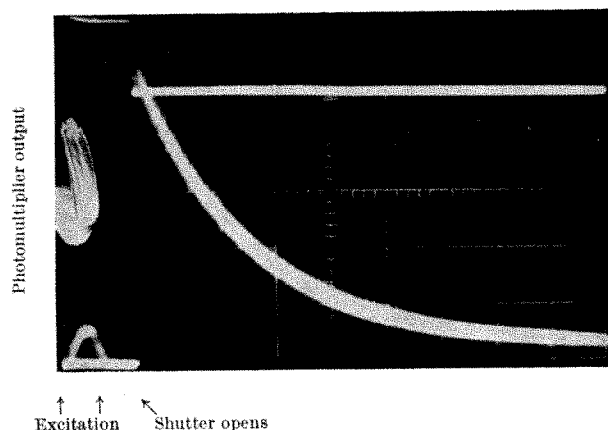


Fig. 1. Decay of RPL. Time, 1 division = 2 sec.

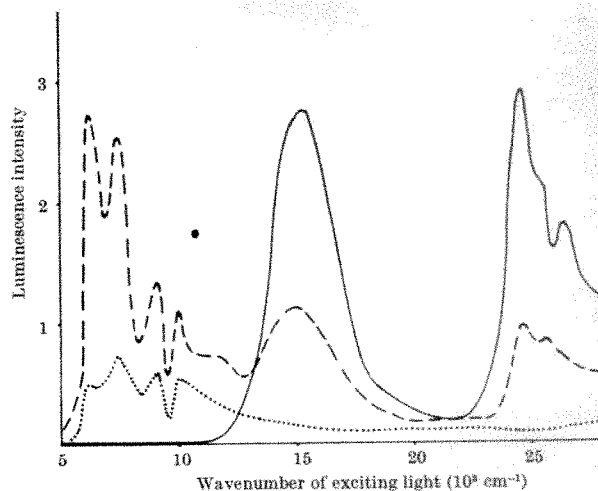


Fig. 2. RPL excitation spectra of biphenyl in methylcyclohexane. Dotted curve,  $10^{-4}$  molar; dashed curve,  $10^{-3}$  molar; full curve,  $10^{-2}$  molar biphenyl.

We have now obtained a complete RPL excitation spectrum of gamma-irradiated glassy solutions of biphenyl in methylcyclohexane, between 360 m $\mu$  and 2.3 $\mu$ .

In this work the solutions were bubbled with helium in order to remove possible traces of carbon dioxide, degassed by the freeze-pump-thaw technique, and then irradiated at 77° K with a cobalt-60 source to a dose of about 60 krad at a dose rate of about 80 rad/min. The sample, placed in liquid nitrogen, was then illuminated with light from a xenon arc, through a monochromator. Slit widths were adjusted at each wavelength to give a constant light flux, as determined by calibration with a thermopile. An electro-mechanical shutter protected the photomultiplier used to detect the luminescence while the exciting light was on (1 sec); it opened 1 sec after the end of excitation. The photomultiplier output was displayed on an oscilloscope and photographed. In this way we obtained the decay of the long-lived component of the luminescence, as is shown in Fig. 1.

The plot of luminescence intensities, at a constant time after the end of illumination, against wavenumbers of the exciting light gave the spectra shown in Fig. 2. The dotted curve, with maxima at  $6.25 \times 10^3$ ,  $7.4 \times 10^3$ ,  $9.1 \times 10^3$   $\text{cm}^{-1}$  and  $10 \times 10^3$   $\text{cm}^{-1}$ , was obtained with a  $10^{-4}$  molar solution of biphenyl. It is similar to the absorption spectra observed by different authors<sup>2,3</sup> for electrons trapped in hydrocarbon matrices. The full curve was obtained with a  $10^{-2}$  molar solution of biphenyl. The trapped electron bands in the infra-red are practically absent, and the bands observed at 0.650, 0.405, 0.393 and 0.380 $\mu$  ( $15 \times 10^3$ ,  $24.6 \times 10^3$ ,  $25.4 \times 10^3$  and  $26.3 \times 10^3$   $\text{cm}^{-1}$ ) coincide with the absorption spectrum of the biphenyl anion<sup>4</sup>:  $\lambda_{\text{max}} = 0.660$ , 0.410, 0.395 and 0.380 $\mu$ . For  $10^{-3}$  molar solutions of biphenyl, both anion and trapped electron bands appear in the excitation spectrum (dashed curve).

The observed effect of solute concentration on the excitation spectrum indicates competition between traps and biphenyl molecules for electrons released during gamma-irradiation. A similar concentration effect was observed by Guarino and Hamill<sup>5</sup> for the absorption spectra of irradiated solutions of biphenyl in 2-methylpentene-1.

From this concentration dependence it can be deduced that an electron can migrate up to a distance of 55 Å from the parent cation with practically no chance of being trapped in the matrix (only biphenyl ion bands are present in the RPL excitation spectrum of a  $10^{-2}$  molar solution), but that within a distance of 250 Å trapping in the matrix is nearly 100 per cent (no biphenyl ion



bands in  $10^{-4}$  molar solution), the average trapping distance being about 120 Å, which agrees with results obtained by other techniques.

From the luminescence decay a lifetime of  $4.05 \pm 0.05$  sec has been determined for the long-lived excited state. This value is in good agreement with the lifetime of biphenyl phosphorescence, 4.0 sec, measured by Azumi and McGlynn<sup>6</sup> at the same temperature.

When infra-red light was used, the protection of the photomultiplier during excitation was unnecessary, and both fluorescence and phosphorescence could be recorded simultaneously in the course of excitation by McClain and Albrecht's technique<sup>7</sup> of chopping the exciting light. McClain and Albrecht analysed the kinetics of the fluorescence and phosphorescence of tetramethyl *p*-phenylenediamine brought about by infra-red light after photo-ionization in glassy 3-methylpentane and showed that the enhancement of phosphorescence could be explained in this case by a direct formation of both triplet and singlet states from a common intermediate formed in the course of the recombination. Their relation seems also to apply to the RPL of biphenyl in methylcyclohexane glass; the lifetime for biphenyl phosphorescence deduced in this way,  $4.5 \pm 0.5$  sec, is in fairly good agreement with the value given by Azumi and McGlynn.

Analogous experiments with other aromatic hydrocarbons are in progress.

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## Origin of the Luminescence in Natural Zircon

NATURAL zircons generally luminesce yellow or orange under ultra-violet light<sup>1,2</sup>. The luminescence is not caused by the tetravalent uranium responsible for the absorption spectra of zircon<sup>1,3</sup>. Because there have been speculations on the origin of the luminescence, I have studied several zircons which gave a yellow luminescence and I have found that there are at least two main types of luminescent

centres in zircon. The varying proportions of these centres give the differences in the colour of the luminescence.

The luminescence spectrum of a zircon crystal which has both types of centres is shown in Fig. 1.

Type 1 centres are most probably trivalent europium,  $\text{Eu}^{3+}$  ions. They give the two groups of lines at 4800 Å and 5800 Å, and these correspond to the transitions  $^5\text{D}_2 \rightarrow ^7\text{F}_2$  and  $^5\text{D}_1 \rightarrow ^7\text{F}_3$ , respectively, of the  $4f^6$  configuration of  $\text{Eu}^{3+}$  in  $\text{LaCl}_3$  (ref. 4). The number of lines in each group is about that expected from selection rules. No other rare earth ion could be responsible because different host lattices have only a very small effect on the transition energies of these ions. Only the intensities of the different transitions are affected by the host lattice. Europium is known to have a very high luminescence efficiency and hence only a small amount is required to give the weak luminescence observed.

Europium lines were found in most of the luminescent spectra observed. Some New Zealand zircon sands gave weak lines while a white zircon gemstone from Siam gave strong europium lines but no trace of the type II centre. Type II centres appear to be colour centres induced by the radioactive decay of the uranium and thorium present. Their luminescent spectrum is a broad band centred around 6000 Å. (In Fig. 1 the structure around 6500 Å is caused by absorption of the luminescence by  $\text{U}^{4+}$  ions.) The zircons giving this luminescence are coloured a yellow-brown, and on heating to about 1,000°C they become clear and the broad band goes from the luminescent spectrum. This type of heat treatment is often applied to coloured zircons to produce the clear gemstone variety<sup>1</sup>.

Zircons which have been subjected to intense radiation become red-brown and have marked colour centres<sup>5</sup>. No luminescence, however, is known from these, though there may be some infra-red luminescence.

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## Photo-annealing of Chemical Radiation Damage

ANNEALING of the chemical effects of nuclear recoil in solid substances by ionizing radiations<sup>1</sup> and by ultra-violet<sup>2</sup> and visible<sup>3</sup> light is well known. In the case of chemical radiation damage, evidence for the existence of radiation annealing, necessarily indirect, is provided by the observation that the rate of damage generation decreases with increase in the radiation dose. The effect is particularly pronounced in lead nitrate for which the steady state nitrite yield under gamma-irradiation, for example, is only about 2 per cent<sup>4,5</sup>. There is no record of photo-annealing of chemical radiation damage in the literature. We have observed the recombination of damage fragments in lead nitrate by light in the visible region.

The experiments were conducted on lead nitrate crystals sieved to between 85 and 100 mesh, vacuum dried and irradiated with a dose of 50 Mrads with cobalt-60 gamma-rays at the dose rate of 1.5 Mrads/h. Samples (100–150 mg) of the irradiated crystals, in thin layers, were illumin-

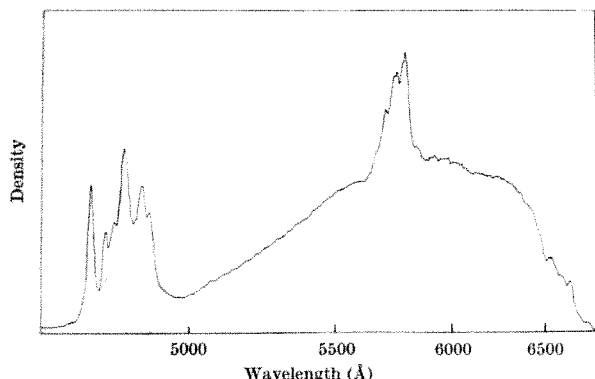


Fig. 1. Densitometer trace from an 'Astra III' plate of the luminescence spectra of a zircon crystal at room temperature. Taken on a Hilger quartz spectrograph.

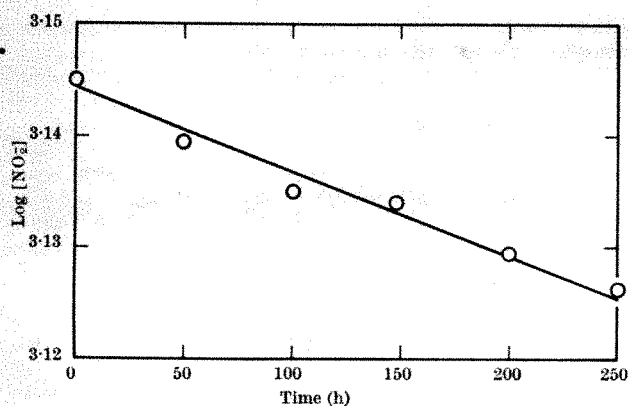


Fig. 1. Photo-annealing of chemical radiation damage in lead nitrate.

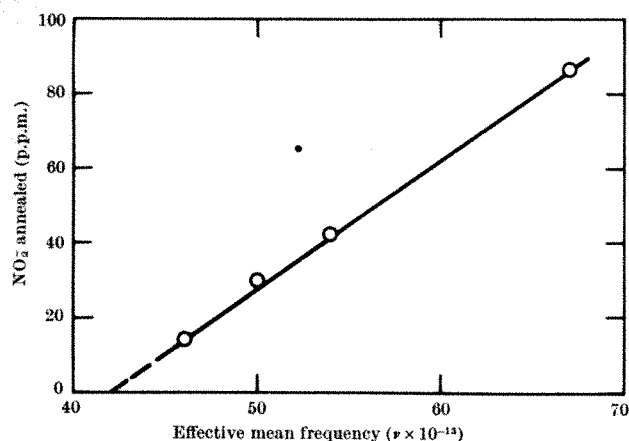


Fig. 2. Dependence of photo-annealing on the light frequency.

ated at 50 cm distance with light from a 200 W tungsten filament lamp condensed by a 10 cm diameter lens system. Care was taken to ensure that the samples did not get heated up during the illumination. The same procedure as that previously described was used for determining the damaged nitrite<sup>4</sup>. Typical plots of the nitrite concentration in p.p.m. versus the time of illumination are given in Fig. 1. The plots lie on a straight line which implies a monomolecular recombination process.

The dependence of the annealing on light frequency, in the visible range, was investigated by exposing samples of the irradiated crystals to different narrow wavebands obtained by using monochromatic coloured glass filters and the same source of light as before. The effective mean frequency of the light bands was determined by standard optical methods. Fig. 2 records a set of data for an exposure of 360 h. It is seen that the annealing increases linearly with increase in the effective mean frequency of the light. Extrapolation to the zero value of nitrite

annealed gives a threshold frequency for annealing, namely,  $42 \times 10^{13} \text{ sec}^{-1}$  (wavelength, 7140 Å) corresponding to an energy of 1.74 eV. The fact that the recovery is caused by light shows that it occurs by an electronic process.

Experiments with ordinary lead nitrate showed that the salt is not susceptible to photolysis by visible light.

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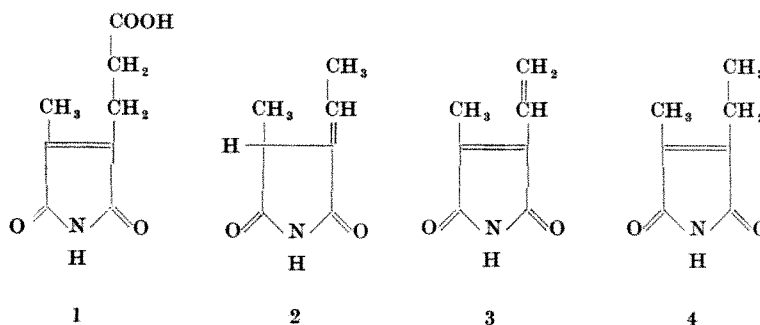
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## CHEMISTRY

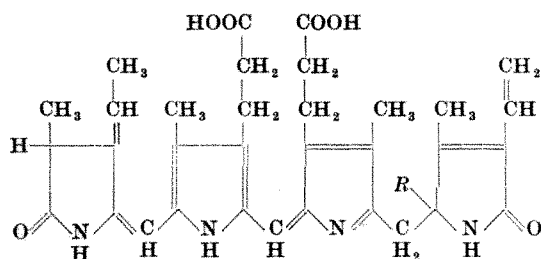
### Structure of Phycoerythrobilin and Phycocyanobilin

DEGRADATIVE work on the phycobilin prosthetic groups of the algal biliproteins (phycoerythrins and phycocyanins) has been hampered by the difficulties involved in splitting off these prosthetic groups in native form and isolating them in sufficiently high yields for classical degradation and analysis. These difficulties have now been avoided by using the chromic acid micro-degradation technique recently refined for the study of aplysiocyanin<sup>1</sup>, the defence pigment of the sea hare. This technique may be used with as little as 100  $\mu\text{g}$  of bilin and is not affected by the presence of protein (Rüdiger and Klose, W., unpublished), thus enabling direct degradation of the phycobilins while still attached to the proteins. This development, together with the improved resolution of the chromatographic techniques used, has led to the identification of side chain groupings not previously detected in these prosthetic groups.

Degradation of the phycobilins *in situ* on the biliproteins and release of the resulting imides was achieved by mixing 2–4 mg of the purified biliprotein<sup>2</sup> in water (0.2 ml.) with acetone (0.1 ml.) and 1 per cent chromic acid in 2 normal sulphuric acid (0.2 ml.) and heating at 90–100° C for 1 h. Free bilins were degraded as described for aplysiocyanin<sup>1</sup>. The imide degradation products were extracted into ether (0.2 ml.) and identified by thin-layer chromatography<sup>1</sup>. The identifications were verified by co-chromatography with authentic imide samples.

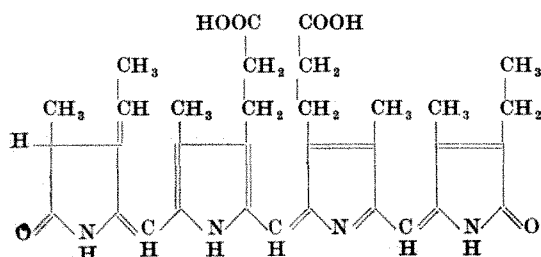


*R*-phycoerythrin, *C*-phycoerythrin, phycoerythrobilin (the bilin isolated after controlled acid hydrolysis of phycoerythrins<sup>3</sup>) and "purple pigment" (the closely related pigment released from phycoerythrins after prolonged refluxing with methanol<sup>4</sup>) all yielded the imides 1, 2 and 3 on oxidation. Phycoerythrobilin and "purple pigment" both contain two free carboxyl groups<sup>3,4</sup> suggesting that they each contain two rings corresponding to 1. Aplysiocyanin, which contains one free and one methylated carboxyl group, gave the imides 1, 2, 3 and the methyl ester of 1 on oxidation<sup>1</sup>. After methylation of their free carboxyl groups, "purple pigment" and aplysiocyanin became indistinguishable by thin-layer chromatography on silica gel in a range of solvent systems, and had identical ultra-violet and visible spectra as free base, hydrochloride and zinc complex. As reported previously for "purple pigment"<sup>4</sup>, aplysiocyanin was converted by cold 10 normal hydrochloric acid first to a rhodin spectrally identical with phycoerythrobilin, and then to a urobilin which formed a chloroform-insoluble adduct with cysteine. The "purple pigment" (in common with phycoerythrobilin and phycoerythrin) gives a transient deep blue colour in alkali, which is also characteristic of aplysiocyanin<sup>1</sup>. This evidence strongly suggests that the "purple pigment" is the unesterified form of aplysiocyanin. Structure 5 is therefore proposed for the "purple pigment", since the structure previously proposed for aplysiocyanin<sup>1</sup> is represented by the mono methyl ester of 5, with *R* representing a hydroxyl group. The assignment of this hydroxyl group was tentative, however<sup>1</sup>, and some recent results (Rüdiger and Ó Carra, unpublished) cast further doubt on it, suggesting that *R* represents a hydrogen atom.



Phycoerythrobilin rather than the "purple pigment" appears to be the native prosthetic group of phycoerythrins<sup>3,4</sup>. The oxidative degradation shows that both compounds have identical side-chains, and as the two are readily interconvertible<sup>4</sup> they must be very closely related.

Oxidation of *C*-phycoerythrin yielded the imides 1, 2 and 4. The same products were obtained on oxidation of phycobilin 630, the bilin isolated after careful acid hydrolysis of phycoerythrin<sup>5</sup>, and of the "blue pigment" released by prolonged refluxing of phycoerythrin in methanol<sup>4</sup>. Both these isolated bilins were also found to contain two acidic side-chains, and because the phycoerythrin chromophore is intermediate between the verdin and violin types<sup>6</sup>, the conjugation must extend through



all four rings, but with one ring incompletely conjugated. Structure 6 is consistent with all these data.

Principally on the basis of nuclear magnetic resonance and mass spectral data, Cole *et al.*<sup>6</sup> have recently proposed the same structure for the "blue pigment" which they termed phycocyanobilin. The term phycocyanobilin, however, is properly applied to the native prosthetic group and it remains to be established whether the "blue pigment" or phycobilin 630 should be regarded as such. Present evidence favours phycobilin 630 (refs. 4 and 5). The precise basis of the spectral differences between these two pigments<sup>4</sup> also remains to be elucidated.

Crespi *et al.*<sup>7</sup> have also recently proposed a structure for the "blue pigment". It is similar to 6 as regards the side-chains, but contains one double bond less in the conjugated system. We consider that this is incompatible with some of the properties of the "blue pigment", in particular its facile isomerization in alkali to mesobiliverdin<sup>8</sup> and the presence of a basic pyrroline ( $-N=$ ) group in its conjugated system<sup>4</sup>.

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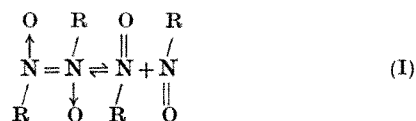
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## Photodissociation of Nitroso Dimers

Most C-nitroso compounds in the crystalline state exist as colourless dimers, though the monomers, often formed by dissociation in solution, are green or blue<sup>1-3</sup>. Heating solutions of the nitroso compounds also shifts the equilibrium so that formation of monomers is enhanced.



We have observed that solutions of some nitroso compounds which are colourless become green or blue on irradiation with ultra-violet light. On removal of the light the colour fades. This photochromic effect may be repeated many times. The colour produced by irradiation is identical to that produced by heating, and apparently is due to the formation of the monomer species.

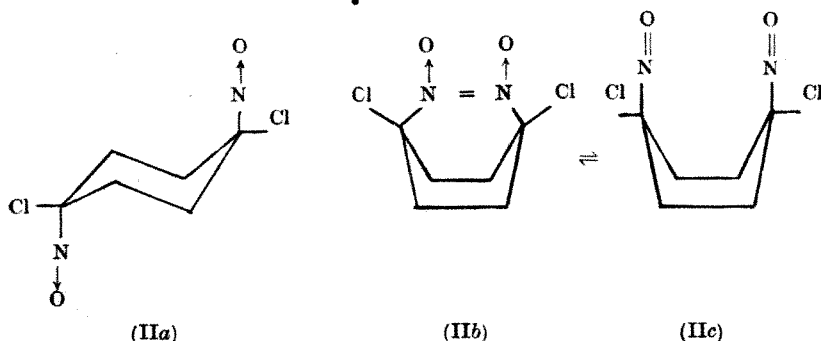
Dimeric 3-methyl-3-nitrosobutan-2-one (I) (ref. 4) when dissolved in ethanol, chloroform, methylene chloride, or benzene yields colourless solutions. These solutions on irradiation at or below room temperature with ultra-violet light, become blue and show a maximum at 6860 Å. The same absorption curve is obtained by heating without irradiation.

Solutions of (I), in a thermostated cell, were irradiated with the unfiltered light from a high-pressure mercury lamp and the disappearance in the dark of the 6860 Å band produced by the irradiation was followed with time. The

kinetics of the fading reaction, which we believe to be the rate of dimerization, were of second order. If we assume an extinction value of 20 at 6860 Å, an approximation which appears reasonable<sup>5</sup>, the estimated respective rates of decay in ethanol and methylene chloride at -25° C were  $12.1 \times 10^{-2}$  and  $3.7 \times 10^{-2}$  l. mole<sup>-1</sup> sec<sup>-1</sup> (ref. 5).

It was also noted that in the infra-red region, irradiation caused a decrease in the intensity of the 1289 cm<sup>-1</sup> band, and formation of a new band at 1561 cm<sup>-1</sup>. On removal of the light the original absorption curve was obtained. The same respective effects were obtained by warming and cooling the same solution. Dimeric C-nitroso compounds have been reported to have an absorption in the region from 1430 to 1000 cm<sup>-1</sup>, and the monomeric form in the region from 1650 to 1495 cm<sup>-1</sup> (ref. 6).

1,4-Dichloro-1,4-dinitrosocyclohexane exists in two stable forms as a solid. The blue solid has been assigned structure (IIa) and the white solid structure (IIb) (refs. 2-7). Compound (IIb) yields a colourless solution, which on warming becomes blue because of the formation of (IIc). Irradiation of the colourless solution with ultra-violet light also produced the same blue colour which faded in the dark. At -26.3° C, the fading rate in methylene chloride measured at 6550 Å was  $0.9 \times 10^{-5}$  sec<sup>-1</sup>, and followed first order kinetics.



The formation of free radicals on irradiation of C-nitroso compounds with ultra-violet and visible light has been reported recently<sup>8-10</sup>. The relationship of this reaction to the dissociation effect observed is not clear at this time.

We have observed the production of a blue or green colour by irradiation of colourless solutions of other tertiary, secondary, and primary nitroso compounds. The kinetics of the dissociation of C-nitroso compounds have been reported<sup>11-15</sup>. By irradiating and following the colour fading rates at several temperatures we are currently investigating the kinetics of the association reaction.

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## IMMUNOLOGY

### Effect of Surfactants on Antigen-Antibody Reactions

In our laboratories bentonite flocculation tests<sup>1</sup> are generally used for the titration of antigens and antisera. To reduce non-specific reactions, surfactants are sometimes added to the bentonite suspensions. Recently a report has appeared suggesting that the surfactants have an adverse effect on biological systems<sup>2</sup>. We therefore decided to examine the effect of surfactants on antigen-antibody reactions.

We have found that two surfactants generally used in our laboratory, 'Tween 80' (polyoxyethylene sorbitan mono-oleate) and 'Gantrez AN-119' (a copolymer of methyl vinyl ether and maleic anhydride), inactivate antibody when added at concentrations used for immunological studies.

Three rabbits were immunized with crystalline bovine serum albumin (BSA). The concentration of antibody in each rabbit serum was determined by the quantitative precipitin test, as described by Kabat<sup>3</sup>. All protein concentrations were determined by the method of Lowry<sup>4</sup>.

Each rabbit serum was tested for inactivating effects of the two surfactants. All rabbit sera were diluted 1:4 with 0.01 molar borate buffered saline, pH 8.2. To 0.5 ml. of the diluted rabbit sera, 1.0 ml. of either 'Tween 80' or 'Gantrez AN-119' was added in decreasing concentrations. The surfactants were previously prepared in the same buffer as the antisera. The mixtures of surfactant and antisera were incubated for 30 min at 37° C before addition of 0.5 ml. of BSA containing sufficient antigen to bring the reactants to the equivalence point. The tubes were then incubated for

another hour at 37° C and placed at 4° C for an additional 48 h. The precipitates were washed and the total protein was determined. The loss of precipitable protein as compared with controls was taken as the degree of inactivation.

Results are shown in Fig. 1. The inactivation caused by the presence of 'Tween 80' seems to increase to a maximum. This may be caused either by inactivation of a particular antibody fraction or by binding of surfact-

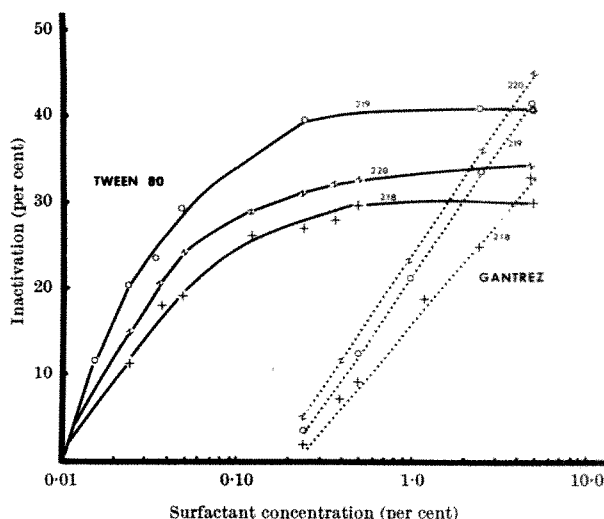


Fig. 1. Antibody inactivation in the presence of surfactants as determined by antigen-antibody precipitation at equivalence for each rabbit.



ant to antigen, which blocks specific antigenic sites. The results obtained with the 'Gantrez AN-119' seem to be completely dependent on concentration and may represent denaturation, antibody dissociation or even soluble antigen-antibody complex formation. No antibody activity could be detected in the supernatants from any of these experiments.

A series of experiments was carried out to determine whether the loss of precipitable protein was caused by (a) antibody dissociation; (b) formation of soluble antigen-antibody complexes; or (c) irreversible denaturation. Antigen adsorbed to bentonite was prepared by modification of the method of Bozicevich<sup>5,6</sup>. Each serum was titred by the antiserum dilution method. Titres were repeated after addition of 'Tween 80' or 'Gantrez AN-119' to the antiserum. If the loss of precipitable antibody, as observed in Fig. 1, were caused by the formation of a soluble complex, the bentonite titre would not be expected to decrease because the antigen is bound to an insoluble matrix and cannot solubilize. Decreased titres were observed (Table 1).

Table 1. BENTONITE FLOCCULATION TITRE VERSUS SURFACTANT CONCENTRATION

Serum No.	Total antibody (mg/ml.)	Original reciprocal titre	Surfactant	Reciprocal titre after addition of surfactant. Concentration of surfactant (per cent)		
				5.0	0.5	0.01
218	1.85	256	'Tween 80'	128	128	256
			'Gantrez AN-119'	64	256	256
219	1.21	128	'Tween 80'	64	64	128
			'Gantrez AN-119'	32	64	128
220	1.40	256	'Tween 80'	128	128	256
			'Gantrez AN-119'	32	64	256

The possibility that the surfactants were causing reversible antigen-antibody dissociation was also considered. Three rabbit sera were pooled and fractionated by precipitation of the globulins with one-third saturated ammonium sulphate. The globulins were adsorbed to bentonite as previously described<sup>5,6</sup>. The sensitized bentonite was titred against BSA before and after addition of either 'Tween 80' or 'Gantrez AN-119'. Loss of titre was observed on the addition of either surfactant. The sensitized bentonite was centrifuged and washed free of the surfactants, resuspended, and retitred. No recovery of antibody activity was observed. Either the surfactants irreversibly inactivated the antibody bound to bentonite or remained adsorbed to the bentonite in some manner, causing continued inhibition. Washing and resuspending sensitized bentonite in the absence of surfactants did not affect the titre.

These experiments strongly indicate the necessity of carefully considering the possible effects a surfactant may have when added to biological systems. It cannot be assumed that a surfactant, if used in low concentrations, will have little or no adverse effects on the system being investigated.

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## Detection of Antibodies against Mitochondria by the Fluorescent Antibody Technique with Smears of Rat Liver Cells

SERA of patients with primary biliary cirrhosis contain autoantibodies against a non-organ specific cytoplasmic component, which are probably mitochondria<sup>1,2</sup>. A convenient way to detect these antibodies is by the fluorescent antibody technique using unfixed cryostat sections of human or rat organs. Sections of renal cortex proved to be the most suitable for the demonstration of cytoplasmic

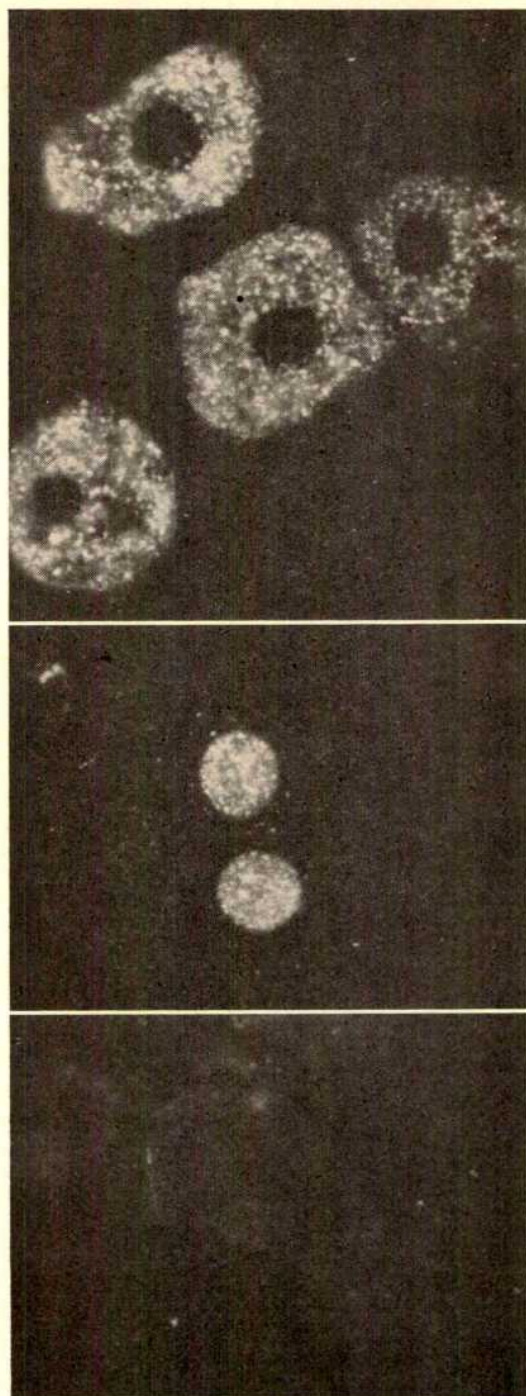


Fig. 1. Indirect fluorescent antibody technique on smears of a rat liver cell suspension with fluorescein conjugated anti-human-complement. (A) Incubated with serum of a patient with primary biliary cirrhosis showing staining of mitochondria; B, incubated with serum containing ANF, showing speckled staining of nuclei of a bi-nuclear liver cell; (C) control with normal serum, no staining.



antibodies. Liver sections showed the same coarsely granular staining, but because of the uniform appearance of this tissue, it was difficult to distinguish the fluorescence obtained with the weaker sera from non-specific artefacts<sup>1</sup>.

This technique is suitable for the detection of these antibodies, but a cryostat is essential. We have tried to show that smears of rat liver cell suspensions can be used to detect antibodies against mitochondria as well as antibodies against nuclei.

Rat liver cell suspensions were prepared as follows: rats were decapitated and 0.04 molar sodium citrate-0.15 molar sucrose (adjusted to pH 7 with citric acid) was perfused through the aorta for 3 min. The liver tissue was minced with scissors and suspended in a hand-driven 'Teflon' pestle in 0.25 molar sucrose at 0°-4° C. After filtration through nylon gauze the suspension was washed twice with 0.25 molar sucrose, after which it consisted of a pure suspension of single cells<sup>2</sup>. Smears were prepared on thoroughly cleaned slides and air dried. If they were not used immediately, they were stored, sometimes for several weeks, at -20° C. The indirect fluorescent antibody technique was used with a fluorescein conjugated rabbit anti-human-complement serum (anti- $\beta_{1A}$ -C and - $\beta_{1E}$ , supplied by the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service) or with various anti-human gamma globulin conjugates. Usually the conjugates were applied in 1:5-1:10 dilutions in buffered saline. Inactivated (30 min, 56° C) sera were incubated for 30 min at room temperature. Thereafter the smears were incubated with fresh human AB serum for 30 min at room temperature, when the anti-complement conjugate was to be used. To avoid aspecific staining of an irregular, stringy appearance in the periphery of the cytoplasm, the fresh AB serum, as well as the conjugate, was absorbed with lyophilized rat liver homogenate (respectively 50 and 100 mg/ml.).

Sera from patients with primary biliary cirrhosis and serum from a patient with breast cancer who may have had immunological tumour resistance<sup>4</sup> showed coarse granular staining in the cytoplasm of the liver cells. The fluorescent granules were seen best when the  $\times 40$  objective was focused on a level just above the nucleus. Sera known to contain anti-nuclear factor (ANF) stained the nuclei. Normal sera stained neither granular cytoplasmic components nor nuclei.

The anti-gamma globulin conjugates showed smooth aspecific staining when the undiluted sera were used. Staining with anti-complement conjugate was much more distinct because there was no background fluorescence (Fig. 1). The results were as good as those from unfixed rat renal cryostat sections. When the smears were compared with renal sections the sera of three patients with mitochondrial antibodies and one with ANF showed no difference in titre, and the staining of the smears was much more clear cut than the staining of the sections. Smears stored for up to 6 weeks showed no diminution of antigenicity, because the pattern and intensity of staining and also the titre of the three anti-mitochondrial sera were the same as those of fresh smears. Fluorescent staining of nuclei with ANF sera was nearly as strong with the stored smears as with fresh ones. As Walker *et al.* found, fluorescent staining of the granules was only positive when the smears were air-dried. Staining was abolished by fixation with ethanol or methanol and decreased by fixation with acetone.

Sera of patients with primary biliary cirrhosis are supposed to contain antibodies to mitochondria, and so it seemed logical to assume that the fluorescent granules represent liver mitochondria. Electron microscopy has shown that isolated rat liver mitochondria incubated for 30 min with one of these sera, and negatively stained by PTA, had a fringe on the outer membrane (Fig. 2). The size and shape of this fringe are typical of antibody deposition<sup>5,6</sup>. The fringe was absent on the fragments of endoplasmic reticulum and plasma membranes which were

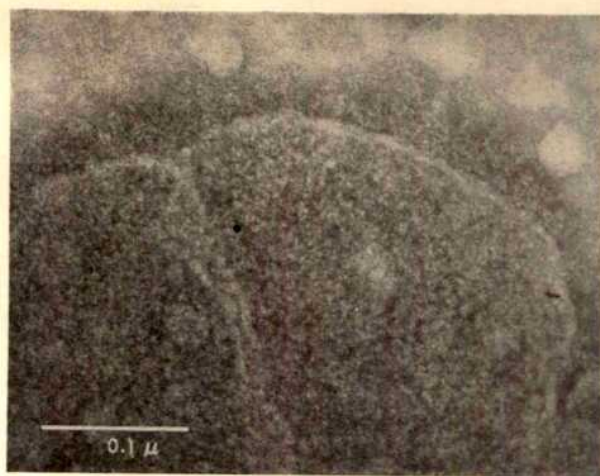


Fig. 2. Electron micrograph of isolated rat liver mitochondrion negatively stained with 1 per cent PTA pH 7, after incubation for 30 min with antiserum. On the outer surface of the mitochondrion a fringe representing antibody deposition is visible. ( $\times 192,700$ .)

occasionally present as contaminants in the same preparation. Control mitochondria incubated with normal human serum did not show fringes.

It can be concluded that smears of rat liver cell suspensions are a suitable substrate for the detection in human sera of antibodies to mitochondria as well as to nuclei. The method is particularly useful for routine purposes in laboratories where cryostats are not available. For the detection of mitochondrial antibodies the results are more clear cut with anti-human complement conjugate than with anti-human gamma-globulin conjugate.

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### Time-dependent Immunosuppressive Effects of Anti-thymocyte Serum

THE effectiveness of immunosuppressive agents depends greatly on the time of their administration in relation to the time of immunization. The immunosuppressive effects of heterologous anti-lymphoid sera are under intensive investigation but little seems to be known about their time-dependence. Monaco *et al.*<sup>1</sup> found that a course of seven daily injections was most effective in prolonging the survival of allogeneic skin grafts if it was given during the week before grafting and was progressively less effective the later it was begun. On the other hand, Levey and Medawar<sup>2</sup> found that a course of two injections was marginally more effective if given on days +2 and +5 relative to the time of grafting than if given on days -1 and +2.

It was therefore of interest to study the effect on the antibody response of anti-thymocyte serum given at different times before or after a single injection of antigen.

Anti-thymocyte serum was produced by the method of Levey and Medawar<sup>2</sup> by injecting rabbits intravenously



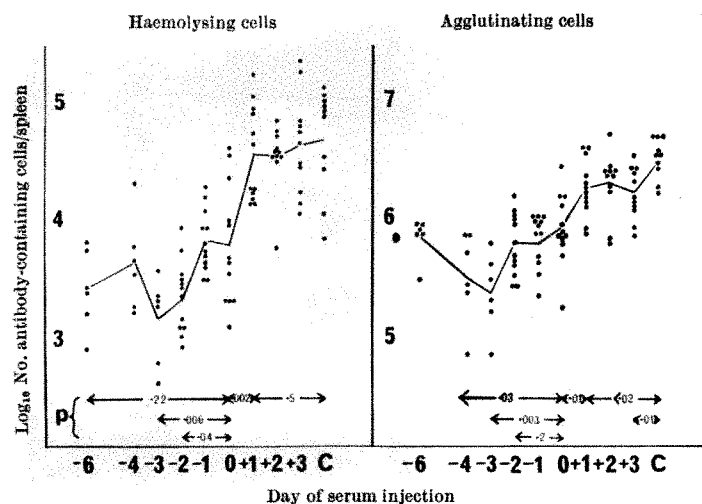


Fig. 1. Effect of a single dose of anti-thymocyte serum on the number of antibody-containing cells in the spleen 4 days after injecting sheep red cells. Curves drawn through geometric means of the values in each group. C, Counts in mice given sheep red cells only; p, probabilities that the differences between the groups indicated were due to chance.

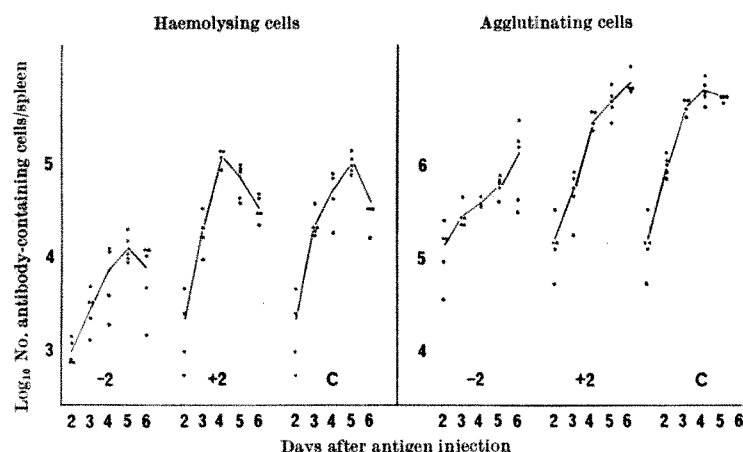


Fig. 2. Effect on number of antibody-containing cells in the spleen of anti-thymocyte serum given on day -2 or day +2 relative to an injection of sheep red cells. C, Mice given sheep red cells only.

with two lots of  $10^6$  CBA mouse thymus cells at an interval of two weeks and bleeding a week after the second injection. Sera were heated at  $56^\circ\text{C}$  for 30 min and stored at  $-20^\circ\text{C}$ .

Male CBA mice, weighing 20–25 g, were injected intraperitoneally with 0.2 ml. 10 per cent (v/v) formalized sheep red cells in saline. Groups of between six and twelve mice were given single injections of anti-thymocyte serum, 0.25 ml./10 g body weight subcutaneously, at various times before or after the injection of red cells. Control mice were given red cells only. The spleens were removed 4 days after the antigen injection and the numbers of cells containing haemolysin or haemagglutinin in the spleens were counted by Jerne's method<sup>3</sup> and a modification of

Zaalberg's method<sup>4</sup>. The results are shown in Fig. 1. The values were compared statistically by a distribution-free ranking method of Wilcoxon<sup>5</sup>.

There was a significant depression in the response ( $P < 0.01$ ) if anti-thymocyte serum was given at any time between days -6 and 0, with a maximum effect on day -3. The haemolysin-cell response was not significantly depressed if the injection of antiserum was delayed to day +1 or later. The agglutinin-cell response was depressed even with late administration, but the depression was minor (to about half the control values).

In a second experiment, mice were given sheep red cells on day 0 and 0.25 ml. anti-thymocyte serum/10 g body weight on day -2 or +2. Counts of cells containing antibody were made from day +2 to +6. Administration on day -2 markedly reduced the response, whereas administration on day +2 had no effect (Fig. 2).

In a third experiment, mice were injected once with sheep red cells and then daily with 0.2 ml. of anti-thymocyte serum or normal rabbit serum/10 g body weight, subcutaneously, from day +2 to +6 inclusive. Spleens were examined on days +7 and +11. The results are shown in Table 1. Depression of haemolysin-cell counts by anti-thymocyte serum was relatively minor (to one-third or one-half of the control values) and the level of cells containing agglutinin was not materially affected.

It therefore seems that the processes leading to production of humoral antibody are sensitive to anti-thymocyte serum if it is given before, or simultaneously with, the antigen, but lose sensitivity to this agent within 24 h of administration of antigen. This sort of timing effect could be caused by a delayed absorption of the agent, but this is not likely in view of the failure of a relatively prolonged course of injections to cause more than a modest depression of the response (experiment 3) and the finding by Brent, Courtenay and Gowland<sup>6</sup> that lymphoid cells taken from mice 24 h after administration of anti-thymocyte serum had a reduced ability to cause a graft-versus-host reaction. It is therefore unlikely that heterologous anti-lymphoid sera act during the stage of cell proliferation induced by antigen. The fact that the greatest effect is produced by giving anti-thymocyte serum 3 days before the antigen makes it difficult to believe that the serum acts directly on the cells which are sensitive to antigen. The serum can scarcely take 3 days longer than the antigen to reach these cells, nor does it seem likely that, once having reached them, it would take 3 days to damage or inactivate them.

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Table 1. EFFECT ON THE NUMBER OF ANTIBODY-CONTAINING CELLS IN THE SPLEEN OF ANTI-THYMOCYTE SERUM (ATS) OR NORMAL RABBIT SERUM (NRS) INJECTED DAILY FROM DAY +2 TO +6 AFTER INJECTION OF SHEEP RED CELLS

	Haemolysing cells/spleen ( $\times 10^{-3}$ )			Agglutinating cells/spleen ( $\times 10^{-4}$ )		
	Controls	ATS	NRS	Controls	ATS	NRS
Day 7						
	73	43	159	444	191	462
	96	47	192	400	324	458
	127	25	99	507	395	391
	87	52	170	304	293	675
	52	29	163	333	195	490
Geometric mean	81	38	153	391	270	487
Day 11						
	24	9	54	57	218	227
	37	18	76	440	120	258
	44	21	43	244	58	344
	79	18	29	160	62	200
	70	20	—	378	315	—
Geometric mean	46	16.5	48	205	124	284

## BIOCHEMISTRY

## Photoreactions of Retinol and Derivatives sensitized by Flavins

CRYSTALLINE riboflavin is present in the eyes of several fishes and mammals (for example, *Galago crassicaudatus*)<sup>1</sup>. Its role in vision, however, is obscure. One possible function may be that of a transducer to convert blue light (efficiently absorbed by the flavin but not by rhodopsin) into energy that can be utilized by rhodopsin producing the chemical signal. This conversion may be achieved by the trivial process of re-emission (the wavelength of fluorescence of riboflavin is at 530 m $\mu$ , which is favourable for absorption by rhodopsin)<sup>2</sup> or by direct energy transfer either of a singlet-singlet or of a triplet-triplet type.

In our studies we have chosen all-*trans*-retinol and the 13-*cis*-derivative as models for the photosensitive part of the visual pigment rhodopsin. This choice was primarily governed by the consideration that the absorption spectra of these compounds involve transitions of higher energy than the longest wavelength absorption of riboflavin. This, then, excludes the possibility of the trivial process or of singlet-singlet energy transfer in our system.

When an anaerobic solution of all-*trans*-retinol ( $1.6 \times 10^{-5}$  molar) and lumiflavin ( $2 \times 10^{-5}$  molar) in 98 per cent methanol/water is illuminated (using light composed of wavelengths above 400 m $\mu$ ) a gradual decrease in the peak height of retinol at 328 m $\mu$  is observed (Fig. 1). No reaction is found for either of the reactants alone under these conditions. Lumiflavin can be replaced by riboflavin or flavin mononucleotide (FMN) and all-*trans*-retinol by 13-*cis*-retinol. In the dark no interaction is observed between retinol and lumiflavin either by spectrophotometry or by fluorescence methods.

The photoreaction is inhibited by  $10^{-2}$  molar nickel sulphate, oxygen and potassium iodide (Fig. 1). Potassium iodide also quenches the fluorescence of lumiflavin but less efficiently than it quenches the photoreaction (Fig. 2).

Thin-layer chromatography of the reaction mixtures of 13-*cis*-retinol and lumiflavin has shown qualitatively the formation of all-*trans*-retinol. The reverse of this reaction was also observed although in this case the analytical procedure did not distinguish the 13-*cis*- from the 11-*cis*-isomer. The thin-layer chromatography was essentially as already described<sup>3</sup>.

Thus the flavins can sensitize a reaction of retinol similar to that observed in the ultra-violet illumination of retinol or retinal alone<sup>4</sup>. The *cis-trans* isomerization is undoubtedly accompanied by some destruction of the compound. We have no direct evidence for the mechanism

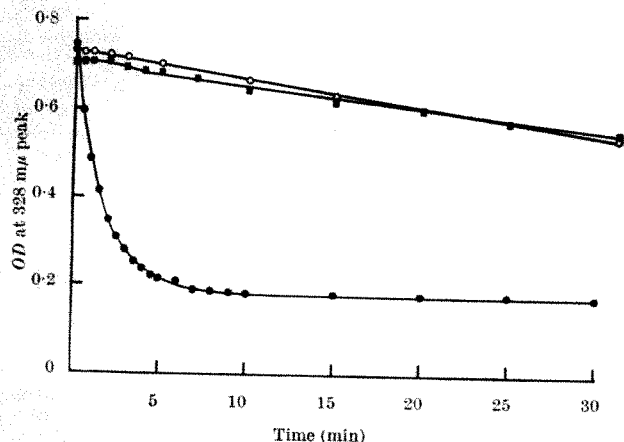


Fig. 1. Sensitized photoreaction of all-*trans*-retinol. ●, Retinol and lumiflavin; ■, retinol, lumiflavin and  $10^{-2}$  molar nickel sulphate; ○, retinol, lumiflavin and oxygen.

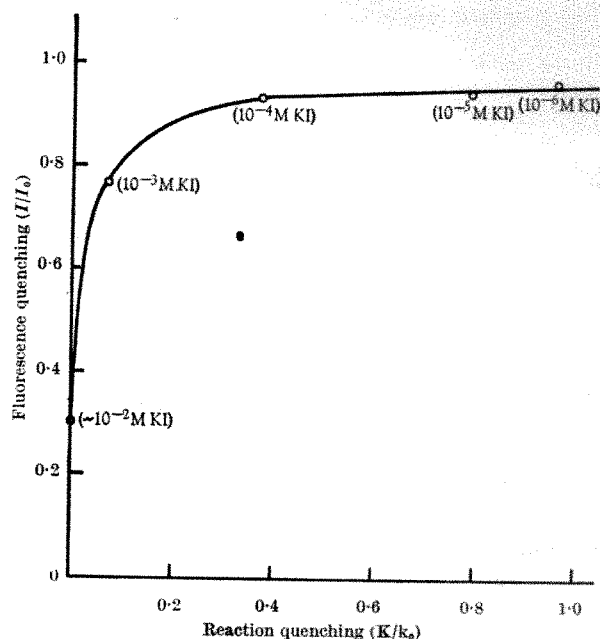


Fig. 2. Inhibition of sensitized photoreaction against fluorescence quenching by potassium iodide (concentrations shown in brackets).

of the photosensitization, but the quenching experiments suggest that the flavin triplet is involved as in other photoreactions of flavins<sup>5</sup>. Triplet-triplet energy transfer has been suggested for the lumichrome-sensitized isomerization of stilbene-4-carboxylic acid<sup>6</sup> and the process described here may involve a similar mechanism. Alternatively the triplet may react directly with retinol to form a covalent labile intermediate similar to that formed in the reaction of phenylacetic acid with 3-methyl-lumiflavin<sup>7</sup>.

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### Localization of Lactic Dehydrogenase Isozymes in Lysosomal Fraction of the Neutrophil of Normal Human Blood

THE lysosomal fraction of the leucocyte is the principal source of peroxidase and hydrolases<sup>1</sup>, and our unpublished results show that it also contains lactic dehydrogenase (LDH) usually found in the soluble compartment of cells. Vesell and Bearn<sup>2</sup> and Vesell and Osterland *et al.*<sup>3</sup> have shown that whole homogenates and sonicates of the leucocyte contain four isozymes of lactic dehydrogenase.



In this communication we shall show that the isozymes of lactic dehydrogenase of the human white cell are bound to the particulate of the lysosomal fraction, and that this accounts for all the isozymes of the white cell.

The preparation of lysosomes of the human leucocytes<sup>1</sup> has been modified by use of a Ficoll gradient. LDH activity was quantitatively analysed spectrophotometrically<sup>4</sup>; however, a Process and Instrument spectrophotometer was used to follow the reaction of the 3400 Å with an expanded scale, wherein 0.10 absorbance is spread over a 12 in. scale. The enzymes were examined on acrylamide gels, using the 470 apparatus manufactured by the E.C. Company of Swarthmore, Pennsylvania. Instead of using the gel preparation described by that company, the stacking gel technique, used on disc electrophoresis<sup>5</sup>, was applied with slight modification in composition and preparation of the gels as follows (the letters in parentheses refer to stock solutions tabulated in ref. 6).

**Lower gel:** One part of (A) which contained 48 ml. 1 normal hydrochloric acid, and 36.3 g *tris* made up to 100 ml. with distilled water, and 2 parts of (C) which contained 30 g of acrylamide monomer and 0.8 g of *bis* (*N,N*-methylene bis acrylamide monomer) made up to 100 ml. with distilled water, and 1 part of distilled water which was added just before mixing lower gel A and B, and 0.1 ml. of TEMED (*N,N,N,N*-tetramethylethylenediamine). Lower gel B consisted of 0.2 g of ammonium persulphate in 100 ml. of distilled water. Equal parts of lower gel A and lower gel B were mixed and poured into the apparatus without air bubbles. The level of the gel was adjusted immediately and it was allowed to set for 20–30 min for polymerization. After polymerization, the upper gel was added.

**Upper gel:** One part of (B) (25.6 ml. 1 molar orthophosphoric acid and 5.7 g *tris*, made up to 100 ml. with distilled water), 2 parts of (D) (10 g of acrylamide and 2.5 g of *bis* made up to 100 ml. with distilled water); 1 part of (E) (4.0 mg of riboflavin in 100 ml. of water), and 4 parts of distilled water. The upper gel was polymerized after putting the teeth in place and exposing the gel to fluorescent light for 20–40 min. After polymerization, the teeth were removed and the apparatus carefully transferred to the cold room where known amounts of the samples were applied into each slot. The buffer used in this experiment was *tris*-glycine buffer (*tris*, 6 g; glycine, 28.8 g; made up

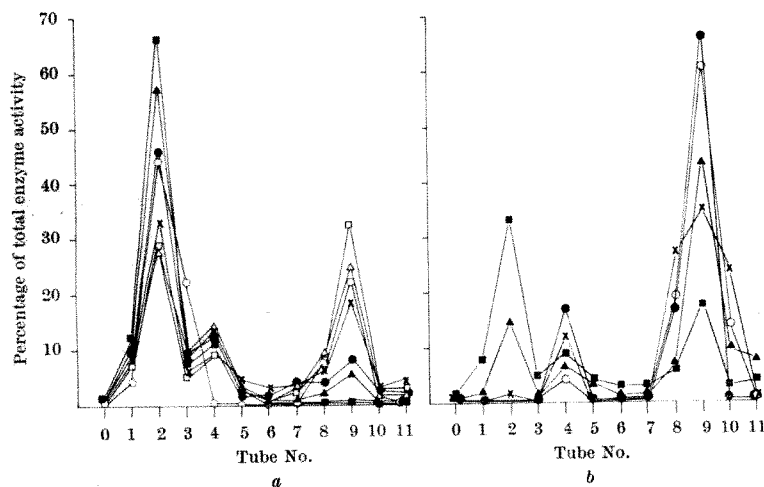


Fig. 1. Enzyme studies representing the results obtained when 4,000 g fraction is separated on a Ficoll gradient and the gradient is sliced into eleven sections from the bottom to the top; 0 representing the bottom slice and 11 representing the top. To avoid confusion, the results are plotted on two separate charts although they are done on the same run. In a, ■ indicates lysozyme; ▲, peroxidase; ●,  $\beta$ -glucuronidase; ○, LDH; ×, protein; □, acid phosphatase; △, amino-peptidase. In b, ■ indicates protein; ▲, catalase; ●, succinate cytochrome-c reductase; ○, DPNH cytochrome-c reductase; ×, cytochrome oxidase.

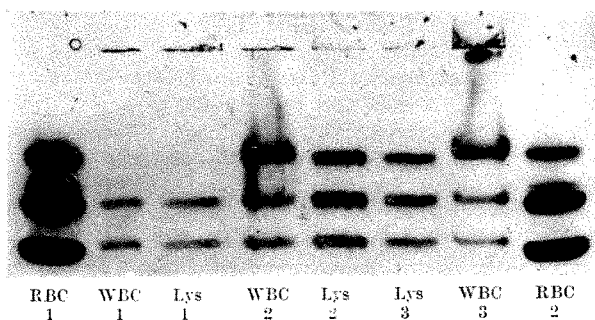


Fig. 2. Acrylamide gel electrophoresis of the lysosomal fraction (Lys), human white blood cell (WBC) showing the presence of LDH isozymes. RBC-1 (1,034  $\mu$ g protein) and RBC-2 (517  $\mu$ g protein) are haemolysed human red blood cells. WBC-1 (193  $\mu$ g protein) and Lys-2 (13  $\mu$ g protein) are sonicated samples of leucocytes and lysosomes (tube 2) in Fig. 1a. WBC-2 (1,161  $\mu$ g protein) represents 6 times as that of WBC-1. In order to increase the amount of material, fraction 2 was concentrated by lyophilizing and resuspending before application. Thus Lys-2 (1,975  $\mu$ g protein) and Lys-3 (1,234  $\mu$ g protein) represent concentrated lyophilized samples; WBC-3 (784  $\mu$ g protein) was treated in a similar fashion. The stained spot near the original over WBC-3 is an insoluble aggregate.

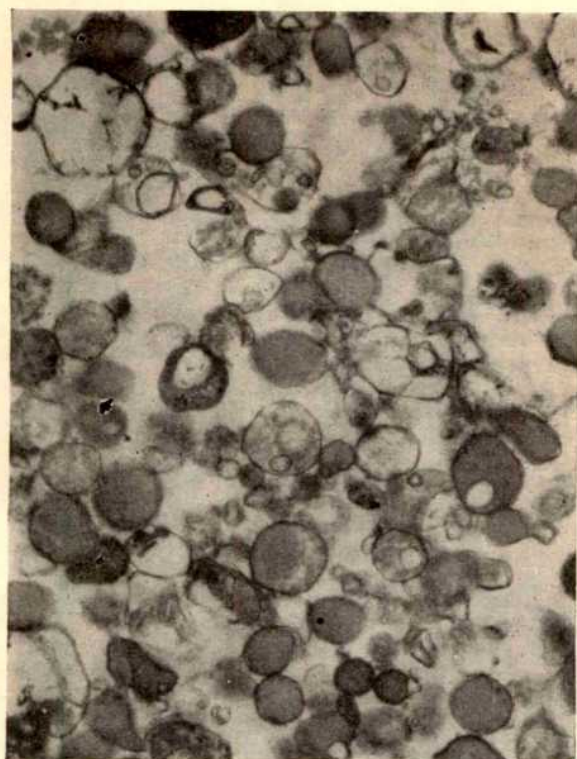
to 1 l., pH 9.0). Electrophoresis was carried out for 7 h at 50 V and 40 m.amp. The gel was transferred to a tray and was incubated with a developing reagent, as described by Vesell<sup>6</sup>, in the dark at room temperature.

From Fig. 1a, it can be seen that most of the LDH of the 4,000 g pellet is found in tubes 1, 2 and 3 respectively, representing 66 per cent of the original enzyme activity of the pellet. Although LDH activity was also found in tubes 8 and 9 (the mitochondrial fraction), one half of the rate of disappearance of DPNH took place in the absence of pyruvate. On this basis, only 11 per cent of true LDH activity can be assigned to this fraction. No LDH activity was observed in tubes 1, 2 and 3, when pyruvate was left out of the test solution. Of the total white cell LDH activity, 43–45 per cent can be accounted for in the lysosomal fractions.

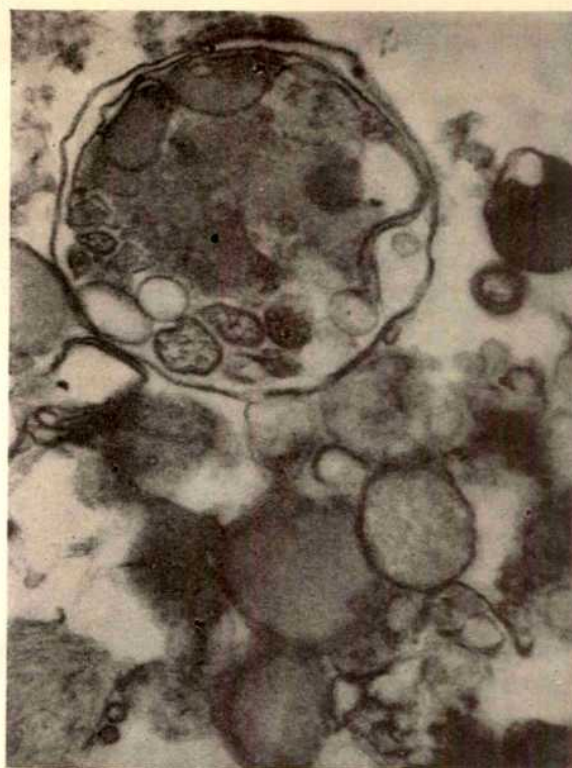
The fact that the LDH isozymes of the whole cell are located principally in the lysosome is indicated in Fig. 2, where whole cell sonicates, red blood cell haemolysates and lysosomal preparations are compared. While the fast-moving A and B isozymes are seen in all fractions, increasing the concentration by lyophilization and resuspension, a third band is observed in the white cells and the lysosomes. Only in the case of lyophilized white cells is a slow-moving fourth band seen; this was seen when increased quantities of the white cell homogenates were applied without lyophilization (WBC-2). Although RBC-1 represents twice the amount of RBC-2, no slow-moving component was observed. As observed by Vesell<sup>7</sup>, experiments by frozen and thawed material showed the slow-moving isozyme. For this reason, sonication was chosen.

The 4,000 g pellet which was the starting material on the Ficoll gradient is described in a previous report<sup>1</sup>. It contains the peroxidase and hydrolytic enzymes and a small amount of mitochondria found in tube 9, similar to that seen on the sucrose gradient reported previously.

Detailed studies of other enzymes along with electron micrographs of the particulates separated in tube 2 are shown in Figs. 1b, 3a and 3b. These data provide the necessary evidence that these particulates are the lysosomal fraction of the white cell. The specific granules and the large multivesicular bodies seen in the electron micrographs are morphologically similar to what is now recognized in lysosomal fractions as described by Novikoff<sup>8</sup>, who suggested that a variety



a



b

Fig. 3. Electron micrographs of material from tube 2 (Fig. 1a). a,  $\times 25,200$ ; b,  $\times c. 76,000$ .

of lysosomes may be produced in the form of coated vesicles, dense bodies, multivesicular bodies, and autophagic vacuoles containing endoplasmic reticulum and ribosomes. These figures can all be seen in Figs. 3a and b, which are probably the purest lysosomal fraction yet obtained from a normal human leucocyte. In more than thirty-five preparations made in this laboratory, the hydrolases characteristic of this fraction are always accompanied by myeloperoxidase.

Although the LDH activity is apparently associated with the particulate containing the peroxidase and hydrolytic enzymes<sup>1</sup>, it cannot be said that the enzyme is within the cell, but rather attached to the membrane. This reasoning is based on the observation that there is no increase in the LDH activity of the particulate of the lysosomal fraction following sonication or destruction of the intact particle. In the case of peroxidase and hydrolytic enzymes, the activity is increased markedly under these conditions. The statement may be made that although LDH is recognized as a soluble enzyme, in the white cell it has a strong affinity for the lysosomal membrane. In regard to the presence of the LDH in the mitochondrial fraction, one may conjecture that light lysosomes may be present as indicated by the enzyme markers found there as indicated here by peroxidase<sup>1</sup>; the finding, however, that one half of the activity is retained in the absence of pyruvate does indicate that a DPNH oxidase could be present in sufficient quantities along with the electron transport system to overcome the amount of cyanide used in the test solution. This was not investigated further. Finally, the specific activity of LDH of the whole cell homogenate was 9.1 units/mg protein, while that of the particulate in tube 2 of Fig. 1a was 311 units/mg protein, a concentration of about 34-fold.

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### Possible Duplication of Haemoglobin $\alpha$ -Chain Locus in the Iru Macaque

THE iru macaque (*Macaca irus*) is polymorphic for a minor haemoglobin component which resembles human Hb-A<sub>2</sub> in electrophoretic mobility and concentration but differs from it in having a common  $\beta$ -chain with the normal major component, Hb-A<sub>1</sub> (ref. 1). Two major haemoglobin variants Hb-P<sub>mi</sub> and Hb-Q<sub>mi</sub> are also frequent in this species and these also differ from Hb-A<sub>1</sub> in their  $\alpha$ -chains. Some animals carry three haemoglobins, for example Hb-P<sub>mi</sub> ( $\alpha_{Pmi}\beta_{A1}$ ), Hb-A<sub>1</sub> ( $\alpha_{A1}\beta_{A1}$ ) and the minor component ( $\alpha_{Xmi}\beta_{A1}$ ), and therefore have three types of  $\alpha$ -chain ( $\alpha_{A1}$ ,  $\alpha_{Pmi}$  and  $\alpha_{Xmi}$ ). Hb-P<sub>mi</sub> and Hb-A<sub>1</sub> are probably determined by allelic genes.



It was therefore suggested<sup>1</sup> that the minor component arose by duplication of the  $\alpha$ -locus in a manner analogous to the proposed origin of Hb-A<sub>2</sub> by a  $\beta$ -locus duplication<sup>2</sup>.

In several animal species a number of different haemoglobin chains are produced during the individual life cycle, for example the  $\delta$ ,  $\beta$ ,  $\gamma$  and  $\epsilon$ -chains in man. These are usually non- $\alpha$  chains which are thought to have originated by gene duplication. On the other hand, in a few cases<sup>3-5</sup> in which individuals of a species carry more than one type of  $\alpha$ -chain it has been argued that this is a result of misreading of a codon during  $\alpha$ -chain synthesis rather than duplication of the locus. In this communication more detailed structural studies of the minor component of *M. irus* are reported. These studies confirm that the minor component differs from the normal major component in the  $\alpha$ -chain and strengthen the view that gene duplication rather than misreading of the genetic code provides the better explanation of the situation.

The minor haemoglobin component (about 4 per cent of total haemoglobin) and Hb-A<sup>mi</sup> were isolated by chromatography on 'Sephadex CM'<sup>6</sup>, followed by chromatography on 'Sephadex DE'<sup>7</sup>, from an animal carrying only these two haemoglobins.

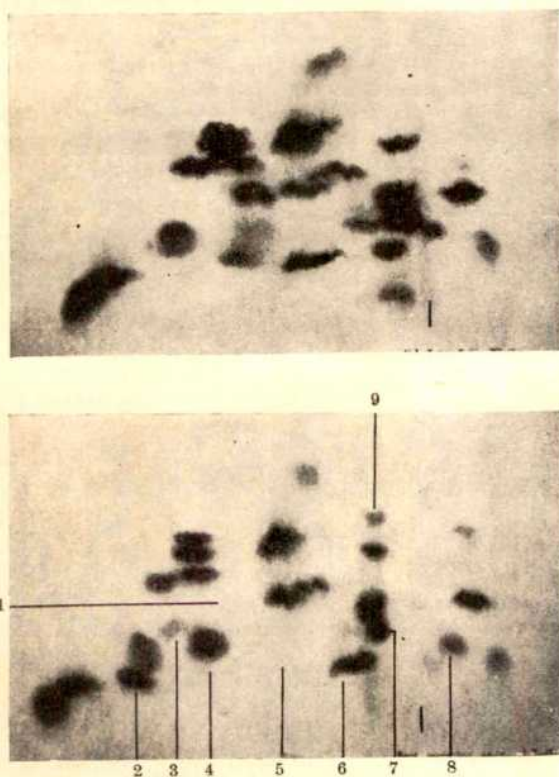


Fig. 1. Top: Fingerprint of *M. irus* haemoglobin Hb-A<sup>mi</sup>. Bottom: Fingerprint of *M. irus* minor haemoglobin component.

Globins, prepared from the purified haemoglobins by precipitation in acid acetone at  $-20^{\circ}\text{C}$ , were digested with trypsin and fingerprinted by paper electrophoresis in pyridine, acetate and water (10:0.4:90) buffer pH 6.4, followed by chromatography in pyridine, isoamyl alcohol, water (35:35:27)<sup>8</sup>. The peptides were stained with 0.2 per cent ninhydrin. The presence of tryptophan was detected with Ehrlich's reagent, histidine with Pauly's reagent, arginine with Sakaguchi's reagent and tyrosine with the nitrosonaphthol reagent<sup>9</sup>.

Several differences between the fingerprints of the minor component and Hb-A<sup>mi</sup> were visible. These are shown in Fig. 1 and are listed below with the same numbering. Two separate samples from the same animal

gave identical results. (1) No peptide was visible in the  $\alpha$  T3 position of the minor component fingerprint; in addition, no tryptophan stain was visible in this position, in contrast to  $\alpha$  T3 of Hb-A<sup>mi</sup>, which is tryptophan positive. (The peptide numbering used in this paper is by analogy with human Hb-A as discussed in ref. 1.) (2) The presence of a new positively charged peptide was noted in the minor component fingerprint; this did not stain for arginine, histidine, tyrosine or tryptophan. (3) An extra peptide, only weakly stained by ninhydrin, was visible; this peptide was positive for arginine. (4) The peptide pattern in this region was clearly different from that found in Hb-A<sup>mi</sup>; instead of a diffuse double spot, a compact single spot was present. (5)  $\alpha$  T1 + 2 was absent in the minor component fingerprint. (6 and 7)  $\alpha$  T8 + 9 ox. and T9 ox. both had a lower chromatographic mobility than the corresponding peptides found in Hb-A<sup>mi</sup>; both peptides were histidine positive. The difference in  $\alpha$  T9 can only be seen with this stain. (8)  $\alpha$  T4 was more negatively charged in the minor component than in the Hb-A<sup>mi</sup> fingerprint. It was identified by being positive for arginine, histidine and tyrosine in both fingerprints. The difference in mobility was confirmed by one dimensional electrophoresis of the tryptic digests of the minor component and Hb-A<sup>mi</sup> in parallel, and subsequent staining for histidine. (9) An extra peptide was visible in the neutral region and was positive for arginine.

Comparison of the  $\beta$ -chain peptides of each fingerprint after staining with ninhydrin, as well as for arginine, histidine, tryptophan and tyrosine, revealed no differences.

These results confirm the previous finding that the  $\beta$ -chains of Hb-A<sup>mi</sup> and the minor component are identical and that they differ in their  $\alpha$ -chains. The peptides  $\alpha$  T1 + 2,  $\alpha$  T3,  $\alpha$  T4 and  $\alpha$  T9 of the latter differ from those of Hb-A<sup>mi</sup>, and so it appears that there are at least four amino-acid differences between these haemoglobins, an overall divergence of the same order as that found between Hb-A and Hb-A<sub>2</sub> using the same technique.

Misreading of the genetic code is a plausible explanation of the occurrence of more than one type of  $\alpha$ -chain in the horse<sup>3</sup>, mouse<sup>4</sup> and rabbit<sup>5</sup>, but the situation in *M. irus* is not readily explained by this hypothesis. Fingerprint evidence indicates that the minor component differs from the normal haemoglobin in more than one substitution. If gene duplication is discarded as an explanation we must suppose that this species is polymorphic for a gene with several mutant codons susceptible to misreading. It is not yet clear whether the charge difference of the minor component can be accounted for by a single substitution. If, however, more than one substitution is involved and any of them are caused by translational errors, more than one minor haemoglobin band should regularly be produced, which is not the case. Our earlier work<sup>1</sup> suggested that the minor component occurs at random among major haemoglobin phenotypes. If, then, a misreading producing a change of charge occurred in the synthesis of the variants Hb-P<sup>mi</sup> or Hb-Q<sup>mi</sup>, which themselves differ in charge from Hb-A<sup>mi</sup>, two minor components should sometimes be seen in the phenotypes A<sup>mi</sup>P<sup>mi</sup> or A<sup>mi</sup>Q<sup>mi</sup>, but this has not so far been observed.

The minor components of Old World monkeys and anthropoid apes present some interesting evolutionary problems. A minor component occurs in chimpanzees, gorillas and orangs and we have observed (unpublished work) two electrophoretically distinct minor components, present either alone or together in a sample of nine gibbons (*Hylobates lar*). Pongids are usually assumed to have a homologue of the human  $\delta$ -chain and it has been shown<sup>10</sup> that, in the orang, the minor components have an  $\alpha$ -chain like that of major components. If this assumption is correct the  $\delta$ -chain presumably arose by the postulated  $\beta$ -locus duplication before separation of pongid and hominid lineages  $3-4 \times 10^7$  yr ago and the human  $\delta$ - and  $\beta$ -chains have since diverged by ten substitutions. In the case of macaques, however, a minor compo-



ment was not found in *Macaca nemestrina*<sup>11</sup>, *M. speciosa*, *M. sinica*, *M. mulatta* or *M. cyclopis* (unpublished work), nor in three species of the related baboons (*Papio*)<sup>12</sup>. On the present incomplete evidence it seems that either a minor component may have been present in some ancestral macaque but has been lost except in *M. irus* or that it arose after this form became specifically distinct, if indeed it is<sup>13</sup>. In either case a time span considerably less than  $3-4 \times 10^7$  yr is likely to be involved and yet at least four substitutional differences from the *M. irus*  $\alpha$ -chain have evidently accumulated.

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### Synthesis of ATP driven by a Potassium Gradient in Mitochondria

THE possibility of reversing energy-dependent ion transport to synthesize ATP<sup>1,2</sup> has apparently been realized in the case of chloroplasts subjected to acid-base transitions<sup>3</sup>. In no other case has net ATP synthesis coupled to ion transport been reported although incorporation of phosphorus-32 into ATP has been shown to be enhanced in erythrocytes by adjusting the sodium and potassium gradients so as to "reverse the Na<sup>+</sup>-pump"<sup>4</sup> and during step-changes in pH applied to mitochondria<sup>5</sup>. The high apparent efficiency of the accumulation of potassium supported by ATP in mitochondria treated with the antibiotic valinomycin<sup>6</sup> suggested that, provided the kinetic barrier was not inordinately large, ATP synthesis should be demonstrable in the absence of respiration if the direction of net potassium movement were reversed (that is, during potassium efflux).

Net synthesis of ATP accompanying stimulation of potassium efflux by valinomycin is shown in Fig. 1A. The increase in ATP is accompanied by an equivalent decrease in ADP and a negligible change in AMP. There is thus a net increase in high energy phosphate indicating that the elevation in ATP is not mediated by adenylate kinase. The presence of the rotenone block prevents synthesis of ATP by means of energy derived from the oxidation of endogenous substrate. Oxygen consumption and fluorescence (as a measure of the pyridine nucleotide redox state), [K<sup>+</sup>] and [H<sup>+</sup>] were recorded simultaneously by means of the appropriate electrodes and apparatus previously described<sup>7</sup>. The observed inhibition of respiration by rotenone substantiated the essentially complete block of electron transfer. If 10 mmolar potassium is added to the medium, instead of potassium efflux and elevation of ATP, a very slight net accumulation of potassium and

decrease in ATP occur on addition of valinomycin (Fig. 1A), which may be attributed to the resultant decrease of the transmembrane potassium gradient. Replacement of the exogenous potassium with 10 mmolar sodium actually slightly enhanced the rise in ATP during potassium efflux compared with the basic system which was free of alkali ions. Reagents which interfere with the communication between ion transport and ATP—oligomycin and 2,4-dinitrophenol—prevent the increase in ATP when valinomycin is added as shown in Fig. 1B. If valinomycin is replaced by nigericin<sup>8</sup>, which catalyses an even more active potassium efflux under these conditions, no concomitant effect on the endogenous level of ATP is observed. The corresponding changes in [K<sup>+</sup>] and [H<sup>+</sup>] of the suspending medium as detected by the appropriate glass electrodes are shown in Figs. 2A and B, respectively.

The rapid initial discharge of potassium when valinomycin is added is followed by a slower, sustained leakage of potassium. Oligomycin decreases the rapid initial discharge of potassium while, if anything, 2,4-dinitrophenol stimulates the initial potassium efflux. This pattern—diminution by an energy transfer inhibitor and enhancement by an energy dissipating agent—is consistent with the initial, rapid component of potassium efflux being energy-linked. The rapid potassium flux, moreover, exhibits the same time course as the rise in ATP. In contrast, the rapid and extensive potassium efflux and hydrogen ion accumulation initiated by nigericin does not appear to be energy-linked: no associated rise in ATP is observed, nor are these ion movements affected by oligomycin. This is consistent with work to be published in detail elsewhere which indicates that nigericin bypasses the energy-linked mechanism, presumably by serving as a negatively charged cation carrier catalysing passive potassium ion for hydrogen ion exchange. It is clear from these results that potassium efflux or hydrogen ion accumulation *per se* is not necessarily linked to the formation of ATP.

These observations support the conclusion that valinomycin increases access of potassium to an energy-linked cation transport system tightly coupled to ATP synthesis.

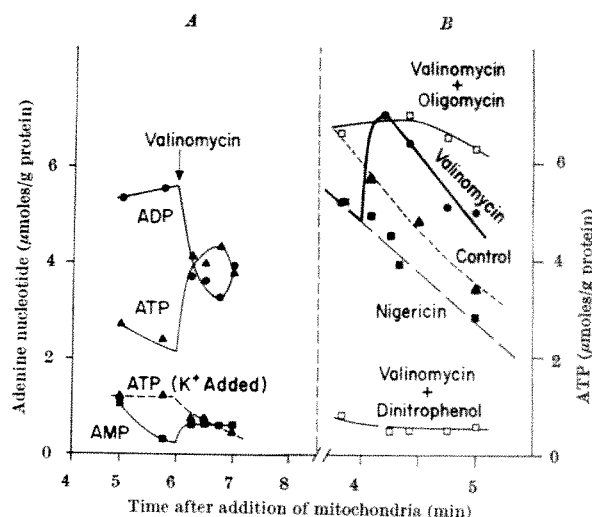


Fig. 1. A, To medium containing 300 mmolar sucrose and 5 mmolar *tris*-phosphate (pH 6.5) at 20° C, rat liver mitochondria equivalent to 6 mg protein/ml. were added followed by rotenone (0.2  $\mu$ g/mg protein) after 1 min. Valinomycin (0.03  $\mu$ g/mg) was added at 6 min either in the absence of added potassium (—) or when the basic medium was supplemented with 10 mmolar potassium chloride (---). Samples were fixed with perchloric acid and analysed for adenine nucleotides enzymatically<sup>10</sup>. Experimental values for ATP, ADP and AMP are represented by  $\Delta$ ,  $\bullet$  and  $\blacksquare$ , respectively. B, Except where noted the conditions were identical to those of A. The curves represent: valinomycin (0.036  $\mu$ g/mg) added at 4 min (—); nigericin (0.07  $\mu$ g/mg) added at 4 min in place of valinomycin (---); control in which neither was added (.....). The complete block of elevation in ATP on addition of valinomycin, 1  $\mu$ g/mg, or 60  $\mu$ molar 2,4-dinitrophenol is also shown ( $\square$ , curves labelled accordingly).



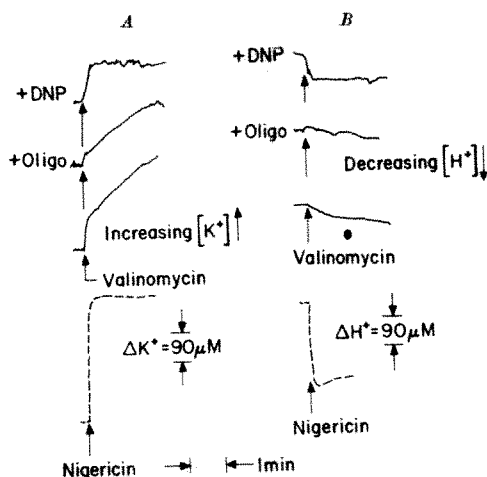


Fig. 2. A, Net efflux of potassium (indicated by an increase in  $[K^+]$  of the medium), and B, the decrease in  $[H^+]$  stimulated by addition of valinomycin (vertical arrows) and the effect of prior addition of either dinitrophenol or oligomycin is shown. The dashed traces are those in which nigericin was added instead of valinomycin; all traces correspond to the experiments of Fig. 1B.

The stoichiometry under optimum conditions for the energy requiring accumulation process has been reported previously as 6.5  $K^+$ /ATP hydrolysed<sup>6</sup>. The stoichiometry between potassium efflux and ATP synthesis under the conditions used here is roughly 10  $K^+$ /ATP formed. The net potassium efflux observed during the formation of ATP is in the range of 20  $\mu$ moles/g protein, while the apparent hydrogen ion accumulation is a variable fraction of this. Under these conditions, the potassium gradient is at least 100 (that is, internal  $[K^+]$  at least 40 mmolar, extra-mitochondrial  $[K^+]$  approximately 0.4 mmolar). Because the membrane pH gradient is in all probability only a fraction of a pH unit even in actively respiring mitochondria<sup>9</sup>, it is unlikely that the movement of hydrogen ion down a pH gradient could supply the energy necessary for driving ATP synthesis rather than the movement of potassium down its concentration gradient.

These results indicate that intact mitochondria possess a highly efficient cation transport system tightly "coupled" to phosphorylation. Under conditions in which gradients highly unfavourable for energy-driven ion translocation are imposed experimentally, reversal of cation transport may drive ATP synthesis in a manner similar to that reported to operate in erythrocytes<sup>4</sup>.

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## Partial Purification of a DNA-polymerase from the Non-histone Chromatin Proteins of Rat Liver

THE basic histones and the acidic proteins constitute all the protein components of the mammalian chromosomes. Although histones have been suggested as possible gene regulators, the functions of these protein components in relation to genetic activity are still poorly understood. It has, however, been suggested that the acidic proteins may be involved in the biosynthesis of DNA<sup>1</sup>. We reported previously that some of the activity of the calf thymus DNA-polymerase is associated with chromatin proteins<sup>2</sup>. With the isolation of the chromatin acidic proteins from rat liver<sup>3</sup>, a direct test of this suggestion is possible. In this communication we report the partial purification of a DNA-polymerase from the solubilized chromatin acidic proteins of rat liver. Results from the study of this chromatin enzyme show that it depends on a complete supplement of four deoxynucleoside triphosphates for its optimal activity and preferentially uses native DNA as the template.

The non-histone chromosomal proteins were prepared as described previously<sup>3</sup>. Rat liver nuclei, isolated and purified according to the method of Chauveau *et al.*<sup>4</sup> as described elsewhere<sup>5</sup>, and rid of the nuclear sap proteins by repeated extractions with *tris* buffer, were extracted with 1 molar sodium chloride to obtain the DNA-protein complex. The extract was diluted to 0.14 molar with respect to sodium chloride, whereupon DNA-histones recombined and precipitated, while the non-histone chromosomal proteins, referred to as the chromatin acidic proteins, remained in the solution. The chromatin acidic proteins thus obtained were precipitated at 80 per cent saturated ammonium sulphate and collected by centrifugation. The pellet (Fraction III) was successively extracted with 60 per cent and 30 per cent saturated ammonium sulphate containing 1 mmolar potassium phosphate buffer, pH 8.0, and 1 mmolar 2-mercaptoethanol. The extract obtained by 30 per cent saturated ammonium sulphate (Fraction IV) was dialysed against the mercaptoethanol-phosphate buffer and absorbed on a hydroxylapatite column. The protein was eluted with a linear gradient of 0.001–0.3 molar potassium phosphate buffer, pH 8.0, containing 1 mmolar mercaptoethanol. Each chromatographic fraction was then assayed for its activity of incorporating tritiated TTP into DNA. The active fractions, eluted between 0.15–0.2 molar phosphate, were pooled (Fraction V) and used as the partially purified DNA-polymerase for the experiments described here.

DNA-polymerase activity was assayed essentially according to the procedure of Mantsavinos<sup>6</sup>. The reaction mixture contained in a volume of 0.5 ml.: 20  $\mu$ moles of glycine buffer, pH 8.0; 5  $\mu$ moles of magnesium chloride; 0.5  $\mu$ moles of 2-mercaptoethanol; 50  $\mu$ g of calf thymus DNA, prepared according to Thomas *et al.*<sup>7</sup>; 20  $\mu$ moles each of dATP, dCTP, dGTP and TTP, one of which was labelled with radioactivity; and 0.05–0.1 mg of the enzyme. The reaction mixture was incubated at 37° for 1 h. At the end of the incubation, the mixture was chilled in ice-water, and 0.3 mg of bovine serum albumin was then added as carrier to the reaction mixture, followed by 0.1 ml. and 2.5 ml. of cold 50 per cent and 5 per cent trichloroacetic acid, respectively. The acid-insoluble precipitate was collected on a 'Millipore' filter (0.45  $\mu$  pore size), washed five times, through filtration with 5 ml. of cold 5 per cent trichloroacetic acid, and counted in a Packard scintillation spectrometer. The counts obtained were corrected for zero-time absorption. Protein concentration was measured from absorbancies at 2800 Å and 2600 Å according to Layne<sup>8</sup>.

Table 1 shows the purification steps for the DNA-polymerase. Using this procedure, a 250-fold purification of the enzyme has been achieved from the starting liver homogenate. Assay of nuclear sap for DNA-polymerase

Table 1. PURIFICATION OF RAT LIVER CHROMATIN DNA-POLYMERASE

Fraction	Specific activity $\mu$ moles tritiated TTP/mg protein
Liver homogenate	0.025
Nuclei	0.26
III Chromatin acidic proteins	0.96
IV Ammonium sulphate	3.6
V Hydroxylapatite	5.2

Table 2. INCORPORATION OF LABELLED DEOXYNUCLEOSIDE-5'-TRIPHOSPHATES INTO DNA

	$\mu$ moles labelled deoxynucleoside triphosphate incorporated	
	Fraction IV	Fraction V
1. Complete system ( $^3$ H-TTP)	476	403
-dATP, -dCTP, -dGTP	290	72
2. Complete system ( $^3$ H-dATP)	503	415
-dCTP, -dGTP, -TTP	138	77
3. Complete system ( $^3$ H-dCTP)	523	317
-dATP, -dGTP, -TTP	342	130
4. Complete system ( $^{14}$ C-dGTP)	446	444
-dATP, -dCTP, -TTP	326	213

activity gave only 10 per cent of that of the chromatin acidic proteins. Partial purification of the enzyme is shown further by the increased dependence of the polymerase system on a complete supplement of all four deoxynucleoside triphosphates (Table 2). The polymerase thus obtained is about three times more active than the DNA-polymerase purified from the soluble proteins of rat liver<sup>9</sup>. The incorporation of isotopic deoxynucleoside triphosphates in the absence of the other three nucleotides suggests some terminal addition activity possibly resulting from 3' —OH termini produced by an endogenous nuclease. The results are, however, comparable with other mammalian preparations.

Table 3. CHARACTERISTICS OF THE RAT LIVER CHROMATIN DNA-POLYMERASE

	Tritiated TTP incorporated ( $\mu$ moles)
1. Complete system	337
2. - DNA, + heat-denatured DNA*	201
3. - DNA	0
4. + Actinomycin D, 100 $\mu$ g	44
5. Complete system, DNA pre-incubated with 50 $\mu$ g DNase	0
6. Complete system, post-incubated with 50 $\mu$ g DNase for 1 h	0
7. Complete system, post-incubated with 50 $\mu$ g RNase for 1 h	300

\* Heat-denatured DNA was prepared by heating 50  $\mu$ g of native DN at 100° C for 10 min and rapidly cooling in ice-water.

That the rat liver chromatin enzyme is characteristic of DNA-polymerase is shown in Table 3. It can be seen that incubation of the labelled reaction product with DNase renders the incorporated radioactivity acid-soluble; whereas treatment with RNase is ineffective. This indicates a DNA-like product. The polymerase-catalysed incorporation of tritiated TTP has an absolute requirement for DNA template. When actinomycin D was included in the reaction mixture, 87 per cent of the incorporation activity for tritiated TTP was abolished. This suggests that the chromatin DNA-polymerase is essentially of the replicative type. Although the enzyme can utilize either native or heat-denatured DNA as the template, it shows a preference for the double-stranded molecule (Table 3). This template specificity is similar to that of the DNA-polymerase purified from rat liver soluble proteins<sup>9</sup>, but differs from the DNA-polymerases of calf thymus<sup>10</sup> and ascites tumour cells<sup>11</sup> which preferentially utilize heat-denatured DNA as template.

DNA-polymerases isolated from various animal tissues and tumours seemingly are not identical with regard to their intracellular localization and the physical state of the DNA template required. Although in bacteria the enzyme has been observed to be associated with DNA<sup>12,13</sup>, most of the mammalian polymerase preparations have been obtained from the soluble proteins of the cell<sup>6,10,14</sup>. Exclusive localization of DNA-polymerase activity in the cell nucleus, however, has been observed in sea urchin embryos<sup>15</sup>, cells which undergo rapid division. In view of the template requirement, it is logical to expect the association of this enzyme with the chromosomal complex

where DNA replication takes place. The partial purification of the DNA-polymerase from the chromatin acidic proteins thus directly demonstrates the chromosomal origin for the enzyme. Perhaps more significantly it shows that one of the functions of the non-histone chromatin acidic proteins is its participation in the biosynthesis of DNA.

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### Ornithine Carbamoyltransferase Activity in Plasma of Mice with Malaria as an Index of Liver Damage

INCREASED concentrations of glutamic oxalacetic and glutamic pyruvic transaminases have been detected in the serum of mice infected with *Plasmodium berghei*, and they are thought to be connected with changes in the liver<sup>1-4</sup>. Transaminases, however, are found in tissues other than liver<sup>5</sup> and are present in high concentrations in erythrocytes. Thus in clinical disorders associated with haemolysis, such as malaria, enzyme from lysed erythrocytes may contribute significantly to serum concentrations<sup>2</sup>.

An increase in carbamoyltransferase (OCT) in the serum reflects liver injury because this enzyme is usually found almost exclusively in the liver<sup>6-11</sup>. Normally, OCT occurs only in small amounts in blood serum, and haemolysis itself, *in vitro* or *in vivo*, does not increase the OCT in the serum<sup>10,11</sup>. The distributions of OCT in various organs of man, the dog and the mouse are similar; the liver shows extremely high activities, the small intestine much less, and other organs only traces of activity<sup>11</sup>. Increases of serum OCT have been demonstrated in a variety of liver disorders of man<sup>8,10,12,13</sup> and after induced liver injury in the dog<sup>7</sup> and the mouse<sup>14</sup>. Study of OCT in the plasma in malaria and other parasitic infections has, however, not been reported, and so we have assayed plasma OCT activity (P-OCT) in mice injected with *P. berghei*.

We assayed P-OCT in mice for 6 days after their inoculation with NYU-2 strain of *P. berghei* maintained through repeated mouse passages. Inocula were prepared by the method of Hillyer and Diggs<sup>15</sup>. ICR donor mice, infected 3 days before with approximately 10<sup>7</sup> parasitized erythrocytes, were exsanguinated, and blood was pooled in ice-cooled sodium citrate. A sample from the pool was withdrawn for the parasites to be counted, and to the rest citrate solution was added to a concentration of about 10<sup>7</sup> parasitized RBC/0.2 ml.

A group of young ICR mice, weighing 15-20 g, was divided into an experimental and a control group. Mice

were freely given baked biscuits and water at all times. All experimental mice were injected peritoneally with  $10^7$  parasitized RBC from the same donor pool and at the same time. On the same morning, control mice were injected intraperitoneally with 0.2 ml. of a similar suspension of non-parasitized erythrocytes derived from non-infected *ICR* donor mice.

Blood for individual determination of parasitaemia and/or P-OCT was obtained concurrently from randomly selected groups of control and experimental mice on each of the 6 days after inoculation. Mice were anaesthetized with carbon dioxide, and exsanguinated by direct heart puncture with pre-heparinized syringes. Heparin does not interfere with OCT assay<sup>10</sup>.

Blood specimens were centrifuged immediately, plasma samples were separated, and chemical analyses were performed immediately after collection<sup>10</sup>.

Baseline plasma citrulline concentration, and that produced by the activity of OCT, were assayed by the method of Ceriotti and Gazzaniga<sup>18</sup>. To measure absorbance, we used the Gilford modification of the Beckman DU spectrophotometer, with rectangular microcells of 10 mm light path.

Parasitaemia levels were determined by thin blood smears stained with Giemsa stain, and the degree of parasitaemia was determined by examining 200 or more erythrocytes and was expressed as percentage of cells parasitized.

On each of 6 days the P-OCT values in infected mice were higher than in controls (Table 1). The differences in mean P-OCT values between infected and control mice were statistically significant ( $P=0.01$  or less) on all days except the second. P-OCT values of infected mice on the fifth and sixth days after inoculation were about five times those of the corresponding control mice. The levels of parasitaemia increased with time after inoculation. As the mean levels of parasitaemia of the groups increased, so did the percentage increases of total plasma citrulline and P-OCT over those of the corresponding control groups (Table 1). Increase in P-OCT after inoculation was in approximate chronological agreement with the development of histopathological changes previously noted in the livers of both germ-free and conventionalized *ICR* mice infected with *P. berghei*<sup>4</sup>. For example, hepatocellular fatty metamorphosis began on the third day, and both numerical increase and hypertrophy of Kupffer cells began on the third or fourth day. Small but distinct increases of P-OCT occurred, however, during the first 2 days of infection, suggesting that hepatocellular derangement occurs before structural damage is detectable by conventional histopathological methods.

There was no significant difference between basal plasma citrulline concentrations in infected and non-infected mice, and no significant change occurred in the infected mice.

Basal citrulline was not essential for detecting increased P-OCT in the malarious mice. This is shown by the increases of total plasma citrulline (Table 1), which include both basal citrulline and the citrulline produced from the ornithine substrate by OCT during incubation. Thus, the relative increases in total plasma citrulline reflect adequately the progressive increases in P-OCT during the course of malaria.

As mentioned, lysis of non-parasitized erythrocytes does not result in an increase in serum OCT activity. Rather, it has been reported that "massive haemolysis" results in a small decrease, presumably by dilution<sup>10</sup>; this contrasts sharply with the eight-fold increase in activity of serum glutamic oxalacetic transaminase which follows haemolysis of whole blood, for example, with water<sup>17</sup>. Nevertheless, in order to ensure that rupture of erythrocytes parasitized by *P. berghei* did not directly contribute to the increase in P-OCT, we devised an *in vitro* test to provide a more extreme lysis than is usual with malaria. This involved intentional disintegration of the erythrocytes and the parasites from infected mice, and we could determine whether the presence of parasite and/or erythrocyte components free in the plasma altered the results of the P-OCT assay.

Using the same materials and methods as described, we infected a separate group of five male *ICR* mice with  $10^7$  parasitized RBC. Three days later the mice were anaesthetized with carbon dioxide and exsanguinated by heart puncture with pre-heparinized syringes. The blood was handled carefully to avoid haemolysis. The blood specimens were pooled, and a stained smear showed that approximately 30 per cent of the RBCs were parasitized. The pooled whole blood was divided into two equal portions. One portion was centrifuged, and the clear, apparently unhaemolysed plasma was collected to assay its OCT activity. The rest of the whole blood was passed twice through a pressure cell<sup>18</sup>, operated at 20,000 lb/in.<sup>2</sup> in a 4° C cold room. A stained thin smear revealed virtually no intact host cells or parasites and centrifugation yielded a clear and very red supernatant portion; only a small pellet of cellular debris remained as the sediment. The haemolysed and non-haemolysed preparations were simultaneously assayed for OCT, and yielded values, respectively, of 41 and 58 units, as defined in Table 1. Thus, even after massive haemolysis and parasite damage we found only a slight, insignificant decrease in OCT. This agrees with the evidence that haemolysis slightly lowers the concentration of P-OCT. It is evident, then, that the marked elevation of P-OCT observed in infected mice is not directly related to haemolysis and/or to the presence of the parasite or its components in the assayed plasma.

Maegraith<sup>2</sup> has recently summarized some of the experimental evidence for the occurrence of mitochondrial

Table 1. P-OCT ACTIVITY OF *ICR* MICE DURING THE FIRST 6 DAYS OF *P. berghei* INFECTION

Time mice observed (day)	No. of mice	Parasitaemia (%)	Plasma citrulline	Percentage difference between C and E total citrulline (%)	P-OCT activity (units)	Percentage difference between C and E P-OCT activity (%)
	C	E	Basal* (μmoles/100 ml.)		C	E
			C	E		
1	10	10	25	23	69 ± 3.0	83 ± 3.7 ‡
2	10	10	22	22	54 ± 8.0	78 ± 14.0
3	10	10	25	25	27 ± 0.4	76 ± 15.6 †
4	10	10	12	15	41 ± 13.0	132 ± 18.0 †
5	10	10	31	36	17 ± 6.9	114 ± 22.5 †
6	7	6	11	27	52 ± 10.6	304 ± 29.2 †
1-6	57	56	21	25	43	131

Experimental mice (E) were infected by intraperitoneal injection of 0.2 ml. of a suspension containing about  $10^7$  parasitized erythrocytes; control mice (C) were similarly injected with 0.2 ml. of a normal, non-parasitized erythrocyte suspension. P-OCT units represent μmoles of citrulline produced/100 ml. of plasma after incubation with L-ornithine substrate at 37° C for 1 h; the production of citrulline *in vitro* is calculated from the total citrulline by subtraction of the basal (preformed) citrulline, assayed on another aliquot of the same plasma specimen. The percentages of the differences between the E and C values are calculated as follows:

$$\frac{(E-C)}{C} \times 100$$

Blood parasite levels are expressed as the percentage of parasitized erythrocytes from 200 (or more) cells examined. Values given are the means, alone or ± the standard error of the mean.  
\* Before incubation. † After incubation. ‡ Difference between mean values of C and E is statistically significant at  $P=0.01$  or less, as determined by Student's *t* test.

dysfunction in liver cells in malaria. In view of our present finding of striking elevations of P-OCT in infected mice, it is pertinent that OCT is found in liver mitochondria<sup>6,19</sup>. Thus, elevated P-OCT may reflect hepatocellular involvement at the level of the mitochondrion. If malaria infection results in dissolution of the well known interdependent structural and functional integrity of this hepatic organelle, then the release of OCT from this source may account for the rise in P-OCT that we observed. The mechanism by which malaria infection produces elevation in P-OCT and the full clinical significance of this finding remain to be determined. Maeraith<sup>2</sup> has pointed out that bilirubin can initiate, in certain circumstances, mitochondrial damage similar to that which he has observed in malaria. Mice infected similarly to ours showed, however, only minor and inconsistent changes in total serum bilirubin<sup>3</sup>, and there was no evidence of obstructive or other changes in the hepatobiliary tree<sup>4</sup>.

If laboratory primates and man also show significant increases in P-OCT during malaria infection, measurement of P-OCT may be useful in the prognosis and diagnosis of malarial infections and in the evaluation of the efficacy of antimalarial agents.

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### Chemical Coupling of Proteins to Agarose

WE have reported a method for transforming polysaccharides into reactive derivatives useful for coupling proteins to carbohydrates<sup>1</sup> which consists in treating the polysaccharide with cyanogen halide. Imino carbonic acid esters are probably formed and these react with the primary amino groups of proteins and other substances. This method of activation and coupling is gentle and therefore particularly useful for the production of immunosorbents and insoluble enzymes.

The biological activity of enzymes, hormones and antibodies is much decreased when these proteins are chemically attached to the matrix of, for example, cellulose and 'Sephadex'. The interaction between the fixed proteins and solutes of specially large molecular weight is obviously sterically strongly impeded. When such interactions are important (for example, in antigen-antibody reactions and for enzyme degradation of proteins) the adsorbents

or insoluble enzymes should be prepared from a matrix of a more open structure.

Agarose was considered to meet the required demands. In the bead form agarose gels prepared according to Hjertén<sup>2</sup> give beds with good flow properties which are retained even after coupling. The cyanogen halide activation method is mild enough to allow the necessary reactions to take place without destroying gel particles. Agarose is composed of residues of anhydrogalactose and galactose rather than of glucose, and so crosslinking is likely to be less important.

In the test of agarose as a coupling partner for proteins, chymotrypsin was chosen and its activity before and after coupling was compared. Agarose beads were prepared from 1 per cent, 2 per cent and 12 per cent agarose solutions, and suspensions of these were prepared containing 3, 10 and 40 mg of agarose/ml. respectively. At room temperature a solution containing 25 mg of cyanogen bromide/ml. (2 ml.) was added to a volume of agarose suspension corresponding to 50 mg agarose in a small container. The pH was rapidly adjusted to 11 and kept constant for 6 min by continuous addition of 2 molar sodium hydroxide using an automatic titrator. The reaction mixture was gently stirred during the process. The gel was immediately washed on a glass filter with ice water, followed by 0.1 molar sodium hydrogen carbonate solution. The activated product was immediately used for the coupling and was for that purpose transferred to a test-tube. A 0.1 molar solution of sodium hydrogen carbonate (11 ml. for 1 per cent agarose, 2 ml. for 2 per cent agarose and 1.5 ml. for 12 per cent agarose) and 25 mg of chymotrypsin were added. The tube was closed and slowly rotated end over end for 20 h at 5° C. The reaction product was very carefully washed in a small column at a rate of 8 ml./h with the following solutions in the order indicated: 0.1 molar sodium borate of pH 8.5 containing 1.0 molar sodium chloride (48 h); 0.1 molar sodium acetate, pH 4.1, containing 1.0 molar sodium chloride (24 h); and finally with 0.01 molar sodium acetate buffer (24 h). A small portion of the gel was prepared for determination of protein by washing with water and then followed by acetone followed by drying. In a similar test with a mixture of chymotrypsin and agarose including all washings no residual absorption of chymotrypsin to the gel was detected.

As blanks for enzyme assay, chymotrypsin and unreacted agarose were mixed and kept in the same conditions as the gel. Esterolytic activity was determined, using *N*-acetyl-L-tyrosine ethyl ester as a substrate. The appropriate amount of coupled chymotrypsin-agarose gel or corresponding free enzyme was added to the substrate solution and the formation of acid was recorded by a titrator. The titration was made at 23° C under nitrogen. Samples of gel suspensions were pipetted out, and proteolytic activity was determined with casein as substrate<sup>3</sup>. The pH optima and the activity ratios for the bound and free enzyme at the optima were determined (Table 1).

To test the stability, chymotrypsin-agarose gel was kept in 0.01 molar acetate solution, pH 4.1, for 2 months at room temperature, then washed for 2 days with 0.1 molar borate buffer, pH 8.5, containing 1 molar sodium chloride. No loss in activity was observed.

The tightness of the gel greatly influences the coupling yield as well as the activity. A tight gel may give a high degree of coupling, but the activity will be lower and the pH optimum is displaced farther than for looser gels. The decrease in activity as a function of the concentration of the matrix is pronounced for casein, which would be expected from sterical hindrance of large molecular size substrates.

The high degree of activity retained in the loose agarose-chymotrypsin gels is noteworthy and makes this a very promising general method for coupling solutes of large molecular size to an insoluble matrix. The chymotrypsin-agarose gel has been used in a series of bed experiments



Table 1. CHEMICAL BINDING OF CHYMOTRYPSIN TO AGAROSE GELS IN BEAD FORM; DETERMINATION OF ENZYME ACTIVITY TOWARD N-ACETYL-L-TYROSINE ETHYL ESTER AND CASEIN

Agarose concentration in the gelation solution (per cent)	Amount of bound protein (mg of protein/g of dried conjugate)	Coupling yield based on added amount of protein (per cent)	pH optimum	Esterolytic activity Activity ratio bound to free enzyme (per cent)	Proteolytic activity pH optimum	Activity ratio bound to free enzyme (per cent)
1	130	30	9.6	50	7.9	45
2	175	42	10.0	45	8.0	35
12	310	90	10.0	50	8.7	10

pH optima for free chymotrypsin toward N-acetyl-L-tyrosine ethyl ester and casein were 7.9 and 8.0 respectively.

("bed reactor") for a wide pH range, and no decrease in activity was seen. Human immunoglobulin of  $\gamma$ G-type has been coupled to agarose in similar conditions. Agarose- $\gamma$ G-gels containing about 350 mg of protein/g of conjugate were obtained.

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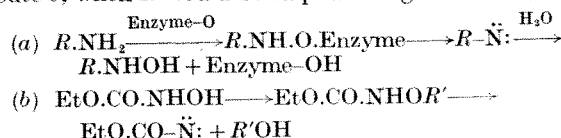
### Possible Cytotoxic Role of Nitrenes

THERE is increasing evidence that the N-oxide structure may be concerned with damage to proliferating cells of a kind which is reflected in carcinogenesis and/or tumour inhibition. Among the compounds recorded in the literature in this respect are 2-N-hydroxyacetamidofluorene<sup>1,2</sup>, 4-N-hydroxyacetamidodiphenyl<sup>3</sup>, 2-N-hydroxyamino-naphthalene and its N-acetyl derivative<sup>1,4</sup>, N-hydroxyurea<sup>5,6</sup>, N-hydroxyurethane<sup>7</sup>, N-hydroxyoxamide and its O-acetyl derivatives<sup>6</sup>. It is now suggested that many of the experimental biological observations may have a common basis, connected with the chemistry of the nitrene (imidogen) group. The purpose of this communication is to draw attention briefly to the manner in which reactive derivatives of this class may be generated, and to speculate on their possible mode of interference with cell structures.

Nitrenes have the general form  $R-\ddot{N}:$ , that is, they show an electron deficient nitrogen atom carrying a sextet of electrons in its outer shell. They are formed, or have been postulated as intermediates (not without controversy), in a number of reactions such as the Curtius, Lossen, Beckmann and Hofmann re-arrangements, so that  $R$  can be said to include H, alkyl, aryl, acyl, sulphonyl, phosphoranyl or amino. They are therefore capable of being generated in a number of ways, but in the present context only two may be relevant. The first involves the abstraction of the molecular species  $R'OH$  from a hydroxylamine derivative; thus  $R-NH-OR' \rightarrow R-\ddot{N}:$ . For example, the chemical reactivity of  $EtO.CO-\ddot{N}:$  has been conveniently studied by its preparation *in situ* through the interaction of triethylamine and the *p*-nitro-sulphonic acid ester derived from N-hydroxyurethane ( $R' = -SO_2.C_6H_4.NO_2$ ). The second experimental preparative method is through oxidation of an amine or amide; thus  $R-NH_2 \xrightarrow{O} R-\ddot{N}:$ . Where  $R$  is acyl, further re-arrangements may occur, as in the oxidation of amides by lead tetra-acetate.

Some of the compounds listed above have been isolated as metabolites after the administration to various animals of the corresponding amines or amides, but from the literature it is not always unequivocally certain whether the manifestations of cytotoxicity *in vivo* are caused by

the parent compound, or its oxidation product, or indeed both. It is now postulated that a nitrene may in fact be the biologically active species, and that this may be generated either as an intermediate step during the metabolic oxidation of the parent amine or amide (for example, route *a*, when  $R$  might be 2-naphthyl) or, by contrast, arise from the already formed N-hydroxy compound or a further metabolic conjugate (for example, route *b*, when  $R'$  could be sulphato or glucuronido).



If the possible enzyme formation of nitrenes in a biological environment is conceded, it is not difficult to foresee how the disorganization of normal cellular processes might then follow from the intervention of these labile substances. For example, they combine with ethylene groups to give aziridines and imino groups to yield hydrazines, with benzene to give azepines (insertion reactions), with aliphatic hydrocarbons to yield alkylamines, and possibly with thio-ethers to form sulphilimines. It can therefore easily be seen how the close juxtaposition within the cell of a nitrene precursor and a nucleic acid strand, for example, could lead to new covalent bond formation as is supposed for the cytotoxic alkylating agents, and with similar sequelae.

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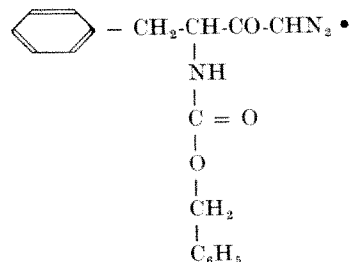
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### Specific Inactivation of Pepsin by Benzyl-oxycarbonyl-L-Phenylalanyldiazomethane

THE report by Delpierre and Fruton<sup>1</sup> on the inactivation of pepsin by L-1-diazo-4-phenyl-3-tosylamidobutanone catalysed by copper ions prompts us to report our results on the inactivation of pepsin by benzyl-oxycarbonyl-L-phenylalanyldiazomethane (ZPDM)



which combines with the enzyme in a 1:1 molar ratio. Our results agree with those of Delpierre and Fruton<sup>1</sup> except that copper ions are not essential for the inactivation of pepsin by ZPDM (compare ref. 2). In addition, we observed that during the attachment of ZPDM to pepsin the optical rotatory properties of the enzyme change in a way similar to that observed for heat-inactivated pepsin<sup>3</sup>.

Crystalline *N*-benzyloxycarbonyl-L-phenylalanine acid chloride (3.9 mmoles) prepared by the procedure of Bergmann *et al.*<sup>4</sup> was added to an ice chilled anhydrous ethereal solution of diazomethane (9 mmoles). After standing, the solvent and excess reagent were removed *in vacuo*. The residue was washed with saturated sodium bicarbonate and water and crystallized from petroleum ether and anhydrous diethylether as yellow crystals (0.41 g, 31 per cent yield), melting point 84–85° C with an infra-red band at 4.77 $\mu$ .

$C_{18}H_{17}O_3N_3$  (323.4)

Calculated: C 66.86, H 5.30, N 13.00

Found: C 67.00, H 5.48, N 11.89

Porcine pepsinogen (lot PG S6GA) and crystalline pepsin (lot 637), Worthington, with 14.60 and 14.84 per cent N, respectively, and the amino-acid composition reported elsewhere<sup>5,6</sup> were used, in addition to pepsin freshly prepared from pepsinogen<sup>7</sup>.

For the reaction of ZPDM with the enzyme 0.2 ml. of ZPDM (2 mg/ml.) in methanol was added to 2.0 mg of pepsin in 1.8 ml. of 0.04 normal sodium acetate buffer, pH 5.16. The reaction mixture was kept at 25° C, and the inactivation in the presence and absence of copper ions was followed by the haemoglobin method of Anson<sup>8</sup>. Thus, at a molar ratio of ZPDM/pepsin = 5 the first order rate constant,  $k$ , in the presence of 1.0 mmolar copper ions is  $1.37 \times 10^{-2} \text{ sec}^{-1}$ ; in the absence of copper ions,  $k = 3.92 \times 10^{-6} \text{ sec}^{-1}$ .

As shown in Fig. 1, pepsin has a pH activity optimum at 1.8 but inactivation of the enzyme occurs at pH 5.3–5.6. ZPDM was stable in our experimental conditions. Thus its inertness toward pepsin at pH 1.0–3.5 cannot be ascribed to instability. Considering that a  $\beta$ -carboxyl of an aspartic acid residue has been implicated as the site of attachment of active site inhibitors to pepsin<sup>9–11</sup>, such a residue with an unusual high  $pK$  may explain the pH optimum of inhibition by ZPDM (ref. 12).

To confirm that in the absence of copper ions ZPDM combines in a 1:1 ratio, a reaction mixture containing a 10 molar excess of ZPDM/mole of pepsin was passed over a 'Sephadex G-25' column, 0.9  $\times$  20 cm, using 0.04 normal acetate buffer, pH 5.04, in 10 per cent methanol and the absorption at 276 m $\mu$  (ref. 2). Inactive pepsin (26.5  $\mu$ mole) 25.0  $\mu$ mole of which was bound to ZPDM was recovered giving a ratio of ZPDM:protein of 0.94.

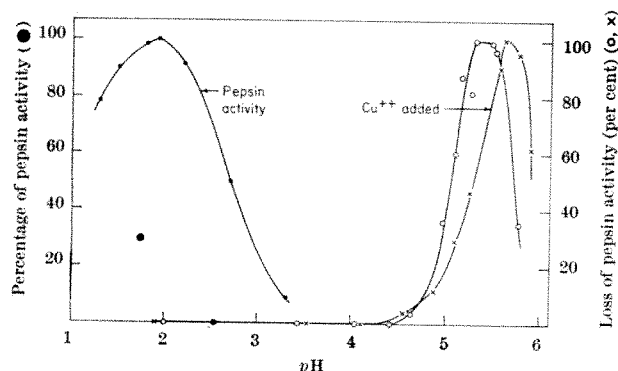


Fig. 1. Dependence on pH of pepsin activity and of inactivation of pepsin by ZPDM. Reaction mixtures were 1 mg/ml. of pepsin in 0.04 normal sodium acetate in methanol at various pH values; 0.20 mg/ml. of ZPDM in the absence of copper ions and 0.05 mg/ml. of ZPDM in the presence of 1 mmolar cupric nitrate. Incubation times were 45 min and 1.5 min respectively.

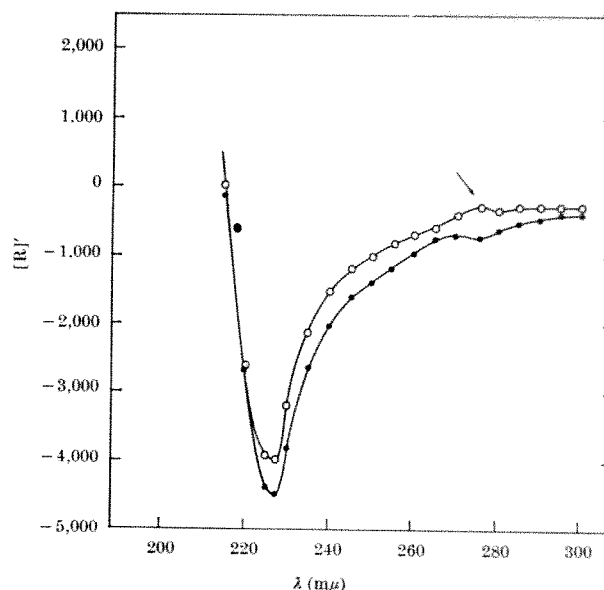


Fig. 2. Far ultra-violet rotatory dispersion of pepsin (○) and ZPDM-pepsin (●) in 0.1 normal sodium acetate buffer, pH 4.9. Arrow indicates the Cotton effects found for wavelength 260–290 m $\mu$ . Protein concentration was 0.025 per cent pepsin and 0.021 per cent ZPDM-pepsin.

The optical rotatory dispersion of ZPDM-pepsin was recorded from 600–200 m $\mu$  in a 'Cary 60' spectropolarimeter. In the spectral region of 600–350 m $\mu$ ,  $[\alpha]$  fits a one-term Drude equation and  $\lambda_c$  is obtained from the plot of  $[\alpha]$  versus  $[\alpha] \cdot \lambda^2$ . As shown in Table 1,  $[\alpha]_{365}$  of ZPDM-pepsin is more laevorotatory and  $\lambda_c$  increases from 217 to 227 m $\mu$ . With both proteins a negative Cotton effect is observed at 225–227 m $\mu$ . In ZPDM, however, the small positive Cotton effect at 260–290 m $\mu$  has disappeared (Fig. 2). As discussed elsewhere, an increase of  $\lambda_c$  from 217 to 227 m $\mu$  is characteristic for heat-inactivated or alkali-denatured pepsin<sup>3,13</sup>. Similarly, changes of the optical rotatory dispersion in the far ultra-violet as those described here take place if the macromolecular conformation of pepsin is altered<sup>14</sup>. Thus we conclude that if ZPDM reacts with a certain amino-acid residue of the polypeptide chain segment necessary for the biological activity of pepsin, that is with its active site, a conformational transition to a less stable conformation takes place which may be accompanied not only by a loss of the enzyme activity but also by a change of the optical rotatory dispersion parameters.

Table 1. SPECIFIC OPTICAL ROTATION AND ROTATORY DISPERSION OF PEPSIN-INHIBITOR AND OF PEPSIN IN 0.1 NORMAL ACETATE BUFFERS

Protein	pH	$-\alpha_{365}^*$	$-\alpha_{365}^*$	$\lambda_c$ (m $\mu$ )	Relative specific activity (per cent)
ZPDM-pepsin	4.3	104	251	225	Not measured
	4.6	102	249	227	6.3
	4.9	105	253	227	6.3
	5.3	98	243	225	5.0
	5.6	109	257	228	5.1
Pepsin	4.6	94	240	218	98.8
	4.9	95	233	218	100.5
	5.3	95	228	217	88.4
	5.6	97	233	217	96.5

The relative specific activity of a freshly prepared pepsin solution in 0.1 normal sodium acetate of pH was taken as 100.

\* Corrected for the refractive index of the solvent.

In summary, the use of substrate-like irreversible inhibitors affords an important tool for the identification of an amino-acid residue involved in the catalytic process<sup>9–11,15–18</sup> and gives information about the conformational changes accompanying interaction of substrate and enzyme.

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### Relation between Thrombosis on Metal Electrodes and the Position of Metal in the Electromotive Series

INTEREST in the electrochemical nature of thrombosis<sup>1,2</sup> has prompted us to study thrombus deposition on metal electrodes inserted through side branches into the carotid and femoral arteries of mongrel dogs. Magnesium, aluminium, cadmium, nickel, copper, gold and platinum, which cover a wide range in the electromotive series, were chosen for the present investigation.

The electrodes were carefully cleaned to ensure the absence of any oxides or other impurities on their surfaces, and their spontaneous potentials were measured in normal (0.9 per cent) saline solution. In the first series of experiments four electrodes of the same metal were inserted through side branches into the carotid and both the femoral arteries in healthy anaesthetized (1/2 ml/kg of 'Diabital' intramuscular injection) mongrel dogs; care was taken to prevent injury to the intimal surface. The spontaneous potential set up by each of these electrodes, after their insertion, was measured with respect to a standard calomel electrode (contained in a beaker with saturated potassium chloride solution). An electrolyte bridge was made between the experimental animal and the beaker containing the calomel electrode by inserting a fine polyethylene tubing into a second branch of one of the femoral arteries. The back flow of blood into this tubing was allowed to clot. The electrodes were kept in position within the lumen of the arteries for a period of 30–40 min.

The dog was killed and the four vessels containing the electrodes were gently clamped both proximally and distally, so as to include the electrode, but without disturbing the position. Formalin was then slowly injected into the portions of the blood vessels between clamps so as to fix any deposits of thrombi on the electrodes. Finally, the vessels were gently slit open and the electrodes within these were examined for any thrombus deposition.

The results were striking. Electrodes of metals establishing a negative interfacial potential (NHE), magnesium, aluminium and cadmium, showed no thrombus deposition (on their electrodes), whereas metals with a positively

charged surface—copper, nickel, gold and platinum—showed a measurable deposition of thrombus along the length of the electrode. The interfacial potentials set up by metals in contact with blood *in vivo*, their corresponding standard electrode potentials and the occurrence or otherwise of thrombus deposition on their surfaces, are summarized in Table 1. As expected, there is a direct correlation between the spontaneous potentials set up by these metals in blood *in vivo* and their respective standard electrode potentials.

Table 1. DEPENDENCE OF THROMBUS DEPOSITION AT METAL ELECTRODES ON POSITION OF METAL IN ELECTROMOTIVE SERIES

Metal	M/M <sup>0+</sup> standard electrode potential (V, NHE)	Resting potential at metal-blood interface (V, NHE)	Occurrence (✓) or non-occurrence (×) of thrombus deposition
Mg	-2.375	-1.360	×
Al	-1.670	-0.750	×
Cd	-0.402	-0.050	×
Cu	+0.346	+0.025	✓
Ni	-0.230	+0.029	✓
Au	+1.420	+0.120	✓
Pt	+1.200	+0.125	✓

In an attempt to confirm these results further, experiments were carried out using electrodes of two different types of metals, one on the electropositive, and the other on the electronegative side. These were inserted into ipsilateral, carotid and femoral arteries in the same animal in the conditions of the previous experiments. The results confirmed the earlier set.

The present series of experiments conclusively proves that thrombus deposition on metals *in vivo* depends, at least partly, on the interfacial potential. The more positively charged interfaces are thrombogenic; those negatively charged are non-thrombogenic. These findings are of considerable fundamental importance in the search for suitable non-thrombogenic surfaces for incorporation into various artificial internal organs.

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### Psychotropic Phenylisopropylamines derived from Apiole and Dillapiole

It is an interesting fact that most of the known psychotropic phenylisopropylamines (amphetamines) possess ring-substitution patterns identical to those of natural essential oils. (The single exception is the active 2-methoxy-4,5-methylenedioxyamphetamine (MMDA-2, II<sup>d</sup>); neither the allyl nor the propenyl counterpart has been observed in plant extracts.) Thus 3,4-methylenedioxyamphetamine (MDA, II<sup>a</sup>) is related to saffrole (II<sup>a</sup>)<sup>2</sup> (Table I), 3,4,5-trimethoxyamphetamine (TMA) to elemicin<sup>3</sup>, 3-methoxy-4,5-methylenedioxyamphetamine (MMDA, II<sup>c</sup>) to myristicin (Ic)<sup>4</sup> (Table I), 2,4,5-trimethoxyamphetamine to asarone, and 2-methoxy-3,4-methylenedioxyamphetamine (MMDA-3a, II<sup>b</sup>) to croweacin (Ib) (Table I). C. F. Barfknecht, of Idaho University, tells us that there is preliminary evidence that these olefines may be aminated in the living organism, and this reaction can be readily performed *in vitro*. There are two additional essential oils known that contain the methylenedioxy ring. These are apiole (Ie) and dillapiole (If) (Table I).

These two naturally occurring aromatic ethers are the two possible ring-methoxylated analogues of myristicin.

We have synthesized the two amphetamines which correspond in structure to these essential oils, that is, 2,5 - dimethoxy - 3,4 - methylenedioxyamphetamine (DMMDA, IIe) and 2,3-dimethoxy-4,5-methylenedioxyamphetamine (DMMDA-2, II f).

Table 1. A COMPARISON OF THE STRUCTURES OF THE NATURAL ESSENTIAL OILS (I) AND THE AMPHETAMINES (II)

	I			II			Potency (mescaline units)
	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	I	II		
a	H	H	H	Safole	MDA		3
b	OCH <sub>3</sub>	H	H	Croweacin	DMMDA-3a		18
c	H	OCH <sub>3</sub>	H	Myristicin	MMDA		2
d	H	H	OCH <sub>3</sub>	(Unknown)	MMDA-2		21
e	OCH <sub>3</sub>	OCH <sub>3</sub>	H	Apiole	DMMDA		12
f	H	OCH <sub>3</sub>	OCH <sub>3</sub>	Dillapiole	DMMDA-2		5

DMMDA was synthesized directly from apiole (obtained from oil of parsley) using the same sequence of steps (isomerization,  $\beta$ -nitration, and hydrogenation) that was successful in the conversion of myristicin to MMDA<sup>4</sup>. It was not possible to isolate useful quantities of dillapiole, so it was obtained synthetically<sup>5</sup> and converted through the above steps to DMMDA-2.

A threshold intoxication with DMMDA in human volunteers was consistently recognized at about 200  $\mu$ g/kg (calculated as the free base and administered orally as the hydrochloride). With most subjects\* concentrations within the range 250–300  $\mu$ g/kg produced a psychotropic episode with the following chronology. The initial 1–1.5 h, preceding the first indications of mental change, were quite free of the signs of the autonomic distress that have frequently been observed with both mescaline and TMA, but only occasionally within the MMDA series. Mild incoordination marked the start of the intoxication period which lasted 2–4 h. During this interval there were only mild perceptual distortions and, in common with MDA, there were increased generalizations of the thought processes, increased emotional affect and empathy, as well as euphoria and a lack of anxiety. The colour exaggerations of mescaline and the eyes-closed images characteristic of MMDA were absent. The gradual disappearance of this syndrome was complete in 8–12 h and the subjects' recall of these events and interpretations was unimpaired, as has been consistently true with the related amphetamines. The syndrome of DMMDA-2 intoxication was qualitatively similar in nature; the threshold was first observed at 400  $\mu$ g/kg and an effective dose range was established as lying between 600–1,000  $\mu$ g/kg. DMMDA-2 has therefore an activity intermediate between DMMDA and MMDA, the latter being active in the vicinity of 2–2.5 mg/kg.

Two arguments must be considered in any explanation of the activity of compounds such as these. First, it has been suggested that  $\beta$ -phenethylamines may participate in central nervous system metabolism through ring closure with the formation of an indole intermediate. This cyclization has been argued as involving an electrophilic attack by the protonated amine on the aromatic ring. In this manner both epinephrine<sup>6</sup> and the demethylation products of mescaline<sup>7</sup> have been oxidatively

cyclized *in vitro*, although no evidence has appeared to support such reactions *in vivo*. At first appearance this argument is supported by the observation that the addition of a methoxyl group to either of the ortho-positions of MMDA (to produce DMMDA or DMMDA-2) increases the potency of the product *in vivo*. Such substitutions would certainly enhance electrophilic ring closure. Specifically, the dose levels of DMMDA and DMMDA-2 reported here allow assignments of potencies of 12 and 5 mescaline units (MU)<sup>1</sup>, respectively, whereas the trisubstituted counterpart MMDA has a rating of about 3 MU.

An alternate indole synthesis route must also be considered. It will be noted that if the *meta*-methoxyl group were removed from either of these tetrasubstituted amphetamines (so actually reducing its theoretical ease of cyclization) MMDA-3a (IIb) would be obtained from DMMDA, and MMDA-2 (II d) from DMMDA-2, yet both of these simpler bases are of still higher potencies (MU of 18 and 21, respectively). Thus it may not be the presence, but rather the position, of the additional group that leads to an enhanced activity. This latter route would then suggest an interaction of the amino-groups with a quinonic intermediate in which the oxygen atom of the ortho-methoxyl group participates.

A second argument is that several phenolic amines are known to act as neurotransmitters. Methylated and methoxylated analogues might function directly (without chemical modification) either as inhibitors or as false transmitters in the specific neural networks served. On the basis of this hypothesis a psychotomimetic molecule should be resistant to chemical attack, rather than sensitive to it, as would be required for conversion to an indole.

Any attempts to understand the mechanisms of action of these materials must still consider the qualitative distinctions that have been noted, however. The two new psychotropic agents reported here, as is true with the two-oxygen methylenedioxy analogue MDA, exhibit changes in affect and empathy and in general are intoxicants, but they should not be classified as psychotomimetics. It seems that this property occurs, at least among the phenylisopropylamines only in those which are trisubstituted.

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## PATHOLOGY

### Effect of Previous Injection of Homologous Embryonic Tissue on the Growth of Certain Transplantable Mouse Tumours

In a previous communication Buttle, Eperon and Menzie<sup>1</sup> reported that injections of suspensions of human embryonic tissues into weanling rats prevented the growth of the transplantable human tumour HS.1 when the

\* All subjects were familiar with the other materials mentioned in these comparisons, MDA, MMDA, DMMDA-3a.



animals were challenged 7 days later with tumour and then conditioned with cortisone. This communication reports similar experiments undertaken to study the inhibition of transplantable mouse tumours by using mouse embryonic tissue.

Mice of several random and inbred strains were used. The mouse tumours used were the imferon induced sarcoma<sup>2</sup>, the Crocker sarcoma S180 and two 20-methyl cholanthrene induced sarcomata, one in BALB/C mice and one in C57 Black/6, obtained from Dr Lloyd Old of the Sloan Kettering Institute. Efforts to induce the imferon sarcoma in a pure line mouse, or in an F1 cross, have so far been unsuccessful.

The embryonic tissues were obtained from mice towards the end of the gestation period. Both embryonic and tumour tissues were minced finely with scalpels and injected subcutaneously with penicillin and streptomycin in 50 per cent suspension by weight of tissue in mammalian Ringer solution.

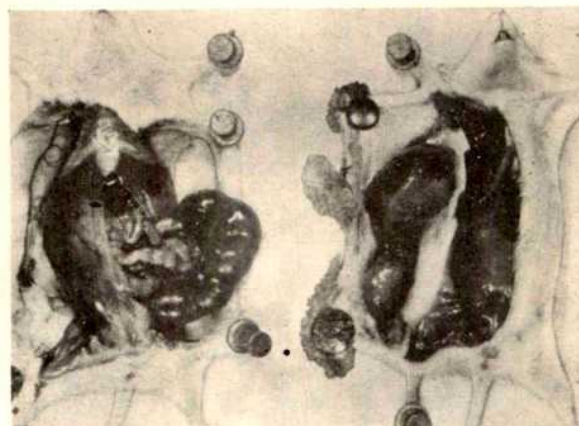
For imferon (iron dextran) sarcoma induced in random Chester Beatty mice, Table 1 shows the degree of inhibition caused by the injection, 7 days before the tumour implants of 0.5 ml. of 50 per cent suspensions of the liver, and of the placenta from 20 day old foetal mice. As controls mice were injected with similar quantities of a suspension of adult mouse liver. It can be seen that suspensions of foetal liver as well as foetal residues after the removal of the liver are effective in completely inhibiting the tumour growth whereas the placenta and adult mouse liver are less effective.

Table 2 shows the effect of early pregnancy in inhibiting growth of the transplantable sarcoma induced by imferon. The growth is negligible when the tumour is inoculated on the eighth day of pregnancy. The tests were carried out in B.S.V.S. mice, in which the tumour grows well; B.S.V.S. mice were also used as donors of embryonic tissue. The imferon tumour has also been induced in the golden hamster, but in this animal neither pregnancy nor the prior injection of embryonic tissue influenced tumour growth.

Experiments with the Crocker mouse sarcoma S180 (Table 3) show that when a suspension of mouse embryonic liver is injected into mice 7 days before tumour challenge, an inhibitory effect on tumour growth is observed. This effect is, however, never as great as that seen with the sarcoma already mentioned which is induced by imferon; the injected mice show tumours of about half the size of those in the controls. Embryonic thymus appears to be as effective as or more effective than foetal liver. Adult mouse liver has little effect while placental suspension is inactive. Fig. 1 shows that the effectiveness of embry-

Table 2. EFFECT OF PREGNANCY ON THE GROWTH OF THE SARCOMA INDUCED BY IMFERON IN B.S.V.S. MICE

Day of gestation Number of animals in group	Non-pregnant females		Pregnant females			
	—	2	8	12	17	20
Average weights of tumours (g) after 12 days growth	22	4	7	2	6	2
	1.94 ± 0.34	2.25 ± 0.15	0.027 ± 0.02	0.31 ± 0.31	0.96 ± 0.5	1.26 ± 1.3



Left mouse inoculated with tumour when 8 days pregnant. Right mouse control, not pregnant.

Table 3. AVERAGE RESULTS OF 23 EXPERIMENTS EACH WITH A GROUP OF SIX MICE

Parke's strain mice used as a source of maternal and foetal tissue

	Mice injected 250 mg foetal liver suspension	Mice injected 250 mg adult liver suspension	Mice injected 10 mg foetal thymus suspension	Mice injected 250 mg placenta suspension
Controls	2.97 ± 0.15	1.52 ± 0.26	1.61 ± 0.31	2.22 ± 0.27
	49 per cent inhibition	28 per cent inhibition	46 per cent inhibition	None

Suspensions injected into B.S.V.S. mice 7 days before challenge with S180. Average tumour weights in grams after 12 days growth.

onic tissue is more obvious if the criterion used is mouse survival time rather than tumour weight.

Fig. 2 shows that mice which react to embryonic tissue by the production of a large subcutaneous swelling at the site of injection show a greater degree of protection against the tumour than those in which no reactions occur; in the latter, tumour weights do not differ significantly from those in the controls. Furthermore, the degree of tumour inhibition is highest if the interval between tissue injections and tumour challenge is 7 days (Table 4). If, on the other hand, the interval is shorter (for example, 3

Table 1. SARCOMA INDUCED BY IMFERON IN B.S.V.S. MICE

Treated mice injected subcutaneously with 0.5 ml. (250 mg wet weight) suspension of:

Date	Controls	Placenta	Foetal liver	Whole foetus after removal of liver	Adult liver
Nov. 20, 1963	0.2	0	0		
	0.3	0	0		
	0.6	0	0		
Nov. 22, 1963	0.9	0	0		
	0.1	0	0		
	0.3	0	0		
Dec. 4, 1963	0.4	0.1	0		
	0.8	0.4	0		
	1.2	0.4	0		
Dec. 13, 1963	1.3	0.7	0		
	1.4				0
	0.6				0
Jan. 29, 1964	1.1				0.1
	2.4				0.8
	2.9				
May 21, 1964	0.2	0.2	0	0	
	3.5	2.0	0		0.75
	3.0	0	0	0	0
	1.25	0.5	0	0	3.5
	1.0	1.75	0	0	
Average weights	1.14 ± 0.20	0.38 ± 0.016	0	0	0.77 ± 0.42

Inhibition of growth caused by subcutaneous injection of 0.5 ml. of 50 per cent embryonic mouse tissue suspension 7 days before tumour implants.

Tumour weights in grams after 12 days growth.

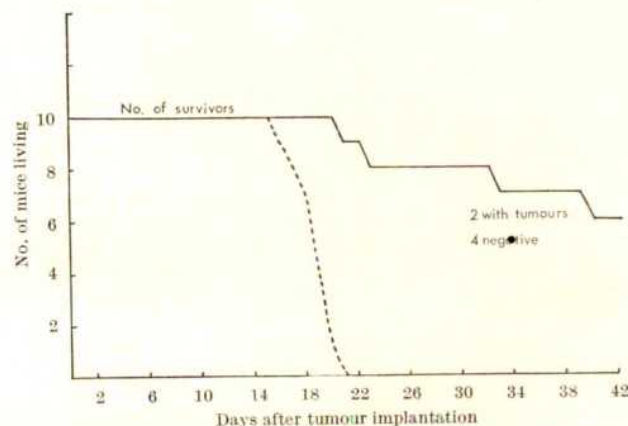


Fig. 2. Mouse survival experiment with sarcoma 180. Tumour hosts are B.S.V.S. mice. Embryonic tissues are from Parke's mice. —, Foetal liver suspension 250 mg subcutaneously injected 10 days before tumour implant; ----, controls.

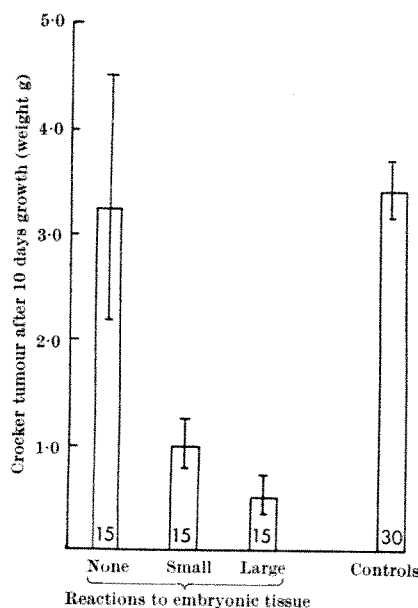


Fig. 2. B.S.V.S. mice injected with Parke's mouse embryonic tissues 7 days before tumour implantation.

days), tumour growth exceeds that in the controls, whereas if the interval is longer than 7 days, the effect is gradually reduced until, after 21 days, there is little difference between injected animals and controls.

While all the foregoing experiments were carried out with mice of different strains as donors of embryonic tissue and as tumour hosts respectively, Table 5 shows that when B.S.V.S. mice are used for both purposes the degree of tumour inhibition is reduced from 49 per cent to 34 per cent.

Table 4. CROCKER TUMOUR S180 IMPLANTED INTO B.S.V.S. MICE AT VARIOUS TIMES AFTER SUBCUTANEOUS INJECTION OF MOUSE EMBRYONIC LIVER TISSUE SUSPENSION FROM MICE OF PARKE'S STRAINS (Six mice in each group)

No. of days between injection of tissue and tumour implant	Control mice tumour weight (g)	Injected mice tumour weight (g)	Percentage difference between tumour weight in injected mice and in controls
3	2.02 ± 0.40	3.8 ± 0.40	+88
7	2.43 ± 0.21	0.68 ± 0.15	-72
10	2.66 ± 0.24	1.25 ± 0.32	-53
14	3.4 ± 0.27	2.25 ± 1.0	-33
21	1.57 ± 0.29	1.94 ± 0.33	-24

Tumour weight compared with controls after 10 days of growth.

Table 5. CROCKER TUMOUR S180 GROWING IN B.S.V.S. MICE

Comparison of the effect of injection of mouse embryonic tissue from mice of B.S.V.S. and of Parke's strains 7 days before tumour implantation

Embryonic tissue used	Controls tumour weight (g)	Injected mice tumour weight (g)	Percentage inhibition
B.S.V.S.	2.73 ± 0.31	1.79 ± 0.45	34.4
Parke's	2.97 ± 0.2	1.39 ± 0.26	53.2

In other experiments pure line *BALB/C* mice were used both as donors of embryonic tissue and as tumour hosts; two different tumours induced by methyl cholanthrene were used. In these instances the growth of the tumours in the mice injected with embryonic tissue did not differ significantly from that in the controls, nor was there any prolonging of the survival time of the treated animals. Similar results were obtained with one tumour induced with methyl cholanthrene in *C57 Black/6* mice.

The inhibitory effect obtained from the injection of embryonic tissue suspensions, 7 days before tumour implantation, is most marked in the case of the imferon induced sarcoma growing in B.S.V.S. mice. The growth of this tumour is also inhibited if it is implanted in early pregnancy (about the eighth day of gestation). A likely explanation of this phenomenon is the possibility that trophoblast cells are mobilized extensively in the circula-

tion early in pregnancy and that these cells may produce an "immunizing" effect. The presence of such cells has been demonstrated in the human subject by Douglas *et al.*<sup>3</sup> and by Canlas<sup>4</sup>. With the Crocker tumour, on the other hand, growth is much less influenced by the prior injection of embryonic tissue, the effect in this case being further reduced if the mice used as donors of embryonic tissue are of the same strain as those used as tumour hosts. The animals which show the greatest tumour inhibitory response are those in which the reactions to the embryonic tissue are most pronounced.

The growth of twenty tumours induced by methyl cholanthrene in pure line *BALB/C* and *C57 Black/6* mice was not influenced by the previous injection of embryonic tissue, derived from animals of the same inbred strains. In every instance where the inhibitory influence of foetal tissue was displayed this was always more pronounced than when adult tissues were used. This could be the result of an antigenic similarity between embryonic and malignant tissues or, alternatively, of the presence of interfering antigens in adult tissues which could then decrease the tumour inhibitory effect. The fact that the methyl cholanthrene tumours in pure line mice do not respond to the injection of embryonic tissue from the same pure line strains suggests that the effect may be similar to that occurring in the homograft reaction and may occur only when there are genetic differences between the host animal and the embryonic tissue used.

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### Effect of Route of Administration on Immunosuppression by DMBA in *CBA* Mice

It has been known for some time that certain chemical carcinogens depress the immune response<sup>1</sup>. When the carcinogen is given neonatally this depression may be long lasting<sup>2</sup>. Stjernswärd has shown that in addition to depressing the serum haemolysin response to heterologous red cells, a number of carcinogens lower the number of antibody forming cells in the spleen as measured by the Jerné technique<sup>3</sup>. No such depression was found in response to non-carcinogenic analogues tested, but whether or not immunological impairment is an integral part of carcinogenesis as suggested by Prehn and Main<sup>4</sup> is not yet known. Tumour-specific antigens have been demonstrated in a variety of chemically induced tumours of both mesodermal and epidermal origin<sup>5,6</sup>. Transformed cells multiplying to form macroscopically recognizable tumours seem to do so despite their antigenicity: they may represent only the proportion of potentially neoplastic cells which have in some way overcome host resistance.

The incidence and sites of tumour production vary with the route of administration of a carcinogen, and it was therefore interesting to see whether immunosuppression induced by a carcinogen is similarly affected. Carcinogenic doses of 9,10-dimethylbenzanthracene (DMBA) were administered orally or subcutaneously to mice, and the subsequent responses to an intraperitoneal injection of sheep erythrocytes were compared.

We used seventy-eight female *CBA/H* mice 6 weeks old, weighing between 15 and 18 g, maintained on a cubed



Table 1. AVERAGE HAEMAGGLUTININ TITRE TO SHEEP ERYTHROCYTES EXPRESSED AS LOG<sub>2</sub> OF LAST POSITIVE SERUM DILUTION, WITH STANDARD DEVIATIONS

Group	Treatment (a.g. = 3% aqueous gelatine)	Time after immunization			
		4 days	6 days	10 days	75 days
1	Untreated	5.8 ± 0.4 (6)	6.6 ± 1.2 (6)	7.0 ± 0.0 (6)	—
2	3% a.g. subcutaneously	4.8 ± 1.1 (12)	5.8 ± 2.0 (12)	6.3 ± 2.0 (11)	7.3 ± 1.2 (6)
3	33.3 µg DMBA/g body weight in a.g. subcutaneously	1.4 ± 0.5 (12)	3.0 ± 2.5 (12)	3.6 ± 2.1 (12)	4.3 ± 1.2 (6)
4	100 µg DMBA/g body weight in a.g. subcutaneously	1.0 ± 0.0 (12)	1.2 ± 0.3 (12)	1.4 ± 2.5 (9)	—
5	3% a.g. orally	5.7 ± 0.8 (11)	6.4 ± 1.8 (11)	7.6 ± 0.7 (11)	—
6	33.3 µg DMBA/g body weight orally in a.g.	5.2 ± 1.1 (11)	6.4 ± 0.8 (12)	7.1 ± 1.1 (12)	—
7	100 µg DMBA/g body weight orally in a.g.	4.3 ± 1.5 (11)	6.1 ± 1.6 (10)	6.5 ± 0.7 (8)	—

Numbers of animals tested are shown in parentheses.

diet (Diet 86, Dixons, Ltd.). They were divided into seven groups which were treated as follows.

Group 1 (six mice) was untreated, group 2 (twelve mice) received 3 per cent aqueous gelatine subcutaneously, group 3 (twelve mice) received 33.3 µg/g body weight (that is, 500 µg/15 g of mouse) of DMBA in aqueous gelatine subcutaneously, group 4 (twelve mice) received 100 µg/g body weight (that is, 1,500 µg/15 g of mouse) of DMBA in aqueous gelatine subcutaneously, group 5 (twelve mice) received 3 per cent aqueous gelatine orally, group 6 (twelve mice) received 33.3 µg/g body weight of DMBA in aqueous gelatine orally, and group 7 (twelve mice) received 100 µg/g body weight of DMBA in aqueous gelatine orally.

DMBA (Koch-Light Laboratories, Ltd.) was suspended by ultrasonication in 3 per cent aqueous gelatine. Neither dose resulted in death or weight loss during preliminary toxicity tests. Before peroral treatment the animals were starved overnight, and the DMBA suspension was passed into the stomach through a cannula. The larger dose was given in approximately 0.4 ml., and the smaller dose in 0.25 ml., of aqueous gelatine, the exact volume being adjusted to give the correct dose/g of body weight for each animal. Subcutaneous injections were given at five sites, the axillae, flanks and central back, and the volume of each injection was approximately 0.15 ml. Equivalent volumes of 3 per cent aqueous gelatine were administered to animals in control groups by each route. There was no loss of weight in any group during the 6 days after treatment.

After 6 days all mice were challenged with an intraperitoneal injection of a 10 per cent solution of washed sheep erythrocytes. Each animal was given 0.1 ml. containing approximately  $1.4 \times 10^8$  cells, and bled from the retro-orbital sinus 4, 6 and 10 days later. The group given the smaller dose of DMBA subcutaneously, and the subcutaneously injected gelatine control group, were also bled 75 days after challenge. Individual sera were titrated for haemagglutinins by the Takátsy microtitration method, and titres expressed as log<sub>2</sub> of the reciprocal of the last serum dilution which showed macroscopically visible agglutination. The results (Tables 1 and 2) indicate a marked depression in response in the subcutaneously injected animals at both doses, but insignificant decreases in the two groups treated orally. The mice are now 33 weeks old. Of the animals injected subcutaneously, those given the larger dose succumbed to generalized infection 2–3 weeks after carcinogen treatment. Three died during the experiment and the remainder had to be killed shortly afterwards because they were sick. At post-mortem all showed lymphoid involution. Early death was not indicated by the preliminary toxicity tests and it may be that the injection of sheep red cells in some way adversely affected recovery from a high dose of DMBA. There were no deaths among animals given the smaller dose of DMBA and each one of these twelve later developed subcutaneous tumours at one or more of the injection sites. None of the animals given DMBA orally has yet developed a tumour although it is likely that occult neoplasms are already present, or will later develop, in some of them<sup>7–15</sup>.

The reason for the difference in response between groups 3 and 4 and groups 6 and 7 is obvious. It is possible that the sensitivity of the system for the estimation of carcinogenic activity exceeds its sensitivity for the estimation

Table 2. STATISTICAL COMPARISON OF RESULTS SHOWN IN TABLE 1

Comparison	P values at following times after immunization			
	4 days	6 days	10 days	75 days
Group 1/2	<0.001	NS	NS	—
Group 1/3	<0.001	<0.01	<0.01	—
Group 2/3	<0.001	<0.01	<0.01	<0.001
Group 1/4	<0.001	<0.001	<0.001	—
Group 2/4	<0.001	<0.001	<0.001	—
Group 1/5	NS	NS	NS	—
Group 5/6	NS	NS	NS	—
Group 1/6	NS	NS	NS	—
Group 1/7	NS	NS	NS	—
Group 5/7	NS	NS	NS	—

NS, Not significant at 5 per cent level.

of immunological impairment. Other work<sup>16</sup> has shown that subcutaneously injected doses of DMBA, smaller than those injected into each site in either of groups 3 or 4 of the present experiment, may induce sarcomas at the site of injection. Another possible explanation of the result is that only part of the orally administered DMBA was absorbed and, of that part, some may have been detoxified in the liver before it reached the reticulo-endothelial system.

Irrespective of the mechanism involved, the results of the experiment clearly show that large doses of DMBA, administered orally to mice, did not affect the haemagglutinin response to sheep erythrocytes, whereas smaller doses of the same agent injected subcutaneously caused marked depression.

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## Respiratory Distress in a Newborn Foal with Failure to form Lung Lining Film

A SYNDROME in newborn thoroughbred foals, characterized by irregular respiration, convulsions, and lung pathology, has been described already<sup>1</sup>, and its resemblance to the respiratory distress syndrome of the newborn

human noted. This latter condition is associated with the absence of the surface active lung lining complex, or lung surfactant<sup>2</sup>; this absence can be investigated by observation of bubbles obtained from the lung<sup>3</sup>. From normal lungs bubbles of very low surface tension which show great stability in air-saturated water can be obtained<sup>4,5</sup>. The present case demonstrates the common factors operating in the two syndromes in the different species.

A thoroughbred foal was delivered at 336 days (average gestational length 340 days<sup>7</sup>). It was normal for about 32 h, after which it became unable to stand without support, and extensor spasms began. Breathing became shallow and rapid, the tidal volume falling from 700 to 320 ml., and the respiration rate rising from 25 to 85 per min, between 32 and 64 h after birth. The arterial oxygen pressure fell from 115 mm of mercury at 32 h to 74 mm of mercury at 57 h, while during the same period the carbon dioxide pressure fell from 53 mm of mercury to 30 mm of mercury. Meanwhile the neurological signs showed deterioration, and in spite of supportive measures including oxygen and intravenous bicarbonate the animal died after 78.5 h.

At autopsy pathological changes were confined to the lungs. These were wholly atelectatic except for small areas along the apical and cardiac borders; their consistency was that of liver. Histological examination showed widespread atelectasis with evidence of oedema, the persistence of cuboidal epithelium lining the alveoli and a general lack of normal alveolar patterns which should be established in the foal very rapidly after birth. There was no genuine hyaline membrane such as is seen in the infant.

The surface properties were compared with those of the lungs of normal still-born and live-born foals. Air injected into the specimen of affected lung caused local inflation, which subsided almost completely when the inflating pressure was removed; normal atelectatic lung shows a persistent white inflated patch. Bubbles squeezed from the partly aerated region into a hanging drop of air-saturated water usually contracted and dissolved; bubbles from the normal lung contracted a little and became stable. The behaviour of two typical bubbles is shown in Fig. 1. The ratio of final to initial surface area (stability ratio) of bubbles initially 30  $\mu$  to 50  $\mu$  in diameter was (means of batches of 30): from affected lung, 0.15, 0.09, 0.07; from normal lung, 0.72. The smallest bubbles from normal lungs (<1  $\mu$  diameter) showed Brownian motion; no such bubbles were obtained from affected lung. Larger bubbles (100  $\mu$  to 200  $\mu$  diameter) from normal lung, when dissolving in de-aerated water, showed the peculiar oscillations of shape known as "clicking"<sup>8</sup>; those from affected lung dissolved steadily. Extracts of both types of lung were formed by injecting saline and squeezing it out again, or by crushing a fragment in a drop of saline. Drops of the extracts were made to froth with the aid of

a hypodermic needle; the stability ratios of the bubbles formed were (means of batches of ten): affected lung, 0.0, 0.0, 0.0, 0.0; normal, 0.8, 0.8, 0.7, 0.5. These results demonstrate almost total absence of surfactant and failure to form a lining film. We think it very likely that, if the specimens had been tested by the trough method of Clements<sup>6</sup> used by Avery and Mead<sup>2</sup>, similar conclusions would have been reached.

The signs of rapid shallow breathing, lung atelectasis at autopsy, tendency of the lung to collapse after inflation, and absence of surfactant resemble those of the respiratory distress syndrome of the newborn human. The delayed onset in the foal suggests that some surfactant was present at birth but was used up more rapidly than it was produced. It is uncertain whether or not the onset of the human syndrome is ever delayed.

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## GENETICS

### Effect of Blood Group on Blood Lipids in Monozygotic Twins

THE total variance of any measurement made in a population of monozygotic twins can be divided into two parts: interpair variance and intrapair variance. Calculation of the interpair/intrapair *F* ratio is informative because if this statistic is significantly large it shows that genotype affects the measured variable. Monozygotic twins of the same pair share a common genotype, and so intrapair variance is caused only by environmental effects. These can be divided into two parts: short term effects and long term effects.

A previous twin study of ours demonstrated significant interpair/intrapair variance ratios for total serum cholesterol, free cholesterol, total lipid phosphorus, and triglyceride in thirty-one pairs of monozygotic twins<sup>1</sup>. Blood for assay in our previous investigation was then obtained twice with a 48 h interval between bleedings. This study also demonstrated that certain twin pairs showed intrapair differences in total cholesterol, free cholesterol, and triglyceride of unexpected magnitude. Twins of blood group O appeared to have the largest intrapair differences of total and free cholesterol, particularly if they also possessed the secretor gene as judged by Lewis blood group typing<sup>2</sup>. We have now carried out a larger and more complete experiment on monozygotic twins to confirm or deny the existence of blood group effects on blood lipids and to examine short and long term environmental effects more fully. Ninety-five additional monozygotic twin pairs were bled three times a week on Monday, Wednesday and Friday. All twins were fasting, and all but a few were bled on the same day as their co-twins. Serum total cholesterol, free cholesterol, phospholipid, and triglyceride was determined by methods described before<sup>1</sup>. Determination of twin zygosity was based on blood typing for factors

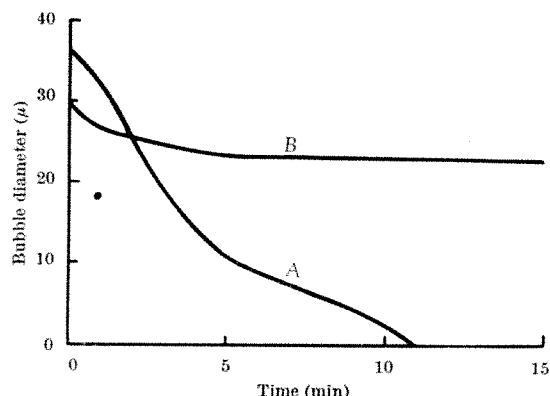


Fig. 1. Time-course of behaviour of typical bubbles in hanging drop. Curve A, from affected lung; curve B, from normal lung, stability ratio of bubble 0.60.



A, B; Lea, Leb; Se, se; D, C, E, c, e; M, N, S, s; Fy<sup>a</sup>, Fy<sup>b</sup>; K, k; P<sub>1</sub>; and Vel by methods described before<sup>1</sup>. Analytical error in the determination of lipids, as shown by a series of blind replicate controls, did not differ in the two experiments.

To analyse short term variation in this experiment, we defined a variable  $S$  = standard deviation of the measurements of 3 days divided by the mean of these measurements (coefficient of variation). We obtained one value of  $S$  for each twin. To analyse long term variation we defined a variable  $D$  = log of absolute value of the difference between means of the two members of a twin pair divided by the standard deviation of the six values obtained from the pair (log  $t$ ). We obtained one value  $D$  for each pair of twins. The variance of these variables was analysed on a 4 (blood groups O, A, B, AB)  $\times$  2 (sex) model with age as a covariate. Only one  $F$  ratio (free cholesterol—blood group) was found to be significant at the 1 per cent level (Table 1). These results do not confirm the hypothesis that blood group affects intrapair differences between twins, but do clearly suggest that blood group affects the day to day change of free cholesterol. In the present experiment twins of blood group B showed more day to day variation in free cholesterol in both males and females than did members of any other blood group ( $P < 0.01$ ) (Table 2). In our previous twin experiment male twins of blood group B also showed greater day to day variation of free cholesterol, but the differences found between blood groups were not significant at the 1 per cent level.

Table 1.  $F$  RATIO TESTS,  $S$  AND  $D$  SCORES

	$F$ ratios for $S$				$F$ ratios for $D$			
	Age (1, 181)*	Sex (1, 181)	Blood group action (3, 181)	Sex- blood group inter- action (3, 181)	Age (1, 86)	Sex (1, 86)	Blood group action (3, 86)	Sex- blood group inter- action (3, 86)
Total cholesterol	0.24	0.14	0.80	0.06	1.67	0.40	1.15	0.01
Free cholesterol	0.01	0.56	4.71†	0.38	1.24	1.79	0.61	0.07
Phospholipid	5.27	0.24	3.57	2.16	0.30	0.07	0.52	0.98
Triglyceride	0.41	1.44	0.18	0.35	0.16	2.46	2.33	0.19
Cholesterol/ phospholipid	3.64	3.03	1.00	2.72	1.43	0.38	1.10	0.75

\* Degrees of freedom.

† Significant at 1 per cent confidence level.

Table 2. MEAN  $S$  FOR FREE CHOLESTEROL IN THE PRESENT EXPERIMENT

Blood group	Male	Female
B	0.095	0.092
O	0.079	0.069
A	0.069	0.054
AB	0.041	0.032

Table 3.  $F$  RATIO AND COEFFICIENTS OF VARIATION IN TWO TWIN EXPERIMENTS

	Tri- glyceride	Total choles- terol	Free choles- terol	Phospho- lipid	Total cho- lesterol/ phospho- lipid
Present experiment, 95 monozygotic twins, 3 determinations:					
Interpair $F$ ratio*	10.4	11.0	11.5	6.33	4.59
Intrapair variance					
Mean	0.130	0.091	0.096	0.098	0.071
Previous experiment, 28 monozygotic twins, 2 determinations (1):					
Interpair $F$ ratio†	3.39	5.01	5.81	6.07	4.35
Intrapair variance					
Mean	0.268	0.117	0.109	0.083	0.063

\* Degrees of freedom (94, 95).

† Degrees of freedom (27, 28).

Interpair/intrapair  $F$  ratios of this and of the previous twin experiment are shown in Table 3. All  $F$  ratios are significant at the 1 per cent level. The intrapair variance divided by mean is also shown for each lipid class in Table 3. Except for triglyceride, this statistic is not appreciably different in the two experiments. The smaller value for triglyceride intrapair variance/mean in the present experiment appears to be the result of increasing

the number of determinations from two in the previous experiment to three in the present one. Triglycerides are known to be the most variable class of lipid. It seems reasonable that this statistic, which is an estimate of long term variation for a set of twin pairs, should show the greatest refinement in the most variable class when more determinations are performed on each twin.

The results of this study contribute new information in two areas of interest: (a) the interrelation of blood group substances and other physiological substances, and (b) the inheritance of blood triglyceride level. Two previous reports suggest that blood group substances may be related to processes involved in lipid metabolism. Arfors, Beckman and Lundin have demonstrated a link between inheritance of blood group substance and a serum isoenzyme of alkaline phosphatase<sup>2</sup>. Inglis *et al.* have shown that this isoenzyme can be altered by ingestion of a fat meal<sup>3</sup>. In addition there is evidence that certain polysaccharides showing the activity of a blood group substance can activate lipoprotein lipase<sup>4</sup>. This report provides additional indication that blood group substances may be linked to processes involved in lipid metabolism. It suggests linkage to a function which controls synthesis and/or degradation of cholesterol before the time that cholesterol esters are formed.

Most current genetic studies of blood lipids are concerned with individuals with gross or abnormal concentrations in the blood. Familial likeness of serum total cholesterol has been demonstrated, however<sup>6,7</sup>, although total cholesterol is in the normal range. These workers studied each family member with a single determination of cholesterol. Familial likeness of normal serum triglyceride has been postulated but not proved to exist. The hypothesis that familial xanthomatous hypercholesterolaemia can be divided into two sub-groups—type II hyperlipoproteinaemia and type III hyperlipoproteinaemia<sup>8</sup>—is affected in several important ways by postulated familial likeness of normal as well as abnormal concentrations of triglyceride. Although studies of families with normal serum triglycerides do not exist, the  $F$  ratios and intrapair/mean ratios in Table 3 suggest that familial likeness of normal serum triglyceride can be of the same relative degree as that of normal serum total cholesterol. They also suggest that to determine likenesses of familial triglyceride it will be necessary to repeat several times the determination of this most variable lipid class in each family member.

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## PHYSIOLOGY

## Minimum Heart Rates of Marsupials

STUDIES of the nutrition and nitrogen metabolism of some macropod marsupials (kangaroos and wallabies) suggested that the level of energy metabolism of marsupials may be lower than that of eutherian mammals<sup>1</sup>.

In the absence of facilities for direct study of the energy metabolism of marsupials, we have sought additional indirect evidence. One readily accessible measure is that of basal or minimum rate of heart beat, which in mature eutherian species has been shown to be related to metabolic body size ( $W_{kg}^{0.75}$ ), as is basal energy metabolism<sup>2,3</sup>. We have observed the minimum heart rates of fourteen species of marsupial.

The leads from an electrocardiograph were attached to the animals by small silver coated safety pins inserted subcutaneously in the chest region. The animals were fasted for at least 24 h and the kangaroos up to 48 h and the heart beat was monitored at intervals during this time. The koala bear was monitored for only 3 h after a light meal, and so it is unlikely that minimum heart rates were recorded. The smaller animals were placed in bags to limit their activity.

Table 1 lists the species used, their body weights and the minimum heart rate recorded. Only small numbers of animals could be tested because many species of marsupials are difficult to obtain. Some species are represented by only one animal.

Table 1. BODY WEIGHTS AND MINIMUM HEART BEAT RATES

Family	Binomen	Common name	Body weight (kg)	Minimum heart rate (beats/min)
Dasyuridae	<i>Antechinus flavipes</i>	Marsupial mouse	0.019	292
	<i>Phascogale tapoatafa</i>	Brush tailed phascogale	0.096	210
	<i>Myrmecobius fasciatus</i>	Numbat	0.36	102
	<i>Dasyurus geoffroyi</i>	Western native cat	0.80	89
	<i>Sarcophilus harrisi</i>	Tasmanian devil	(1) 4.26 (2) 5.93	54 66
Pera- melidae	<i>Isodon obesulus</i>	Short nosed bandicoot	1.31	84
Phalan- geridae	<i>Petaurus breviceps</i>	Sugar glider	(1) 0.112 (2) 0.119	183 312
	<i>Pseudocheirus occidentalis</i>	Ring-tail possum	0.695	102
	<i>Trichosurus vulpecula arnhemensis</i>	Kimberley brush-tail possum	0.950	99
	<i>Trichosurus vulpecula</i>	Brush-tail possum	(1) 1.36 (2) 1.51	62 98
	<i>Phascogale cinereus</i>	Koala	6.84	87
Macro- podidae	<i>Setonix brachyurus</i>	Quokka	(1) 2.72 (2) 2.38	119 121
	<i>Macropus eugenii</i>	Tammar	(1) 3.55 (2) 4.60 (3) 5.30	73 73 63
	<i>Petrogale lateralis</i>	Rock wallaby	5.45	86
	<i>Macropus robustus</i>	Euro or hill kangaroo	(1) 12.7 (2) 17.1 (3) 19.8	40 40 39
	<i>Macropus giganteus</i>	West Australian grey kangaroo	18.7	47
	<i>Macropus rufus</i>	Red kangaroo	19.8	40

The relationship between minimum heart rate and body weight (line B, Fig. 1) when plotted on a logarithmic grid was:

$$F = 106 W^{-0.27}$$

where  $F$  is the minimum heart beat rate and  $W$  is the body weight in kg.

This relationship is compared, in Fig. 1, with the relationship between heart beat rate and body weight for eutherian species given by Brody<sup>2</sup> of  $F = 217.8 W^{-0.27}$  (line A). The average minimum heart beat rate of the fourteen species of marsupials observed is a little less than half that given for eutherian species on the basis of metabolic body size.

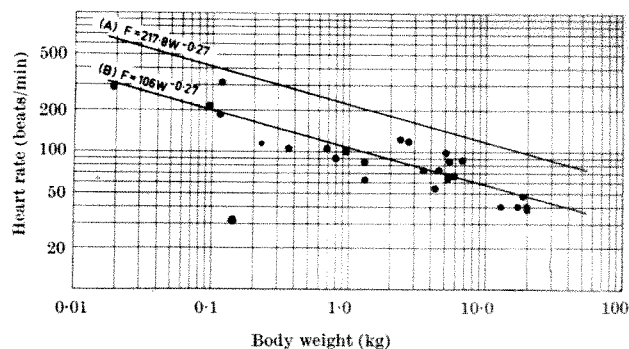


Fig. 1. Relationship between minimum heart rate and body weight in marsupials (line B), compared with eutherian mammals (line A).

Bartholomew and Hudson<sup>4</sup> have reported a minimum heart rate of 300 beats/min for Tasmanian pigmy possums (*Cercartus nanus*) of about 50 g body weight, which correlates with the results presented here (line B).

The marked reduction in the heart rate of marsupials, compared with that of eutherians of similar body weight, suggests either a difference in metabolic activity or an equally marked difference between the haemodynamics of marsupials and eutherian mammals. Maxwell *et al.*<sup>5</sup> reported that the cardiac output of two macropod marsupials investigated under anaesthesia was similar to that of a dog when corrected for differences in body weight. The authors point out, however, that under anaesthesia the heart rates of the macropods were elevated when compared with the resting conscious state (no values given).

Handling and restraint can substantially increase the heart rate of an animal, but the use of an electrocardiograph over an extended period can minimize such effects. Brody's<sup>2</sup> curve is derived from data collected before electrocardiography came into widespread use; it is possible that line A may not be basal for eutherians.

Other parameters related to the level of energy metabolism are, however, lower, at least for some macropods studied. Brown and Main<sup>1</sup> have shown that the minimum output of urinary nitrogen of macropod marsupials is markedly less for each unit of metabolic body size than that of eutherian species, and that the voluntary feed intakes of these macropods were also less than those of ruminants. The water requirement and insensible water loss of the tammar wallaby (*Macropus eugenii*) are low in relation to those of eutherians (our unpublished results).

In the light of the available evidence, therefore, it seems probable that the low heart rates of the marsupials observed here imply a lower level of energy metabolism compared with that of most eutherian species.

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## Inhibition of Spinal Neurones by Glycine

THERE is now much evidence to support the role of glycine as a mammalian spinal inhibitory transmitter. The distribution of glycine in the spinal cord of the cat has been related to the presence of inhibitory interneurons<sup>1</sup>; glycine hyperpolarizes spinal motoneurons<sup>2</sup>; and strychnine, an antagonist of spinal postsynaptic inhibition, blocks the effects of glycine on spinal motoneurons, interneurons and Renshaw cells<sup>3</sup>.

There are considerable difficulties in determining whether the postsynaptic action of glycine is identical with that of the inhibitory transmitter, although both substances clearly hyperpolarize motoneurons. The measurement of "reversal" potentials by passing current through an intracellular micro-electrode may not necessarily establish the true relationship between the "equilibrium" potentials for ionic conductance increases which occur at sites unequally distributed over the cell membrane<sup>4</sup>. The reversal potential for inhibitory postsynaptic potentials (IPSP) of motoneurons<sup>5</sup>, however, has occasionally been almost identical with that of the hyperpolarization induced by the electrophoretic administration of glycine from the extracellularly located micropipette of a parallel electrode<sup>3</sup> (Fig. 1A). In other cases the reversal potential for the glycine effect was at a less hyperpolarized level than that of the IPSP, as might be expected if the ionic mechanisms were identical but the majority of inhibitory synapses were further from the site of intracellular polarization than membrane regions influenced by glycine. The finding that intracellular injections of anions (see also ref. 2), potassium or sodium ions influence both the IPSP and the glycine hyperpolarization in an identical fashion also suggests that these hyperpolarizations are produced by similar alterations in membrane conductance. It is noteworthy that  $\gamma$ -aminobutyric acid (GABA) apparently has the same postsynaptic action as glycine (Fig. 1B), although in general this amino-acid was a much less effective hyperpolarizing agent than glycine<sup>3</sup>.

Although it has been reported that glycine lacks effect on Renshaw cells<sup>2</sup>, it has been invariably found in this laboratory that glycine depresses the firing of these and other spinal interneurons<sup>3,6</sup>. Furthermore, this depression is reversibly blocked by strychnine, but that produced by GABA is not<sup>3</sup>. The firing of Renshaw cells can be inhibited by volleys in spinal and descending pathways and this inhibition is of the type which is sensitive to strychnine<sup>7</sup>.

Antagonism between strychnine and glycine has also been shown on spinal motoneurons, and an example is illustrated in Fig. 2. After administration of strychnine

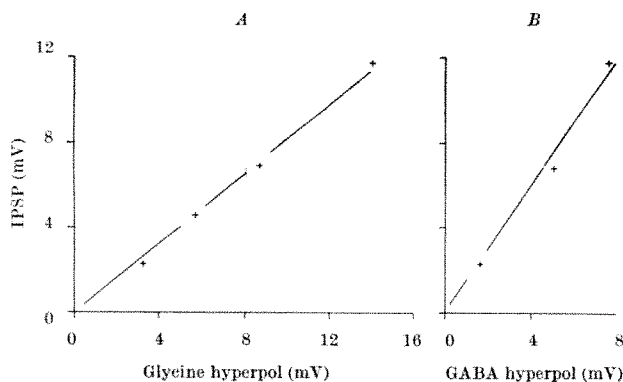


Fig. 1. Potentials were recorded from a peroneal motoneurone (resting potential 65 mV) with one barrel of a double-barrel intracellular electrode ( $K_2SO_4$ ) while the membrane potential was altered by passing current through the other barrel. Amino-acids were ejected electrophoretically from separate barrels of an extracellular five-barrel micropipette (glycine, 50 n.amp; GABA, 125 n.amp). Ordinates, amplitude of an inhibitory postsynaptic potential evoked by stimulation of the flexor digitorum longus nerve, 2.5 mV at the resting potential. Abscissae, amplitude of the hyperpolarization produced by glycine (A) and GABA (B). Cat anaesthetized with pentobarbitone sodium.

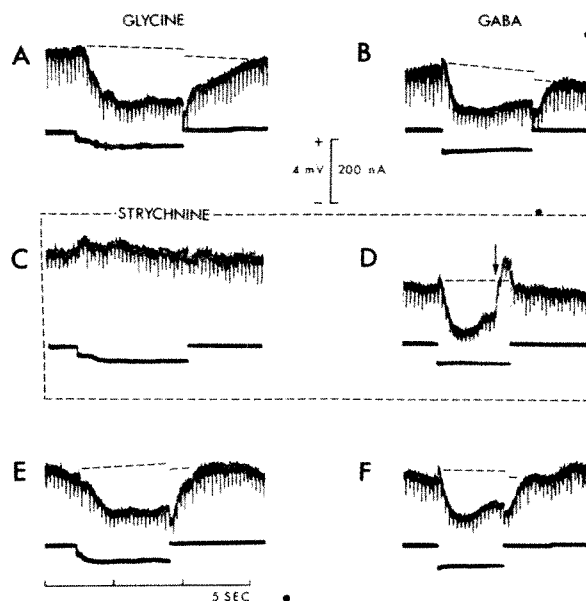


Fig. 2. Potentials recorded intracellularly from an anterior tibial motoneurone (resting potential 63-68 mV) in response to the extracellular electrophoretic administration of glycine (30 n.amp) and GABA (60 n.amp); the lower traces indicate the appropriate electrophoretic current. The brief downward deflexions are IPSPs evoked by stimulating the ipsilateral sural nerve. A, B, Control. C, D, During the electrophoretic ejection of strychnine from another barrel of the five-barrel extracellular micropipette (20 n.amp, 2 mmolar solution of strychnine hydrochloride in 165 mmolar NaCl). E, F, 4 min after the termination of the strychnine ejection. Calibrations, 4 mV for the intracellular recording, negativity down; 200 n.amp for amino-acid currents. Time, 5 sec.

for 7.5 min the hyperpolarizing effect of glycine was considerably reduced (C) whereas GABA (D) remained as effective as during the control period (B), apart from the late occurrence of a depolarization (arrow), the significance of which is being investigated further. Within 4 min of terminating the administration of strychnine, glycine again hyperpolarized the membrane (E). Strychnine reduced the IPSP studied in this cell by approximately 30 per cent, presumably because most of the activated synapses were not affected by the alkaloid, the distribution of which after electrophoretic administration would approximate that of glycine and GABA. Intravenously administered strychnine hydrochloride (0.1-0.2 mg/kg), which would influence inhibitory synapses in a more uniform manner, effectively reduced the glycine hyperpolarization of several other motoneurons.

Other substances like strychnine which reduce spinal postsynaptic inhibition<sup>8</sup> also diminish the depressant action of glycine, but not that of GABA, on spinal interneurons and Renshaw cells. These were administered electrophoretically and include bruceine, thebaine, 5,7-diphenyl-1,3-diazadamantan-6-ol, 4-phenyl-4-formyl-N-methylpiperidine and hexahydro-2'-methyl-spiro [cyclohexane-1,8'(6H)-oxazino(3,4-A)pyrazine].

The failure of strychnine to affect the action of GABA indicates that glycine and GABA apparently interact with different postsynaptic receptors, as was previously inferred from a study of the relationships between structure and activity of excitant and depressant amino-acids<sup>6</sup>. On the basis of strychnine antagonism, the depressant amino-acids can be separated into two types: "glycine-like" including glycine, L- $\alpha$ -alanine,  $\beta$ -amino-*iso*-butyric acid, D- and L-serine; and "GABA-like" including GABA,  $\delta$ -aminovaleric acid,  $\epsilon$ -aminocaproic acid,  $\gamma$ -amino- $\beta$ -hydroxybutyric acid and 3-aminopropane sulphonate.

If strychnine blocks the access of inhibitory transmitter to subsynaptic receptors, this pharmacological evidence strongly favours structural similarity between the transmitter and "glycine-like" amino-acids, of which glycine, L- $\alpha$ -alanine, taurine and L-serine are found in feline spinal tissue. On the other hand, if strychnine

merely reduces the potassium conductance at activated inhibitory synapses<sup>8</sup>, these amino-acids may not necessarily interact with the same receptor as the transmitter. The failure of strychnine to reduce the hyperpolarizing postsynaptic inhibition of certain supra-spinal neurones<sup>10</sup>, which presumably also involves an increased permeability to potassium ions, however, suggests that the interfering effect on spinal inhibition is most probably at the transmitter receptor site.

Glycine is rapidly metabolized in the spinal cord<sup>11</sup> and the distribution of incorporated glycine is remarkably similar to that of glycine as extracted by 5 per cent trichloroacetic acid<sup>12</sup>. Observations on the lack of action of a variety of enzyme inhibitors, including amino-oxoacetic acid, hydroxylamine, 2-hydroxy-ethyl-hydrazine, *p*-amino-hippuric acid and aminopterin on the glycine-induced depression of interneurons and Renshaw cells may indicate that glycine is effectively removed from the extraneuronal environment by rapid intracellular transfer, rather than by enzyme inactivation. This mechanism may be similar to the active accumulation of amino-acids by tumour cells<sup>13</sup> and brain slices<sup>14</sup>. It is perhaps significant that different membrane sites have been proposed for the transfer of "glycine-like" and "GABA-like" amino-acids into brain slices<sup>14</sup>. It is possible that the actual hyperpolarizing action of glycine is produced by intracellular transfer and the consequent re-distribution of potassium and chloride ions: such a mechanism is not incompatible with a function of glycine as a spinal inhibitory transmitter. If this glycine-induced ionic transfer was not solely restricted to inhibitory synapses which are sensitive to strychnine, this proposal provides an explanation of the finding that glycine depresses the firing of cortical neurones in the apparent absence of a post-synaptic inhibitory process affected by strychnine<sup>3</sup>.

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## Acetylcholine, Strychnine and Spinal Inhibition

It was recently proposed<sup>1</sup> that the action of strychnine at spinal inhibitory synapses may be explained by an antagonism between strychnine and acetylcholine, an interaction between acetylcholine and presynaptically located receptors which are sensitive to strychnine being an essential requirement for the release of the inhibitory transmitter. This proposal, which would "eliminate blockade by strychnine as an essential qualification for the identification of a putative inhibitory transmitter" in the mammalian spinal cord, is based on the

finding that strychnine, in concentrations of the order of  $3-300 \times 10^{-6}$  molar, reduces the release of acetylcholine by impulses in the preganglionic terminals of the perfused feline superior cervical ganglion.

An opportunity has been taken to test this hypothesis on spinal neurones for which there is very good evidence that acetylcholine is an excitatory transmitter. The pharmacology of the excitation of Renshaw cells by impulses in motor axon collaterals bears some resemblance to that of cholinergic excitation in sympathetic ganglia<sup>2-4</sup>. Furthermore, Renshaw cells can be inhibited by appropriate spinal volleys<sup>5,6</sup>, and this inhibition is reduced by strychnine administered micro-electrophoretically<sup>7</sup>.

It has invariably been found that concentrations of strychnine, adequate to suppress the inhibition of lumbar Renshaw cells evoked by hind limb afferent volleys, enhance rather than reduce the excitation of these neurones by sub-maximal ventral root volleys. This facilitation might be explained as a blockade of a simultaneously occurring inhibition but, in view of the associated increase in the sensitivity of Renshaw cells to both acetylcholine and excitant amino-acids, a direct excitation of these cells by strychnine is a more likely explanation. Under conditions of comparatively high strychnine concentrations there is a reduction in the number of synaptically evoked spikes. This is, however, presumably indicative of a postsynaptic, rather than presynaptic, depressant action because it is accompanied by changes in the shape and size of action potentials recorded outside the cells and a diminution in the sensitivity of Renshaw cells to electrophoretically administered excitants<sup>7</sup>.

Thus no evidence has been obtained which would indicate that concentrations of strychnine sufficient to block the inhibition of Renshaw cells diminish the amount of acetylcholine released from axon collateral terminals. Unless it can be shown that there are differences in the susceptibility to strychnine of the acetylcholine-releasing mechanisms at the terminals of motor axon collaterals, the postsynaptic acetylcholine receptors on Renshaw cells and the proposed acetylcholine receptors on the terminals of inhibitory nerve fibres, the postulate that acetylcholine interaction with these latter receptors is necessary to initiate the release of inhibitory transmitter appears unnecessary. Furthermore, the recent demonstration that glycine hyperpolarizes spinal neurones, and that this effect is blocked by strychnine<sup>8</sup>, provides a more satisfactory explanation of the action of strychnine in terms of postsynaptic antagonism at inhibitory synapses.

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## Pharmacological Characteristics and Ionic Bases of a Two Component Postsynaptic Inhibition

SEVERAL types of postsynaptic inhibition have been observed in molluscan neurones; in *Aplysia*, the most common is a rapidly decaying inhibitory postsynaptic potential (IPSP) with pharmacological characteristics which have been described by Taue and Gerschenfeld<sup>1</sup>.



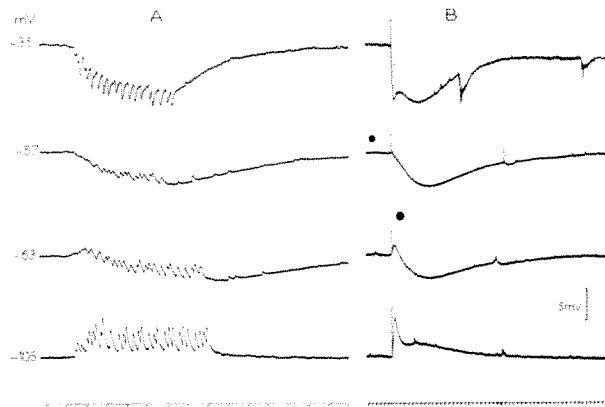


Fig. 1. Successive inversion of the first and second phases of the synaptic (A) and ACh (B) potentials with progressive hyperpolarization of the postsynaptic membrane. One barrel of a double-barrelled microelectrode filled with 0.5 molar potassium sulphate was used for intracellular recording; the other, for applying currents to displace the membrane potential to values indicated in mV. ACh ( $10^{-3}$  g/ml.) was injected ionophoretically on the cell soma by passing currents of approximately 300 namp during 400 msec. Time: second marker.

In their study of the role of acetylcholine (ACh) in the nervous system of *Aplysia*, they noted that cells receiving this inhibitory input are hyperpolarized by ionophoretic injection of ACh, and that both the ACh potential and the IPSP are blocked by *d*-tubocurarine. They also demonstrated that the polarization at which the ACh potential reverses is similar to that of the equilibrium potential for the IPSP, implying that the two potentials have the same underlying ionic mechanism. They concluded, on the basis of these and other data, that these IPSPs are cholinergic. Further work on similar cholinergic IPSPs in the land snail indicated that these potentials are the result of changes in the permeability of the postsynaptic membrane to chloride<sup>2</sup> with some possible contribution from potassium ions<sup>3</sup>.

I have observed in certain cells of *Aplysia californica* IPSPs similar to those described by Tauc and Gerschenfeld, but which are coupled with a more slowly decaying inhibition. The coexistence of two different types of postsynaptic inhibition in the same neurone has been described before<sup>4,5</sup>, but because the ionic and pharmacological characteristics of these inhibitory potentials were not thoroughly studied it is impossible to ascertain whether they are the same as those described here.

The dual component inhibition described here can be observed, using standard intracellular recording techniques, in an identifiable group of neurones in the pleural ganglia. This inhibitory input can be selectively activated when using the isolated-head preparation of Bruner and Tauc<sup>6</sup>. In a modification of this preparation, the head, which remains connected to the peri-oesophageal ring by the cerebral nerves, is isolated from these ganglia in such a manner that the tentacles can be stimulated mechanically without causing artefacts in the electrophysiological recording. Also, the solution bathing the ganglia is isolated from that (natural seawater) bathing the head, thus permitting changes in the ionic concentrations of, or addition of drugs to, the seawater bathing the ganglia without mechanically or chemically stimulating the head.

Drops of water falling on the tentacles produce within these pleural neurones a series of rapidly decaying IPSPs superimposed on a longer latency, more slowly decaying hyperpolarizing wave. A double-barrelled microelectrode was used to polarize the membrane progressively and to measure the inversion potentials of these two components. The earlier, short-decay potential was found to reverse between  $-55$  and  $-60$  mV; the later, long-decay potential, at approximately  $-80$  mV.

This dual-phased synaptic inhibition is particularly interesting because the cells receiving this inhibitory input

respond to ionophoretic injection of  $10^{-2}$  g/ml. of ACh with a similar two-component hyperpolarization. Fig. 1 shows that the two phases of this ACh potential show inversion potentials essentially identical to those of the first and second components, respectively, of the synaptic potential.

The disparity of the inversion potentials of the first and second component of both the ACh and synaptic potentials indicates that two different ionic mechanisms are involved. Changes in the concentration of potassium in the seawater bathing the ganglia markedly affect the inversion potential of the second component, and have no effect on that of the first. The inversion potential of the second phase ( $-80$  mV in normal seawater) is reduced to approximately  $-65$  mV when the external potassium concentration is doubled, and is increased to approximately  $-97$  mV when the potassium concentration is halved. The effects of an increase in the external potassium from 10 to 20 mmoles/l. can be seen in Fig. 2. Note that the inversion potential of the second phase is markedly reduced, whereas that of the first is left unchanged. Similar experiments in which the external chloride concentration has been varied indicate that the first component, on the other hand, is caused by an increase in the permeability of the membrane to chloride ions.

The synaptic and ACh potentials again demonstrate marked similarity in their response to the addition of *d*TC to the bathing solution. This substance readily blocks the first component of both the ACh and synaptic potentials, but fails—even at very high concentrations ( $10^{-3}$  g/ml.)—to block the second. This can be seen in Figs. 3 and 4, showing the effects of *d*TC  $10^{-3}$  g/ml. on the synaptic and ACh potentials, respectively. Note that whereas the first phase is completely blocked by the curare, the second phase is accentuated, showing a more abrupt decrease and a more prolonged action.

The first and second phase of the ACh potential cannot be explained on the basis of the selective activation of nicotinic and muscarinic receptors, respectively. Different cholinomimetics were injected on the cell soma, and the

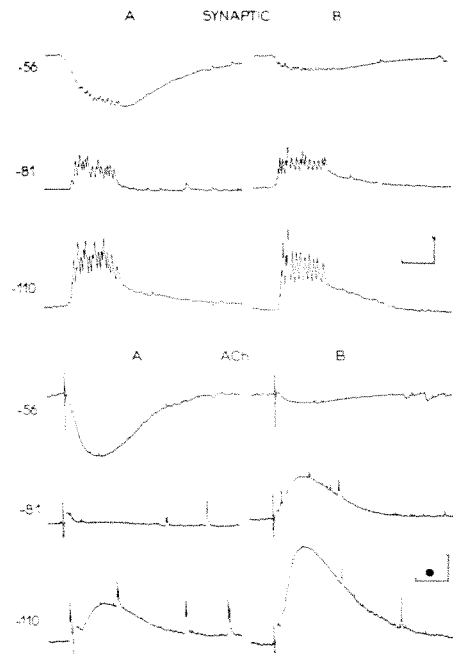


Fig. 2. Effects of doubling the external potassium concentration (10 mmoles/l. in A; 20 mmoles/l. in B) on the inversion potential of the second phase of the synaptic (upper half) and ACh (lower half) potentials. Note that the inversion potential of the second phase, which was  $-80$  mV in normal potassium concentration (A), was reduced to approximately  $-65$  mV when the external potassium was doubled (B). The inversion potential of the first phase remained unchanged. Calibration: synaptic potentials, 5 mV, 2 sec; ACh potentials, 5 mV, 10 sec. See Fig. 1 for description of recording and injection electrodes.

only agent—other than ACh—that was capable of producing a biphasic inhibitory potential was carbachol. All others—whether nicotinic (nicotine, propionyleholine, tetra-methyl-ammonium, and dimethyl-phenyl-piperazinium) or muscarinic (methacholine and oxotremorine)—mimicked only the early, rapidly decaying phase of the ACh inhibition. None of these substances provoked the longer latency, potassium dependent phase. Similarly it has not been possible to block either component of either the synaptic or ACh potentials with classical anti-muscarinic agents such as atropine or lachesine, even with concentrations as high as  $10^{-3}$  g/ml.

The possibility that the two components of the inhibition are caused by the action of two different transmitters cannot be excluded. An ionophoretic injection of dopamine on these cells causes a single phased, prolonged hyperpolarization, which—like the second phase of the ACh potential—reverses at  $-80$  mV. The existence in molluscan neurones of non-cholinergic potassium dependent inhibitory potentials has been established already<sup>7</sup>, and such data suggest the possibility that ACh causes the second phase indirectly by stimulating the release of another transmitter (for example, dopamine) from the presynaptic terminals. Such presynaptic action might also seem to be suggested by the long latency of the second component of the ACh potential. It seems that the ACh diffuses a considerable distance before reaching the receptor zone responsible for the second phase of the potential. Because the synaptic region of the neurones of *Aplysia* is located on the axon far from the soma<sup>8</sup>, however, diffusion to and selective activation of the sub-synaptic membrane could equally well account for such a delay. This interpretation is supported by the fact that both phases of the ACh potential persist in a calcium free solution ( $10^{-3}$  molar EGTA added) which presumably blocks transmitter release<sup>9</sup> and effectively blocks the synaptic input. These data suggest that the second phase as well as the first results from direct postsynaptic action of the injected ACh.

Two ACh receptor types seem to be necessary to account for the two successive states of membrane permeability. Neither of the two can readily be placed in classical pharmacological categories. The ACh receptors responsible for producing the second phase of the injection potential, whether pre- or post-synaptic, are of a particular interest because of their remarkable resistance to all standard blocking agents.

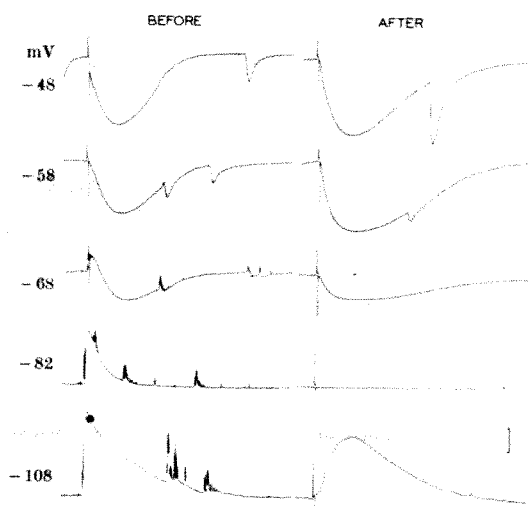


Fig. 3. Effects of dTC ( $10^{-3}$  g/ml.) on the two component ACh potential. Note that under curare (see AFTER) the first component is no longer visible, whereas the second component is accentuated. Calibration: 5 mV; time base, second marker. Recording and polarizing electrodes filled with 2.5 molar potassium chloride. See Fig. 1 for further specifications.

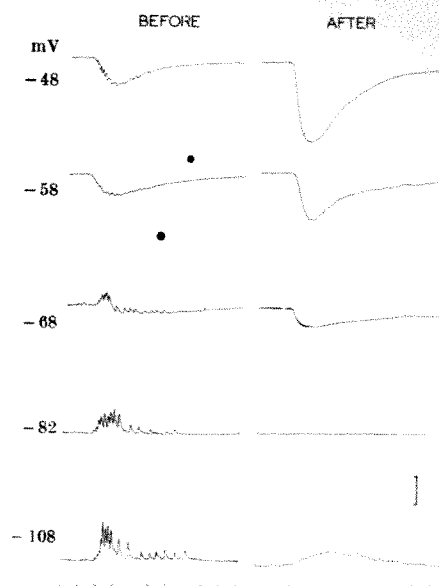


Fig. 4. Effects of dTC ( $10^{-3}$  g/ml.) on the two component synaptic inhibition. Note that under curare (see AFTER) the first component is no longer visible, whereas the second component is accentuated. Calibration: 5 mV; time base: second marker. Record taken simultaneously with that of Fig. 3.

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### Evidence for Intracellular Transport of Radioactive Protein in Alveolar Cells

HISTOCHEMICAL studies have suggested that the acellular lining layer of pulmonary alveoli as well as the cytoplasmic inclusions (lamellar bodies) of alveolar cells contain carbohydrate-protein moieties<sup>1-3</sup>. These findings led me to investigate the subcellular site of incorporation of D-glucosamine-1-<sup>14</sup>C by alveolar cells induced by *M. bovis* (B.C.G.<sup>4</sup>). This designation does not imply that these cells are type II granular pneumocytes, but rather that they originate predominantly from cells that line normal alveoli<sup>5</sup>. The kinetics<sup>6</sup> of the subcellular distribution of radioactivity in cells which have been pulse labelled with glucosamine-1-<sup>14</sup>C suggest attachment of glucosamine to protein at the microsomal level followed by transfer of the labelled protein to particles which sediment at 15,000g. These data are consistent with the morphological studies of Sorokin<sup>7</sup>.

Twenty ml. of cells suspended in 5 volumes of Hanks solution (v/wet weight) were pulse labelled at 37° C with 25  $\mu$ Ci of D-glucosamine-1-<sup>14</sup>C, specific activity 10.9, for 40 min. The reaction was stopped by adding ice cold Hanks solution and the cells washed three times and resuspended in this medium. Equal volumes were then

reincubated for various times and the reactions stopped by chilling. The cells were collected by centrifugation and resuspended in 10 volumes (v/wet weight) of medium A (ref. 8). The cells were homogenized in a Potter-Elvehjem tissue grinder, a sample of the homogenate removed and the remainder centrifuged at 1,500g for 10 min. The supernatant fluid was centrifuged at 15,000g for 10 min and the sediment designated 15,000g fraction. The supernatant material was centrifuged at 105,000g for 1 h to sediment the microsomes. The remaining fluid was designated the supernatant fraction. The 15,000g and microsomal fractions were resuspended in medium A. Protein<sup>9</sup> and ribonucleic acid<sup>10</sup> were measured on all fractions. The samples were prepared for radioactivity assay and counted as previously described<sup>11</sup>.

Fig. 1 reveals the time course of the protein specific activity in the homogenate and various subcellular fractions. The specific activity of the homogenate indicates that net incorporation of radioactivity ceased after the first hour after the pulse. This time corresponds with the peak of microsomal protein specific activity. Thereafter, the protein specific activity in the microsomal fraction fell below that of the 15,000g fraction while the protein specific activity of the latter rose for 2 h after the pulse and remained above that of the microsomal fraction. These curves clearly fit a precursor-product relationship<sup>6</sup>. The protein specific activity in the supernatant fluid remained relatively constant throughout most of the experiment.

Table 1. PROTEIN-RNA RATIO OF SUBCELLULAR FRACTIONS

Incubation time (min)	15,000 g	Subcellular fraction Microsomes	Supernatant
0	0.04	0.23	0.02
30	0.05	0.20	0.04
60	0.08	0.21	0.02
120	0.06	0.19	0.03
180	0.07	0.18	0.02
240	0.05	0.19	0.03
300	0.04	0.13	0.04
360	0.06	0.10	0.04
Mean $\pm$ SD	0.05 $\pm$ 0.02	0.17 $\pm$ 0.03	0.03 $\pm$ 0.02

Table 1 shows the ratio of protein to RNA of each subcellular fraction. The ratio is about three times greater in the microsomal fraction than the 15,000g or supernatant fractions.

These data may be interpreted as incorporation of radioactivity into protein on the microsome followed by intracellular transport of the labelled protein to a particle which sediments when the cell homogenate is centrifuged at 15,000g; an alternative possibility is synthesis at more than one site but at different rates. This alternative would entail the unlikely possibility that synthesis by particles sedimenting at 15,000g increases at the same rate as microsomal synthesis falls, to maintain constant net synthesis in the cell as indicated by the constancy of protein specific activity in the cell homogenate. Further-

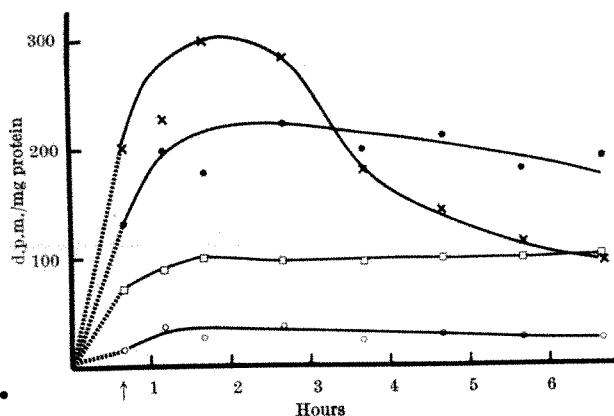


Fig. 1. Time course of protein specific activity in the homogenate (□), and in the 15,000g (●), microsomal (×) and supernatant (○) fractions.

more, in all systems studied so far carbohydrate incorporation into protein has been shown to be a microsomal function. Thus, all things considered, these data more strongly favour intracellular protein transport. This interpretation is consistent with the morphological data presented by Sorokin<sup>7</sup>.

These findings, which may be entirely unrelated to the biosynthesis of surfactant, as may the coincidental development of alveolar cell inclusions (lamellar bodies) and the appearance of surfactant in foetal lungs, assume potential significance when it is recalled that the 15,000g fraction probably contains many lamellar bodies<sup>12,13</sup>. Further, this fraction also has the greatest ability to lower surface tension<sup>12</sup>. Finally, when one considers that the enzymes required for the final stages of lecithin biosynthesis are microsomal enzymes, some type of intracellular transport is necessary for localization of surfactant in the 15,000g sediment<sup>14,15</sup>.

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### Increase in Permeability of the Postsynaptic Membrane to Potassium produced by 'Nembutal'

'NEMBUTAL' (pentobarbital sodium) is known to block synaptic transmission, although its actual site of blockade is not well established<sup>1-5</sup>. On the other hand, the effect of 'Nembutal' and other barbiturates on the electrogenic membrane, which is capable of generating the conducted impulse, is generally considered to be less remarkable<sup>1,6</sup>. Neither the membrane resting potential nor the threshold firing level of cat motoneurons is significantly altered by barbiturates applied intravenously at a concentration of 10<sup>-3</sup> g/kg<sup>1,6</sup>.

Abdominal ganglion cells of *Aplysia* have been regarded as having little direct synaptic contact on their somata<sup>7</sup>, although the soma membrane is quite sensitive to acetylcholine, suggesting a structure analogous to the postsynaptic membrane<sup>8</sup>. When dilute 'Nembutal' (10<sup>-5</sup> g/ml.) is applied directly on the exposed ganglion cells by constant rate perfusion, the response induced by 10<sup>-6</sup> g/ml. of acetylcholine in both D- and H-type cells is readily blocked before the resting potential begins to be slightly hyperpolarized (1-3 mV). When concentrated 'Nembutal' of 10<sup>-3</sup> g/ml. is applied, both D- and H-type cells are equally hyperpolarized often as much as 20 mV during application, then gradually recover as 'Nembutal' is washed away (Fig. 3). Repetitive application of concentrated 'Nembutal' results in almost irreversible hyperpolarization. It is significant that the membrane resist-

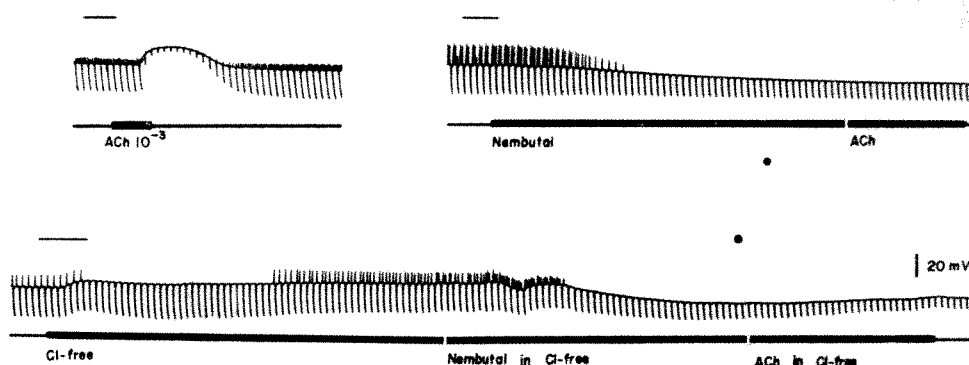


Fig. 1. 'Nembutal'-induced hyperpolarization in perfusing media free of chloride ions. The cell is of a D-type which is depolarized by acetylcholine, as shown in top left. Repetitive downward shifts of the resting potential level are the monitoring of membrane resistance change in response to drugs. This was made by feeding a constant current pulse through one microelectrode inserted within a cell, and the potential drop across the membrane was recorded from another microelectrode inserted in the same cell. Pulse interval is 5 sec. Chloride ions were replaced by propionate in solution free of the ions. Top right is the control of 'Nembutal'-induced hyperpolarization in normal Ringer solution. Bottom trace is the same response in Ringer solution free of chloride ions. Acetylcholine and 'Nembutal' concentrations are 1 mg/ml. and 6 mg/ml., respectively. Horizontal bars on each trace indicate the extracellular potential levels.

ance markedly decreases during the 'Nembutal'-induced hyperpolarization, suggesting an increase in membrane permeability to certain ions. The observed hyperpolarization is unlikely to be produced simply by removal of excitatory background activities. If that were the case, the membrane resistance should increase during the hyperpolarization as the result of inactivated postsynaptic excitation.

The possible ions responsible for the observed hyperpolarization are chloride and potassium, because their equilibrium potentials are more negative than the resting potential<sup>3,8</sup>. If the 'Nembutal'-induced hyperpolarization were caused by an increase in the permeability of the membrane to chloride ions, the amplitude of hyperpolarization should be varied by changes in the extracellular concentration of these ions. It should be reversed into depolarization when all extracellular chloride ions are replaced by large impermeant anions. By contrast, the 'Nembutal'-induced response is not affected by the external concentration of chloride ions, as shown in Fig. 1. This excludes the possibility that chloride ions are responsible for the observed hyperpolarization. On the other hand, the 'Nembutal' response is greatly influenced by the external concentration of potassium ions. Some ganglion cells are not readily depolarized by a high external concentration of potassium ions because of intense inhibitory bombardment arriving at the cell as a result of the primary depolarizing effect of Ringer solution containing a high concentration of potassium ions on presynaptic fibres. Once treated with 'Nembutal', however, these cells are readily depolarized as much as would be expected from the Nernst equation. The destruction of inhibitory postsynaptic activities by 'Nembutal' is an important factor to explain the enhancement of the depolarization caused by potassium ions<sup>9</sup>. In addition, however, the augmented potassium ion depolarization may also be caused by the increase in permeability of the membrane to these ions produced by 'Nembutal'. When 'Nembutal' ( $6 \times 10^{-3}$  g/ml.) is applied simultaneously with a concentration of 220 mmoles/l. of potassium ions the usual hyperpolarizing response to 'Nembutal' is completely masked by enhanced potassium ion depolarization, as shown in Fig. 2. The increase in conductance during potassium ion

depolarization is greatly augmented with 'Nembutal'. When an adequate concentration of external potassium ions (50–60 mmoles/l.) is chosen, the usual 'Nembutal' hyperpolarization can be completely abolished despite the marked increase in membrane conductance. These observations strongly suggest that 'Nembutal' in a relatively high concentration actively increases the permeability of the postsynaptic membrane to potassium ions. The enhanced potassium ion depolarization gradually returns to a normal response to these ions as 'Nembutal' is washed away. The response to acetylcholine, which is completely blocked during the period of enhanced potassium ion depolarization, gradually recovers as the latter returns to normal. This parallelism suggests that the increase in permeability to potassium ions is maintained as long as 'Nembutal' occupies the acetylcholine receptors in the postsynaptic membrane. Fig. 3 shows the determination of the reversal potential of the 'Nembutal'-induced hyperpolarization. The reversal potential was found to be

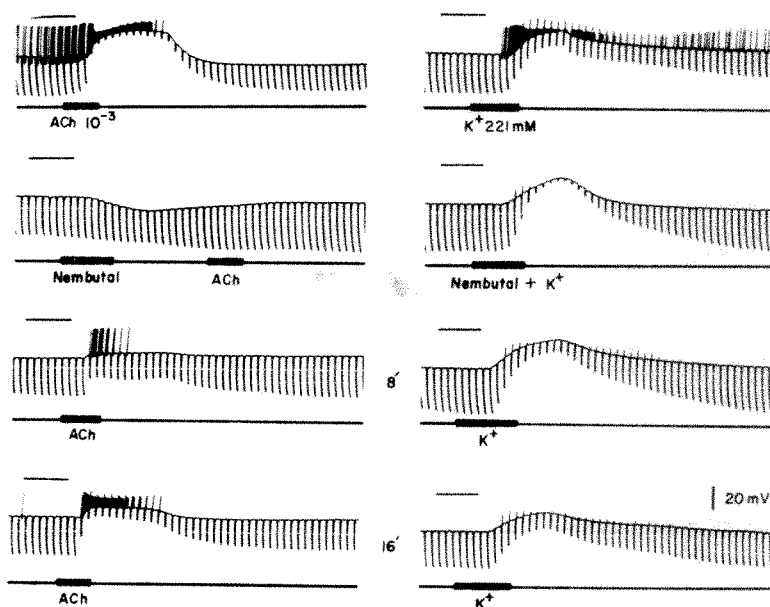


Fig. 2. Recovery of response to acetylcholine and the enhanced potassium ion depolarization of the same cell (D-type) after application of 'Nembutal'. Acetylcholine and 'Nembutal' concentrations are the same as shown in Fig. 1. Top traces are controls before 'Nembutal' is applied. The third and fourth rows were obtained 8 and 16 min after the second row. Note the marked decrease in membrane resistance during the potassium ion depolarization when 'Nembutal' was applied at the same time (right in the second row). Interval of resistance monitoring pulse is 5 sec.



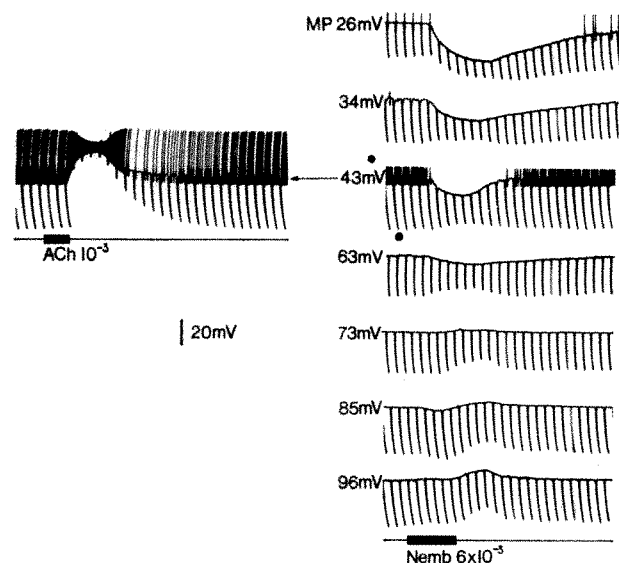


Fig. 3. Determination of reversal potential of 'Nembutal'-induced hyperpolarization. The cell was of a D-type with a resting potential of 43 mV. The membrane potential was artificially shifted to various levels described in mV by passing inward or outward current through a micro-electrode during the recording of the response to 'Nembutal'. Concentrations are shown in g/ml. Interval of resistance monitoring pulse is 5 sec.

–72mV, which is approximately 10mV more negative than the chloride ion equilibrium potential<sup>8</sup> but corresponds with the potassium ion equilibrium potential calculated from the intracellular concentration of potassium ions

$$E_K = -\frac{RT}{F} \log_e \frac{[K^+]_i}{[K^+]_o} = -56 \log_{10} \frac{232}{11.6} = -72 \text{ mV}$$

where  $[K^+]_i$  and  $[K^+]_o$  are the intracellular and extracellular concentrations of potassium ions, respectively. The former was measured by an atomic energy absorption spectrophotometer and the mean value of 400 cells was 232 mmoles/l. The latter is the concentration of potassium ions of our artificial *Aplysia* Ringer solution, 11.6 mmoles/l. The temperature of the preparation was kept constant at 10° C.

We conclude from the above findings that 'Nembutal' combines with acetylcholine receptors in the postsynaptic membrane and causes a specific permeability increase to potassium ions when the concentration of 'Nembutal' is sufficiently high. As described here, however, the responses of D- and H-type membranes to acetylcholine are blocked by more dilute 'Nembutal' which does not appreciably affect the permeability of their membranes to potassium ions. Apart from this dose dependence on the blocking action of 'Nembutal' on synaptic transmission, it is interesting that this compound has a molecular affinity for the receptors in the postsynaptic membrane and that its binding can cause a specific permeability increase toward a certain ion in a manner similar to the action of transmitter substance. The molecular basis to account for this ionic specificity awaits further investigation.

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### Sequence Theories of Blood Coagulation re-evaluated with reference to Lipid-Protein Interactions

THE well-known "sequence" theories of blood coagulation<sup>1,2</sup> attempt to rationalize the participation of a dozen or so clotting factors in the formation and consolidation of a fibrin clot. It has been proposed<sup>3</sup> that "normal" prothrombin activation involves a number of functionally and perhaps physically distinct entities (clotting factors) present in the plasma, that these react one upon the other in a preferential sequence during normal clotting and that most, if not all, are proenzymes each yielding an enzyme capable of activating the next component of the sequence". Several investigations have been concerned with the last of these propositions<sup>4-11</sup>. Each of these has involved the assumption that a linear response in reaction velocity with changing concentration is indicative of an enzyme while a saturation response is indicative of a substrate. The validity of these assumptions and their consequences will now be considered.

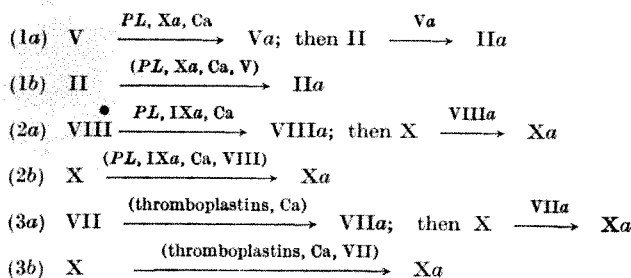
The steps necessary to establish an enzyme-substrate relationship are as follows. (a) To construct a reasonable model for the interactions of known participants. (b) To predict the kinetic behaviour of the proposed model. (c) To show that the kinetic behaviour of the real system is consistent with that predicted for the model.

This argument is based on inductive reasoning as opposed to deductive reasoning. Assuming that (c) is satisfied, this does not constitute proof of the reality of the model but merely indicates that the model is feasible. Kinetic analysis of a reaction mechanism is therefore of value only where a reasonable model can first be proposed. For each of the following reactions involving clotting factors we shall have to consider whether the application of the general Michaelis-Menten treatment can be justified. The reactions are: the interaction of plasma factors V, Xa, phospholipids and calcium ions; the interaction of factors VIII, IXa, phospholipids and calcium ions; the interaction of factor VII, tissue thromboplastins and calcium ions.

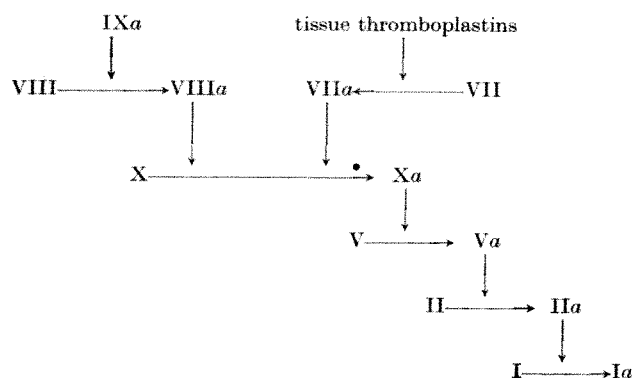
In each of these reactions the various components apparently combine to form a macromolecular complex which possesses the activity necessary to initiate a subsequent reaction<sup>12-15</sup>. Phospholipids in aqueous dispersion exist in the form of aggregates in which the charged groups are arranged at the lipid-water interface. It has been suggested therefore that phospholipids participate in blood coagulation by providing a catalytic surface for the interactions of the protein clotting factors<sup>16-18</sup>. The participation of a biphasic system (heterogeneous catalysis) introduces additional rate components related to diffusion constants of the reactants and products and to their adsorption and desorption isotherms. Depending on the adsorption characteristics of the particular proteins involved it may well be possible to seem to demonstrate an "enzyme-substrate" relationship where one does not in fact exist<sup>19</sup>. Consequently, the kinetics of such reactions would not be amenable to interpretation on the basis of a classical Michaelis-Menten model. In this light the sequence designations of the clotting factors, based on such interpretations, must also be suspect.

We have therefore to consider alternative experimental approaches to test the "cascade-waterfall" hypothesis.

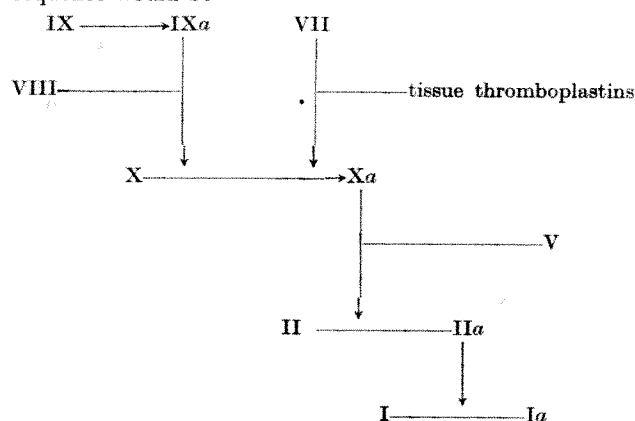
In each of reactions 1-3 two models are possible; (a) involves the formation of an entirely new intermediate (Va, VIIIa, VIIa) while (b) is catalysed by a lipoprotein complex.



If model (a) is applicable in every case, the partial sequence must be



whereas if model (b) is applicable in every case the partial sequence would be



(V, VII, VIII and X are plasma factors; a represents an activated form. I is fibrinogen, Ia is fibrin, II is prothrombin, and IIa is thrombin.) It is not, of course, necessary to assume a uniform model for all three reactions, but because of their superficial similarity<sup>13</sup> it is tempting to do so. The crucial experiment in each case is to determine whether or not a distinctly new entity (Va; VIIIa; VIIa) is formed which can be separated from the other reactants and which can then participate with other materials in the subsequent interaction in the sequence. In the first example selected (reaction 1) no activated factor V is formed, but the reaction with factor Xa, phospholipids and calcium ions is reversible and factor V can be recovered apparently unchanged<sup>20, 21</sup>. Furthermore, there is direct evidence that activated factor X alone will convert prothrombin to thrombin in a reaction which can then be accelerated by the addition of factor V, phospholipids and calcium ions<sup>20-22</sup>. Thus, for reaction 1 there is compelling evidence for model (b) which is in direct conflict with the conclusion inferred from kinetic measurements<sup>8, 9</sup>. Concerning reaction 2, the existence of

a lipoprotein complex (VIII, IXa, phospholipids, Ca) capable of activating factor X has been demonstrated by the gel filtration technique<sup>13</sup>. We have now found that when the complex is rechromatographed on 'Sephadex G-200' in the absence of calcium, factors VIII and IXa can be recovered unchanged and that no component can be eluted from the columns which will activate factor X without first adding back the complementary factors. Experimental details of this work will be published elsewhere. Thus there is again strong evidence that significant activity resides only in the intact complex. With regard to reaction 3 the existence of a thromboplastin-factor VII complex is well documented, but attempts to demonstrate the hypothetical VIIa free from the lipoprotein complex have been inconclusive<sup>14, 15</sup>. Treatment of the complex with a variety of reagents designed to disrupt different types of intramolecular bonds either failed to dissociate the complex or resulted in a loss of activity.

In summary, it seems evident that the evaluation of the kinetic data concerned with these reactions has been based on the preconceived assumption that the "cascade-waterfall" hypothesis is correct. The sequences with regard to factors V and Xa, factors VIII and IXa and factor VII and the tissue thromboplastins therefore still remain to be firmly established.

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### Transformation of the Histochemical Profile of Skeletal Muscle by "Foreign" Innervation

EVIDENCE that long term neuronal influence has a decisive role in determining the cytochemical features of skeletal muscle cells is presented in this report.

The soleus muscle of the adult guinea-pig is histochemically completely "uniform", containing only type I extrafusal muscle fibres. Type I fibres display low activity of myofibrillar ATPase, menadione-mediated  $\alpha$ -glycero-phosphate dehydrogenase and amylophosphorylase, and high activity of most dehydrogenases and EDTA activated myofibrillar ATPase<sup>1</sup>. (In type II fibres, the relation of the activities of these enzymes is reversed.) All other limb muscles examined in the guinea-pig are histochemically "mixed", containing type I and type II fibres. The histochemically "uniform" character of the

adult soleus makes it an excellent subject for the present experiments, for even a few "transformed" muscle fibres can be readily discerned.

Male albino guinea-pigs 3 months of age were used. "Foreign innervation" was produced on one side of each animal by one of three methods; the other side of the animal served as a control. The three methods are: (a) implanting the common peroneal nerve, which normally innervates mixed muscles, to the denervated soleus (12 animals); (b) complete section, rotation and re-suture of the sciatic or posterior tibial nerve (6 animals); (c) vigorous, multiple crush of the sciatic or posterior tibial nerve (6 animals). It was thus planned that at least some nerve fibres which normally innervate histochemically "mixed" muscles would regenerate into the soleus and innervate some of its muscle fibres. In six control animals, self re-innervation of the soleus was produced by vigorous crush of its small nerve. The experimental and control animals were killed at four and six months (a few at two months) after treatment. The muscles were examined by preparing serial cryostat sections<sup>2</sup>, 10 $\mu$  thick, of the entire cross section of the muscle at its mid-point. A series of histochemical tests was applied to the fresh, frozen sections, as described elsewhere<sup>1</sup>.

All experimental and control solei become virtually completely re-innervated, as shown by the normal size and architecture of nearly all the muscle fibres and the normal excitability of the muscle through the nerve.

In all experimental soleus muscles, a number of typical type II fibres were present in small and large groups and were best displayed with the myofibrillar ATPase reaction which is our preferred method for identifying histochemical fibre types<sup>3</sup> (Fig. 2). In the experimental groups (a) and (b), the amount of type II fibres was estimated to be 5-40 per cent of the total cross-sectional area in the muscles of different animals. In animals of group (c), they were only 1-3 per cent of the area. Variation in the quantity of type II fibres appearing in the experimental solei is probably attributable to technical factors. In the solei on the control side of experimental animals and in the self re-innervated solei, no type II fibres were seen (Fig. 1). It appears that a portion of the original type I fibres of the soleus was "transformed" into type II fibres as a result of innervation by "foreign" nerve fibres. It is suggested that the ability to transform muscle fibres is a feature of the trophic function of the motor nerve. The present experiments confirm the findings of Romanul and Van der Muelen<sup>4</sup>, who demonstrated the appearance of type II fibres (though not using the myofibrillar ATPase reaction), in "cross innervated" solei of cats and rats. Results of self re-innervation were not reported by these authors.

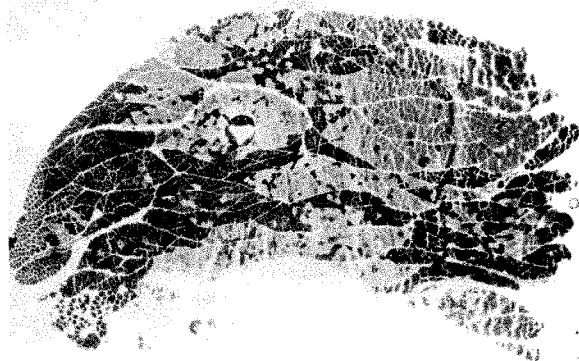


Fig. 1. Entire cross section of a self-innervated soleus muscle, 6 months after treatment. Myofibrillar ATPase reaction,  $\times c. 10$ . Only light, type I fibres are present, as in unoperated control adult soleus muscles.

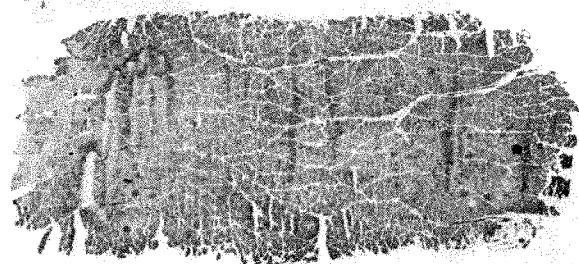


Fig. 2. Entire cross section of a soleus muscle, 6 months after "foreign" innervation. Myofibrillar ATPase reaction,  $\times c. 10$ . Numerous dark, type II fibres are present and occupy about 40 per cent of total cross-sectional area.

It has been reported that in cats<sup>5,6</sup> and rats<sup>7</sup>, the speed of the slow soleus is increased when it is innervated by a nerve normally supplying a fast muscle. A direct relationship of this physiological change to the appearance of type II fibres in foreign innervated solei has not yet been demonstrated, although the two changes are concurrent. One possible method of determining a direct relationship, if it exists, would be the demonstration of a quantitative correlation between the amount of type II fibres (as percentage of cross-sectional area), and the changes in the various physiological parameters within each foreign innervated muscle.

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### Interactions of DDT with the Nervous System of the Resistant and Susceptible German Cockroaches

DDT is regarded as a nerve poison in insects and mammals chiefly from the symptomological, electrophysiological and biochemical points of view<sup>1,2</sup>. The actual mechanism of DDT poisoning at the target, however, is not known.

Recently a hypothesis was proposed to explain the mode of action of DDT on the basis of charge-transfer complex formation of nerve components with DDT<sup>3</sup>. The first obvious step toward providing evidence for this hypothesis is to confirm the formation of a DDT complex with nerve components through physical separation of the complex. Matsumura and O'Brien were able to do so by showing that DDT was quickly adsorbed by the intact nerve cord and the various nerve components of the homogenates of the American cockroach<sup>4</sup>. They also found that this binding process was accompanied by the shift in the ultra-violet absorption spectrum<sup>5</sup>. These results alone, however, may not be sufficient to prove or disprove the hypothesis, for it cannot provide evidence that such complex formation is causally related to the actual process of DDT poisoning. That is, DDT is a very apolar compound, and it is only

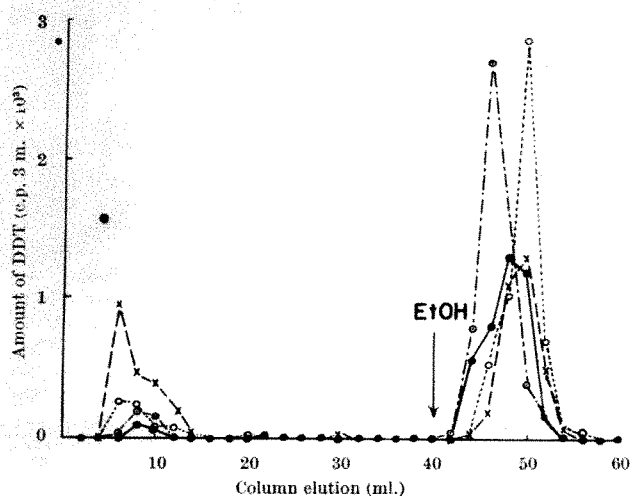


Fig. 1. 'Sephadex' column elution of DDT complexes from the resistant and susceptible homogenates which had been incubated with free  $^{14}\text{C}$  DDT.  $\circ$  ---  $\circ$ , VPI;  $\circ$  - - -  $\circ$ , VPI-DDTR;  $\times$  - - -  $\times$ , London;  $\bullet$  -  $\bullet$ , CSMA.

natural that it is either absorbed or bound to lipid containing neural substances such as lipoproteins in an aqueous system. To prove that such a binding phenomenon is genuinely related to the process of poisoning, it is necessary to find a specific system, in which the increase or decrease in the amount of DDT binding to a particular nerve component causes a marked change in the susceptibility of the test organisms. Such an example may be found in the insect colonies resistant to DDT, provided that the mechanism of resistance in those insects is not simply related to an increased elimination system of the toxicant before it reaches the target site.

The strains used to test this possibility were the CSMA susceptible and the Fort Rucker strain (both  $LC_{50}$  at 0.0032 per cent, as determined by a dipping method<sup>6</sup>), and the London ( $LC_{50}$  at 0.06 per cent) and the VPI strain ( $LC_{50}$  over 2 per cent) respectively. Preliminary experiments first established that the London strain had an identical rate of degradation and excretion of DDT as compared with the CSMA strain. The head parts from the male cockroaches were collected and homogenized in 0.25 molar sucrose solution at a concentration of three heads/ml. by using a small 'Teflon' Potter-Elvehjem homogenizer at 0°C. The weight of the head samples from each

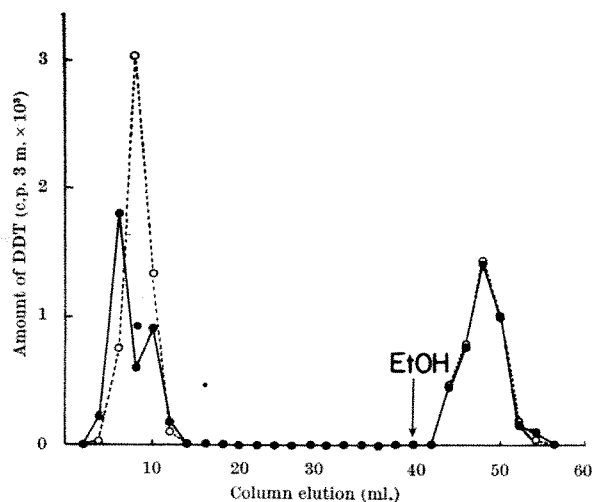


Fig. 2. 'Sephadex' column elution of DDT complexes from the VPI DDT-resistant and CSMA susceptible supernatant which had been incubated with  $^{14}\text{C}$  DDT.  $\circ$  ---  $\circ$ , VPI;  $\bullet$  -  $\bullet$ , CSMA.

strain was carefully measured to ascertain the equality of the homogenate concentration among the strains.

A binding experiment similar to those of previously reported dieldrin cases<sup>7</sup> was then conducted with the head homogenates. The results, given in Table 1, indicated that the DDT molecule indeed bound with various subcellular components of the roach head; that the resistant crude nucleus fractions had less binding capacity than did the susceptible counterparts; and that the resistant supernatants contained more DDT.

Table 1. ABSORPTION OF  $^{14}\text{C}$  DDT TO VARIOUS SUBCELLULAR COMPONENTS

Fractions	VPI	Strains		
		London	Ft. Rucker	CSMA
Crude nucleus	66.4	65.8	72.7	71.2
Mitochondria	6.3	6.8	4.7	8.3
Microsome	4.9	4.6	2.8	5.3
Supernatant	22.4	21.8	19.9	14.9

Absorption data are expressed in percentage of DDT added.

The supernatant fraction may still contain unknown amounts of free DDT and therefore the fraction from each strain was analysed chromatographically with 'Sephadex G-50' gels as before<sup>7</sup>. The resulting chromatograms indicated that under the conditions tested—that is 7.5 mg/ml. of homogenate concentration—no elutable free DDT existed. Nor was there any DDT that was bound to true soluble proteins. Instead all DDT was found to be associated with very small particles (or with the gel particles), and it could only be eluted by denaturing the 'Sephadex' gel with 95 per cent ethanol. Only when the homogenate concentration was increased to 100 mg/ml. was a portion of administered  $^{14}\text{C}$  DDT eluted with soluble proteins (Fig. 1). It was also found that the binding capacities of the resistant supernatants were considerably higher than those of the susceptible strains. The difference was not caused by the presence of other subcellular fraction in the homogenate, because an experiment with the supernatant alone showed a similar result (Fig. 2).

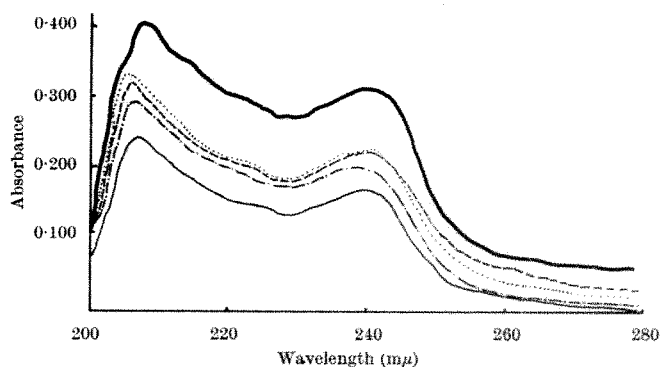


Fig. 3. Ultra-violet absorption spectra of DDT and DDT administered cockroach brain homogenates. —, DDT; . . . , VPI; - - - , London; - - - - , Fort Rucker; —, CSMA.

To investigate further the nature of DDT binding in relation to the formation of possible charge-transfer complexes with the roaches' nerve components, ultra-violet spectra of these DDT-complexes were then studied. Fig. 3 illustrates the change in the ultra-violet spectra<sup>8</sup> of DDT in an aqueous condition on reaction with the brain homogenate (exclusive of large particles which sediment at 600g). It can be seen that in all cases the shape, the positions and the heights of the main peaks shifted, and that the degree of shifting was highest with the S-homogenate and the lowest with the VPI-homogenate. To ascertain that this large shift in the susceptible homogenate is caused by the fine subcellular particles in the homogenate, it was centrifuged for 4 h at 140,000g with 0.06 molar sodium phosphate buffer to eliminate all particles. The resulting supernatant was treated with



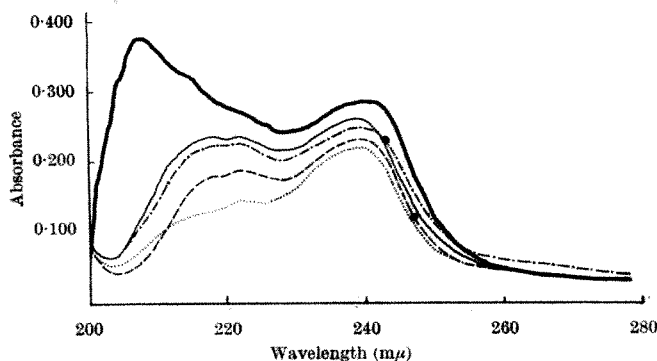


Fig. 4. Ultra-violet absorption spectra of DDT and DDT administered cockroach brain supernatants. —, DDT; ·····, VPI; ---, London; - · - · -, Fort Rucker; ———, CSMA.

DDT as before. Fig. 4 indicates similar shifts in the ultra-violet spectra of DDT aqueous suspensions on addition of various supernatants. The principal difference here is that the order of binding was the complete reverse of the previously observed data with homogenates.

The results described here indicate that the resistant particulate components indeed have less binding capacity with DDT than do the susceptible components. This finding is in agreement with the previously predicted view that there should be a binding difference between the strains at a specific binding site which is related to the susceptibility of the test organisms, if such a site is actively engaged in the process of attaining a high level of DDT resistance by the roaches. Despite this additional piece of evidence in favour of the hypothesis, there are a number of problems remaining unsolved. One is the lack of information on the identity of this particulate component(s) which causes the interstrain difference, and another is the absence of genetical study on the above roach strains. Unfortunately the amount of the binding substances present in the nerve preparation was too small to permit any chemical analysis, and the relatively long life span of the German cockroach does not permit the genetic cleaning experiments<sup>9</sup> required to eliminate strain differences due to geographical variations at this time. In conclusion, we have found that DDT does indeed bind with various components of the German roach nerve, and that the particulate nerve components from the cockroaches resistant to DDT have less binding capacity with DDT than do the susceptible components, implying that these resistant cockroaches could be utilizing this vital process for their advantage. Whether this binding substance can play an indispensable part in DDT poisoning is, however, a problem requiring further study.

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## Effect of Cold on the Vitamin E Requirement of Rats

SINGH<sup>1</sup> has suggested that man's vitamin E requirement increases at high altitudes. Vitamin E is known to be involved in the transport of oxygen in the respiratory chain, and it could facilitate oxygen transport during enzyme adaptation to hypoxia. At an altitude of 3,450 m and a temperature of 20° C, rats have shown neither an increased requirement for vitamin E nor an improved fitness when given vitamin E<sup>2,3</sup>. These negative results were found at constant conditions of ambient temperature at which the oxygen consumption at a high altitude is known to be low<sup>2</sup>. The turnover of vitamin E would be expected to increase with any increase in oxygen consumption, and because temperature decreases with altitude and oxygen consumption increases with decreasing temperature, the greater requirement for vitamin E postulated by Singh<sup>1</sup> could be caused by exposure to cold and not by hypoxia at a high altitude. We have investigated the vitamin E requirement of rats at high and low temperatures independent of altitude.

Female Wistar rats of the conventional breeding colony Füllisdorf, 4 weeks old at the onset of the experiment, were used. They were given freely a diet containing no vitamin E<sup>4</sup>. Animals were kept at room temperature (28° C) in wire cages, four to a cage. Vitamin E depletion was determined by haemolysis induced by dialuric acid, by the method of Friedman *et al.*<sup>5</sup>. As soon as the blood had reached 95 per cent haemolysis in all animals, each received daily, for 15 consecutive days (without weekends), 1 ml. of *dl*- $\alpha$ -tocopherol acetate dissolved in 0.2 ml. of peroxide and vitamin E-free olive oil. With this treatment the haemolysis values returned to zero, which indicated an adequate provision of vitamin E. The animals were then divided randomly into two groups of twelve; one group was transferred to a cold room at 1° C while the other group remained at 28° C. In both groups the progressive depletion of vitamin E was determined at regular intervals by the increase in percentage haemolysis. Food consumption was calculated from the total food consumed by each group during the experimental period.

		Table 1			
		1° C		28° C	
Days after application of vitamin E	N	Mean haemolysis (per cent)	N	Mean haemolysis (per cent)	
0	12	0	12	0	
8	12	7.2	12	10.5	
17	12	23.4	12	21.9	
22	12	38.2	12	25.3	
30	12	51.5	12	41.0	
38	12	88.1	12	81.2	
45	12	92.7	12	82.4	
g of food/animal/day		17.6		15.5	

The mean values of haemolysis for both groups showed a steady depletion of vitamin E (Table 1). The linear regression was computed by the maximum likelihood method for probit lines. This was  $y = 3.2061x + 0.3892$  for the cold group and  $y = 3.8794x - 0.3333$  for the warm group. The exact end-point of the experiment was determined by the crossing point of the regression line with the probit line of 6.64 (95 per cent haemolysis) and was found to be 62.7 days for the cold and 95.4 days for the warm group. The mean daily vitamin E consumption by an animal of the cold group was 0.24 mg and by the warm group 0.16 mg. The daily food consumption by each rat during the experimental period was 17.6 g in the cold and 15.5 g in the warm group, the increase after exposure to cold being 13.5 per cent.

The results of the investigation show that in the experimental conditions the vitamin E requirement of the rats in the cold was 50 per cent greater than in the warm. We do not believe that this is an absolute figure. In this experiment, rats were housed in groups of four, which

enabled them to protect themselves from extreme heat loss by huddling together. According to the findings of Cottle and Carlson<sup>6</sup> with individually housed rats, the production of heat by the animals and later their intake of energy should have increased by more than 100 per cent. We saw a moderate increase of only 13.5 per cent with exposure to cold and believe that this low figure is an expression of a protection from severe heat loss brought about by the behaviour of the animals. If protection from heat loss is avoided and animals become fully exposed to the cold, higher daily requirements of vitamin E must be anticipated.

These results suggest that if an increased vitamin E requirement exists at high altitudes, it is more likely to be caused by the cold exposure than by hypoxia. There is no information about the vitamin E requirement of man in a cold environment<sup>7</sup>. There is evidence that vitamin E depletion is caused by exposure to cold when no supplementary vitamin is given in the frequent and sudden occurrence of muscular dystrophy among calves and lambs during the cold season<sup>8-11</sup>. It is not certain, however, that this form of dystrophy is entirely caused by vitamin E deficiency.

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## BIOLOGY

### Production of Plant Growth Inhibitors from Xanthophylls: a Possible Source of Dormin

THE plant growth inhibitor, dormin, shows similarities in structure to certain carotenoids and, because there is evidence that light stimulates the production of inhibitor  $\beta$  (refs. 1 and 2), the principal activity of which is probably caused by dormin<sup>3,4</sup>, we have studied the possible formation of inhibitory substances by illumination of these pigments.

Carotenoids, extracted from dried nettle (*Urtica dioica* L.) leaves<sup>5</sup>, were stored in the dark under vacuum. Samples (50 mg) were dissolved in peroxide-free ether (100 ml.), washed with 2 per cent aqueous sodium bicarbonate to remove endogenous inhibitors and then with water. Samples (2 ml.) of the ether solution were added to disks of Whatman No. 1 filter paper in 5 cm Petri dishes, and the solvent allowed to evaporate. All these processes were carried out in weak green light. The open Petri dishes were then kept in the dark or illuminated 30 cm from a 500 W tungsten lamp for 3 h. The light was filtered through water (5 cm path) and a stream of air at about 25° C was directed over the dishes. The paper in each dish was then moistened with water (1 ml.) and

twenty cress seeds (*Lepidium sativum* L.) were placed on it. The dishes were closed and transferred to a darkened humidity box at 23.5° C. After 60 h the germination of the seeds on the illuminated filter papers was negligible compared with that on the papers which had not been exposed to light. The mixed carotenoids (100  $\mu$ g) irradiated in this way gave an inhibition comparable with that shown by synthetic ( $\pm$ )-dormin (1  $\mu$ g).

Heating the pigment-treated papers in the dark for 3 h at 55° C did not reproduce the light treatment, although higher temperatures were partially effective. The inhibition was less when the pigments were illuminated over phosphorus pentoxide or alkaline pyrogallol solution, which suggests that water and oxygen are necessary in this conversion. The inhibitor eluted from filter paper with ether was shown to partition in the non-acid fraction and may be an alcohol or aldehyde, perhaps being converted to a carboxylic acid, like dormin, within the cress seeds.

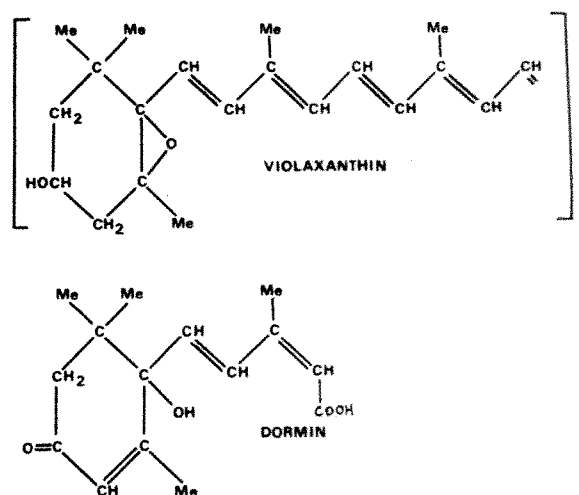


Fig. 1. Structures of violaxanthin and dormin.

The carotenes, which had been shown to be inert, were removed by partitioning between petroleum ether and 90 per cent aqueous methanol. The lower methanol layer was then diluted with water, and ether extracted to recover the xanthophylls. Thin-layer chromatography in the dark on silica gel *G* using benzene: ethyl acetate (3:1) as solvent, separated at least five pigments. The bands of silica gel were immediately eluted with ether to minimize the oxidation which occurs on the dry adsorbent. On illumination, these eluted pigments did not produce inhibition in proportion to the intensity of their initial yellow colours, but the greatest inhibitory effect was obtained with one of the minor xanthophylls present in the nettle leaf extract. This has been tentatively identified as violaxanthin.

These findings may explain many of the responses to blue light which are reported to occur in plants; in particular, phototropism in *Avena coleoptiles*<sup>6</sup> in which tissue violaxanthin has already been shown to be the principal carotenoid<sup>7</sup>. It is also known that blue light inhibits root elongation<sup>8,9</sup> and seed germination<sup>10,11</sup> in certain plants. Moreover, the production of dormin in deciduous leaves may be related to the exposure of the xanthophylls to light in the autumn, a time when the chlorophyll is lost.

It is thus possible that the photo-oxidation of certain xanthophylls is a mechanism by which growth inhibitors are produced in plants. Because more than one xanthophyll gave rise to inhibition, more than one dormin-like inhibitor may be produced in this way.

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### Effect of Benzyl Adenine on Isolated Apple Shoots

EVIDENCE for export from roots to shoots by way of the xylem of substances with biological activity similar to that of kinetin has accumulated in recent years. The significance of these substances in relation to shoot growth is, however, not fully understood. Evidence suggests that cytokinins from roots have beneficial effects on the protein and chlorophyll of leaves<sup>1-4</sup>, and results obtained with vine cuttings show that the effects of roots on inflorescence retention are simulated by supplies of synthetic cytokinins<sup>5</sup>.

The possibility that apple roots supply cytokinins to the shoots was raised when my preliminary experiments<sup>6</sup> indicated cytokinin-like activity in apple stem xylem sap. Luckwill and Whyte<sup>7</sup> confirmed this and found the highest kinin activity in sap collected in April which had a concentration equivalent to approximately 0.1 µg kinetin/ml. of crude sap. Chromatography of butanol extracts indicated the presence of at least two active substances.

If the cytokinins of apple xylem sap have an important role in the growth of the shoots, then some indication of this role might be gained by supplying the cytokinins to isolated apple shoots. This communication reports the effects of the synthetic cytokinin benzyl adenine (donated by Shell Chemical Co., Ltd., as SD4901) on apple shoot cultures—work preliminary to studies of the effects of the natural cytokinins of the xylem sap.

M.26 apple rootstock shoots were taken shortly after bud-break, when they were about 1.5 cm long. The bud scales and between one and three of the largest leaves were removed—a requisite for sterility—and the shoots then sterilized<sup>8</sup>. Cultures were also made of 10 mm shoot tips of older vigorously growing M.26 and M.VII rootstock shoots which were about 15 cm long. The tips were grown under sterile conditions in 6 in. × 1 in. test-tubes on "wicks" of Whatman No. 1 filter paper which dipped into 10 ml. of liquid culture medium<sup>9</sup>. Cultures were kept at 25° ± 2° C and illuminated for 16 h daily with fluorescent tubes which gave 500–700 ft.-candles at the tube surface.

The basic medium used was Knop's solution with the minor element solution of Murishige and Skoog<sup>10</sup>, 20 mg/l. NaFeEDTA, and 3 per cent sucrose. The medium was adjusted to pH 5.8 and sterilized by autoclaving at 15 lb./in.<sup>2</sup> for 5 min. The shoots grew to a limited extent in this medium (Fig 1B). Occasionally the shoots rooted, but roots did not develop extensively and never reached lengths beyond about 5 cm. The rooted shoots were very variable, but all were still green after 10 weeks while rootless shoots were turning brown by this time. Furthermore, rooted shoots sometimes produced between three and six more leaves and greater internode extension and leaf expansion than unrooted shoots.

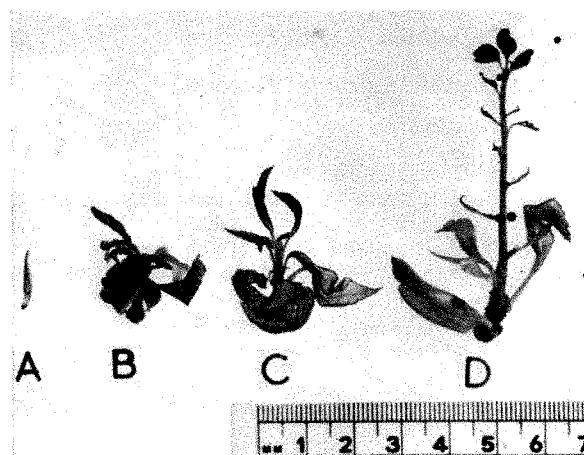


Fig. 1. Effect of benzyl adenine on M.26 shoot cultures (culture period, 5 weeks). A, Inoculum (from shoots about 1.5 cm long); B, basic medium; C, basic medium + 0.1 mg of benzyl adenine/l.; D, basic medium + 1.0 mg of benzyl adenine/l.

The addition of 1 mg of benzyl adenine/l. to the basic medium resulted in sizable increases in leaf production and internode extension (Table 1, Fig. 1). Shoots never rooted when growing with benzyl adenine. Shoot growth with benzyl adenine was greater than with rooted cultures, but this may be caused by the lack of extensive root development when shoots rooted. The benzyl adenine delayed senescence of the shoots, as did roots. Cultures did not grow appreciably after 5 weeks, but those which received benzyl adenine were, in contrast to the controls, still green after 10 weeks.

Table 1. EFFECT OF BENZYL ADENINE ON ISOLATED APPLE SHOOT CULTURES

Source of tips	Benzyl adenine in medium (mg/l.)	No. of replicates	Growth values ± standard errors after 5 weeks		
			Fresh wt. (mg)	Stem length (mm)	No. of leaves and primordia
About 1.5 cm M.26 shoots	—	6	132 ± 12	10 ± 2	13 ± 2
	1	7	562 ± 30	47 ± 7	21 ± 3
About 15 cm M.26 shoots	—	7	222 ± 40	10 ± 3	15 ± 1
	1	6	400 ± 46	18 ± 6	23 ± 3
About 15 cm M.VII shoots	—	6	94 ± 15	5 ± 3	11 ± 1
	1	8	310 ± 38	27 ± 6	16 ± 2

Many of the leaves of shoots with benzyl adenine were small with poorly developed laminae. Anatomical study of internodes indicated that all shoots, whether receiving benzyl adenine or not, had secondary thickening.

The effects of benzyl adenine were greatest on the cultures initiated from the 1.5 cm shoots. These different responses may be caused by differences in reserves of essential metabolites in shoots of different ages. At 0.1 mg/l., benzyl adenine had no marked effect on shoot growth apart from increased callus production in the basal regions. At 10 and 50 mg/l. the cytokinin inhibited shoot extension but produced profuse callus growth.

The isolated shoots seem to require an external supply of cytokinin for growth and the effects of benzyl adenine simulate to some extent the effects of roots. These findings, together with the evidence for cytokinins in the ascending sap, support the view that cytokinins from the roots may be essential for continued shoot growth in apple. Compared with rooted shoots, however, shoots grown with 1 mg of benzyl adenine/l. of culture medium had poorly developed leaves. These abnormal leaves may reflect the possibility that the shoots depend on roots for other organic substances in addition to cytokinins. Analyses of apple xylem sap suggest that the roots supply amino compounds<sup>10</sup> and gibberellin-like substances to the shoots (Jones and Lacey, unpublished results), and possibly these substances are also required for normal shoot development. It is also possible that the cytokinins

of the xylem sap produce effects on shoot growth in addition to those observed with the synthetic cytokinin. These possibilities are under investigation by further experiments with isolated apple shoot cultures.

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### Effect of DDT on Photosynthesis in Certain Varieties of Barley

APPARENTLY uniform varieties of barley show striking differences in their reaction to DDT (1,1,1-trichloro-2,2-bis-(*p*-chlorophenyl)-ethane) or certain of its analogues (Fig. 1). Some varieties are resistant to DDT whereas others are susceptible and develop a severe chlorosis some 4–10 days after treatment with sprays containing DDT (ref. 1). DDT is not translocated in the plant to any extent and similar amounts of the insecticide are absorbed by resistant and susceptible varieties<sup>2,3</sup>. The reaction to DDT is controlled by a single major gene with susceptibility dominant to resistance<sup>1,4</sup>.

We have investigated the effect of DDT on two varieties of barley; 'Rika' (susceptible) and 'Proctor' (resistant). To determine whether photosynthesis or respiration is affected by DDT in the susceptible barley, gas exchange in DDT-treated barley leaf tips during alternating periods of illumination (580 ft.-candles) and darkness was studied<sup>5</sup>. In the centre well of a Warburg flask we placed 0.1 ml. of water and 100 mg of leaf tips; the outer compartment contained 2.0 ml. of bicarbonate buffer (0.2 M NaHCO<sub>3</sub>, 0.2 M Na<sub>2</sub>CO<sub>3</sub>; 9:1 v/v). Gas<sup>2</sup> evolution or uptake was recorded in the normal way. Leaves of untreated varieties and of resistant barley treated with DDT reached a peak of photosynthetic activity (oxygen evolution) as seed stores were used up and chloroplasts matured. Senescence of the leaves was accompanied by a progressive fall in photosynthetic activity. In contrast, leaves of susceptible barley treated with DDT showed a decrease in oxygen evolution within 12 h of spraying the plants with DDT. The leaves never reached their normal peak of photosynthetic activity and eventually died as chlorotic symptoms developed (Fig. 2).

The Hill activity of chloroplasts isolated from treated barley was measured by the rate of reduction at pH 6.5 of

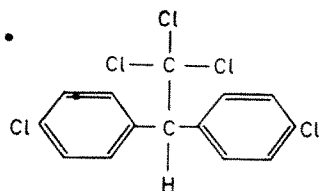


Fig. 1. Structure of DDT. Analogues of DDT possessing (a) —OCH<sub>3</sub>, Br, Cl or F in the *para*-positions of the phenyl rings; (b) the C2 ethane hydrogen; and (c) the trichloro- or tribromo-ethane grouping is also toxic to some varieties of barley<sup>2</sup>.

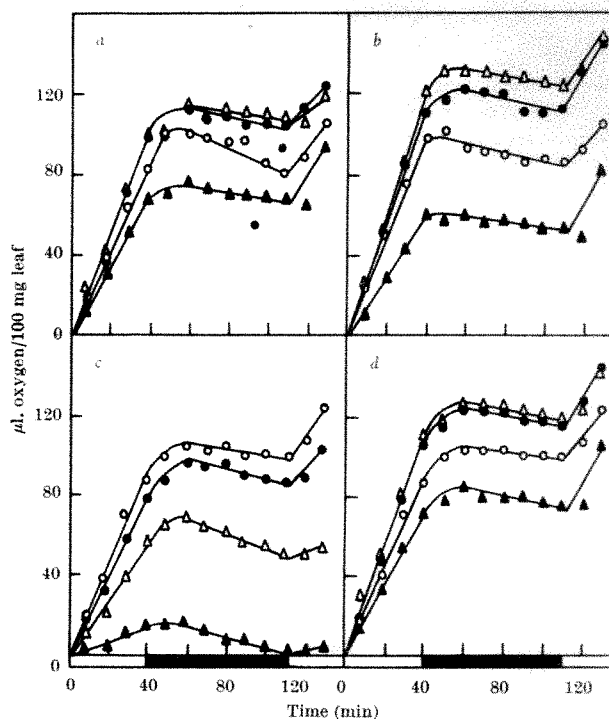


Fig. 2. Gas exchange during successive periods of illumination and darkness after treatment of 8-day-old barley plants with DDT. Experimental details as described in the text. ○, ●, △, and ▲ represent results obtained 0, 4, 7 and 15 days after treatment of plants with DDT. Results from equivalent plants not treated with DDT are shown for comparison. a, Resistant barley, treated with DDT; b, control. c, Susceptible barley, treated with DDT; d, control.

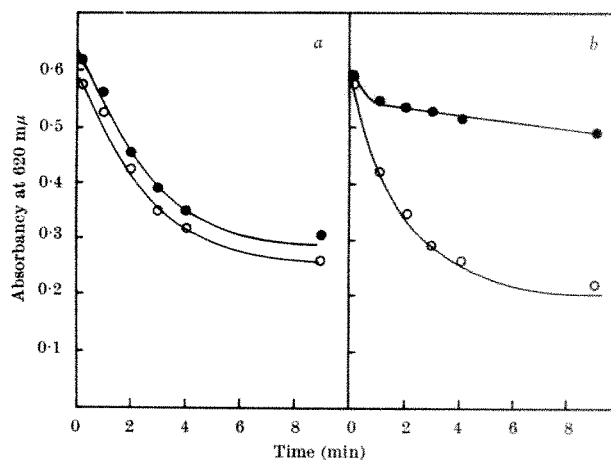


Fig. 3. Photoreduction of 2,6-dichlorophenolindophenol by chloroplasts isolated from barley treated with DDT. Experimental details as described in the text. ○, Barley not treated with DDT; ●, barley 4 days after treatment with DDT. a, 'Proctor' (resistant); b, 'Rika' (susceptible).

the redox dye 2,6-dichlorophenolindophenol (DCIP). The reaction medium, made up in 1 cm cuvettes, contained (per 3.0 ml.): KH<sub>2</sub>PO<sub>4</sub>, 125 μmoles; KCl, 25 μmoles; DCIP, 0.10 μmole; chloroplast preparation containing 30γ chlorophyll. Illumination (14,000 ft.-candles) was provided by two 500 W 'Argaphoto' lamps and reduction of DCIP was followed by the fall in absorption at 620 mμ. Chloroplasts isolated from susceptible barley treated with DDT showed a marked reduction in Hill activity (Fig. 3) whereas those from treated resistant barley showed Hill activity characteristic of unsprayed plants. Four days after spraying the plants with DDT the chloroplasts isolated from susceptible barley showed only about 25 per cent of the Hill activity of chloroplasts from unsprayed plants.



When chloroplasts isolated from unsprayed plants were suspended for 45–60 min in an aqueous suspension of DDT (75–200  $\mu\text{g}$  DDT/mg chlorophyll) chloroplasts from susceptible barley showed a reduction in Hill activity whereas those from resistant barley were not affected. The incubation of chloroplasts in the suspension of DDT for 30–60 min before estimation of Hill activity was essential for this effect to be shown. If chloroplasts isolated from susceptible barley were assayed for Hill activity immediately they contacted the DDT suspension<sup>2</sup> no reduction in Hill activity was observed. The effect of the DDT could be significantly reduced by prior suspension of the chloroplasts in a medium containing DDE [1,1-dichloro-2,2-bis-(*p*-chlorophenyl)ethylene], an analogue of DDT which is not toxic to either variety of barley.

Fixation of  $^{14}\text{CO}_2$  into ethanol soluble products of photosynthesis<sup>6</sup> by treated plants or into phosphoglyceric acid by carboxydismutase preparations<sup>7</sup> from treated plants was quantitatively the same in both susceptible and resistant varieties of barley. The proportion of soluble material from susceptible barley 8 days after treatment was, however, increased some 35–40 per cent compared with the similarly treated resistant variety. The material accumulating in the susceptible barley treated with DDT seems to be sugars (personal communication from J. D. Hayes).

The results suggest that in susceptible varieties of barley DDT affects the light reaction in photosynthesis. Further investigations to find the precise site of action of DDT on the photosynthesis of susceptible barley are now in progress.

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### Effects of Actinomycin D on Development in Pupae of *Tenebrio molitor*

Ilan, Ilan and Quastel have reported<sup>1</sup> that modified adults emerge when 0.16  $\mu\text{g}$  of actinomycin D is injected into the pupae of *Tenebrio molitor* at any time during the pupal period. The duration of pupation is said to be unaffected. The adults which emerge are reported to have an adult head and thorax, but the abdomen has pupal gin traps, urogomphi and pupal cuticle on the posterior segments. Injected pupae do not develop wings. The interest in the effects of actinomycin D on development stems from the way in which the drug is known to interfere with DNA dependent RNA synthesis. The pupal development of insects must involve the transcription of the genetic information coded in DNA into the specific proteins of the various tissues by the formation of messenger RNA (*mRNA*). Ilan *et al.* interpret their observations by supposing that the *mRNA* for the development of the head and thorax is present from the first day of pupation and that abdominal development is sensitive to actinomycin D until the last day of the pupal period.

My own observations, made during a detailed study of the effects of drugs on the developmental pattern of *Tenebrio molitor* pupae, do not agree with the results of Ilan *et al.* Pupae weighing  $100 \pm 10$  mg, kept at 31° C, emerge on the seventh day after pupation. Injection into pupae of different ages of 0.04  $\mu\text{g}$ , 0.08  $\mu\text{g}$ , 0.16  $\mu\text{g}$ , and 0.32  $\mu\text{g}$  of actinomycin D in 1  $\mu\text{l}$ . of 0.9 per cent sodium chloride produced six characteristic patterns of epidermal differentiation (Table 1). Controls were injected with 1  $\mu\text{l}$ . of 0.9 per cent sodium chloride. The control animals and those injected with 0.04  $\mu\text{g}$  of actinomycin D all emerged as normal adults on the seventh day (class VI). When the pupae injected with 0.16  $\mu\text{g}$  were inspected on the seventh day, four classes were observed (classes I, II, III and V). By the ninth day after pupation however, some of Class II had completed cuticle formation, the abdomen forming a second cuticle of pupal type (class IV).

The degree of inhibition of epidermal differentiation is dependent on both the dose and the day on which the actinomycin D is injected. Injection of any dose after the fourth day, or injection of 0.08  $\mu\text{g}$  on any day, results in class V or class VI animals only (Table 2).

The description given by Ilan *et al.* of their "modified adult" suggests at first, that these animals might correspond with my class IV, in which the abdomen forms a second cuticle of pupal type. They state, however, that a new cuticle is not formed over the abdomen of modified adults, which excludes my class IV and suggests my class III. I believe, however, that these authors were in fact observing class V animals in which the adult cuticle is formed, but is still enclosed by the pupal cuticle. Their photograph of a modified adult appears to show a tanned abdominal cuticle; a pupal cuticle does not tan. Also, although the abdomen has not shortened to the adult

Table 1. CLASSES OF RESULTS OBTAINED AFTER THE INJECTION OF ACTINOMYCIN D INTO THE PUPAE OF *Tenebrio molitor*, IN DECREASING ORDER OF INHIBITION OF EPIDERMAL DIFFERENTIATION

Class I	No adult differentiation of epidermis.
Class II	Sclerotized adult cuticle formed on the limbs and head appendages only.
Class III	Head and thorax form adult cuticle, the abdomen forms no new cuticle.
Class IV	Head and thorax form adult cuticle, the abdomen forms a second pupal cuticle. External genitalia are present.
Class V	Complete adult cuticle formed, but animals fail to emerge from pupal cuticle over abdomen. Abdomen does not shorten fully. Wings do not expand. Some tanning of ventral abdominal cuticle always occurs.
Class VI	No inhibition occurs; full emergence of normal adults on the seventh day.

Table 2. RELATION BETWEEN THE DOSE AND THE DAY OF INJECTION AND THE CLASS OF RESULT OBTAINED AFTER THE INJECTION OF ACTINOMYCIN D INTO THE PUPAE OF *Tenebrio molitor*

Day of pupal period when injected	1	2	3	4 and over
$\mu\text{g}$ of actinomycin D in 1 $\mu\text{l}$ . of 0.9 per cent sodium chloride or water	Percentage in each class	Percentage in each class	Percentage in each class	Percentage in each class
None	I II III IV V VI	— — — — — —	— — — — — —	— — — — — —
0.04	I II III IV V VI	— — — — — —	— — — — — —	— — — — — —
0.08	I II III IV V VI	— — — — — —	— — — — — —	— — — — — —
0.16	I II III IV V VI	— — — — — —	— — — — — —	— — — — — —
0.32	I II III IV V VI	— — — — — —	— — — — — —	— — — — — —

Total number injected for each group in brackets.

form, it is not pupal in shape, and external genitalia appear to be present. The suggestion that their modified adults are indeed class V animals is supported by the fact that I have obtained class V animals after the injection of pupae of any age, but I could not produce class III animals either by injection after the fourth day or by injection of doses less than 0.16  $\mu$ g. In my experiments, the injection of 0.08  $\mu$ g of actinomycin D produced 60 per cent class V animals and the rest emerged normally which compares with the 70 per cent of "modified adults" obtained by Ilan *et al.* after injection of the same dose.

Ilan *et al.* used pupae heavier than mine, and it seemed possible that 0.16  $\mu$ g is below the critical dose in such pupae for inhibition of the formation of adult abdominal cuticle. To check this, fifty animals of different ages which weighed between 110 and 120 mg were injected with 0.16  $\mu$ g of actinomycin D and incubated at 28° C—conditions which correspond exactly with those of Ilan *et al.* All but five formed an adult abdominal cuticle. It is worth noting that, by the time of emergence of the adult cuticle in control pupae, much of the pupal cuticle has been digested and only a fine transparent sheath remains to be shed. In most of the injected pupae in which emergence is inhibited, the pupal cuticle remains undigested and opaque, giving the superficial appearance of a lack of adult cuticle over the abdomen. I therefore suggest that Ilan *et al.* did not observe the inhibition of some process concerned with cuticle formation, but the inhibition of one of these later events leading to the final successful eclosion of the adult.

My results, shown in Table 2, seem to indicate separate determinations for the formation of a second cuticle on the limbs, the head and general thorax, and the abdomen, because, depending on the time of injection and the dose of actinomycin D, development of a new cuticle in one or more of the three regions can be inhibited or modified. Ilan *et al.*, on the other hand, claim to have shown two stages; the formation of an adult cuticle on the head and thorax was said to be insensitive to actinomycin D throughout pupation, while the formation of an adult cuticle on the abdomen was sensitive to actinomycin D until the last day of pupation. It is tempting to suggest that the three stages in the formation of the cuticle, which I have found to be independently sensitive to actinomycin D, indicate that three separate mRNAs are involved.

This work was carried out during the tenure of an SRC studentship and under the supervision of Dr Janet Harker. The actinomycin D was a gift from Merck, Sharp and Dohme.

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### High Concentrations of Haemoglobin A<sub>2</sub> in Malaria Patients

THE A<sub>2</sub> fraction of normal haemoglobin (Hb-A<sub>2</sub>) has been described by Kunkel and Wallenius in 1955<sup>1</sup> and soon after it was confirmed that this fraction doubled its normal values or reached even greater concentrations in  $\beta$ -thalassaemia minor<sup>2</sup>, and it has been found to be increased in pernicious anaemia in relapse<sup>3</sup>, in patients with Hb-Zürich<sup>4</sup>, in recipients of foetal haemopoietic tissue<sup>5</sup> and in a single case of hereditary spherocytosis<sup>6</sup>. It is easy to exclude the last four conditions, and so greater concentrations of Hb-A<sub>2</sub> than normal are considered specific for the diagnosis of  $\beta$ -thalassaemia minor.

In 1960, while studying Hb-A<sub>2</sub> in starch block electrophoresis on 118 samples of Venezuelan Paraujano Indians,

105 were found with a mean value of  $2.32 \pm 0.58$  per cent of the total haemoglobin. The other thirteen samples showed larger values than the upper normal level, but less than the smallest concentration found in thalassaemia minor. Deficiency of serum vitamin B<sub>12</sub> was excluded in this population<sup>7</sup>.

In 1965 another group of the same Indians was investigated. Again high concentrations of Hb-A<sub>2</sub> were found in twenty-five out of 140 people:  $3.79 \pm 0.45$  per cent of the total haemoglobin (my work, in preparation). If these have thalassaemia minor we would expect about 3 per cent of thalassaemia major, which does not correspond with information given by local physicians. Thus there ought to be another cause producing high concentrations of Hb-A<sub>2</sub>.

Results of X-ray studies on ancient skulls<sup>8</sup> have suggested that the Peruvian Incas, as well as the Mayas of Central America, could have disappeared because of a fatal osteoporotic process similar to thalassaemia. Concentrations of Hb-A<sub>2</sub> studied in various Indian populations of South America (Table 1), however, have not confirmed this hypothesis, which indicates that if  $\beta$ -thalassaemia is prevalent among the still living Indians of the American continent it is in a very small proportion and never as great as suggested by that report. Cabannes, Bourrier and Larrouy<sup>9</sup> have raised this question again when describing a very high percentage of what they considered to be  $\beta$ -thalassaemia and hereditary persistency of high Hb-F in French Guiana Indians.

The opportunity arose recently to study the concentrations of Hb-F and Hb-A<sub>2</sub> in several cases of acute and chronic *Plasmodium vivax* malaria (including those of myself and my wife, whose Hb-A<sub>2</sub> concentrations had been previously within normal limits) and the conclusion was reached that malaria infection produces significant changes in the pattern of haemoglobin, especially the increase of Hb-A<sub>2</sub>, which could very easily be mistaken for  $\beta$ -thalassaemia minor. This indicates the need for a new interpretation and further studies on the subject.

Blood was collected by venipuncture and haemoglobin solution was prepared by the method of Houchin and Robinette<sup>10</sup>. Starch block electrophoresis for Hb-A<sub>2</sub> quantification was carried out according to the technique of Kunkel and Wallenius<sup>1</sup>. Concentrations of Hb-F were determined using the technique of Betke *et al.*<sup>11</sup> and Singer *et al.*<sup>12</sup>.

Results obtained for Hb-A<sub>2</sub> (Table 2) are significantly larger ( $P < 0.001$ ) than the mean value of normal subjects obtained in our laboratory (Table 1). Even by simple inspection, the starch block electrophoresis plate shows high Hb-A<sub>2</sub> in malaria patients (Fig. 1).

Table 1. Hb-A<sub>2</sub> VALUES IN VARIOUS NORMAL SOUTH AMERICAN POPULATIONS

Population	No. tested	Range	Hb-A <sub>2</sub> mean	S.D.	Reference
Venezuela					
Natives non-Indian	76	1.47-3.37	2.46	$\pm 0.48$	
European residents	19	1.25-3.14	2.25	$\pm 0.57$	7
Paraujano Indians	105	1.25-3.49	2.32	$\pm 0.58$	
Yaruro Indians	44	1.32-3.34	2.38	$\pm 0.62$	
Guyana					
Wapishana Indians	29	1.50-3.35	2.46	$\pm 0.45$	17
Colombia					
Inca Indians	74	1.34-3.44	2.59	$\pm 0.52$	18

Values were calculated by starch block electrophoresis as percentage of the total haemoglobin pigment.

Table 2. HAEMOGLOBIN A<sub>2</sub> VALUES FOUND IN MALARIA PATIENTS (*Plasmodium vivax*)

Subject	Hb-A <sub>2</sub> (% of the total Hb)	Hb-F		Malaria clinical type
		After Betke <i>et al.</i> technique (%)	After Singer <i>et al.</i> technique (%)	
T.A.	3.2	0.8	2.5	Acute
T.R.A.	3.2	0.8	2.9	Acute
H.R.	3.0	0.4	—	Acute
D.R.	3.7	1.5	—	Induced
J.O.R.	3.2	0.4	1.0	Acute
Ya 22	3.6	—	—	Chronic
Ya 24	3.4	0.5	—	Chronic
Ya 25	4.0	0.4	—	Chronic



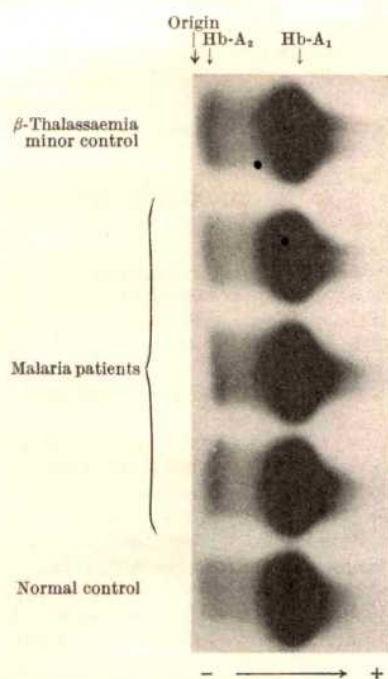


Fig. 1. Visualization of Hb-A<sub>2</sub> by means of starch block electrophoresis using barbital buffer pH 8.6, 0.1 molar, 400 V, 75 m.amp, for 18 h at 4° C. By comparison with both normal and  $\beta$ -thalassaemia minor controls the Hb-A<sub>2</sub> fraction of patients with malaria is clearly increased.

At the moment there is no clear physiopathological explanation of the increase of Hb-A<sub>2</sub> in malaria, as there is also no valid interpretation of the increase occurring in the other conditions mentioned. Immunologically, malaria infection is an extraordinary stimulus<sup>13</sup>. The increase of Hb-A<sub>2</sub> could possibly indicate a more generalized body reaction in the presence of malaria, including other than the immunological system, in this case the haemoglobin synthesis. Another possibility is that not all cells have an equal amount of Hb-A<sub>2</sub> and that *Plasmodium* could have a selective preference for cells with a lower amount of Hb-A<sub>2</sub>. The protective effect of Hb-S in African children<sup>14</sup> is known and it has been suggested that congenital immunity against malaria disappears at the same time as Hb-F (ref. 15). Our finding may represent another proof that *Plasmodium* only metabolizes Hb-A efficiently.

Studies on sheep haemoglobin have shown that animals with Hb-A are more resistant to infection in the small bowel by *Haemonchus contortus*, a blood sucker nematode, than those having Hb-AB (ref. 16). This characteristic, the physiopathological mechanism of which has not been clarified, is a good example of possible interaction between a type of haemoglobin and a typical environmental disease. This apparently may also occur in malaria infection.

On the other hand, it is justified in view of these findings to re-investigate certain populations where malaria infection is or has been endemic and which also show an excessive proportion of  $\beta$ -thalassaemia minor, such as Italy, Greece, Sardinia and French Guiana. The population of Paraujano Indians in which high concentrations of Hb-A<sub>2</sub> have repeatedly been found is known to have had periodical sieges of malaria, having positive cases of *Plasmodium vivax* as recently as 1957.

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## PSYCHOPHARMACOLOGY

### Factors determining the Effect of Chlorpromazine on the Food Intake of Rats

CHLORPROMAZINE often alters body weight and food intake in patients<sup>1</sup> and animals<sup>2-5</sup>, but the results of the animal studies conflict. Actions of chlorpromazine on food intake are also interesting because there has been much work on the effects of this drug on animal learning and performance with food used as the reward, and these could be confounded with effects on hunger.

Experiments in which rats were maintained on daily cycles of severe food deprivation have indicated that chlorpromazine decreases the food intake<sup>2,3</sup>. When unlimited food was available, or when the animals could at least partially satisfy their appetites, chlorpromazine increased the food intake<sup>4,5</sup>. Some authors<sup>6</sup> have suggested that food deprivation and the consequent level of hunger determines whether a rise or a fall of food intake occurs after chlorpromazine is given. The falls occurred in rats which had become accustomed to eat a large amount at a time of day when normally they ate little, and so it seems necessary to consider whether the effect of the drug was related to this.

In none of these experiments, however, were direct comparisons made between rats maintained on different feeding cycles, so other differences between the various experiments might account for the conflicting results. The experiments described here were planned so that the same measures of food intake could be obtained, regardless of the cycle of food deprivation on which the rats had previously been maintained.

One group of rats, the "limited time" of feeding group, was allowed to eat as much as it could, but only during 2 hours each day during the week before testing. A second group ("limited amount" of feeding) had its food intake reduced in a different way; the possibility of learning to eat a large amount in a short time was eliminated by limiting the quantity of food supplied to that needed to maintain body weights at 80 per cent of their initial weight. A third group of rats was given unlimited food at all times—the "satiated" group. All groups had free access to water throughout.

Each rat was tested twice, once after subcutaneous injection of 5 mg/kg chlorpromazine and once after saline injection. The order of the treatments, which were separated by 2 clear days during which the appropriate feeding cycles were reimposed, was counter-balanced within each group. On the test days, food and water were made available in unlimited quantities 30 min after injection to all groups of rats for 24 hours, and food and water intake and the amount of food spilt were measured at intervals. Male hooded rats were used, 70–100 days old, housed singly at temperatures of 70°–74° F. Powdered rat diet was available from open cups.

The feeding regimes described reduced the body weights of the limited time and limited amount of feeding groups to 75.5 per cent and 74.5 per cent, respectively, of their initial free-feeding weights, so that these two groups were at effectively the same level of food deprivation when tested. After saline injection, there was very little difference in the intake of food and water between the two groups (Table 1). Thus there was no evidence that the rats had learned to eat more during the 2 h feeding period than would be expected from the high level of hunger.

Table 1. FOOD AND WATER CONSUMED DURING ACCESS FOR 2 H TO UNLIMITED FOOD AND WATER BY RATS PREVIOUSLY MAINTAINED ON DIFFERENT CYCLES OF FOOD DEPRIVATION

	Limited time of feeding group	Limited amount of feeding group	Satiated group
Food intake	14.1 g	13.2 g	2.6 g
Water intake	12.7 ml.	11.1 ml.	2.3 ml.

As one would expect, the satiated group ( $n=7$ ) ate and drank significantly less ( $P<0.001$ , Mann-Whitney U-test) than the deprived groups. The differences between the limited amount ( $n=6$ ) and limited time ( $n=7$ ) groups were not significant ( $P>0.2$ ).

Fig. 1 shows the food intake after the administration of chlorpromazine as a percentage of the intake after saline injection. The Walsh test<sup>4</sup> for related samples was used to test the significance of drug effects because homogeneity of variance need not be assumed. The overall pattern is clear; during the first 2 h of testing the intake of food by satiated and both types of deprived rats was depressed by chlorpromazine, but after that the intake rose and remained relatively high for 22 h. The overall food intake during the 24 h of the tests rose by about 15 per cent ( $P<0.05$ ), so that the rises more than compensated for the initial falls. Chlorpromazine also increased the amount of food spilt. In another experiment, neither smaller nor larger doses stimulated food consumption during the period of 2 h after injection, which suggests that the time course described was not merely a consequence of changing concentrations of the drug in the tissues.

These results were unexpected: the time after injection was an important factor which determined whether chlorpromazine inhibited or stimulated food intake. Screening tests for appetite-reducing drugs are usually carried out within 2-3 h of administration, and may thus give misleading results with chlorpromazine. In patients<sup>5</sup>, chlorpromazine usually causes a gain in weight and sometimes even obesity, and it is also used in the treatment of anorexia nervosa, but these effects may be due to water retention<sup>7</sup> or other factors.

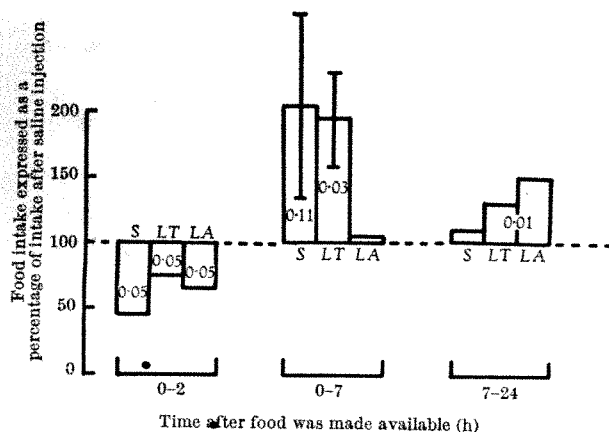


Fig. 1. Changes in food intake of three groups of rats during three consecutive time intervals after 5 mg/kg chlorpromazine. LT, Limited time of feeding group ( $n=7$ ); LA, limited amount of feeding group ( $n=6$ ); S, satiated group ( $n=7$ ). The dashed line at 100 per cent is food intake after saline injection. The levels of statistical significance of the changes from food intake after saline are shown (Walsh test, two-tailed). Measurements of food intake began 30 min after injection. It can be seen that food intake first fell and then rose, but was also influenced by the particular feeding cycles on which the rats had been maintained for 1 week before the drug experiments began.

Surprising differences between the groups emerged 2-7 h after the start of testing. The satiated group showed very variable responses. The large standard deviation is shown in Fig. 1; according to the Walsh test the effect of the drug was not significant, although the mean rose. The rise in the limited time of feeding group was consistent and statistically significant, but there was no effect in the limited amount group. Thus appropriate deprivation of food made it easier to obtain statistically significant increases, but with only one particular type of deprivation did the drug have any effect at all. This is a case where the overt behaviour of two groups of rats with different previous experiences was not detectably different after saline injection (Table 1), but where previous experience was apparently a factor in determining responsiveness to a drug. Previous experience also modifies reactions to psychoactive drugs in other behavioural tests<sup>8</sup>.

Water intake also depended on the time of testing after injection. At first chlorpromazine inhibited the water intake of all groups, but later the water intake rose, at about the same time as food intake rose.

Because 5 mg/kg of chlorpromazine is a fairly large dose, a general depressant action could have produced the fall in consumption of food and water which occurred soon after injection. This could hardly apply to the subsequent increases which more than compensated for the fall, despite the fact that general activity is usually found to be depressed for several hours after chlorpromazine is given. Furthermore, drugs which produce heavy sedation may at the same time stimulate eating<sup>4</sup>.

The experiments described here therefore suggest that the food and water intakes of rats treated with chlorpromazine are mainly dependent on the time of testing after injection, but can also be influenced by previous experience of particular types of feeding cycles. Hunger induced by both limited time and limited amount feeding cycles is widely used to motivate rats used for learning experiments. Although one should be cautious in inferring effects on motivation from changes in the intake of food or water<sup>9</sup>, the present experiments show that fluctuating levels of hunger and thirst may be expected up to at least 7 h after injection of chlorpromazine and should be considered when interpreting the results of experiments on learning in animals.

I should like to thank Dr Hannah Steinberg for advice and discussion. This work was supported by a Smith, Kline and French studentship and a research grant from the National Institute of Mental Health, US Public Health Service.

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## PSYCHOLOGY

### Time required for Judgements of Numerical Inequality

AN educated adult can tell which of two digits is the larger with virtually no uncertainty. By what process is this accomplished? On the one hand, it is conceivable that such judgements are made in the same way as judgements of stimuli varying along physical continua. On the other hand, numerical judgements may be made at a



different, less perceptual and more cognitive, level. For instance, the task may be one of memory access, each possible pair of numerals being stored with a corresponding inequality sign; or perhaps some sort of digital computation is performed, such as counting the space between the two numerical values.

One way of exploring the nature of such processes is by examining the time which they require. Judgements of inequality for length of lines, pitch and colour are known to require longer time the smaller the difference between two stimuli<sup>1</sup>. Moreover, such judgements tend to show a psychophysical function—a given difference between two stimuli evokes a quicker reaction the smaller their absolute values<sup>2</sup>. Thus, if humans judge differences between numerals in the same way as differences along physical continua, reaction times should be inversely proportional to the difference between two numbers and smaller for smaller numbers. If, instead, the process involves direct memory look-up, there is little reason to expect such relations. Finally, if the process involves counting of the number space between the two numerals, then reaction time should be directly proportional to the difference. To explore this question, we have measured the time required for judging which of two single digit numerals is the larger.

Ten female undergraduates of Stanford University served as subjects. Each stimulus consisted of two numerals each of one digit typed 2.5 cm apart on a white background. Every non-repeating pairing of the numerals 1 to 9 was used. Each digit appeared twenty-four times on the left and twenty-four times on the right; each pair three times as  $x \dots y$  and three times as  $y \dots x$ . The order was random. Subjects were instructed to throw the left-hand or right-hand of two switches according to whether the digit on the left or right was the larger. They were told to respond as quickly as possible without making errors.

The stimuli were presented through a half-silvered mirror, and were not visible until a light and timer were simultaneously activated.

Fig. 1 shows that the decision time was an approximately linear inverse function of the numerical differences between the two stimulus digits. The negative correlation is significant ( $r = -0.63$ ) ( $P < 0.01$ ). Error data (Fig. 2) show a similar trend.

We explored a number of possible equations for describing these data, and found that a reasonable fit is of the same general class as those usually found to describe discrimination reaction times for differences between physical quantities such as pitch of tone and length of line (much of the relevant literature is reviewed in ref. 3). For example, the equation  $RT = K \log$  (larger/larger-smaller) which Welford<sup>3</sup> proposes as a general model for

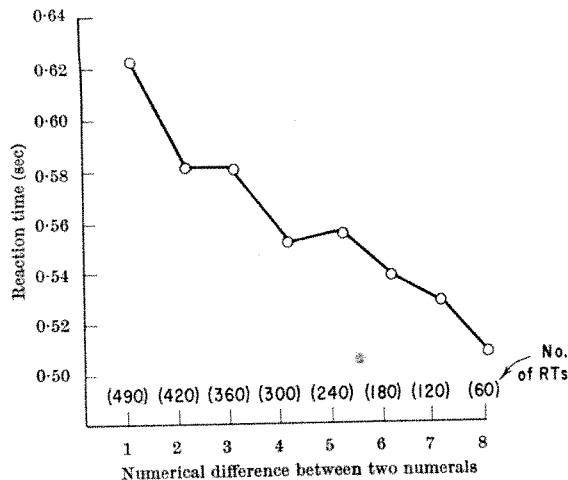


Fig. 1. Reaction time as a function of numerical difference between the two stimulus digits.

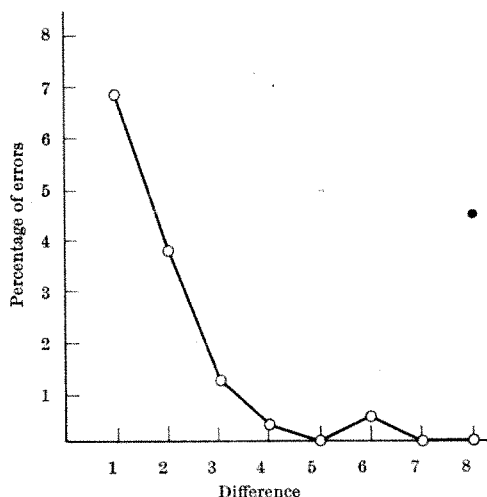


Fig. 2. Distribution of errors as a function of numerical difference between the two stimulus digits.

such situations yields a product-moment correlation coefficient of 0.75 for our data. Thus the function resembles classical psychophysical functions, in that the ratio of the two stimulus numerals is more closely related to  $RT$  than is the absolute difference between them.

These results strongly suggest that the process used in judgements of differences in magnitude between numerals is the same as, or analogous to, the process involved in judgements of inequality for physical continua.

A possible artefact in these results arises from the fact that in the set of pairs used the numerically larger digits were more often "correct" (that is, they corresponded to the proper switch) and were also more often found in pairs with larger differences. If subjects respond more quickly to single digits with higher probabilities of being "correct", this correlation among the stimulus materials would produce a spurious appearance of shorter times for pairs with larger differences. To control for this possibility, a second experiment was run in exactly the same manner as the first, except that a new set of pairs was used. For comparisons between certain pairs of this set the size of numerical differences between the two members varied while the likelihood of the component single digits being correct over the entire set was constant. For comparisons between certain other pairs the effect of probability of their members being "correct" over the entire set varied while the numerical difference between the numbers was constant. Numerical differences between pair members produced a statistically significant effect ( $P < 0.05$ ), while variations in probability correct did not ( $P < 0.10$ ). It thus seems safe to assume that numerical difference between pair members was the critical variable in the first experiment.

The decision process which these data suggest is one in which the displayed numerals are converted to analogue magnitudes, and a comparison is then made between these magnitudes in much the same way that comparisons are made between physical stimuli such as loudness or length of line.

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## BOOK REVIEWS

### HEROIC ANTARCTIC EXPLORATION

#### South to the Pole

The Early History of the Ross Sea Sector, Antarctica. By L. B. Quartermain. Pp. xx+481+26 plates. (London: Oxford University Press, 1967.) 75s. net.

MR LESLIE QUARTERMAIN is a scholar and Antarctic enthusiast who has delved deeply into published and unpublished records of the heroic era of Antarctic exploration. He is also a practical historian who, in his capacity as information officer with the Antarctic Division of New Zealand's DSIR, has restored huts used by Scott and Shackleton so that all who visit McMurdo Sound can see how these expeditions lived.

*South to the Pole* tells a story which runs from the time of the early Polynesians to the 1920s but has the unusual geographical limitations of 160° E. to 150° W. and south of 60° S. Within this area, the lands and islands form the Ross Dependency which is administered by New Zealand. Especially in these days of the Antarctic Treaty, one could criticize the division of Antarctic history into sectors, but the later history of the sector does in fact have a unity of its own. The earlier history consists of relevant sections of the voyages of Cook, Bellingshausen, Wilkes, Balleny and one or two others, plus the first major exploration of the Ross Sea under Sir James Clarke Ross in 1840-42.

After Ross, little exploration of the sector took place until the turn of the century, although many scientists pressed for action, as we see from articles in *Nature* by the German hydrographer in 1872, by H. R. Mill in 1896, and by resolutions of the International Geographical Congress in 1895. This pressure culminated in a series of national expeditions starting in 1901, Scott's British Antarctic Expedition of 1901-04 being the first major one to explore to the south of New Zealand. Before this took place, however, a Norwegian schoolteacher from Australia, Borchgrevink, secured the backing of the press baron Sir George Newnes and organized the Southern Cross expedition of 1898-1900. The party had its shortcomings, but nevertheless was the first to overwinter on the Antarctic continent and it seems to have received less than due recognition from the President of the Royal Geographical Society, Sir Clements Markham, and from Scott.

While this expedition was in the field, the Royal Geographical Society in co-operation with the Royal Society succeeded in raising funds from private and government sources to send out the British Antarctic Expedition of 1901-04. Relations between the two societies did not stay happy, because of differences over finance and the leadership of the land parties. These disputes seem to have had some lasting effect, for although the two societies have co-operated in many ways since, no further expeditions have taken the field with the two societies accepting joint responsibility for their organization until the Royal Society/Royal Geographical Society Brazil Expedition of 1967.

The stories of the period 1901-14 are well known, but they are retold in an interesting way with ample references to original source material. The assessment of the person-

alities and respective merits of the leaders of this period seems very fair and free of the more usual emotional assessment. Due prominence is given to the little known but heroic story of the Ross Sea section of Shackleton's Imperial Trans-Antarctic Expedition of 1914-17. Their shore party was equivalent to Hilary's section of Fuchs' subsequent Trans-Antarctic Expedition. Radio communications were too primitive to establish communication with the outside world. The ship which was intended to stay with the wintering party in McMurdo Sound was driven to sea in a storm in early autumn, leaving those on shore with inadequate stores and clothing. These men, not knowing that Shackleton had failed to land from the *Endurance* in the Weddell Sea, succeeded against gross odds in laying the depots expected by the trans-Antarctic group. One of the shore party died during the return journey, and two more lost their lives by attempting the last stage between huts in McMurdo Sound before the sea ice was firm enough. Finally, Captain J. K. Davis on the *Aurora*, with Shackleton on board, rescued the survivors in January 1917.

The history is taken into the 1920s with an account of the development of early whaling operations in the Ross Sea. It stops before the opening of the mechanical era which began with Byrd's first expedition.

The book does not attempt to provide a history of early scientific research in Antarctica in relation to the present day efforts. Quartermain provides instead an interesting, accurate and balanced assessment of the various expeditions and their leaders in the light of present day information on Antarctica. As a volume it will clearly join the classics of the history of general Antarctic exploration.

G. DE Q. ROBIN

### HIGH ENERGY PHYSICS

#### High Energy Physics

Vol. 1. Edited by E. H. S. Burhop. (Pure and Applied Physics; a Series of Monographs and Textbooks. Vol. 25.) Pp. xi+499. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1967.) 176s.

High energy physics is the title which, for want of a more appropriate description, is nowadays assigned to that branch of physics which formerly went under the name of elementary particle physics. It is the frontier of physics, concerned with the most basic laws, and it is not surprising that it has attracted and continues to attract the most talented and ambitious among physicists. Although high energy physics is still far from answering the questions which it poses for itself, it has made great progress in the thirty or forty years of its existence and continues to do so. It is now a vast subject embracing a wide class of phenomena ranging from very low energies of the order familiar to low-temperature physicists to the very great energies obtainable only from cosmic rays and from the giant accelerators. It has an army of practitioners and its demands on the exchequer are now the subject for national and international debate. I am therefore inclined to agree with Professor Burhop that "it is now extremely difficult to envisage a work both comprehensive and up to date, written by a single author" on this subject.

Professor Burhop has sought to cover the main branches of high energy physics by collecting seventeen chapters on various subjects contributed by experts in their respective fields. The choice of subjects and the manner in which they are treated necessarily reflect the preferences of the editor and of the various authors.

Of the five chapters in the present volume, the introductory chapter by V. F. Weisskopf is a popular account, reprinted from *Science*, of the present status of elementary

particle theory. In simple language, in a few pages, Professor Weisskopf explains the questions which must be answered and describes current attempts to do so.

The first substantive chapter (170 pages) by G. Breit and R. D. Haracz is appropriately devoted to "Nucleon-Nucleon Scattering". It was precisely in order to learn more about nuclear forces that the first accelerators were built. It was believed that all that one needed to ascertain the detailed properties of nuclear forces was to perform nucleon-nucleon scattering experiments at sufficiently high energy. We now know that this is frustrated by production of  $\pi$ -mesons or pions, and of the totally unexpected "strange" particles. Even at energies low enough for meson production to be neglected, the nucleon-nucleon interaction has turned out to be a very complicated one, at least according to the current description. This chapter describes, with the thoroughness which we associate with Professor Breit, the great amount of work, much of it by Breit and his collaborators, which has gone into the analysis of nucleon-nucleon scattering experiments.

The next chapter (146 pages) on "Pion-Nucleon Interactions" is by J. Hamilton. The discovery of pions, predicted by Yukawa as the quanta of the nuclear force-field, was a great triumph of theoretical physics which earned Nobel Prizes both for its prophet and for its discoverer. A knowledge of pion-nucleon interactions is essential for a fundamental understanding of the nucleon-nucleon force and much of the credit for our present comprehension of the pion-nucleon interaction goes to Professor Hamilton and his school. It was suggested in the mid-fifties that the dispersion relations which express the condition of relativistic causality could provide the basis for a dynamical theory of strong interactions. Since then many exaggerated claims have been made on its behalf, but almost the only quantitative application of these relations has been to elastic pion-nucleon scattering, of which this chapter gives an authoritative account.

A much shorter chapter (50 pages) by T. A. Griffy and L. I. Schiff is entitled "Electromagnetic Form Factors". The authors have been closely associated with the high energy electron scattering experiments, first systematically studied at Stanford, which have played an important part in the determination of nuclear and nucleon structure. The interaction is known so that information is directly available on nuclear charge and current distributions, the parameters describing which are usually called form factors. Most of the chapter is devoted to the analysis of elastic electron scattering from nucleons, deuterons and trineutrons, and to a discussion of the dynamical origin of the nucleon form factors. Electro-production of pions is briefly mentioned.

The concluding chapter (90 pages) by P. T. Matthews is on "Unitary Symmetry". The first half, together with an excellent appendix, provides a clear and elegant exposition, assuming no previous knowledge of group theory, of the SU(2) and SU(3) groups and their application to particle physics, the high point of which was the discovery of the theoretically predicted  $\Omega^-$  particle in 1964. The latter half of the chapter describes theories, which were the sensation of 1964-65, which attempted to combine internal and space-time symmetries. As stated briefly in a note added in proof, it is now clear from the work of Professor Matthews and others that these theories all violate one or other of our most basic theoretical expectations. This part of the chapter is therefore mainly of pedagogical or historical interest.

The editor states that this is primarily a reference book but expresses the hope that the individual chapters provide sufficient basic material to be useful to graduate students entering the field. This is certainly correct but, because the price may deter some students, this purpose might be better served by re-issuing the main articles as separate small books.

P. K. KABIR

## MÖSSBAUER INDEX

### Mössbauer Effect Data Index 1958-1965

By Arthur H. Muir, jun., Ken J. Ando and H. M. Coogan. Pp. xviii+351. (New York and London: Interscience Publishers, a Division of John Wiley and Sons, 1966.) 72s.

THE implications of Mössbauer's discovery, in 1958, of recoilless emission and absorption of  $\gamma$ -rays were rapidly recognized. Here is a spectroscopic technique with sufficient but not too much resolution for studying the interactions between nuclei and their electronic environments. Workers and results have multiplied explosively. A "nine year wonder" is an inappropriate description of the situation, because interest shows no sign of diminishing.

In this volume, members of the North American Aviation Science Center have published a complete index of Mössbauer work for the period 1958-65. Limited, privately circulated editions have appeared previously. An IBM computer has been used for information storage and retrieval and the body of the work consists of reprinted computer output. As a result, there are many oddities of notation and abbreviation. These do not, however, make the index difficult to use—indeed, it is of outstanding value.

The references to journals are first classified by experiment—nuclear transition involved, source and absorber materials, temperatures and so on. References to theoretical work, instrumentation, books and reviews are listed at the end of this section.

In the "Master Reference Section", all references are listed by their code numbers, involving year of publication and the initial letter of the first author's name. The effect is to produce a more or less chronological ordering. In the final section, references are listed under the names of each author.

Much additional useful information is to be found tucked away in this volume, which will surely find its way on to the shelves of all Mössbauer workers.

R. E. MEADS

## DEVELOPMENT OF A TECHNIQUE

### Electron Spin Resonance

A Comprehensive Treatise on Experimental Techniques. By Charles P. Poole, jun. Pp. xxvii+922. (New York and London: Interscience Publishers, a Division of John Wiley and Sons, 1967.) 240s.

THE sub-title of this book, "A Comprehensive Treatise on Experimental Techniques", is a correct description. The work is reasonably comprehensive and the accent is on experimental techniques.

We are told in the preface that the book is primarily intended for those who might wish to build their own spectrometers or to modify existing spectrometers. Much less attention is given to the results which have been obtained or to their theoretical interpretation. Nevertheless, nearly 1,000 pages have been written and the book will be of great use to practitioners in the field of electron spin resonance instrumentation. This will mean that physicists will be more interested than chemists because most chemical laboratories interested in this technique are now equipped with commercial instruments of very advanced design which few people would hope to better, or even equal, on their own.

Had the book appeared some twelve years ago at a time when chemists were becoming really interested in this technique, it would have been invaluable, and most of what has been said now could indeed have been said at that time.

In covering this field at great depth, the author has been somewhat indiscriminate and many of the chapters

seem to be unnecessarily long and parts of them are unnecessarily elementary. Many of the circuits are of historical interest and would not be used in practice, and those areas where instrumentalists are currently making very important advances, such as ENDOR, are, unfortunately, neglected. So, for example, ENDOR is given about thirty-four pages, which is about the same space allocated to vacuum systems. This means that some recent important work in this field has been overlooked. In short, this book, despite its high price, can be strongly recommended to physicists who wish to learn about the historical development of this technique and who wish to construct their own instruments for special purposes, but will be of little use to the experimentalist who wishes to use electron spin resonance in a routine manner.

M. C. R. SYMONS

## ABSTRACT ANALYSIS

### Functional Analysis

(Academic Press Textbooks in Mathematics.) By George Bachman and Lawrence Narici. Pp. xiv + 530. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1966.) 116s.

THIS book is intended as an introduction to functional analysis, the part of mathematics concerned with infinite dimensional vector spaces. After some preliminary material on finite-dimensional spaces and on metric and general topology, there is a thorough discussion of the fundamental properties of Banach spaces, including the Hahn-Banach theorem, the principle of uniform boundedness, the closely related continuous inversion and closed graph theorems, and related questions. A brief section on spectral theory is followed by an introduction to Banach algebras, going as far as the Gelfand representation. The last third of the book is devoted to spectral theory in Hilbert space; a number of proofs of the spectral theorem for self-adjoint operators (bounded and unbounded) are discussed in detail. The treatment of normal operators contains some novel features derived from recent research.

The book is long for the material it covers; proofs are spelled out in great detail, and there is much incidental comment. There are numerous exercises for the reader, mainly of a quite straightforward character. The book will be useful for reference to student and teacher alike, but might be found rather heavy going by a beginner wishing to acquaint himself with the elements of the subject.

F. SMITHIES

## METAL-METAL COMPOUNDS

### Intermetallic Compounds

Edited by J. H. Westbrook. (Wiley Series on the Science and Technology of Materials.) Pp. xvii + 663. (New York and London: John Wiley and Sons, 1967.) 235s.

DR WESTBROOK claims, rightly, that this is the first comprehensive treatment of this subject in fifty years. It is therefore all the more welcome.

Considerable effort is now being directed towards metallic compounds of all types, largely as a result of the state of near exhaustion in the further development of metallic alloys. This volume deals mainly with the structure, transformations and properties of metal-metal compounds, but because many of these are operative agents in improvement of the properties of metallic alloys, they are of interest to both metallurgists and materials scientists.

The editor presents a historical sketch in the first chapter, concise and informative, and rather unusual for a

book originating in the United States, where history tends to be more introverted. Excellent reviews of bonding theory and experimental evidence for bonding characteristics are given in the second part, and although one or two are a little too brief, they form an excellent introduction to the subject for the research student. The chapters in the third part, on crystal structure, incorporate some well executed illustrations to assist understanding of the text in a way which puts the average undergraduate textbook to shame. Many matters of metallurgical interest are featured here.

The fourth part of the book, on micro- and sub-structure, is short, and perhaps the least successful is the chapter entitled "Lattice Defects", which adds little and omits much which is relevant. Formation and stability are well covered in the fifth part and so are kinetics and transformations in the next part. The final part of the book comprises seven chapters on properties, from mechanical to super conductivity, and much of this will be well thumbed in library copies. It is disappointing, however, to find little reference to thermal properties except for thermoelectricity, but in recent years this has become almost a constant feature of any book dealing with the properties of materials. It would appear that only mechanical strength and electronic applications are of any significance to industrial and academic research alike.

I welcome an index including not only author and subject entries but also a complete list of the compounds mentioned. The star-studded international cast includes twenty-four authors from the United States and nine from other countries, all in Europe, but the price of roughly 4d. a page is, by the standards of research costs, very cheap.

C. R. TITTLE

## RULES FOR CHEMISTS

### Chemists and the Law

By F. A. Robinson and F. A. Amies. Edited by H. M. Bunbury. Pp. xx + 231. (London: E. and F. N. Spon, Ltd., 1967.) 40s. net.

THE term "chemist" in the context of this recent publication must very properly be accepted as covering a multitude of scientific disciplines, for there is much in this book which will prove of the greatest value and interest, not only to academic and industrial chemists, but to biochemists, physicists, pharmacists, engineers, factory managers and many others. Indeed, this book can also be strongly recommended for the earnest attention of the intelligent layman, because it provides a wealth of legal information regarding such diverse matters as "Contracts of Employment", "Negligence", "Pollution", "Food Statutes", "Agricultural Substances", "Explosives", "Medicines and Dangerous Drugs", "Radioactive Substances", "Patents", "Copyright" and so on. The legislative aspects of all these matters are discussed most authoritatively and comprehensively, particular attention being drawn to specific differences between the statute law of England and Wales as compared with the corresponding provisions of the Scotland and Northern Ireland Acts. Numerous legal cases are cited which constitute precedents and which provide the reader with some prognostication of the judgments which might conceivably be given when legal cases of a certain pattern come before the courts.

The organization of the British courts system is clearly outlined; details are given of the preparation for trial and hearing, of the duties and responsibilities of such people as expert witnesses, assessors and scientific advisers. The fundamental items of statute law, for example, Food and Drugs Act, Patents Act, and the provisions of a host of other important acts of parliament and statutory instruments are given in the greatest detail, accompanied



by extensive bibliographical references and explanatory notes. Public analysts, inspectors, and governmental scientific officers who have specific duties in the administration of the regulations of such acts will find this comprehensive book indispensable. Similarly, those individuals and organizations who have obligations in common law or under various statutes will find this publication an essential *vade-mecum*. In this complex technological age, it can be all too easy to become ignorant of the law, sometimes with dire consequences.

A well bound book with legible text, it is very reasonably priced at 40s. There has been a real need for a generic, specialist work of this kind and the demand is certain to be such that the first issue must soon be exhausted.

D. T. LEWIS

## ENZYME KINETICS FOR MEDICS

### Enzyme Kinetics

A Learning Program for Students of the Biological and Medical Sciences. By Halvor N. Christensen and Graham A. Palmer. Pp. viii + 124. (Philadelphia and London: W. B. Saunders Company, 1967.) 24s. 6d.

THIS is an excellent introductory book on enzyme kinetics. Its excellence stems from the fact that it explains in a simple and lucid manner the relationship between enzyme kinetics and physicochemical principles. Moreover, the basic principles of enzyme kinetics are explained in simple terms—it is not assumed, as it is in many books, that basic principles are so well understood that they do not have to be explained. This is particularly important in teaching enzyme kinetics to medical students whose background knowledge of physical chemistry is usually poorer than those of other biological students. The book concentrates almost exclusively on the effects of substrate and inhibitors on enzyme activity with brief reference to pH and temperature effects. But this severe restriction to specific topics is advantageous, in that with a few hours concentrated effort the elementary basis of enzyme kinetics should be clear.

My impression from teaching preclinical medical students, who are so busy with their major subjects of anatomy and physiology that they have little time for anything else, is that unless a particular subject is written clearly and concisely they prefer to learn the essentials in a "parrot-fashion" without understanding the subject. This book by Christensen and Palmer should go a long way in solving this problem with respect to enzyme kinetics.

It is interesting that the authors touch on the subjects of biological transport and chemotherapy, hinting at the possible future applications of enzyme kinetics. I think this is a particularly relevant point to the importance of enzyme kinetics for medical students: in the not too distant future knowledge of metabolic regulation of enzyme catalysis may lead to a more rational approach to chemotherapy, and the medical practitioner may require some knowledge of enzyme kinetics in order to understand the mode of action of his drugs. It is perhaps important to stress that medical students being taught at the present time will be practising their medicine well into the twenty-first century.

The book is, in fact, a learning programme, but the contents are so well organized that it makes excellent reading material. The judicious repetition provided by the programme aids the retention of the subject matter, whereas the question-answer routine of the programme continually stimulates one's interest.

Finally, it should be stressed that it is only an introductory book, but serves this purpose admirably, both for explanation of the subject and for stimulation of interest in enzyme kinetics.

E. A. NEWSHOLME

## VIROLOGY DOUBLE

### General Virology

By Salvador E. Luria and James E. Darnell, jun. Second edition. Pp. xv + 512. (New York and London: John Wiley and Sons, 1967.) 100s.

### Perspectives in Virology

Virus-Directed Host Response. Edited by Morris Pollard. (The Gustav Stern Symposium.) Pp. xxvi + 344. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1967.) 84s.

LURIA's *General Virology* first appeared in 1953, and it is indicative of the quality of this work that it should have held its position as an excellent textbook for fourteen years. The second edition, written with a collaborator, follows the same general plan, using many of the same chapter headings, but it is extensively rewritten and is some eighty pages longer. The book is well illustrated with many photographs and clear diagrams, and several large tables help to bring out the underlying theme of the book, which is the essential unity underlying animal, plant and bacterial viruses.

The definition of a virus is more complex than that given in the earlier edition, and emphasizes the concept of parasitism at the genetic level. Every virus has two essential attributes, the genome, the genetic material of a virus, either DNA or RNA, which reproduces inside living cells and behaves as part of the host cell; and the virion, the specialized particle produced in the cell and used to transfer the genome to other cells. An extreme case of parasitism at the genetic level is represented by the genome of a temperate bacteriophage which may become physically integrated into the chromosomes of the host bacterial cells, its presence being carried through generations of bacterial cell divisions in a non-infectious form. A related form of parasitism occurs when animal cells are transformed by polyoma virus, the dividing cells continuing to produce at least part of the polyoma genome but no complete polyoma virions. Rous sarcoma virus is apparently a defective virus in that the genome is unable to produce mature virions unless the cell is also infected with a helper virus which completes the maturation process of RSV virions in addition to forming more helper virions. The experimental work underlying these different forms of parasitism is summarized in the text, and the interpretation of laboratory results is set out with commendable clarity. Students of virology, whatever their particular discipline or vintage, should benefit from reading this excellent book.

The second book, the proceedings of a symposium, is entirely different in character. Of sixteen papers presented on the theme of virus-directed host response, those by W. P. Rowe and P. K. Vogt are particularly notable. Rowe's paper on defective animal viruses is rather speculative, presenting preliminary results on four examples of helper-sensitive defective animal viruses. This is one of the growing points of virology at the present time, and it is clear from the published discussion which followed the paper that there is great interest and activity in this field. Vogt analyses the meaning of the title of the symposium and recognizes two different types of virus-directed host responses, which he terms "submissive" and "defensive". The first implies control and direction of the host metabolism by the viral genome, which may lead to malignant transformation of the cell. The second type does not require viral genetic activity in the host cell, and is exemplified by host reactions such as antibody and interferon production. This paper contains a mass of detail, and provoked active discussion by the other participants. This reporting of the discussion is one of the most valuable aspects of the proceedings of symposia, which too often tend to be re-presentations of previously published work.

J. S. PORTERFIELD

## NUCLEIC ACIDS AND PROTEINS

### Genetic Elements

**Properties and Functions.** Edited by D. Shugar. (Symposium held on the occasion of the Third Meeting of the Federation of European Biochemical Societies, Warsaw, April 4-7, 1966.) Pp. ix+361. (London: Academic Press, Inc. (London), Ltd.; New York: Academic Press, Inc.; Warsaw: PWN-Polish Scientific Publishers, 1967.) 84s.; \$15.

THE book is a fascinating collection of fifteen articles concerned with recent developments in the structure of nucleic acids, their replication, the genetic code and the mechanism of protein synthesis. The organizers of the symposium attempted to select topics which were of direct interest not only to the specialists but also to as many of the participating biochemists as could be envisaged. It is perhaps partly because of this policy that, despite the title, there is little concern with chromosomes, mutation, recombination, genetic suppression, regulatory mechanisms or the transcription of DNA into RNA. Nevertheless, the limited range has enabled the editor and contributors to produce a valuable and coherent book.

Several papers are concerned with the structure of nucleic acids. Fuller reviews the conclusions obtained from X-ray work on solid materials and includes an interesting study of the interaction between DNA and certain antibiotics. Luzzati, Witz and Mathis describe the value of X-ray techniques in determining the conformation of nucleic acids in solution. G. L. Brown, Lee and Metz discuss the study of active sites in RNA by chemical modification. Zachau, Dütting and Feldmann give an account of the techniques used in their determination of the primary sequence of serine transfer RNA and undertaken simultaneously with Holley's work on alanine RNA. Engelhardt and his collaborators describe important parallel Russian work on the valine transfer RNA leading to a probable primary structure which is essentially confirmed by the results of further work added at the proof stage. Perhaps because of a shortage of time at the meeting, neither of these two papers refers to conceivable secondary structures of transfer RNA such as the clover-leaf model that is now receiving much attention. In an important short paper, Sanger and Brownlee describe their extremely powerful methods for the fractionation and identification of radioactive oligonucleotides. These methods have more recently (*Nature*, 215, 735; 1967) led to the determination of the primary sequence of the 5S ribosomal RNA.

Borst, Kroon and Ruttenberg describe their own and other work on mitochondrial DNA and consider the relation between mitochondrial and nuclear DNA and the genetic function of cytoplasmic DNA in general. Bollum rather briefly discusses the replication of DNA while Martin attempts to reconcile the large volume of work on the replication of viral RNA in various systems. The genetic code and mechanisms of protein synthesis are considered in four papers given by Khorana, by Matthaei and his colleagues, by Ochoa and his collaborators, and by Monro, Maden and Traut. Littauer and Revel describe their work on the possible function of the methyl groups in transfer RNA. The only genetic map in the book is given by Yaniv and Gros in their fascinating paper on modified aminoacyl RNA synthetases in temperature conditional mutants of *Escherichia coli*. The discussion which followed each paper is recorded and this includes some pertinent invited comments by Leder and Clark on synonymous codewords and on phasing mechanisms.

The book has been produced well, although the print is more difficult to read than the styles of type that are usually used by Academic Press. Although the numbering of pyrimidine rings is only referred to occasionally, it is unfortunate that among the various papers two different numbering conventions have been used.

As a whole, the book gives an impression of high scientific quality. New information is presented and discussed in a stimulating and balanced manner in relation to pertinent work from other laboratories. Many useful references are given and there is both a subject and an author index. It should prove to be a valuable guide to many aspects of nucleic acid biochemistry.

K. BURTON

## INVERTEBRATE NEUROPHYSIOLOGY

### Invertebrate Nervous Systems: their Significance for Mammalian Neurophysiology

Edited by C. A. G. Wiersma. Pp. ix+370. (Chicago and London: The University of Chicago Press, 1967.) \$10; 72s. net.

THIS symposium volume contains twenty-seven papers on various aspects of invertebrate neurophysiology. About half of these, comprising just over two-thirds of the book, review recent progress in limited parts of the field. This type of article seems to be particularly suited to inclusion in a symposium volume, because it is less extensive and more detailed than the orthodox review article. The remainder of the book consists of brief reports on particular investigations, the matter of which, I suspect, will shortly appear at greater length in their proper place, in journals devoted to the publication of original observations.

The longer articles between them cover a considerable slice of invertebrate neurophysiology. G. A. Kerkut gives a useful account of recent work on the pharmacology and ionic bases of synaptic transmission in molluscan neurones, and F. Strumwasser considers the types of information that may be stored in them. M. J. Cohen describes his work on the RNA metabolism of regenerating insect neurones. Some of the ways in which the nervous system controls the development of arthropods are described by J. S. Edwards. I. R. Hagadorn gives a brief review of neurosecretory mechanisms, and I. M. Cooke describes his experiments on the relation between electrical activity and secretion in neurosecretory axons. Non-striated muscles are examined by C. L. Prosser, and G. Hoyle delivers a polemic against the assumption that all striated muscles are like the frog sartorius.

All these different approaches are concerned, more or less, with physiological analysis at the cellular level. The usefulness of invertebrate material for this type of study is two-fold. First, many individual invertebrate neurones are larger and more accessible than those of vertebrates. Consequently, certain physiological problems may be much more easily solved by the use of invertebrate material; our knowledge of the mechanism of nervous conduction, to pick the most obvious example, would be much less advanced than it is if it were not for the giant axons of squids. Second, the study of a variety of different types of cells serves to indicate the variety of possible types of neuronal activity. An example of this is provided by the discovery of electrically transmitting synapses in invertebrates; the possibility of such electrical transmission must now be borne in mind by anyone investigating neuronal interaction in vertebrates.

Other approaches are concerned with the properties of systems rather than individual units. Here the great advantage of most invertebrate nervous systems is their relative simplicity in comparison with those of vertebrates. Their investigation, therefore, may provide us with models of how parts of the vertebrate nervous system work. The remainder of this volume is concerned with studies of this type.

G. M. Hughes and W. D. Chapple describe the interconnexions of neurones in the ventral nerve cords of crustaceans and the ganglia of gastropod molluscs. The organization of central ganglia is discussed by D. M. Maynard in an article which includes a most interesting comparison of the control of cephalopod colour patterns

with the visual perception of pattern. C. A. G. Wiersma describes the processing of visual information in crustacea. D. M. Wilson discusses the control of rhythmic behaviour in flying locusts and running cockroaches; the central control of more complex behaviour is reviewed by F. Huber. J. Z. Young compares the nervous systems of cephalopod molluscs and mammals, especially with reference to his views on the mechanism of learning.

This volume, then, gives a good account of many of the problems which interest invertebrate neurophysiologists at the present time. Its stated aim, however, is more than this, in that it claims to deal with the significance of its subject in relation to mammalian neurophysiology. It is disappointing to have to state that it does not succeed in this, because the majority of the articles (those by Maynard, Young and Wiersma are exceptions) make little attempt at the comparison. The mammalian neurophysiologist may well find the contents interesting, but for the most part he will have to work out their significance for himself.

D. J. AIDLEY

## FOREST ECONOMY

### Forest Planning

By D. R. Johnston, A. J. Grayson and R. T. Bradley. Pp. 541. (London: Faber and Faber, Ltd., 1967.) 126s.

THE outstanding interest of this book is that it is based on a study of the principles, policy and main methods used by the Forestry Commission of Great Britain which is now engaged not merely in afforestation but in the full management of the extensive forests created since 1919. Consequently, the authors have shown a new and more realistic, commercial approach to forest management. Classic forest management has been chiefly concerned with the capital value and structure of the forest growing stock and its silvicultural management, whereas now the authors are also greatly concerned with labour costs which constitute so large a part of the capital invested in a managed forest. Forestry must be competitive with other enterprises and must therefore reduce labour intensity, analyse and respond to cost/benefit ratios and to price and demand, and organize and time the thinnings, final fellings and other operations in relation to profit.

The first part of the book deals with policy, in particular the past and present role of forestry in Britain. Here, in an otherwise valuable section, there is perhaps some confusion between basic purpose and policy—the means of accomplishing a purpose—and it seems a pity that basic forest values which provide the fundamental purposes of forestry to various types of owner are not studied and analysed before policy is considered.

The second part of the book deals with planning and analyses many of the practices and problems of forestry. There are particularly valuable studies of investment criteria, costs and benefits, the influence of time and consequent interest charges on nearly all forest operations and their effect on the internal rate of return. The calculation of net discounted revenue is clearly presented and a chapter is devoted to its use in the particularly important matter of deciding the time of final fellings, and there is another valuable chapter on the economics of thinnings. Some foresters may, however, think that the ecological values of mixed, and even irregular, and mainly indigenous naturally regenerated crops are too easily rejected in favour of pure, artificially regenerated and often exotic stands with their apparent substantial commercial advantages without undue risk.

The third and fourth parts of the book describe the application of planning, by administrative organization and working plans, and the collection of data. There is a very useful chapter on incentives for and movement of labour and an appendix on the productivity of labour. The chapter on accounts and cost accounting could have been improved by a study of the problems of presenting a

proper balance sheet for a forest enterprise and its growing stock.

The book is excellent, thought provoking yet practical and informative for all professional foresters and for more advanced students. The book is well produced, with many diagrams, tabular statements and a host of interspersed references.

F. C. OSMASTON

## TEACH YOURSELF BIOLOGY

### The Science of Biology

By Paul B. Weisz. Third edition. Pp. xxiii + 886. (New York: McGraw-Hill Book Company, Inc.; Maidenhead: McGraw-Hill Publishing Company, Ltd., 1967.) 80s.

### Bioscience

By Robert B. Platt and George K. Reid. (Reinhold Books in the Biological Sciences.) Pp. xvi + 528. (New York and London: Reinhold Publishing Corporation, 1967.) 76s.

BOTH these volumes are designed for the first year American college student. They are comprehensive surveys of current biology theory, doubtless intended to accompany parallel courses of laboratory work. There are many such books on the market and they need continual revision to keep abreast of the extraordinarily rapid development of their subject. These two books are reminiscent of an admirable symposium, *The Science of Life*, by Wells, Huxley and Wells, which was published in Great Britain in the 1930s and presented an equally encyclopaedic view of "biology" as it then was. This comparison emphasizes the many discoveries and new concepts which have changed our biological outlook in the three intervening decades.

*The Science of Biology* is an attractively produced, comprehensive post-A level primer, lucid and readable. It comprises two well-balanced parts, roughly analogous to the traditional "Structure and Function", "The Organization of Life" and "The Operations of Life". After a brief preamble on the meaning of biological science, the four sections of the first part proceed in logical sequence from the basic physical and chemical detail of matter through cell structure to organism, species, population, ecosystem, community, biosphere and habitat, culminating in a classification of living forms. The second part covers metabolism, self-perpetuation and the controls and co-ordination involved in the maintenance of steady states, reproduction, heredity and evolution. Verbal statements are supplemented and reinforced with a great deal of original diagrammatic presentation and the work is pleasingly illustrated throughout by line drawings and coloured photographs. At the end of each chapter are searching review questions on its content and very full lists of references for collateral reading; there are a useful glossary and a functional index. The book is a fully revised version of two previous editions and has a scholarly sophistication and objectivity which appeal to the initiated.

*Bioscience*, though similar in scope and intention, is perhaps more a beginner's book and presents its subject matter in more traditional sequence—a "blue" as contrasted with a "yellow" version. The editor claims that it is a modern, realistic account of the nature, structure, function and interactions of the organisms of this planet with their environment, with each other and, particularly, with man. The parts of the book survey, in succession, the diversity of life, organization into populations, communities and ecosystems, molecular and cellular biology, organ systems, plant and animal organisms, genetics and evolution. The book concludes with a chapter on man's place in the world ecosystem. The authors maintain that their arrangement is flexible and that different teachers could adapt it to suit their own courses. The text is illustrated by line diagrams and black-and-white photographs. There is a reading list, correlated with the various sections,

at the end of the book, and a useful index. A "statement of principle" and a historical orientation precede each chapter.

E. J. VINNICOMBE

## SCHOLASTIC COSMOGONY

### Summa Theologiae

(Latin Text and English Translation, Introductions, Notes, Appendices and Glossaries.) By St. Thomas Aquinas. Edited and translated by William A. Wallace. Pp. xxiii + 255. (London: Eyre and Spottiswoode; New York: McGraw-Hill Book Company, 1967.) 42s.

THE editor and publishers have done a notable service to both men of science and historians in producing this volume in their series of translations of the celebrated *Summa* of St Thomas Aquinas (1225-74). In all fairness, as the text is at pains to point out, certain reservations must be accepted if this example of mediaeval writing is to be appreciated at its true value. The first is that it is in the nature of a museum piece. Nobody, at any stage of sophistication, would rate it very highly, either for its originality or as a major contribution to scientific thought. That much was obvious to later commentators. Neither was it intended as an all-embracing treatment of Aristotelian physics such as that provided for the monks by Albertus Magnus. Its author was a theologian first and foremost, resolved upon an attempt, as heroic as it was profound, to fit the cosmogony of his age to the tenets of the Catholic Church. But St Thomas exhibits one characteristic different from the outlook of the patristic doctors: he shows great reluctance to commit himself to any one theory of creation (and there were many); he preserves a remarkably open mind as he examines in detail the *hexaemeron*, namely, the six (active) days of creation of the Genesis story.

The editor provides the Latin text and English translation on opposite pages—not a completely word for word translation, but one in careful harmony with the original syntax. From the whole assemblage there emerges a deep tension which St Thomas realizes with devastating honesty. It is that the unfolding of creation as presented in the Book of Genesis was a kind of metaphysical exercise, the primary purpose of which seems to have been to discover the Creator. (In any case, it is unlikely that we shall ever know exactly what the inspired writer was trying to convey.) The chasm between that view and ours is stupendous. Nevertheless, odd as it may appear, the development of subsequent theories, bizarre and irrational as most of them were, paved the way for Galileo and all who followed. St Thomas himself notes, with marked erudition, all the controversies of his time and those of former epochs. In one respect at least he is a mild rebel in preferring the more "scientific" Aristotle to the purely philosophical Plato. In this we discern the precursor of descriptive and taxonomic methods, later to fructify into medical research. There was an acute awareness of "foreign" influences, even the *Guide for the Perplexed* by Maimonides.

If this were all, however, we should not be much the wiser in 1967, and the editor's efforts would be judged as sadly unrewarding. But luckily, that is far from the case. The fact is that St Thomas Aquinas stands revealed as the forerunner—by about six and a half centuries—of D'Arcy Wentworth Thompson and his *On Growth and Form*, now available as a Cambridge paperback. For example, the early fathers knew the essentials of the social habits of bees, centuries later to become a focus of mathematical research into problems of symmetry and close-packing. Again, engineers make allowance, even during construction, for the stresses and strains inseparable from those which the finished edifice will have to sustain, an analogue of an expanding creation of which we find traces in primitive thought.

In brief, nobody is likely to derive very much profit from reading St Thomas's *Cosmogony* in isolation. If, however, one takes it in one hand, and a copy of *On Growth and Form* in the other, then there is a treat in store.

The Queen of Sheba, at the court of King Solomon, is said to have exclaimed that "the half was not told me". Even today, she would not be far wrong.

F. I. G. RAWLINS

## RESTRICTED COGNITIONS

### Cognitive Consistency

Motivational Antecedents and Behavioral Consequents. Edited by Shel Feldman. Pp. xiii + 312. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1966.) 68s.

TEN American social psychologists wrote the nine chapters of this volume. Their theme, stated in a useful introductory review by W. J. McGuire, is the notion that a person tends to minimize internal inconsistencies among his beliefs, feelings and actions. The material is not well presented. Much of the writing is wordy and obscure. Some of the factual information would have been better published in original papers in specialist journals. For example, S. Feldman, in a chapter entitled "Motivational Aspects of Attitudinal Elements, and their Place in Cognitive Interaction", describes an investigation to test "whether belief strength or importance is a better predictor as to which of the belief statements will be retained in the response set from one test to the next". This was done by asking people at the Pennsylvania State Farm Show about their views on whole milk and skim milk. The figures obtained are given complete with a value for *t* and other statistics. The results "seem to confirm the hypothesis that the measurement of the importance of a cognitive element may be differentiated from the measurement of its belief strength, as well as from its salience".

Some important topics are touched on. E. Aronson ("The Psychology of Insufficient Justification") describes an experiment with children on the effects of threat either of severe punishment or of mild punishment. The latter had the greater influence on behaviour. Other important questions include the character of responses to skin colour, discussed by M. Fishbein ("Beliefs, Attitudes, and Behavior"), and the effects of large, propagandist generalizations, discussed by R. P. Abelson and D. E. Kanouse ("Subjective Acceptance of Verbal Generalization"). These passages lead, however, to no decisive conclusions or guides to social action.

There is surprisingly little comment on relevant matters outside the range of social psychology in its narrowest sense. J. E. Singer ("Motivation for Consistency") does raise some physiological questions, in a chapter which is on a consistently high level of abstraction. At the other end of the psychological spectrum, a reader might well look here for a coherent review of the role, if any, of psychoanalytic concepts in this field, but he would find only a single paragraph by A. Pepitone ("Problems of Consistency Models") and a mention by Feldman. Other cognate topics barely mentioned include the effects of sensory deprivation and the related notion of the "drive for stimulation".

The general impression is of a great deal of talent trapped in an unnecessarily restricting theoretical framework. The authors themselves are obviously discontented with their own achievements. K. E. Weick, at the end of his chapter ("Task Acceptance Dilemmas"), writes: "Whether... the approach will be productive remains to be seen". M. J. Rosenberg ("Some Limits of Dissonance") describes his conclusions as "debatable propositions". And W. J. McGuire suggests "that perhaps we have not answered any question to anyone's satisfaction". S. A. BARNETT



## University News :

### Australian National

PROFESSOR F. FENNER, at present professor of microbiology in the John Curtin School of Medical Research, has been appointed head of the school in succession to Sir Hugh Ennor, who was appointed secretary to the Department of Science and Education of the Commonwealth of Australia earlier this year.

### Loughborough

PROFESSOR E. J. RICHARDS, professor of applied acoustics and director of the Institute of Sound and Vibration Research in the University of Southampton, has been appointed vice-chancellor of the university in succession to Dr H. L. Haslegrave, who retires on September 30.

### Memorial

DR K. B. ROBERTS, at present reader in physiology in the London Hospital Medical College, has been appointed associate dean of medicine and head of the Department of Physiology in the Memorial University of Newfoundland, and will take up his appointment in September 1968.

### Nottingham

PROFESSOR E. MAURICE BACKETT, at present professor of social medicine and head of the Department of Public Health and Social Medicine in the University of Aberdeen, has been appointed to the chair of community health in the medical school.

## Appointments

DR S. FAWCETT, at present director of Battelle-Northwest, has been appointed executive vice-president of Battelle Memorial Institute, and Dr F. Albaugh has been appointed director of the Pacific Northwest Laboratory of the Battelle Memorial Institute.

DR E. E. FERGUSON has been appointed director of the Aeronomy Laboratory of the US Environmental Science Services Administration's research laboratories in Boulder, in succession to Dr E. K. Smith.

## Announcements

THE following awards have been announced by the Gairdner Foundation: DR C. R. DeDUVE, professor of chemistry in the Rockefeller University, a \$20,000 special award of merit for the discovery of minute structures inside cells called lysosomes; DR G. E. PALADE, professor and head of the Laboratory of Cell Biology in the Rockefeller University, a \$20,000 special award of merit for his contribution to the understanding of the synthesis and movement of protein destined for export in cells; DR M. NIRENBERG, chief of the Laboratory of Biochemical Genetics, US National Institutes of Health, a \$20,000 special award of merit for demonstrating the method of manufacture of protein within cells, and some of the ways in which the rate of production and the composition of various proteins are controlled inside the cell; DR D. H. COPP, professor and head of physiology in the University of British Columbia, and DR I. MACINTYRE, professor of endocrine chemistry and joint director of the Endocrine Unit, Royal Postgraduate Medical School, London, share equally a \$5,000 annual award, Dr Copp for his discovery of a new hormone called thyrocalcitonin and Dr MacIntyre for demonstrating that this hormone is produced by cells present in the thyroid gland; DR P. J. MOLONEY, a consultant at Connaught Medical Research Laboratories, Toronto, a \$5,000 annual award for his study of the structure of insulin; DR J. AXELROD, chief of the section on pharmacology, Laboratory of Clinical Science, US National Institutes of Health, and DR S. UDENFRIEND, chief of the Laboratory of Clinical Biochemistry, US National Institutes of Health,

share equally a \$5,000 annual award for their study of chemical reactions in the body which lead to the detoxication of drugs and the handling of certain active chemicals which may be concerned with the control of blood pressure and the activity of the cardiovascular system; DR J. FRASER MUSTARD, professor of pathology in McMaster University, a \$5,000 annual award for his work in arteriosclerosis.

THE exhibition "The History and Development of Geological Cartography", which opened on July 28, is on display to the public until October 27, Monday to Friday, 9-5 pm, in the University Library, Whiteknights, Reading. This exhibition of geological maps is in honour of the eightieth birthday of Emeritus Professor H. L. Hawkins.

ERRATUM. In the article "Half-lives of Peptides and Amines in the Circulation" (*Nature*, 215, 1237; 1967), the first sentence of the third paragraph on page 1238 should read: "The design of the experiments to find the half-life of bradykinin in the circulation of the cat was as follows".

# CORRESPONDENCE

## What is Science Policy?

SIR,—On my return from an overseas business tour attempting the difficult task of selling European aircraft in the United States, I have seen in your issue of September 2 your leading article "What is Science Policy?" (*Nature*, 215, 1013; 1967), and I would like to challenge the point you make about Concord.

It has never been suggested that the full cost of Concord research and development will be recovered through aircraft sales, although I believe personally that at least a very substantial recovery will be made. With Concord Britain and France have a real opportunity of securing a worthwhile share in the civil aircraft market, a major growth market.

Our detailed market assessments based on realistic even pessimistic premises (such as, for example, the assumption that there will be a complete ban on overland supersonic cruise) indicate a minimum market of 200 Concorde by 1975, rising to a possible 350/400 by 1980. These estimates are borne out by independent American market research. The sale of 200 Concorde would represent a contribution of at least £100 million annually for seven years to Britain's balance of payments, and you would probably concede that this would be "some kind of economic return".

As a direct result of Concord research and development expenditure, advanced technological activities have been initiated and pressed forward in many and diverse fields in materials, electronics, miniaturization, automation machine tools, computerized operational research and structural test techniques, to give a random selection. The greater part of this work has a direct applicability and value for industrial fields other than aviation, but, as you will appreciate, this value cannot be expressly stated in money terms.

In a recent speech Mr. Wedgwood Benn said: "Foreign defence expenditure is strengthening the advanced technology of our competitors and losing us export markets". Everyone who is trying to sell in international markets knows this is true. Concord expenditure is already doing much, and will do more, to improve our competitive position.

Yours faithfully,

E. H. BURGESS

British Aircraft Corporation (Operating), Limited,  
Filton Division,  
G.P.O. Box No. 77,  
Filton House, Bristol.

## FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

## Monday, October 2

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 9.30 a.m.—Colloquium on "Definition, Realization and Use of Time and Frequency".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 5.30 p.m.—Mr. D. K. S. Bain: "Power System Model".

INSTITUTION OF MECHANICAL ENGINEERS, STEAM PLANT GROUP (at 1 Birdcage Walk, Westminster, London, SW1), at 6.30 p.m.—Discussion Meeting on "The Combination of the Gas Turbine and Industrial Steam Production".

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (at Shell Centre Theatre, Shell Centre, London, SE1), at 6.30 p.m.—Scientific Films.

## Tuesday, October 3

BRITISH BIOPHYSICAL SOCIETY, PHYSICAL BIOCHEMISTRY GROUP (at Queen Elizabeth College, Campden Hill Road, London, W8)—Meeting on "Dielectric Properties of Biological Macromolecules".

SOCIETY OF CHEMICAL INDUSTRY, PESTICIDES GROUP—PHYSIOCHEMICAL AND BIOPHYSICAL PANEL (at 14 Belgrave Square, London, SW1), at 5 p.m.—Dr E. J. Crisp: "Physicochemical Aspects of the Settlement of Marine Larvae".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 5.30 p.m.—Dr P. R. Thornton: "The Use of the Scanning Electron Microscope in Device Physics".

INSTITUTION OF ELECTRICAL ENGINEERS (joint meeting with the Automatic Control Group of the Institution of Mechanical Engineers, at 1 Birdcage Walk, Westminster, London, SW1), at 6 p.m.—Discussion meeting on "Instruments and the Mechanical Engineer", opened by Mr L. Finkelstein.

SOCIETY OF CHEMICAL INDUSTRY, PLASTICS AND POLYMER GROUP (in association with the Chemistry Department, Birkbeck College, at Birkbeck College, London, WC1), at 6 p.m.—Dr E. Horowitz: "Synthesis and Properties of Co-ordination of Polymers containing Transition Metals".

INSTITUTION OF MECHANICAL ENGINEERS, AUTOMATIC CONTROL GROUP (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p.m.—Discussion Meeting on "Instruments and the Mechanical Engineer".

POLAROGRAPHIC SOCIETY (in Room 111, The University of Surrey, Battersea Park Road, London, SW11), at 6.30 p.m.—Dr D. Inman: "Some Recent Applications of Chronopotentiometry".

## Wednesday, October 4

SOCIETY OF CHEMICAL INDUSTRY, PLASTICS AND POLYMER GROUP (joint meeting with the Manchester Section, at the University of Manchester Institute of Science and Technology), from 10.30 a.m. to 4.30 p.m.—Meeting on "Plastics in Building".

COLOUR GROUP (Great Britain) (in the Physics Department, Imperial College, London, SW7), at 2.30 p.m.—Forty-fifth Science Meeting. Report on the 36th Annual Meeting of the Inter-Society Colour Council in New York—June 1967. Report on the 16th Session of the Commission Internationale de l'Eclairage held in Washington—June 1967.

INSTITUTE OF PETROLEUM (at 61 New Cavendish Street, London, W1), at 5.30 p.m.—Mr K. Reiser: "The Control of Corrosion in Refinery Distillation Units".

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS (at 8-9 Bedford Square, London, WC1), at 6 p.m.—Dr David Pye: "Animal Sonar".

SOCIETY OF CHEMICAL INDUSTRY, OILS AND FATS GROUP (at 14 Belgrave Square, London, SW1), at 6.15 p.m.—Dr F. B. Padley: "The Use of a Flame-ionization Detector to Detect Components Separated by T.L.C."; Dr P. A. T. Swoboda: "Gas Chromatographic Analysis of Food Fats and Flavour".

SOCIETY FOR ANALYTICAL CHEMISTRY (in the Department of Mechanical Engineering, Imperial College, London, SW7), at 7 p.m.—Professor R. Belcher: "Some New Reagents and Reactions in Analytical Chemistry".

## Wednesday, October 4—Thursday, October 5

INSTITUTION OF MECHANICAL ENGINEERS, THERMODYNAMICS AND FLUID MECHANICS GROUP (at 1 Birdcage Walk, Westminster, London, SW1)—Symposium on "Subsonic Fluid Flow Losses in Complex Passages and Ducts".

## Thursday, October 5

ROYAL INSTITUTION (at 21 Albemarle Street, London, W1), at 2.30 p.m.—Professor George Porter, F.R.S.: "Matter and Motion" (Civil Service Lecture).

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 5.30 p.m.—Sir Stanley Brown, C.B.E.: "The Fascination of Electrical Power Engineering" (President's Inaugural Address).

## Friday, October 6

CARWORTH EUROPE (in the Large Lecture Theatre, Physiological Laboratory, Downing Street, Cambridge), at 9.30 a.m.—Second Annual Symposium on "The Interaction of the Laboratory Animal with its Microflora".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 5.30 p.m.—Dr H. P. Williams and Mr A. N. Ince: "The Range of L.F. Transmissions Using Digital Modulation".

SOCIETY OF CHEMICAL INDUSTRY, FINE CHEMICALS GROUP (at 14 Belgrave Square, London, SW1), at 6.30 p.m.—Professor Sir Ewart Jones, F.R.S.: "Biosynthetic Studies on Natural Acetylenes".

## Monday, October 9

INSTITUTION OF MECHANICAL ENGINEERS, EDUCATION AND TRAINING GROUP (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p.m.—Discussion Meeting on "C.E.I. Examination Requirements—a Progress Report".

SOCIETY OF INSTRUMENT TECHNOLOGY (at the Royal Institution, 21 Albemarle Street, London, W1), at 6 p.m.—Sir Henry Jones, K.B.E.: "Sources and Uses of Energy" (Thomson Lecture).

## Monday, October 9—Thursday, October 12

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2)—Conference on "The Economics of the Reliability of Supply—a Comparison of Standards Adopted in Various Countries".

## APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

LECTURER (mechanical engineer or physicist with a good honours degree and preferably some experience in experimental research and an interest in instrumentation) in the DEPARTMENT OF MECHANICAL ENGINEERING—The Registrar, King's College (University of London), Strand, London, WC2 (October 6).

ASSISTANT LECTURER IN PHILOSOPHY—The Registrar, University College, Cathays Park, Cardiff (October 7).

SENIOR EXPERIMENTAL OFFICER or EXPERIMENTAL OFFICER in the DEPARTMENT OF METALLURGY, to take charge of the electron microprobe analysis equipment, and to participate in the departmental research programmes that need microanalysis—The Registrar, The University of Manchester Institute of Science and Technology, Sackville Street, Manchester 1 (October 7).

ASSISTANT LECTURER (preferably with an interest in the field of psycholinguistics)—The Registrar, University Senate House, Bristol 2 (October 9).

CHAIR OF EDUCATION—The Secretary and Registrar, The University, Southampton (October 9).

RESEARCH ASSISTANT (with a degree or equivalent qualification and some knowledge of chemical methods) in the DEPARTMENT OF PHYSIOLOGY, to work with Professor G. R. Hervey on a project concerned with food intake and body composition in animals—The Registrar and Secretary, The University, Leeds, 2 (October 9).

HEAD (with suitable academic qualifications and preferably some industrial experience) of the DEPARTMENT OF MATHEMATICS—The Clerk to the Governing Body, Woolwich Polytechnic, Wellington Street, London, SE18 (October 11).

LECTURER IN THE PHILOSOPHY OF SCIENCE at St. Salvator's College—The Secretary, University of St. Andrews, College Gate, St. Andrews, Fife, Scotland (October 14).

READER (with research interests in some branch of fluid mechanics such as gas dynamics or plasma dynamics) in APPLIED MATHEMATICS—The Registrar, University of Strathclyde, George Street, Glasgow, C1 (October 14).

SCIENTIFIC INFORMATION OFFICER (with a degree in the biological or agricultural sciences and a knowledge of at least one other European language)—The Director, Commonwealth Bureau of Helminthology, The White House, 103 St. Peter's Street, St. Albans, Herts (October 15).

ASSISTANT KEEPER (with some training and interest in invertebrate zoology) in ZOOLOGY at the Manchester Museum—The Registrar, The University of Manchester, Manchester, 13, quoting Ref 138/67 (October 16).

RESEARCH FELLOW (with research experience) in the DEPARTMENT OF Natural History, to join a team of ecologists studying rook populations—The Secretary, The University, Aberdeen, AB9 1AS (October 16).

LECTURERS or ASSISTANT LECTURERS (2) (pure mathematicians) in MATHEMATICS—The Assistant Secretary, The London School of Economics and Political Science, Houghton Street, London, WC2 (October 19).

SENIOR LECTURER/LECTURER IN INORGANIC CHEMISTRY at Rhodes University, Grahamstown, South Africa—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (South Africa and London, October 23).

RESEARCH FELLOWS in the DEPARTMENTS OF PATHOLOGY AND SURGERY, University of Ibadan, Nigeria—The Inter-University Council, 33 Bedford Place, London, WC1 (October 26).

ASSISTANT LECTURER or RESEARCH ASSISTANT in MATERIALS SCIENCE in the DEPARTMENT OF MECHANICAL ENGINEERING—The Secretary of the University Court, The University, Glasgow (October 27).

LECTURER/SENIOR LECTURER IN PHILOSOPHY at the University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (Australia and London, October 27).

CHAIR OF ANATOMY; and CHAIR OF PHYSIOLOGY in the School of Medicine, University of Auckland, New Zealand—The Secretary-General, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (New Zealand and London, October 31).

CHAIR OF PHYSICS—The Registrar, University College of Swansea, Singleton Park, Swansea (October 31).

SENIOR LECTURER (with experience in geological dating or other isotope studies) in the ISOTOPE GEOLOGY UNIT—The Director, Scottish Research Reactor Centre, East Kilbride, Glasgow (October 31).

CHAIR OF VETERINARY MEDICINE at the University of Sydney, Australia—The Secretary-General, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (Australia and London, November 13).

SENIOR PROFESSORS in the SCHOOL OF THEORETICAL PHYSICS—The Registrar, Dublin Institute for Advanced Studies, 10 Burlington Road, Dublin 4, Republic of Ireland (November 30).

ASSISTANT EXPERIMENTAL OFFICER (preferably with experience with chromatographic, electrophoretic and radioactive tracer techniques as applied to nucleic acids and proteins) to assist in chemical and biochemical studies of the structure and multiplication of animal viruses—The Secretary, Animal Virus Research Institute, Pirbright, Surrey.

BIOCHEMICAL ASSISTANT (with a general degree in biology) for research work on metabolism in renal disease—Professor H. E. de Wardener, Fulham Hospital, London, W6.

CHAIR OF ELECTRICAL ENGINEERING—The Academic Registrar, The City University, St. John Street, London, EC1.

MASTER TO TEACH MATHEMATICS to all levels, including Sixth Form and Open Scholarship work—The Master, Dulwich College, London, SE21.

RESEARCH ASSISTANT in the DEPARTMENT OF OCEANOGRAPHY, to work in a team engaged in a three year study of the physical processes of sediment transport in the ocean—The Deputy Secretary, The University, Southampton, SO9 5NH.

RESEARCH TECHNICIAN at the TENOVUS INSTITUTE FOR CANCER RESEARCH, Welsh National School of Medicine for cytodiagnosis—The Pathologist, The Group Laboratory, St. David's Hospital, Cardiff.

SENIOR LECTURERS or LECTURERS (2) (with special interests in analysis geometry or numerical analysis) in MATHEMATICS; a SENIOR LECTURER or LECTURER in STATISTICS; and a SENIOR LECTURER or LECTURER in COM-

**PUTTING—The Clerk to the Governing Body, Northern Polytechnic, Holloway, London, N7.**

**TECHNICIAN** (with G.C.E. 'O' level in at least four subjects which should include English language, mathematics and another science subject, preferably physics, plus at least five years laboratory experience) in the **PAST NEUTRON GROUP** to assist in experimental work concerned with problems of radiation protection and neutron studies in the field of health physics. The Director, Radiological Protection Service, Clifton Avenue, Belmont, Sutton, Surrey.

## REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

### Great Britain and Ireland

**National Institute of Agricultural Engineering.** Experimental Farm Buildings. Report No. 10: A Refrigerated Chilling House. By H. J. M. Messer, G. P. Franghiadi and Dr J. T. B. Sharrock. Pp. 15. (Slusoe, Bedford: British Society for Research in Agricultural Engineering, National Institute of Agricultural Engineering, 1967. Obtainable from H.M. Stationery Office.) 4s. 6d. [108]

**Chemico-Medical Abstracts.** Section D: Endocrinology, Vol. 1, No. 1. Edited by A. W. Saunderson and J. A. Duncan. Pp. 1-74. (D0001-D0304.) Annual subscription: £55; \$155. (London: Derwent Publications, Ltd., 1967.) [118]

**The Library Association.** Special Subject List No. 48: The History of Science and Technology—a Select Bibliography for Students. By K. J. Rider. Pp. 60. (London: The Library Association, 1967.) 20s. (L.A. members 15s.) [118]

**Scientific Principles and Moral Conduct.** By James B. Conant. (The Twentieth Arthur Stanley Eddington Memorial Lecture delivered at Princeton University, 15 November 1966.) Pp. 48. (London: Cambridge University Press, 1967.) 6s. net. [118]

**University of Southampton.** Annual Report of the Appointments Board, 1965-1966. Pp. 20. (Southampton: The University, 1967.) [118]

**BBC Engineering Monograph No. 68:** Recent Research on Studio Sound Problems. Part 1: A Subjective Investigation into Preferred Microphone Balances. By D. K. Jones. Part 2: The Design of a Low-Frequency Unit for Monitoring Loudspeakers. By H. D. Harwood. Pp. 23. (London: British Broadcasting Corporation, 1967.) 5s. [118]

### Other Countries

**Sveriges Geologiska Undersökning.** Översiktskartor med Beskrivningar. Berggrundskarta över Gävleborgs Län. (Map of Solid Rocks of the Gävleborg County.) Södra Bladet, Norra Bladet. By P. H. Lungegårdh. 60 kr. Avhandlingar och Uppsatser. Ser. C. No. 605: Geological Data from the Kristianstad Plain, Southern Sweden. By Kaj Nilsson. Pp. 32+9 plates. 15 kr. Ser. C. No. 610: Lower Viruan (Middle Ordovician) Conodonts from the Gullhögen Quarry, Southern Central Sweden. By Lars E. Fahraeus. Pp. 40 (4 plates). 6 kr. Ser. C. No. 613: On the Genus *Leptylaria* Wedekind 1937. By Erik Norling. Pp. 24 (4 plates). 6 kr. Ser. C. No. 614: The Minerals of the Silver Mines of Hällefors. By Nils Sundius, Alexander Parwel and Benita Rajandi. Pp. 20. 15 kr. Ser. C. No. 619: The Precambrian Quartzite in the Norberg District, Central Sweden, and Its Iron-Sand Bed, with Some Aspects of the Evolution of the Fennoscandian Supracrustals in Central Sweden. By Per Geijer. Pp. 36. 8 kr. Ser. C. No. 620: The Stora Sjöfjärds Iron Ore Deposit, Kaunisvaara, Northern Sweden. By Bo Lundberg. Pp. 37. 8 kr. Ser. Ca. No. 40: Beskrivning till Berggrundskarta över Kopparbergs Län. (Summary Description to Map of the Pre-Quaternary Rocks of the Kopparberg County, Central Sweden.) By Sven Hjeltnqvist. Pp. 217. Karta, 2 blad, med beskrivning, 90 kr. (Stockholm 50: Sveriges Geologiska

Undersökning, 1966 and 1967. Distribution: Svenska Reproduktions AB, Fack Vällingby 1.) [75]

**Board of Grain Commissioners for Canada, Winnipeg.** Grain Research Laboratory—1966 Report. Pp. v+89. (Ottawa: Queen's Printer, 1967.) [88]

**Serial Atlas of the Marine Environment.** Folio 13: Distribution of the Euphausiid Crustacean *Meganyctiphanes norvegica* (M. Sars). By John Mauchline and Leonard R. Fisher. Pp. 3+3 plates. \$5. Folio 14: Distribution of North Atlantic Pelagic Birds. By Robert Cushman Murphy. Pp. 4+8 plates. \$8. (New York: American Geographical Society, 1967.) [88]

**Sveriges Geologiska Undersökning.** Översiktskartor med Beskrivningar. Ser. Ba, Nr. 22: Berggrunden i Gävleborgs Län. (Petroleum of the Gävleborg County in Central Sweden.) By Per H. Lungegårdh. Pp. 303. 30 kr. Avhandlingar och Uppsatser. Ser. C, Nr. 615: Die Geologie der Sulidierz-lagerstätte Kalvbacken, Mittelschweden. (Geology of the Kalvbacken Sulphide Ore Deposit, Central Sweden.) By Helmut Hübner. Pp. 85+1 plates. 25 kr. (Stockholm 50: Sveriges Geologiska Undersökning, 1966 and 1967. Distribution: Svenska Reproduktions AB, Fack Vällingby 1.) [8-]

**Annual Report of the Department of Mines and Technical Surveys—Ottawa, for the Calendar Year 1965.** Pp. iv+51. (Ottawa: Queen's Printer, 1966.) \$1.50. [98]

**Commonwealth of Australia: Department of National Development.** Geological Map—Georgina Basin. Sheet 2—North-East, Queensland; Northern Territory. Sheet 3—South-West, Northern Territory. Sheet 4—South-East, Queensland; Northern Territory. 1:250,000 Geological Series Explanatory Notes. Avon Downs, N.T. Sheet SF/53—4 International Index. Compiled by M. A. Randal. Pp. 15. (Parkes, A.C.T.: Department of National Development, Bureau of Mineral Resources, Geology and Geophysics, 1966.) [98]

**Department of Mines, New South Wales.** Memoirs of the Geological Survey of New South Wales. Palaeontology, No. 15: Lower Carboniferous Corral Faunas from the New England District of New South Wales. By John Pickett. Pp. 38+20 plates. (Sydney: Government Printer, 1966.) [98]

**Australia: Commonwealth Scientific and Industrial Research Organization.** Annual Report of the Division of Soils, 1965-66. Pp. 137. (Adelaide: Commonwealth Scientific and Industrial Research Organization, 1967.) [98]

**National Science Foundation.** NSF 67-11: The Prospective Manpower Situation for Science and Engineering Staff in Universities and Colleges, 1965-75. Pp. x+25. (Washington, D.C.: National Science Foundation, 1967. Available from US Government Printing Office, Washington. \$0.30.) [98]

**Canada: Department of Energy, Mines and Resources.** Geological Survey of Canada. Bulletin 158: Hettangian Ammonite Faunas of the Taseko Lakes Area, British Columbia. By Hans Frebold. Pp. 35+9 plates. \$2. Paper 66-39: Descriptions of Devonian Sections in Northern Yukon Territory and Northwestern District of Mackenzie. By A. W. Norris. Pp. v+298. \$1. Paper 67-24: Stratigraphic Sections of Palaeozoic Rocks on Prince of Wales and Somerset Islands, District of Franklin, Northwest Territories. By R. L. Christie. Pp. vi+21. \$1. Geological Maps—Preliminary Series. Map 1-1967: Geological Survey of Canada Field Parties, 1967. (Ottawa: Queen's Printer, 1967.) [98]

**Metropolitan Life Insurance Company.** Statistical Bulletin, Vol. 48 (May, 1967): Infant Mortality in the United States and Abroad. Fatalities in Hazardous Sports. Population and Families. Pp. 12. (New York: Metropolitan Life Insurance Company, 1967.) [108]

**Scientific Reports of the Research Institute for Theoretical Physics, Hiroshima University.** No. 5 (1966): The Theory of Spherically Symmetric Space-Times. By H. Takeno. Revised edition. Pp. 303. (Hiroshima-ken: Research Institute for Theoretical Physics, Hiroshima University, 1966.) [108]

**Commonwealth of Australia.** Department of National Development: Bureau of Mineral Resources, Geology and Geophysics. Report No. 88: Geology of the South-Eastern Part of the Amadeus Basin, Northern Territory. By A. T. Wells, A. J. Stewart and S. K. Skwarko. Pp. 59+11 plates. Report No. 111: Magnetic Mean Hourly Values from Watheroo Observatory, Western Australia, 1951-1952. By P. M. McGregor. Pp. vi+116. Report No. 120: Magnetic Mean Hourly Values from Watheroo Observatory Western Australia, 1957-1958. By P. M. McGregor. Pp. vi+116. (Parkes A.C.T.: Bureau of Mineral Resources, Geology and Geophysics, 1966.) [108]

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